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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

A TYPOLOGY OF THE EFFECTS OF (TRANS)GENE FLOW ON THE CONSERVATION AND SUSTAINABLE USE OF GENETIC RESOURCES

by

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This document was prepared at the request of the Secretariat of the Commission on Genetic Resources for Food and Agriculture, in order to provide background information on the the topic of GMO gene flow which the Commission, at its Tenth Regular Session, when considering the draft Code of Conduct on Biotechnology, had identified as one of the most appropriate topics for further work.

The content of this document is entirely the responsibility of the author, and does not necessarily represent the views of the FAO, or its Members.

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EXECUTIVE SUMMARY

Introduction

At its Ninth Session, the Commission on Genetic Resources for Food and Agriculture "recognized the need to go ahead with the Draft Code of Conduct on Biotechnology as it relates to Genetic Resources for Food and Agriculture, with the aim to maximize the positive effects of biotechnologies and minimize any potential negative effects or risks (…)". At its Tenth Session, when considering the Document, *Progress on the Draft Code of Conduct on Biotechnology as it Relates to Genetic Resources for Food and Agriculture: policy issues, gaps and duplications,* Members of the Commission identified amongst others the field of "GMO gene flow and the question of liability" as one of the most appropriate for further work and decided that it should be taken into consideration when designing the Commission's Multi-Year Programme of Work.

This background study paper gives an overview of current research regarding the full range of possible effects of transgene flow on human health, the environment, the various stakeholders in the food and feed production chain and on the *in situ* and *ex situ* conservation of plant genetic resources for food and agriculture. The study further describes responses that governments and/or stakeholders have made, or which could be envisioned, in order to address some or all of these effects.

This background study paper considers transgene flow according to the effects it may have, as the basis for an objective evaluation of transgene flow and possible ways to deal with it. Chapters II-IV constitute the evaluation of transgene flow and Chapters V-VI are discussions on management and further scientific and policy work that might deal with this flow. In Chapter II, gene flow and transgene flow are placed into a scientific context. This is followed by a typology of transgene flow according to its different effects as biological, social and legal in Chapters III-IV. Chapter V considers transgene containment options. Finally, Chapter VI highlights various priority areas for policy, including technical and regulatory policy.

Gene flow – What is it?

Gene flow is a serious issue in the assessment and management of risks created by genetically modified organisms (GMOs) and the transgenes their genomes carry. There is consensus in the scientific community that genes flow whether they are transgenes produced by recombinant DNA techniques, or genes that arose without human intervention or management. Nevertheless, the flow of transgenes from plants derived from recombinant DNA techniques (GM plants) has specific or special impacts on biology, ecology, agriculture, society and culture.

Transgenes flow with normal reproductive processes; this is called vertical gene transfer. Transgenes also may be transferred by infectious processes using microbial vectors such as viruses; this is called horizontal gene transfer. Transgenes also move when a plant carrying a transgene moves to a new environment, via seeds or propagules.

Gene flow is often discussed in terms of limiting reproductive barriers. The barriers include spacial and temporal proximity conducive to mating, and floral and mating system compatibility to achieve mating. Plants have two types of mating systems, asexual and sexual. Asexual reproduction does not require them to produce or receive pollen. Sexually reproducing plants may be primarily self pollinating or outcrossing. The former use their own pollen or pollen from extremely close neighbors and the latter preferentially use pollen from other populations. In addition, some plants attract animal pollinators while others rely on the wind to move pollen. Likewise, some plants have

well-developed animal networks for distributing their seeds and others disperse seeds using physical vectors such as the wind or waterways. All crop plants have highly specialized animal vectors in the form of humans for distributing their seeds or other reproductive parts.

In biology, barriers are almost always incomplete or spatially and/or temporally variable. Corn seed is transported in viable form from the United States across the Pacific Ocean to New Zealand, where it is now routinely grown and harvested even though it is neither native nor apparently capable of surviving without human assistance. Fertility, as a measure of overall sexual compatibility, can be lost, developed and improved depending on a host of factors both genetic and environmental. Therefore, plants that may normally fall outside of the necessary proximities and compatibilities are not definitively denied access to genes, but they may receive them less frequently (Jenczewski et al., 2003).

Reproductive and physical barriers have their limits when it comes to containing genes and plants. Gene flow is a powerful force for genetic change. Modern commercial agriculture and field trials accentuate the natural power of gene flow because of the enormous scales upon which they are conducted and the constant re-introduction of transgenes and transgenic plants through trade. "Classical theory of evolution points out that gene flow is capable of counteracting other evolutionary forces like mutation, drift and selection. In modern industrial agriculture a stand of maize could contain a million plants, and under these conditions gene flow from transgenic maize to local landraces is expected to be very high. Under these conditions, the rate of incorporation of foreign alleles after hybridization is likely to be orders of magnitude higher than typical mutation rates" (p. 154-155 Serratos-Hernández et al., 2004).

Optimistically, a combination of biological and/or socially constructed barriers may inhibit transgene flow and thus permit a sustainable state of co-existence between GM and non-GM plants. However, the scale of agriculture, particularly with certain transgenic crops, and the scale at which flow can occur, is sometimes enough to overcome large quantitative barriers to gene flow. The limits of reproductive barriers and the scale of the application are critical knowledge for estimating the likelihood that barriers will remain effective.

Possible effects of transgene flow on agriculture, biological diversity and human health

To be considered in this typology, the effect of transgene flow had to be judged to be either unique or specific and not a general effect attributed to GM plants. This focus is consistent with the label "transcendent risks", those that derive from the use of GM plants (Adi, 2006). Every attempt has been made to stay within those criteria. Of course, the perceived border between a general effect of GM plants and one specific to transgene flow may differ between individuals.

The consequences of transgene flow are difficult to generalize. This is because of the variety of transgenes being developed, plants being made transgenic, environments in which GM plants are being introduced, legal systems operating worldwide, and stakeholder motivations. The only generalization that is possible is that transgene flow offers no *intended benefits*. Gene flow may not always be harmful, but it is highly unlikely to offer a fortuitous or designed advantage for those in the biotechnology industry, farmers that adopt GM crops, farmers that choose not to, those who value the present biodiversity of plants and wildlife, or those who monitor GM presence for safety or regulatory reasons. Gene flow potentially undermines the revenue of developers when those who do not buy transgenic seed nevertheless benefit from its agronomic properties. Simultaneously the industry may have increased costs from protecting their intellectual property, or exposure to additional liabilities. Farmers who do or do not adopt GM crops gain nothing from the flow of transgenes to wild relatives or to neighbors' farms. They may even incur liabilities if transgenes do flow. Non-GM farmers also risk losing differentiated market certifications.

Presently, there is too much variability in how gene flow studies have been designed and conducted to permit a ubiquitous power of prediction on the pathways, rates and effects of transgene flow in any particular environment in which a particular crop is growing in a given year. For example, while many studies have been conducted on pollen flow using different configurations of plots and other variables, their use in risk assessment is limited by:

- local variation in environmental conditions including wind currents, pollinator behavior and topography;
- the difficulty of extrapolating results from small-scale, short-term trials to the parameters of general release;
- different criteria for estimating gene flow; and
- limitations in identifying effects on wild relatives.

However, two consensus opinions have formed. The first is that transgene flow is a highly likely event. The second is that each assessment requires a case-specific study.

There is less agreement on the likelihood of gene introgression (Hails and Morley, 2005). Introgression requires additional events to maintain an exotic gene and cause it to enter a pathway where it continues to increase in frequency in either a self-sustaining (e.g., wild) population or within a seed production system. However, once a gene has made the transition from occasional flow to environmentally- (including human)-supported maintenance, the frequency at which it is found in unintended genomes is expected to rapidly increase.*Agriculture*Transgenes that confer an advantage upon a plant are generally expected to introgress in plants growing in environments that reward the trait. Selective advantages can make the plant more competitive or fecund in its current environment, or adapt it to a new environment. An example of the former is a transgene that makes a weed pest-resistant and thus more competitive with crops. An example of the latter is a gene that adapts the plant to more arid conditions and thus allows the plant to colonize new environments. What remains unanswered is how to predict in what environments and in what genomes a transgene, or even a component of a transgene (such as an exotic promoter, intron or selectable marker), might confer a selective advantage. Some genes that are deleterious to one organism can be of use to others; this may only be discovered long after the genes have introgressed.

Whereas conferring a selective advantage significantly increases the probability of flow, it is not absolutely essential. Transgenes may flow because they are physically linked to other genes that confer advantages or by drift. That is, they may increase or persist due to random events. Finally, and importantly for commercialized transgenes, their repeated introduction on large scales can simply sustain them in the wild.

Agriculture

The effects on agriculture could include development of new weeds, loss of genetic resources, loss of valuable agronomic and commercial options and unanticipated or unintended effects on agronomic traits.

Weeds are already a large burden on agriculture, so any transgene flow that augmented this burden would be significant. Wild plants could be converted into more effective weeds by the flow of transgenes from GM crops when those new genes make the wild plant more effective at growing where it is not desired. Similarly, GM crops can become weeds by flow of genes to them from wild plants, particularly if those genes restore the GM crops' characteristics that reduce their dependency on humans. GM crops can also become weeds when they cannot be eradicated at will from agricultural land, perhaps because they have accumulated multiple herbicide tolerances, or frequent

introductions of the same species of plant into the environment contributes to the invasiveness of the species in an environment in which the species previously did not grow (Novak, 2007).

Crop diversity
Considerable effort is devoted worldwide to maintaining a diversity of genetic resources for crop improvement. What genes will be beneficial in the future cannot with accuracy be predicted now. So both *in situ* and *ex situ* collections of wild relatives of present day crops, as well as different cultivars of crops, are secured and managed. The purity of accessions is specifically threatened by unintended gene flow.

Gene flow creates potential heterogeneity of traits. This heterogeneity may compromise management strategies such as the use of refuges surrounding pest-resistant plants. For traits such as pesticides, heterogeneity may promote the evolution of resistance among damaging insect pests.

Wild biodiversity
The effects on wild biodiversity could be a reduction in the number of species on local and global scales. In plants, some existing genes might be replaced by transgenes, or unmodified plants might be replaced by transgenic plants. Both outcomes can cause sweeps of the gene pool and lower its diversity.

Animal biodiversity may be affected by the expression of compounds in plants that have a direct toxicity, allergenicity or anti-nutrient quality in consuming herbivores, or indirectly as they move up the food chain. Diversity may also be affected by the secondary loss of food sources due to the elimination of particular kinds of insects or other animals. Neither of the above effects is special to transgene flow. However, the animals that are affected may be different from those expected due to the movement of the transgene into different populations of plants or the plants into new environments.

Human and animal health
Crops that are being modified to serve as “biofactories” for the production of pharmaceutical products (PMPs: plant-made pharmaceuticals) and industrial chemicals (PMIPs: plant-made industrial products), or altered nutritional value, pose special risks to human and animal health. Such crops may be considered non-food, because they have no history of safe use or because they are expected to be unsuitable as human food. They may also be considered non-food/non-feed, if their use is to be further restricted.

Those risks may carry over to plants that unintentionally receive those transgenes through gene flow. For example, a gene for the production of a vaccine protein may be expressed in a non-GM crop through gene flow, with the same spectrum of concerns surrounding the inclusion of either the original or the hybrid crop entering the human food supply. Alternatively, novel hazards might arise from transgene flow. The expression of a protein in one food crop may be significantly different from its expression in another. This was illustrated using the example of a protein from beans with a history of safe use as human food being a potential allergen when produced in peas (Prescott et al., 2005).

Possible legal and economic effects of transgene flow
A quantitatively new level of legal exposure for “biotech” seed producers and farmers that produce plants and plant products has been created by a combination of new international legal frameworks and the inherent biological capacity of crop plants to mix at all levels of their lifecycles (from pollen movement to co-mingling of seed in silos). As transgenes are the basis of international agreements such as the Cartagena Protocol on Biosafety, their presence and not just their impact is

the level at which they have legal consequences. This creates new challenges for countries that enter into international trade of organisms that are meant to be free of transgenes.

National laws and international agreements allow for transgenes and transgenic crops to be protected as intellectual property (IP) (Rosendal et al., 2006, Tvedt, 2005). IP is an especially potent issue for farmers who do not wish to use transgenic crops or transgenes. "The point is illustrated through the example of property rights in agricultural biotechnology, and specifically Monsanto Canada Inc *v.* Schmeiser. In that case, the Canadian Patent Act was interpreted to bestow expansive IP protection for a molecularly engineered gene, effectively nullifying the farmer's classic property rights in his plants and seeds" (p. 6 DeBeer, 2005).

In addition, many states are bound through international agreements on plant genetic resources and IP, such as the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGFRA, Correa, 2006), the UPOV Conventions of 1978 and 1991 (UPOV, Sechley and Schroeder, 2002) and TRIPS, for Trade Related Aspects of Intellectual Property Rights Agreement (Sechley and Schroeder, 2002).

Those who grow transgenic crops either on purpose or by accident could become exposed to legal actions or market rejections. Those growing crops with transgenes may be prosecuted or sued if they fail to properly acknowledge the seed producer's IP. Liability extends to damages to property, human health, the environment or loss of earnings. This is particularly poignant in light of recent market rejections of some GM crops based on perceptions of an inability to segregate GM and non-GM material.

Long-term effects of transgene spread in the context of internationally enforced IP laws could threaten different agri-ecosystems. These form the basis of traditional and subsistence farming systems which often include reliance on seed saving and sharing.

Managing transgene flow

Some effects of transgene flow have already been realized, such as multi-herbicide tolerant canola in Canada, recurrent discoveries of illegal GM corn in New Zealand, and trade disruptions from mixing regulated GM crops (e.g., Starlink corn) with unregulated crops. Other effects are hypothetical, but plausible. This leaves us with the question of whether some effects, should they arise at all, can be managed to acceptable levels. Using existing transgenic crops as examples, transgene flow may be managed in some environments to a level that meets quantitative safety, legal and cultural requirements. The flow of transgenes can be restricted using physical and/or biological containment strategies, with transgene-based containment a possible future option. Abstinence from the release of transgenes outside of contained laboratories, is another option. It appears at this time that no single containment strategy, other than abstinence, can be considered foolproof, and possibly no combination of methods from all strategies would prevent occasional escapes (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Thus, the reasons for containing a transgene(s) in question will dictate whether containment is an appropriate means to meet ecological and social goals. For example, relying on containment to prevent a particular outcome may not be appropriate when a GM crop contains a transgene that would result in unacceptable human health or environmental outcomes if it were to transfer to wild relatives.

Managing transgene flow is considered by some to be the same challenge as managing the flow of any exotic or other gene that is considered to be a threat to human health, the environment or IP. In terms of the physical and biological strategies for managing gene flow, this view is probably correct. In terms of the types of harms that might result from the failure of management, however,

this view is not generally agreed. In part, it cannot be true for some genes, such as those that produce potent human pharmaceutical agents, dsRNA with the ability to silence human genes, or allergens the genes for which would not be present in the twelve plants that supply 95% of crop-based foods for humans (Adi, 2006). Such genes may never have been part of their genomes or if they had been, would long ago have been eliminated by human selection against such plants.

Uncertainties in gene flow

Uncertainties in gene flow remain concentrated in these areas:

- what will be the harms or benefits that transgene flow may create?
- can management reduce the frequency or impact of any potential harms that may result from transgene flow to acceptable levels for the most dangerous commercialized or field tested transgenes?
- what are the cumulative effects of transgene flow on species, crops, conservation areas, and soci-cultural frameworks?
- what are the consequences of transgene flow when considered in the context of liability laws, international trade and market expectations, intellectual property (IP) rights and differentiated market certification programs?

Conclusion

Future research priorities should focus on key scientific uncertainties about the impacts of transgene flow. These include identifying the characteristics of transgenes that may contribute to their introgression via fitness-enhancing effects and in introgression pathways that do not depend on the immediate selective value of the transgene to plants. This will require a combination of evolutionary and population genetics and researchers with expertise in global genetic change. It is worth stressing the point that introgression is not necessary for some hypothetical harms to derive from transgene flow. Even local, single flow events could cause harm. For example, the escape into another crop of a transgene that makes a human allergen, or the escape of a transgene that makes seeds sterile into an endangered wild relative. Therefore, the focus should remain on the potential for transgene flow for some transgenes, not just the effects of introgression.

Complex and unanticipated effects of gene stacking should be explored. Most importantly, the potentially destructive effects of transgene flow from PMPs and PMIPs on human health and biodiversity require discussion of policies or invention of containment options that will prevent transgene flow.

Future policy priorities should focus on creating a more uniform and constructive approach to distributing the benefits of biotechnology without imposing penalties on those farmers and consumers who chose not to use the products of biotechnology. In particular, changes should be encouraged where necessary to legal systems that place responsibility for containing GM crops on those who sell them and their products.

I. GENE FLOW—WHAT IS IT?

Gene flow is the movement of genes from one location to another (*Figure 1*). In molecular biological terms, that includes the movement of genes into a genome regardless of whether the receiving genome is of the same or different species as the donor genome. In ecological and agricultural terms, gene flow includes the movement of plants (or their propagules) into a new environment.

Plant genes flow from genome to genome by natural and human-engineered vectors (*Table 1*). An example of the former is pollen, and genetic modification is an example of the latter. Genes flow from environment to environment also by natural and human-engineered vectors. An example of the former is migratory birds that expel seeds in their feces, and ships that carry consignments of corn seed is an example of the latter.

Gene flow occurs by natural processes often associated with reproduction. The units of movement are frequently the reproductive components of the plant itself, i.e. pollen and seeds or vegetative

***Figure 1*: Definition of gene flow**.

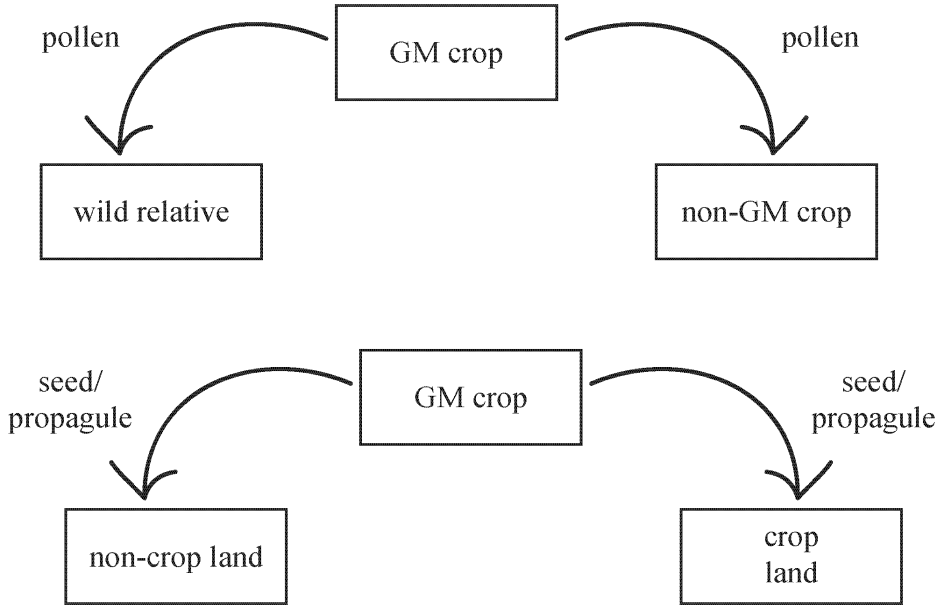

Figure 1: For this report, 'gene flow' is considered as the movement of genes from one location into another. (Top) This movement may be through normal reproductive processes, from the production of gametes, through fertilization to development of offspring, and onto the establishment of a gene in a genetically, legally or culturally distinguishable species or plant line. For GM crops, transgenes may flow to non-GM crops (right) or to wild relatives (left) using pollen, and pollen from either non-GM crops or wild relatives may flow to seeds of GM crops. (Bottom) This movement may also be through vectors of seed and vegetative propagules into new environments. These environments may be agricultural (right) or other lands (left).

propagules (*e.g.* tubers, grafts). However, the reproduction of genes is not always limited to times when organisms reproduce. Gene flow can occur concomitantly with reproduction of organisms or can occur separately. The former is called vertical gene flow and the latter horizontal (or lateral) gene flow. The emphasis in this report is on vertical gene flow according to the definitions of gene flow used by others (*e.g.*, Andow and Zwahlen, 2006, Ellstrand, 2003a, Stewart Jr. et al., 2003a).

While gene flow between organisms is a natural process, the focus of this report is the outcomes associated with transgene flow (i.e., the flow of products of recombinant DNA techniques). Since gene flow is a natural process, conventional (natural) genes also flow and can have genetic and ecological effects. In the case of both recombinant DNA (transgenes) and conventional genes, the

***Table 1*: Examples of plant gene flow vectors**

Gene flow	Vectors	
	Natural	Human-engineered
genome to genome	pollen viruses bacteria hybridization bridges	genetic engineering cell fusion grafting
environment to environment	animals (e.g. birds, bees) moving water wind	contaminated equipment shipping and trade food aid

effects will vary by gene and environmental, legal and social context in which the plant may be found. There also are reported effects that are special to the way in which transgenes are constructed and inserted into a recipient genome (to create a transgenic plant) (Wilson et al., 2006).

In this chapter, pathways of gene flow, gene introgression and plant invasion are introduced and described.

1. PATHWAYS OF GENE FLOW

The pathways of gene flow are:

- pollen-mediated;
- seed/propagule-mediated; and
- horizontal transfer.

By definition transgenic plants carry transgenes in their genome. Those transgenes, like any genes in a genome, are propagated through reproductive processes. When genes flow vertically from parents to offspring, the flow is governed by the rules of meiosis, mitosis, or cell division, depending on the organism that is reproducing and the location of the genetic material in the cell. For example, a gene located in a chromosome of the nucleus of plant cells is sorted by the rules of meiosis during the production of sex cells. The genes from the paternal and maternal parents are distributed according to recognized patterns among the offspring. Meanwhile, genes located in the mitochondria of plant cells, for example, are in many species often only passed through the egg. These genes are not sorted by meiosis, a process that only involves the chromosomes found in the nucleus, and this creates a very different pattern of inheritance in offspring.

The sex cells of plants are the egg and sperm. The sperm is carried by grains of pollen (male gametes) and the egg is part of the embryo sac (female gametes) of the ovule. An embryo is the

product of a fertilized egg and the mature seed develops from the ovule. Both pollen and seed carry one or more copies of the plant's genome, thus each can be vectors of genes including transgenes. It is useful, therefore, to organize the pathways of gene flow predominantly around these two primary vectors.

Genes may also be carried by other types of vectors, such as viruses and the plasmids found in bacteria. The movement of genes via these vectors is an infectious process and thus is not associated with the production of gametes or seeds. The stochastic nature of such gene movements makes them difficult to detect and quantify.

Pollen-mediated gene flow

Genes carried by pollen can flow in two directions, from a transgenic crop to similar conventional crops, other GMO crops and to wild relatives, and from conventional and GMO crops and wild relatives to a GMO crop. The implications of gene flow in both directions are similar if not always identical (Chèvre et al., 2000, Lu, 2003).

Several approaches can be used to attempt to estimate the likelihood of gene flow via pollen. Mechanistic approaches include determining pollen flow and synchronized flowering times. Each approach has a degree of uncertainty (discussed below). Complementing these approaches with estimates of spatial distributions of hybridization or introgression events can help to reduce, but not eliminate (e.g. see Burczyk et al., 2006, Burczyk et al., 2004), the uncertainty.

Pollen-mediated gene flow is influenced by:

- spatial proximity: the GM crop and its sexually-compatible relatives occur within their respective pollination distances;
- temporal proximity: the GM crop and the relative have overlapping flowering periods, to allow the viable material to be released and received, and fertilization to occur;
- floral compatibility: pollen vectors that visit and transfer pollen effectively between both donor and recipient plants are present;
- mating system compatibility and fertility: selfing vs. outcrossing influences characteristics such as pollen production and likelihood of receiving pollen from another population.

The outcome of pollen-mediated gene flow can be a conspecific (parents of the same species, but differentiated by at least the gene of interest) outcross or interspecies hybrid. There is disagreement among specialists on the description of these intermediate types (i.e. the outcross or hybrid population). To avoid terminology debates, usages are here declared.

Outcrossing to hybridization
Fertilization of an egg by the pollen of a plant considered to be of the same or a very close species is called outcrossing (or cross-fertilization). Hybridization results from crosses between plants that are generally regarded as belonging to different species or genera. These two classes are generically referred to in this report as intermediate types.

A broad definition of hybridization is used in this report.

> *"Hybridization can have several different meanings for evolutionary biologists. The term `hybrid' can be restricted to organisms formed by cross-fertilization between individuals of different species. Alternatively, hybrids can be defined more broadly as the offspring*

> *between individuals from populations `which are distinguishable on the basis of one or more heritable characters' (Harrison, 1990)" (p. 600 Rieseberg and Carney, 1998).*

For the purposes of assessing transgene flow, the focus is on the difference brought to genomes by inclusion of the transgene. Therefore, any cross involving a transgenic and conventional plant (wild or crop) results in a hybrid from the view of legally or culturally distinguishable varieties, or individuals distinguishable on the basis of one or more heritable characters. When it aids clarity, near iso-genic relatives that cross-fertilize will be called "outcrosses" instead of hybrids.

Hybridization to introgression
Introgression can be definded narrowly or more broadly. A broad definition of introgression (*Figure 2*) is used in this report.

> *"Similarly, introgression can be defined narrowly as the movement of genes between species mediated by backcrossing or more broadly defined as the transfer of genes between genetically distinguishable populations" (p. 600 Rieseberg and Carney, 1998).*

Some commentators consider introgression to mean the "permanent incorporation of genes from one set of differentiated populations (species, subspecies, races and so on) into another" (p. 806 Stewart Jr. et al., 2003a). The focus of that definition is not on the intermediate type, but on the stable change to one or both parental populations of the intermediate type due to the flow of one or more genes into the parental population via backcrossing with the intermediate type (Newstrom et al., 2003). This endpoint is considered by others to be impractical for the purposes of a typology on the effects of gene flow, both because it is difficult to prove and because some effects of gene flow can appear well before the parental population demonstrates changes. Thus, a gene will be considered to be introgressing at the stage where it is stable during backcrosses between a plant with either of its parental lines or siblings.

While there is agreement that one endpoint of gene flow is introgression (e.g., Cleveland et al., 2005), it is not assumed that hybridization or introgression are the only ways for gene flow to have relevant impacts. Gene flow may have impacts, in contrast to what some authors have argued (e.g., Flannery et al., 2005, Lee and Natesan, 2006), even if viable or fertile offspring do not result. This would be the case if plants modified with certain sterility or fitness mitigation genes were to cross with conventional crops or landraces: the impact would be felt immediately in agro-ecosystems where seeds are saved or shared, or in ecosystems where non-crop plants of cultural or environmental value were harmed, because they might suffer from extremely high frequencies of infertility (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004, FAO, 2001, Moore et al., 2005). Another example is where the intermediate type is unable to reproduce sexually but creates a new population through apomixes, or asexual reproduction.

Spatial proximity
Different plant species have different levels of pollen production, dispersal and rates of cross-pollination, affecting the risk of gene flow. There is a greater possibility of gene flow if potential pollen recipients are adapted to, or grow in, the environment in which the GM crop will be grown (Daniels et al., 2005). This occurs with special concern in a crop's center of origin or centers of diversity (Gepts and Papa, 2003), and for "more common weedy relatives, because there will be closer and more numerous zones of co-occurrence" (p. 11 Jenczewski et al., 2003).

Pollen dispersal by physical vectors will be affected by a number of variables including phenotypic variation, and the speed and direction of wind currents. It will also be affected by the length of time the pollen remains viable (able to fertilize). For example, below 30% moisture content, maize

***Figure 2*: Definition of introgression.**

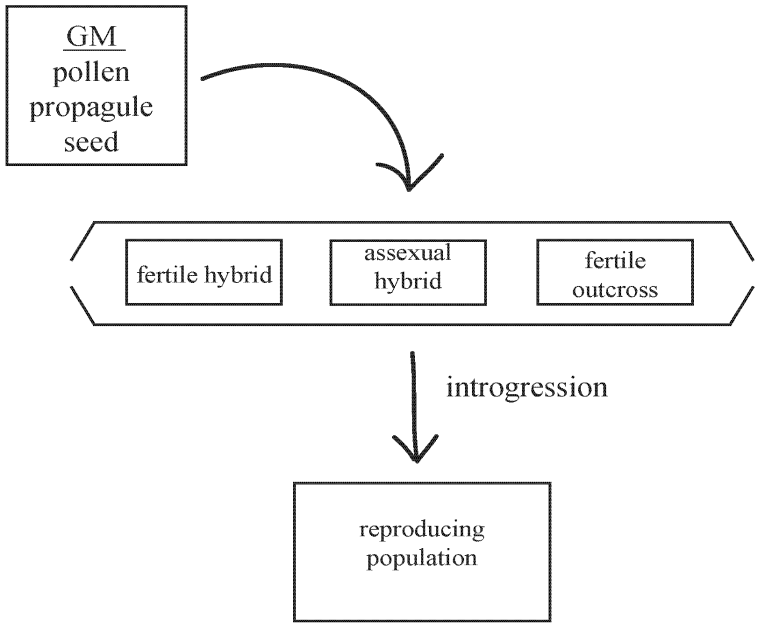

Figure 2: The consequences of gene flow may be the creation of fertile outcrosses or hybrids, or asexual hybrid populations, or death, as could be the case when sterility-causing transgenes flow. At times the units of flow are DNA, chromosomes, pollen, seeds or propagules. One outcome of gene flow may be introgression, beginning with the maintenance of the transgene through backcrosses with parents or siblings to a population of organisms that are sustained by reproduction in the wild or assisted by human activities.

pollen will not germinate (Fonseca and Westgate, 2005). In most studies, pollen longevity overall has ranged from hours to days (Fonseca and Westgate, 2005).

Pollen dispersal from insect-pollinated crops is influenced by the number, type, behaviour and range of pollinators (Gurian-Sherman, 2006). If insects are the primary pollinator, pollen dispersal will vary with location, kind of pollinator and its abundance. Insect pollination will depend on flight paths, which will be influenced by surrounding terrain and flora. Environmental conditions, planting configuration and plant density can affect how many pollinators approach a plant population and how far pollen is carried (Wrubel et al., 1992).

Pollen spread generally has a leptokurtic distribution (*Figure 3*), with most pollen spreading close to the plant, and only a small amount moving over longer distances. For example, most maize pollen falls within about 30m of the pollen source (Devos et al., 2005).

It may occur very infrequently, but long-distance hybridization may allow new free-living populations to establish (Ammann and Jacot, 2003). Creeping bentgrass (*Agrostis stolonifera* L.) pollen-flow was recorded at maximum distances of up to 21km in a study monitoring the flow of

transgenes in the US state of Oregon (Watrud et al., 2004). Most gene flow occurred within the surrounding 2km (Gurian-Sherman, 2006).

The leptokurtic pattern of dispersal makes it effectively "impossible to attain the distance up to which 100% of deposited pollen is contained within, especially when insect-mediated transfer is considered" (p. 32 Flannery et al., 2005). The rate of long-distance hybridization may be significant when the large numbers of transgenic crops potentially being cultivated is considered. Each field can be part of an archepelgo of GM crops in mixed landscapes—increasing the chances of long-distance pollen flow occurring.

While the leptokurtic distribution of pollen is commonly cited, it may not always apply. *Rieger et al.* did not see a leptokurtic decline in pollen dispersal in their study of commercial canola fields in Australia. They surmised that "[t]he multiple pollinating agents (wind and insects) of canola and the large size of the source may contribute to the randomness of long-distance pollination events" (p. 2388 Rieger et al., 2002). Therefore, there may be difficulties in scaling confidence levels for data from small field tests to large farms (Rieger et al., 2002).

A self-pollinating plant uses its own pollen to create seeds and reproduce itself, while a cross-pollinating plant relies on pollen from other plants. When GM crops and their relatives are mostly self-pollinating, there is a lower frequency of hybridization. However, a self-pollinating plant may still cross-pollinate at a low level, so gene flow will remain a concern.

Measuring pollen dispersal
Pollen dispersal may be studied by measuring distances of pollen spread, measuring distances of cross-fertilization, or using mathematical modeling (Devos et al., 2005, Eastham and Sweet, 2002). Measuring pollen spread alone may miss vital parameters such as whether the pollen is viable or silks are receptive, potentially leading to overly high estimates of pollen-mediated gene flow distances (Devos et al., 2005). These problems may be overcome by instead measuring distances at which cross-fertilization has occurred. This may be measured by testing for transgenic DNA or proteins, or by testing for traits, such as herbicide resistance in plants at various distances from a GM crop (Devos et al., 2005, Llewellyn et al., 2007, Yamamoto et al., 2006).

It is not sufficient to simply discount the significance of small amounts of long distance gene flow; instead, "[i]f any hybridization is detected within distances that are typical of those occurring between the crop and the weed, the appropriate interpretation is 'crop genes will move into weedy populations'" (p. 555 Ellstrand et al., 1999).

Pollen dispersal distance and hybridization rates must be considered alongside species inventories. Assessing the risk of gene flow based on surveys of sexually compatible species is difficult, however. Initially sterile (F_1 generation) hybrids can eventually regain fertility, provided that they can continue to reproduce despite the effects on fertility.

Moreover, climate conditions and other environmental variables can have a significant impact on study results. Studies that look at effective dispersal may only give accurate results for one set of climate conditions (Jarosz et al., 2005). As more data emerges on pollen behavior and environmental variables, modeling may be useful to take into account changes in climate and terrain. For example, wind currents can play a significant part in the maximum pollination distances of cultivated rice. Pollen dispersal range increased with wind speed increase, reaching a distance of 110m at a wind speed of 10m/s (Lu and Snow, 2005). Variables could include humidity because as pollen dries it settles more slowly so may travel further (Devos et al., 2005). Variables may be social as well as physical. In the case of gene flow from transgenic cotton in tropical Australia, the

***Figure 3*: Crop/wild gene flow.**

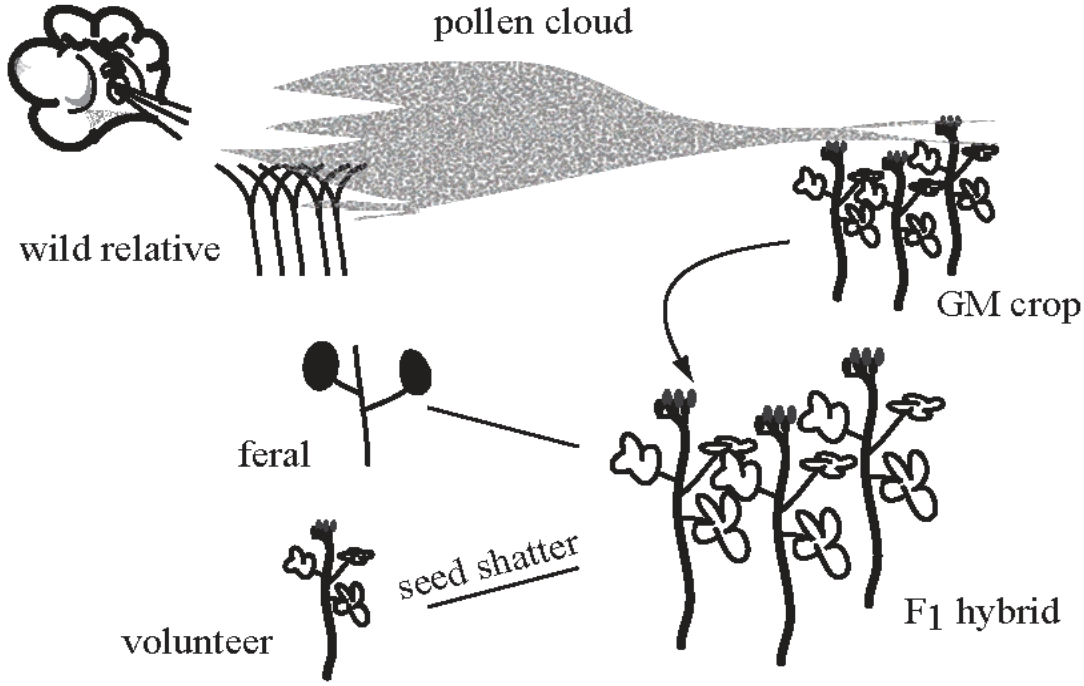

Figure 3: Crop/wild gene flow. Gene flow from cultivated GM crops to wild relatives, and relatives to GM crops, is illustrated. For some crops, volunteers are a significant problem (Ellstrand, 2006).

measured effective buffer zone was influenced by the bee population and variances in that population were due to independent decisions made in other horticultural industries (Llewellyn et al., 2007).

Specific studies of the GM crop in appropriate environmental and agricultural conditions are desirable for risk assessment. In the absence of case-by-case studies, the extrapolation of data to new cases should be done with care.

Temporal proximity
An overlap in flowering times, or phenology, of GM plants increases opportunity for cross-pollination. Wild species with more variation in flowering time are more likely to have flowering dates overlap with the GM crop. The extent of this overlap can indicate the likelihood of hybridization (Jenczewski et al., 2003).

For hybridization via pollination to occur, flowering or stigma-ripening must coincide with pollen production in a GM crop, conventional crop or wild donor. The rate of hybridization may change if the GM crop's phenology (flowering time) has been changed. Genetic modification that alters flowering periods must be considered in the context of other plant species in the surrounding environments.

For some crops, cross-pollination risk is reduced because of agricultural practices. Sugar beet is usually harvested before the crop flowers, because it is grown for its vegetative root. However, a

small risk remains from 'bolters' that flower during cultivation. For other crops, it may be possible to sow GM and non-GM varieties at different times in an attempt to avoid flowering synchrony, although to date, attempts to do this have not been uniformly successful (Devos et al., 2005).

Floral compatibility

Floral compatibility relates to both the preferences of animal pollinators and structural compatibility for crops that are wind pollinated (Rieseberg and Carney, 1998). It is probable that many countries, like New Zealand (Newstrom et al., 2003), lack comprehensive knowledge of overlaps in pollinator preference for transgenic and potential recipient plants and the maximum overlapping ranges of pollinators.

Plants that are wind-pollinated have physical structures that funnel and capture pollen (Newstrom et al., 2003). Wind pollination is described by relatively few parameters, such as wind speed and direction, pollen weight, plant height and the physical terrain or other barriers.

Animal-pollination is described by both physical and behavioral parameters (Rieseberg and Carney, 1998). For example, studies in the early 1990s concluded that the behavior of pollinators was not affected by transgenic oilseed rape and tobacco. However, it is possible that changes in morphological characteristics through genetic modification, such as flower color, could make plants more or less attractive to insect pollinators (Ammann and Jacot, 2003).

Plants that are animal pollinated can sometimes be more resistant to pollen flow from other populations, depending on the strength of the pollinators' preferences for the plant and average travel distance. In Australia, for example, GM cotton may be surrounded by 50m buffer zones of bare ground in an attempt to discourage pollinators from travelling to near-by conventional fields (Llewellyn et al., 2007). The effective resistance achieved by concentrating pollinators within the GM crop can be compromised, however, when hybrids form in zones between two populations and then serve as a bridge for the pollinators (Rieseberg and Carney, 1998).

Ammann and Jacot also argue that some instances of crop genetic modification may encourage pollinators (Ammann and Jacot, 2003). Alfalfa treated with pesticides has been found to retain it in low levels in pollen and nectar, making it toxic to bees. Crops that might use engineered pest resistance that substitute for conventional pest control may have less or no effect on the pollinator and therefore increase the effectiveness of pollinators for entomophilous plants (Ammann and Jacot, 2003).

Mating compatibility and fertility

Mating system, or sexual compatibility is determined by the effectiveness of pre- and post-fertilization barriers (Rieseberg and Carney, 1998). In addition, the mating system determines whether new intermediate types will be sexually compatible and effectively breed with their parental populations or with other potential mating partners (in which the intermediate type forms a "hybridization bridge" (*Figure 4*) between two previously genetically isolated populations). For example, intermediate types could adopt a selfing mating system even though the parental types outcross (Rieseberg and Carney, 1998).

Different authors, agencies and stakeholders use different definitions of compatibility that also produce different assessments of the potential for gene flow hazards. More importantly, there seems to be no precise biological meaning. "It is difficult to predict…the precise limits of sexual barriers between individual crop types and their related species, or the likelihood of hybrids forming and persisting in agricultural or natural habitats" (p. 59 Eastham and Sweet, 2002). From a conservative

viewpoint, the task is to determine if there is enough sexual *in*compatibility to achieve a level of safety.

Pre-fertilization barriers include occupation of common or adjacent habitats, the production of viable and competitive pollen (Rieseberg and Carney, 1998), flowering times, the structure of style and surface of the stigma (Chapman and Burke, 2006, Eastham and Sweet, 2002, Legere, 2005, Newstrom et al., 2003).

Post-fertilization barriers are commonly revealed through hybrid inviability or weakness, sterility and lingering effects, such as initial vitality and fertility that degrades over subsequent generations (Rieseberg and Carney, 1998).

Probably the most effective post-fertilization barrier is meiosis, the process of DNA replication and chromosome sorting that yields gametes. Meiosis requires chromosomes to be "collinear", that is to match as closely as possible their gene order (synteny) and orientation, and overall DNA sequence similarity, to pair and recombine for the process to be completed. Deviations from collinearity can affect meiosis and the plant will exhibit limited fertility or outright sterility (Rieseberg and Carney, 1998). An interesting ramification of this fact is that different chromosomes may be more or less susceptible to incorporating a new gene, and thus gene introgression may be influenced by the

***Figure 4*: Genetic bridges**

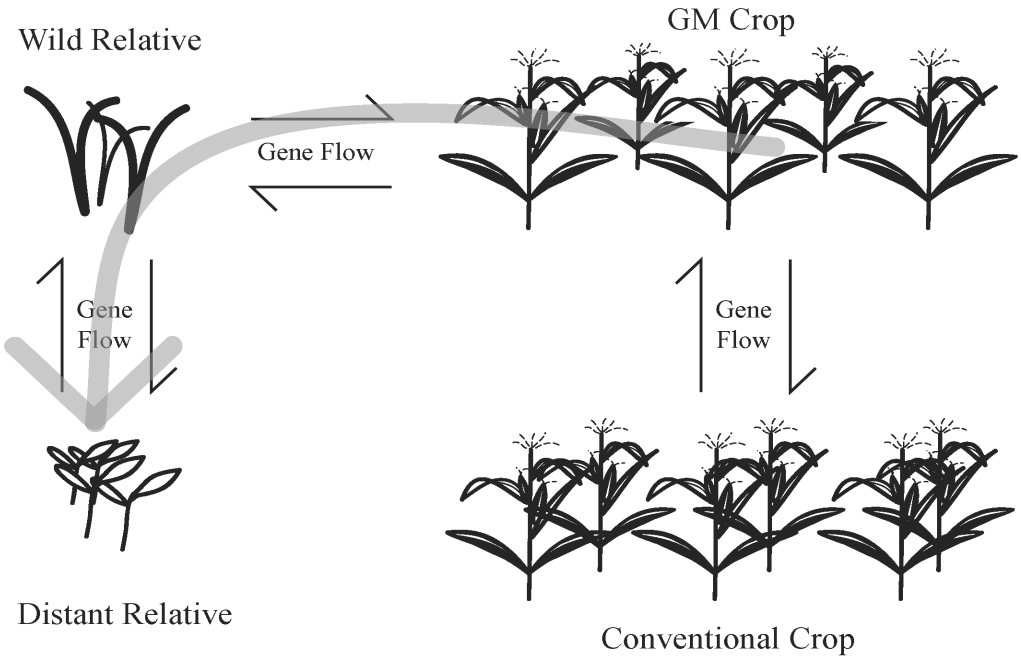

Figure 4: Incremental bi-directional gene exchanges between GM crop plants and conventional crops or wild relatives (single line arrows) may form long-range hybrid bridges that link distant relatives (wild or cropped, grey arrow).

location and structure of a transgene, and particular combinations of transgene donor and potential

gene recipients.

Hybridization can result in a different number of chromosomes being donated by the two different parents (leaving some to be unmatched), or matched chromosomes that are sufficiently different (homeologous) at the DNA level for the pairing process to be inefficient (creating apparently unmatched chromosomes).

While this makes a plant infertile, or reduces fertility, "[i]t is now evident that certain crops (such as oilseed rape) can pass genes to a wild relative even when those genes are carried on unshared (nonhomologous) chromosomes" (p. 542 Snow, 2002). In addition, "[f]ertility can be restored by repeated hybrid backcrossing to parental taxa, which can produce increasingly more-fertile hybrids and result in introgression" (p. 27 Newstrom et al., 2003). So over indefinite amounts of time small hybrid populations can develop into fertile hybrid populations that are capable of spreading via sexual reproduction.

The merger of distinct genomes by hybridization is called allopolyploidy (for multiples of a normal number of genomes). Many current diploids probably have allopolyploid ancestors who returned to sexual compatibility through genome duplication (diploidization of chromosomes) and loss of DNA from various chromosomes.

This process is not just evolutionary. It can occur very rapidly, with the first major steps occurring within a single generation (Ozkan et al., 2001, Shaked et al., 2001).

Throughout this process, near-match, homeologous chromosomes may compete during meiosis with some potentially being displaced from a population. This is illustrated well with reference to a series of reports where transgenic oilseed rape x wild radish hybrids were monitored for multiple generations (Chèvre et al., 1998, Chèvre et al., 1997). First generation female hybrids were found to produce significantly fewer seeds than radishes. However, second generation females produced 10-times more seed and third generation plants produced seed numbers in the same range as wild radishes. Increased fertility correlated with a significant change in the number of chromosomes in the hybrids with each generation, until the number of chromosomes came to align with the wild type radish.

Regardless of how the polyploidy is resolved, the transgene would still have opportunity to 'jump', by recombination or association with a transposon, into another chromosome and thus could spread after a backcross to either of the original parental species (Stewart Jr. et al., 2003a).

Each opportunity, therefore, for fertilization (regardless of whether the cross results in fertile offspring) creates opportunities for gene flow and possibly introgression. Should infertile populations regain fertility, the rate of flow and introgression could rise.

In addition to the molecular barriers to fertility just described, combinations of genes may result in sterility or an increase in fertility. This can be due to separating networked genes that are present on different chromosomes, gene-gene interactions themselves or specific infertility factors (e.g. Baltazar et al., 2005). These types of interactions are not as well understood nor are they comprehensively catalogued in a way that would allow regulators or researchers to predict the fertility of novel hybrids.

Finally, infertility does not rule out eventual impacts nor is it automatically a genetic dead-end. Some species of grasses switch to a form of asexual seed production (called apomixis) that allows them to reproduce even if the hybrids are sexually sterile (Snow, 2002). Some asexually

reproducing hybrids reproduce faster than their sexual parents (Ellstrand et al., 1996). Given enough time, it is possible, through mechanisms such as chromosome doubling (diploidization), that the hybrid will regain sexual fertility. Diploidization and sequence elimination are processes common among plants, and might occur animals, too (Eckardt, 2001, Lu, 2003, Wendel, 2000).

Seed/propagule-mediated gene flow

Seed/propagules or diaspores (these include seeds, fruits, or vegetative units capable of reproduction) are considered in seed-mediated gene flow. Seed-mediated gene flow is influenced by:

- mobility: the seed or the seed bank is mobile. Mobility can establish new point sources of pollen and introduce the plant into a new environment. Mobile seeds/propagules can also compromise temporal barriers when intermediate types have flowering times closer to that of either parental type and produce large numbers of seeds (Arnaud et al., 2003).
- propagule pressure;
- dormancy: the seed persists for long periods before germinating.

Seed and propagule mobility
Like pollen, seeds and propagules have a degree of mobility and a range of vectors. For example, in one study up to 45% of pine seedlings were attributed to seeds travelling over 40 meters (Burczyk et al., 2006). Seeds may be borne by wind, water, animals and human activities (Andow and Zwahlen, 2006, Newstrom et al., 2003).

In most cases, seed and propagule dispersal is leptokurtic, with most spreading close to the source, and only a small amount moving over longer distances (Newstrom et al., 2003). However, the occasional violation of this rule can also be expected. “Together with stochastic founder effects, occasional long-distance seed dispersal, without regard to the extent of geographical separation, also means that gene flow would not necessarily decline regularly as an exponential function of distance from the genetically engineered crop” (p. 1570 Arnaud et al., 2003). For seeds, this is known as the “seed shadow”. The dispersal characteristics of seeds contributes significantly to uncertainty over the contribution of different parental sources of propagules. Seeds may be so small and light, for instance orchid seeds, that wind dominates their dispersal. Some orchids growing in New Zealand are thought to be from seeds originating in Australia that blew across the Tasman sea (Newstrom et al., 2003). The seed bank also can be mobile. For example, seed may be transported by human activities that include soil movements (Arnaud et al., 2003). Thus seed mobility may be competitive under certain circumstances with the distances achieved by pollen.

Seeds may have multiple vectors. It is well known that seeds consumed by frugivorous birds are excreted or regurgitated at varying distances from the parent plant. Some of these seeds may then germinate. Recent work has shown that other animals contribute to a secondary pathway by which such seeds find new locations. These animals not only move the seeds from fecal deposits or the vomit of primary dispersers (*e.g.* birds) to more hospitable locations with lower seed densities, but aid in embedding the seed into the soil. “These secondary seed movements often result in patterns of seedling recruitment that are quite different from the patterns of primary seed dispersal generated by frugivorous animals” (p. 282-283 Vander Wall et al., 2005). Rodents move relatively large seeds, >25mg, a few meters and burry them to a germination range of about 8mm deep. Ants demonstrate a preference for smaller seeds and tend to burry them deep within the nest (Vander Wall et al., 2005). “Caching by rodents does not move seeds far relative to that achieved by bird dispersal, so the colonization of new patches is unlikely to be an important benefit of phase-two dispersal. However, caching places seeds in conditions that often favor seedling establishment. Once cached, seeds are relatively safe from other sources of seed mortality such as ants, beetles,

and birds that act as seed predators. Two-phase seed dispersal has the potential to increase the overall effectiveness of seed dispersal over any single means of seed dispersal" (p. 286 Vander Wall et al., 2005).

Proximity of transgene donors to receptive plants influences the frequency of successful fertilization events, so processes that increase the exposure of non-transgenic plants to transgenic plants influence a risk assessment. The establishment of a plant at some distance from the main population is a point source of pollen from a transgenic parent, and a potential recipient of pollen from non-transgenic plants (as both can produce hybrid or outcrossed offspring).

Progagule pressure
Propagule pressure may contribute to the flow of transgenes. Propagule pressure is influenced by the size and frequency of releases of a species (or a transgene) into a new location (of either seed or propagules). Depending on the magnitude of pressure, transgenes may establish in a new location even if the propagules bearing them are marginally uncompetitive (Lockwood et al., 2005). "Propagule pressure (the number of propagules arriving in the new range) is now recognized as one of the key factors influencing the establishment of nonindigenous species and ultimately whether an invasion occurs" (p. 3671 Novak, 2007). This would also be true of any distinguishable variety, as in the variety of plants within a species that carry transgenes.

Dormancy
The seed bank is also an important reservoir for hybrid transgenic organisms, GM crop volunteers, GM crops with inherently weedy qualities, and GM crops that have gone feral (Legere, 2005). "Seeds may be distributed in time through their dormancy mechanisms as well as in space. The importance of the latter was highlighted recently when an import of conventional rapeseed from Canada to Europe was found to contain traces of adventitious GM material which has not been approved for planting in Europe" (p. 14 Eastham and Sweet, 2002).

Harvesting contributes significantly to the seed bank and extends the temporal distributions of GM crop volunteers and GM crop hybrids or outcrosses. Bean (including kidney, dutch brown, black, white navy, cranberry and white kidney) seed losses during harvest in Canada are estimated at 3-5% (MAFRA, 2002). Oilseed rape losses in the UK are frequently up to 10 million per hectare (IOR-HDRA, 2006).

The seed bank serves as a reservoir, extending opportunity for further flow and aiding introgression. For example, hybrids of GM and weedy rice may "be very difficult to control, because [weedy rice] seeds disperse before the crop is harvested and then accumulate in the soil seed bank. Also, undispersed seeds on weedy rice plants can be collected by farmers and inadvertently planted with the next generation of crop seeds" (p. 672 Lu and Snow, 2005).

Horizontal gene transfer

Horizontal gene transfer is influenced by both biological and human vectors. Vectors of genes between plants and between bacteria and plants are viruses and the soil bacterium *Agrobacterium tumefaciens,* but the process may also be facilitated by parasitic plants and fungi (Heinemann and Bungard, 2005, Hoenicka and Fladung, 2006).

When considering the implications of transgenes flowing from genetically modified plants, both horizontal and vertical pathways are relevant and important. Genes may also transmit between cells of an organism, between members of the same species and between species by a collection of

processes that result in horizontal gene transfer (Heinemann and Bungard, 2005, Heinemann and Traavik, 2004, Syvanen and Kado, 2002).

A virus may enter a single cell of its host, but transfer to multiple cells of the same host independent of cell division. A parent can pass a cold virus to a child that is already born, and a child can pass a virus to a parent. Viruses can cross species, as is demonstrated by the current bird flu outbreak in various parts of the world, and thus are clearly not associated with breeding and the production of offspring. The genes, which in this case are those that define the virus and any genes that the virus happens to acquire from the genomes of its hosts, are reproducing, but the cells, parent and child are not required to also reproduce.

Proteins may be involved in the inter-cellular transport of nucleic acids within plants. The movement of tobacco mosaic virus (TMV) from infected cells to contiguous cells requires a virus-encoded movement protein (MP). One model for TMV inter-cellular transport is that MP binds to and unfolds viral RNA, transports the RNA to, and through, the plasmodesmata (Citovsky and Zambryski, 1993, Weld and Heinemann, 2002).

A. tumefaciens causes tumorous galls to form on dicotyledonous plants. This is achieved by the transfer of a segment of DNA that originates in the bacterium and transfers to the plant, integrating into the recipient's genome (Ferguson and Heinemann, 2002, Thomashow et al., 1980). This bacterium is now one of the two most common tools, along with bioballistics, for making transgenic plants. Other soil bacteria have similar potential to serve as vectors (Broothaerts et al., 2005). DNA can also transfer to monocotyledonous plants and to other bacteria from *A. tumefaciens* (Grimsley *et al.*, 1986, Heinemann, 1991).

Around 10% of all flowering plants (angiosperms) are parasitic, attaching to host plant species from which they extract some, if not all, of the carbon, nutrients and water they require for growth. It has long been known that parasitic plants not only exchange carbon and nutrients with their host but that they also exchange pathogens including viruses, bacteria and phytoplasmas. This intimate relationship may also facilitate gene transfer between plant species. A multigene phylogenetic analysis of the parasitic Rafflesiaceae has shown that some genes from both the nuclear and the mitochondrial genomes are distinct to the order Malpighiales while other mitochondrial genes are closely associated with their obligate host *Tetrastigma*. The Rafflesiaceae are obligate host parasites, meaning that they only parasitize one host species. However, other parasitic plant species like the *Cuscuta* can attach to a broad range of hosts and can even simultaneously parasitize multiple hosts. This raises the possibility that any one host species may be continually exposed to DNA (either naked or within vectors like viruses and bacteria) from multiple, unrelated species. It will not be surprising if introgressions of many genes are due to the long and continuing history of plant-plant parasitism (Heinemann and Bungard, 2005).

For issues special to horizontal gene transfer that will not be directly discussed in this report, the reader is directed to various recent reviews (Heinemann and Bungard, 2005, Syvanen and Kado, 2002).

2. PATHWAYS OF GENE INTROGRESSION

Introgression may not be essential for gene flow to have important environmental, economic, cultural or human health impacts, but the scale and persistence of these impacts can be expected to be much larger if the gene establishes in populations that maintain the transgene despite human intervention.

Introgression is not the automatic outcome of cross-fertilization or hybridization. Even F_1 hybrids that display vigor can produce F_2 plants that fail to thrive (Newstrom et al., 2003, Rieseberg and Carney, 1998). Introgression will be influenced by a number of factors, including the effect the transgene has on fitness in a particular given environment (and this must be determined on a case-by-case basis), the frequency and scale of its introduction into an environment, and on intrinsic factors such as its location in a chromosome. The relative importance of each of these factors is unknown and probably not deterministic. Therefore, the factor that explains an introgression or failure of a gene to introgress may only be identified in retrospect.

Hybrid vigor and genetic assimilation

"F_1 hybrids, particularly between geographic races or closely related species, tend to exceed their parents in vegetative vigour or robustness. This phenomenon - hybrid vigour (heterosis) - is often used to maximize yields in crop plants" (p. 605 Rieseberg and Carney, 1998). Vigor and assimilation can increase the frequency of a transgene in wild or hybrid populations. Hybrid vigor was seen when a transgene that flowed from modified sunflower to wild sunflower dramatically increased the fitness of hybrids as measured by seed production (Snow et al., 2003). As discussed above, fitness advantages will "drive" gene introgression, eroding the purity of existing wild varieties by amplifying those genotypes that benefit from early incorporation of the gene first (Ellstrand, 2003a, Soleri et al., 2006). A fitness advantage can also make hybrids more invasive, driving out parental varieties or species.

Outbreeding depression and demographic swamping

Frequently, however, hybridization results in outbreeding depression (Hails and Morley, 2005). Continual replenishment of the environment with a crop that incurs a cost on hybrids derived from crosses with wild populations could, in time, drive the most frequent recipients of the gene into local decline, perhaps into extinction. Swamping occurs when the rate of flow of a transgene into a population is more important than its effects on the fitness of the hybrids/outcrosses (see "Flow replaces selection" below). In those cases, the gene will increase in frequency and continue to erode the fitness or fertility of the recipient population (Pilson and Prendeville, 2004).

Demographic swamping and genetic assimilation can work together to accelerate the loss of diversity. When swamping reduces the total pollen from wild (non-transgenic) plants, the proportion of pollen from crop plants increases (Haygood et al., 2003).

Selection is usually sufficient to increase the probability of introgression.

The likelihood that transgenes will introgress is greatly increased when there is at least a small advantage to the plant or vector (*e.g.*, virus) that contains the gene (*e.g.* Committee on Environmental Effects of Transgenic Plants, 2002). While saying that the "rate at which genes move into new populations will depend upon the relative fitness of first and subsequent generations of hybrids" (p. 245 Hails and Morley, 2005), may be a simplified overstatement, it is true that few biological factors can interfere with introgression of a gene that contributes to the reproductive success of the plant.

It remains difficult, however, to accurately assign a selective value to any gene or gene element, including transgenes. Thus, the known purpose and function of a transgene may not be easily extrapolated to estimations of its effect in new genetic backgrounds, such as in wild relatives, or the effect it may have on a plant that is in a new ecological context. The concern from a precautionary viewpoint is when selection pressures are underestimated.

Selective value may not be known
Significant advances have been made in bioinformatics through the cataloguing of genes in the genomes of icon organisms and in assigning to them putative biochemical functions, based either on observed activities *in vitro* or *in vivo* or extrapolated from their structural similarities to proteins that have been directly observed. While the explosion in bioinformatics knowledge helps to group genes that have a common activity, it does not identify novel activities in genes for which an activity has never before been associated. Thus, it is possible to overlook an activity of a transgene that will be important for assessing its propensity for introgression (*Box 1*). A gene may have a fitness mitigating effect on one organism but a fitness enhancing effect on another, or may only have a fitness enhancing effect in some environments.

Environment-specificity
Bleeker's study of introgression of genes from *Rorippa austriaca* into *Rorippa sylvestris* showed that different levels of hybrid fitness were environment-specific (Bleeker, 2004). Birdseed rape hybrids carrying *cry* genes taken from plasmids found in *Bacillus thuringiensis*, routinely referred to as Bt crops (de Maagd et al., 2005, Heinemann and Traavik, 2004, Heinemann and Traavik, 2005), were less fit relative to wild relatives under low pressure from herbivory, but significantly more fit under higher pressures (Vacher et al., 2004). Thus, the environment in which the effect of the gene is measured, and not the absolute activity of the gene, determines whether it will spread. For estimates of selective value, case-by-case, environment-by-environment assessments are necessary (Bleeker, 2004).

***Box 1*: New genome, new value.**

All probable gene transmission routes may not be obvious from an analysis of the biology of the organism from which the gene was originally sourced. For example, an analysis of the genome of *Yersinia pestis*, the causative agent of bubonic plague, reveals major portions of its genome have been acquired by horizontal gene transfer. Although not all members of Yersinia have the ability to cycle between insects and humans, *Y. pestis* acquired the ability to transfer from fleas to mammals to cause disease in people. That *Y. pestis* was to become such an effective human pathogen probably could not be foreseen from an analysis of each of the contributing genes considered individually and from knowledge of their activities in the source organism. *Y. pestis* acquired from infectious vectors ((plasmids and transposons) Radnedge et al., 2002) genes that appear to be insect toxins, with activities similar to the Cry toxins of *B. thuringiensis* used in Bt plants (Lindler et al., 1998), that adapt it to its lifecycle in the flea, and genes for causing disease in mammals. It is the combination of insect toxin and other genes that combined to create an effective pathogen of humans (Cole and Buchrieser, 2001). One of the key genes, which allows *Y. pestis* to colonize the flea mid-gut, was probably acquired from a baculovirus (Parkhill et al., 2001).

Underestimating functional diversity
Bleeker's findings are perhaps self-evident given that the purpose of Bt transgenes is to impose a penalty on pests. The outcome may not always be obvious, however. For example, proteins with functions in metabolism may have other functions as well, or may acquire new functions in a new context. Many now agree that the future of GM crops will be dominated not by the agronomic modifications of the past, such as pest resistance, but by metabolic engineering (e.g., Daniell and

Dhingra, 2002). Thus, assumptions about gene function and the strength of selection on transgenes will likely become more important in the near future.

Bhardwaj and *Wilkinson* reviewed proteins with what previously would have been considered to be highly unlikely combinations of activities (Bhardwaj and Wilkinson, 2005, Hall et al., 2004). One of these, a protein called *Arg5,6*, got its name for being part of the biochemical pathway for the synthesis of the amino acid arginine. Mutants of the gene for *Arg5,6* required arginine supplements in their diets unless the mutation was complemented using the DNA for the *ARG5,6* gene (NCBI, 2007); thus the assay unequivocally identified a function of the gene. Using an entirely different assay, a global search for proteins that bind DNA, Arg5,6 was isolated as a DNA binding protein. Further, the protein bound very discreet parts of the genome. A re-analysis of *Arg5,6* gene mutants also found that expression of the genes corresponding to the DNA binding preferences of *Arg5,6* was changed, thus identifying *Arg5,6* as a transcriptional regulator. Thus the assay again unequivocally identified a gene function. The extremely different functions identified depended on the assays used.

> *"Our concept of genes and proteins, and of their functions has evolved over the past 50 years. Initially, the simple idea of 'one gene→one protein→one function' was embraced by most biologists. Then the discovery of alternative splicing and alternative translation initiation led to the idea of 'one gene→multiple proteins→multiple functions.' Now it is clear that some genes follow another paradigm: 'one gene→one protein→multiple functions'…We speculate that, because several metabolic enzymes are known to bind RNA, it would not be surprising if there were also many metabolic enzymes that bind DNA and regulate transcription" (p. 469 Bhardwaj and Wilkinson, 2005).*

This raises other challenging questions. Transgenes in existing GM crops, and those likely in the near future, are composed of "on average, eight genetic elements derived from viruses, bacteria, or plants that are not sexually compatible with the target crop" (p. 421 Rommens et al., 2004). While most of those genetic elements are not associated with a particular protein product in the source organism, their range of functions in the recipient organism may be different. When these elements integrate separately into the genome of the recipient, which is common during plant transformation (e.g. Makarevitch et al., 2003, Svitashev et al., 2002), each may become a unit of selection in some hybrids, in some environments, at some time.

Currently, there is discussion of using transgenes isolated from plants rather then using transgenes from very different kinds of organisms, such as bacteria. This type of genetic engineering is speculated to have fewer of the potential impacts of sourcing transgenes from different species (Nielsen, 2003, Rommens et al., 2004). The source of the gene, however, is also not always sufficient information to assume that using it as a transgene will have the same risk spectrum as the gene in its natural form and location. Even products of the same gene in the same genome but localized to different parts of the cell through genetic engineering might produce different metabolic products (*Box 2*).

In addition, genes considered to have a single biochemical activity can have unknown effects on physiology. The gene *Atwbc19* from the plant *Arabidopsis* confers resistance to the antibiotic kanamycin, and therefore is considered as a desirable alternative to using genes with the same function, but sourced from bacteria, to select transgenic plants. The protein is an ABC transporter, probably used by the plant to transport a variety of normal toxins out of the cell (into the cell vacuole). That *Atwbc19* protein was a kanamycin resistance factor was neither anticipated nor predicted, but found by accident when a mutation in the gene made *Arabidopsis* particularly susceptible to killing by kanamycin (Rommens). This example illustrates that knowing a

biochemical activity conferred by a transgene is not the same as knowing all biochemical functions that may be conferred by the transgene, and thus predictions about its value in the general environment may be highly inaccurate.

Effects of context and concentration also apply to the concept of intragenesis or cis-genics, proffered as a way to ameliorate concerns arising from the use of trangenes (de Cock Buning et al., 2006, Schouten et al., 2006b). Cis-genics is where the "transgene" is sourced from DNA already within the germplasm (that accessible by breeding) of the modified plant (Schouten et al., 2006a, Schouten et al., 2006b). There is debate about whether such approaches would make products more similar to traditional breeding or to the products of recombinant DNA techniques which are behind the term transgene (de Cock Buning et al., 2006, Schubert and Williams, 2006), at least as far as assessing the product for human food. Missing in this debate, however, are considerations of the flow-on effects that might derive from gene flow where the cis-genic construct enters new and unanticipated genomes because of: 1. the human-engineered scale and frequency of the release of the cis-genic plants; 2. propagule pressure; and 3. location of a cis-gene on a chromosome that is more likely to introgress than the chromosome upon which the original gene resides. Wherever this highly terminological debate comes to rest, context and concentration, two variables directly affected by gene flow, will remain important considerations for cis-genes.

Selection may not be necessary for introgression

It is tempting to argue that a fitness advantage is necessary for introgression. But while selection favoring a transgene may be sufficient to expect introgression, it may not in all cases be necessary for it. Genes may persist and even introgress even if they are neutral or deleterious. The

***Box 2*: Function varies with location**.

The characteristics of transgenes that make them desirable are first noticed in the context of the original donor organism. Those characteristics are not necessarily transmitted by the DNA alone. A study by *Zhang et al.* demonstrated that a single metabolite difference between cells can result in changing the properties of a protein (Zhang et al., 2004). Another study has demonstrated that the location of the protein, that is, what species it is expressed in or whether it is in the cytoplasm or chloroplast, can also dramatically change its properties (Heilmann et al., 2004). "The functionality, substrate specificity, and regiospecificity of enzymes typically evolve by the accumulation of mutations in the catalytic portion of the enzyme until new properties arise. However, emerging evidence suggests enzyme functionality can also be influenced by metabolic context" (p. 10266 Heilmann et al., 2004).

Heilmann et al. found that the protein product of a transgene can have different biochemical properties in different transgenic organisms. When yeast were engineered to express enzymes called desaturases originally sourced from the Arabidopsis plant, they produced fatty acids of a different composition and in different proportions than when the same enzymes were produced in Arabidopsis.

They also found that the protein product of a transgene can have different biochemical properties in different parts of the same cell, a property called regiospecificity. "While characterizing the Arabidopsis desaturase (ADS) enzyme family, we discovered that ADS specificity could be controlled by alternate substrate presentation in different subcellular compartments [cytoplasm and chloroplast] rather than by changes to the catalytic portion of the enzymes" (p. 10266 Heilmann et al., 2004).

mechanisms for this are well known and include genetic drift, founder-effect and linkage.

Some genes spread by horizontal gene transfer despite their effects on the organism, but then later may adapt the organism to a new or changing environment. This has been the lesson learned, for example, from the spread by viruses of new virulence determinants used by bacteria, and the success of antibiotic resistance and so-called post-segregational killing (psk) genes in bacteria (Cooper and Heinemann, 2000, Cooper and Heinemann, 2005, Heinemann, 1999, Heinemann and Bungard, 2005, Heinemann and Silby, 2003). The psk genes are actually two-gene cassettes with one gene producing a cell toxin and the other an antidote. Infectious elements, such as plasmids, use them to ward off competitors and thus the genes quickly accumulate on genetic parasites.

Linkage
A gene of neutral or even deleterious effect on the plant may still persist or even increase in frequency if it is linked to a gene that is introgressing. An example of this scenario was discussed by the Committee on Environmental Effects of the Trangenic Plants of the US National Research Council. In their scenario, cotton engineered for tolerance to the herbicide bromoxynil is managed by the use of the herbicide. Weeds that acquire the tolerance gene and a closely linked pesticide gene (Bt) increase in frequency due to clearing of competitors through the farmer's use of the herbicide (Committee on Environmental Effects of Transgenic Plants, 2002). Selection action on bromoxynil tolerance amplifies both transgenes simultaneously even if Bt offers no selective advantage.

Flow replaces selection
If the source of a gene is kept artificially high, it may spread despite being neutral (Andow and Zwahlen, 2006). "Population genetics theory has demonstrated that even very low levels of gene flow (two successful pollinations per generation) are sufficient to maintain a *neutral* crop allele at substantial frequencies in a natural population" (p. 133 Committee on Environmental Effects of Transgenic Plants, 2002).

Even deleterious genes may be maintained under cropping conditions. "Selectively neutral transgenes could reach significant frequencies in a wild population as a result of gene flow. In fact, even slightly deleterious transgenes could become established in a wild population if gene flow is high" (p. 109 Lee and Natesan, 2006). The reason is the persistent re-introduction of the gene into the environment through the cultivation of GM crops (Haygood et al., 2003, Pilson and Prendeville, 2004), and the relative scale of GM crop cultivation, currently estimated at nearly 100 million hectares (Bhalla, 2006).

The absence of known selective values is not sufficient information upon which to draw general conclusions of safety when gene flow is thought to be a likely mechanism for distributing a transgene that might cause health or environmental harms. Even genes known to have deleterious effects can be expected to flow under some conditions, possibly to introgress, into wild plants or non-GM crops.

3. PATHWAYS OF PLANT INVASION

"Biological invasions are believed to be the second largest cause of current biodiversity loss, after habitat destruction" (p. 164 Keane and Crawley, 2002). The outcome of plant invasions is exotic, naturalized populations or weeds that may cause extinction of species in extreme cases, alter ecosystem processes such as nutrient cycling, and reduce economic and agricultural productivity (Novak, 2007). GM plants and intermediate types formed from crosses between GM and other plants that can both invade and persist in an environment where they are not desired may become weeds.

Weed risk assessment is limited because predicting when a plant, or what kinds of plants, will be invasive and then become weeds is difficult (Novak, 2007). Past attempts (e.g. Baker, 1974) should be used only as very rough guides (Rogers et al., 2007). Assessing the potential for weediness involves extrapolation from previous experience with the species in different environments and situations (Rejmanek, 2001). For example, an important factor in many plant invasions has been their release from predation by a herbivore (Keane and Crawley, 2002). This so-called "enemy release" can be achieved by transporting the plant to a new environment that lacks specific herbivorous enemies (e.g. eucalypts in New Zealand as discussed by Withers, 2001), or by making the plant resistant to herbivory through the expression of pesticides (e.g. Bt plants). While caution should be taken when extrapolating information and applying it to a new case, knowledge of previous introductions of species in other places can be the best indicator of potential invasiveness (Committee on Environmental Effects of Transgenic Plants, 2002). Identifying instances of the species spreading can suggest that the GM crop may be predisposed to weediness (Newstrom et al., 2003, Reichard, 2001).

4. PROPAGULE PRESSURE

Invasion begins when a propagule (either a seed or a tissue for plants) is transported to a new location. Under natural conditions, the immigrant propagule will often die during transport or in the new environment with its different combination of competitors and biological and physical conditions. However, commercial distribution of GM plants introduces human-caused propagule pressure because the propagules are transported in large quantities through trade, and are repeatedly introduced into the new environment.

New research suggests that the single most important factor behind past invasions may be propagule pressure created by recurrent introductions (Lavergne and Molofsky, 2007, Novak, 2007). "Multiple introductions can result in nonindigenous populations with similar or even greater genetic diversity (and evolutionary potential) than native populations. Thus, investigations of evolutionary aspects of biological invasions must take into account the introduction dynamics of an invasive species, which directly influences the amount and distribution of genetic diversity of a species in its new range" (p. 3671 Novak, 2007).

This is the case for the perennial grass *Phalaris arundinacea*, which has successfully invaded North American wetlands from its native Europe. Recurrent introductions contributed to invasion by helping relatively small founding populations to access adaptive traits. Continued transport of transgenic plants into environments incubating founder populations of plant invader species derived from similar earlier introductions of GM plants has the potential to accelerate the process. As Novak (2007, p. 3672) observed: "multiple introductions, even of species that are already present in a new range, should be avoided because the new immigrants might contribute to invasiveness."

Traits that may influence invasiveness include fertility, vegetative vigor, tolerance of a wide range of environmental conditions, the quality and dispersal range of viable material and persistence (Lavergne and Molofsky, 2007). The latter describes a plant's tendency to remain in the soil over time. A plant displaying persistence is difficult to eradicate from an area once it is planted. The growth of volunteer plants can indicate a high degree of persistence.

In addition to uncertainties of predicting if a plant will become a weed, there are uncertainties about whether a plant is already a weed. How long a plant takes to become a noticed weed is referred to as the "lag time". Lag times can vary from months to hundreds of years, depending on many and unknown factors relating to the plant species and environment. *Wolfenbarger* and *Philfer*

catalogued the time lag between introduction of 18 exotic trees into Germany and the start of a confirmed invasion. These times ranged from 29 to 415 years (Wolfenbarger and Phifer, 2000). "The lag-time factor has very important implications for the evaluation of risk factors related to forest trees" (p. 132 Hoenicka and Fladung, 2006).

5. SCALE OF RELEASE

The scale of the release of the GM crop can also add to difficulties in assessing the potential for gene flow (Groot et al., 2003). Scale is a multidimensional issue. It refers to the size of a release, but also the pattern (frequency) of areas that harbour the plant of interest. Scale can also include mass effects, where larger populations differentially subsidize small populations.

Unfortunately, the sophistication of modelling scale effects is limited by the number of appropriate experiments that can be drawn upon. "Most field-based and theoretical studies of gene flow have been limited to simple source–sink spatial arrangements due to the difficulty of considering complex spatial configurations that may in fact more accurately reflect real field scenarios" (p. 47 Newstrom et al., 2003). Landscape configurations are not necessarily accurately integrated into small scale tests and therefore cannot be measured, and their effects extrapolated, to large scale predictions. Landscape takes into account the way crops are cultivated; the size, shape and density of fields may have an effect on hybridization and outcrossing. In studies where the recipient field is elongated and the longer side faces the pollen source, cross-fertilization can double. This rise can be avoided if the field is made deeper so that more plants are further away from the source (Devos et al., 2005).

Scale issues affect estimates of pollen-mediated gene flow. The greater the number of plants, the greater the amount of pollen being produced, increasing the likelihood of successful fertilization, and the likelihood that long-distance gene flow may occur. Large-scale release also constrains containment options. For instance, measures such as flower covers to prevent pollen dispersal are impractical for individual plants at the scale of some trials, let alone commercial agriculture (Gurian-Sherman, 2006).

But also asymmetries in gene flow potential are created by asymmetries in the size of source and sink populations. The extrapolation of pollen dispersal or cross-fertilization measurements taken from small-scale trials to general GM crop release should be done with caution, as "such a design does not reflect the real agricultural situation and is not suited to quantify the cross-fertilization levels of recipient fields of commercial size" (p. 74 Devos et al., 2005). For example, successful cross-pollination can depend on the amount of competing pollen generated by the recipient crop. Larger fields will have larger pollen clouds overhead, acting as a competitor to pollen spreading from neighboring fields. The larger the field, the more impact this pollen cloud will have at minimizing cross-fertilization (Devos et al., 2005). The further into the center of a field, the more dominant and protective a maize crop's pollen cloud becomes (Burris, 2003). In contrast, a small sink may be disproportionately vulnerable to the pollen from a large field (Newstrom et al., 2003).

The landscape distribution of transgenic plants can also influence animal pollinators. While the general characteristics of pollen flow can be described from knowing the pollinators' average ranges and preferences, occasionally animal pollinators will move much further than average in what is called "jump" dispersal. The configuration of donor and recipient populations may accentuate the impact of jump dispersal.

Similarly, large outputs of commercial seed being distributed through national distribution centers and international trade will also dramatically increase the complexity of estimating gene flow. The

recall of *LLRICE 601* and Starlink *adn BT10* corn in recent years after findings of global distribution (discussed later in this report) serve as examples.

Scale can also affect introgression of a gene between species and within species. The frequency of a gene can increase by a number of mechanisms such as selection and genetic drift. For example, the simple action of genetic drift can maintain many traits in a population by a random nature, which is more powerful in small population than a large one. If the exotic gene does provide a benefit to the recombinant organism, then it is expected to increase in a deterministic fashion, at a rate proportional to reproductive time and the strength of the advantage relative to balancing factors.

6. CONCLUSION

The above are not requirements for gene flow, but they may significantly affect the frequency of flow and probability of introgression (Jenczewski et al., 2003). Gene flow is a powerful force for genetic change. Modern commercial agriculture accentuates the natural power of gene flow because of the enormous scales upon which it is conducted, and because it provides an avenue for frequent re-introductions of transgenic material and transgenic plants.

The frequency of the flow will vary as a function of pollen characteristics, breeding styles, proximity between donor and recipient plants, coordination of their sexual characteristics, seed dormancy and mobility, human activity and a host of factors that are only beginning to be addressed.

Presently, there is too much variability in how gene flow studies have been designed, and in what they measure, to draw quantitative conclusions about flow and fertilization rates. For example, while many studies have been conducted on pollen flow using different configurations of plots and other variables, their use in risk assessment is limited by:

- local variation in environmental conditions including wind currents, pollinator behavior and topography;
- the difficulty of extrapolating results from small-scale, short-term trials to the parameters of general release;
- different measurements of GM adventitious presence; and
- limitations in identifying effects on wild relatives.

However, consensus has formed on the topic of gene flow and that consensus is that transgene flow is a highly likely event.

There is less agreement on the likelihood of introgression (Hails and Morley, 2005). Introgression requires additional events to maintain an exotic gene and cause it to enter a pathway where it continues to increase in frequency in either a self-sustaining (e.g., wild) population or within a seed production system. However, once a gene has made the transition from occasional flow to environmentally-supported maintenance, the frequency at which it is found in unintended genomes is expected to rapidly increase.

What remains unanswered is how to predict in what environments and in what genomes a transgene, or even a component of a transgene (such as an exotic promoter, intron or selectable marker), might confer a selective advantage. Some genes that are deleterious to one organism can be of use to others.

Research is therefore needed on gene introgression processes. This will require a combination of evolutionary and population genetics and researchers with expertise in global genetic change.

II. POSSIBLE EFFECTS OF (TRANS)GENE FLOW ON AGRICULTURE, PLANT AND ANIMAL BIODIVERSITY AND HUMAN AND ANIMAL HEALTH

Transgene flow, just like gene flow, could have good, adverse or benign effects as measured from biological, agricultural, social or cultural perspectives. This chapter is divided into three parts corresponding to an analysis of the possible effects of transgene flow on agriculture (including crop diversity), wild plant and animal biodiversity and human and (domestic and wild) animal health.

Effects of transgenes that are unique or special to their flow are discussed in this chapter. Effects that are generic to GM plants are considered outside the scope of this typology. As an example, the effects of Bt toxin genes are included from the viewpoint that their expression in plants that were not the primary product of genetic engineering and testing creates potential unintended effects that derive specifically from gene flow. On the other hand, general non-target effects have not been reviewed. Likewise, while there is a literature on impacts of certain GM plants on soil and gut microbes, there is no identified unique or special impact on soil microbial biodiversity from the flow of transgenes between plants. Moreover, the ubiquitous distribution of microbes and the enormous numbers of species (not to mention individuals) (Whitman et al., 1998) makes it difficult to determine whether gene flow between plants would significantly affect the range of microbes that would be exposed.

The determination of whether an effect is unique or special to gene flow can be a matter of judgement. No typology on the effects of transgene flow is likely, at this time, to be universally agreed.

1. AGRICULTURE

Key concerns for agriculture are the generation of new weeds or more fit weeds, loss of genetic resources, loss of valuable agronomic traits, and biological and legal challenges (see Chapter III) of coexistence between GM and conventional agriculture.

Weeds

Concerns of gene flow with respect to weediness are augmenting a wild species' ability to become a more effective weed and a GM crop or hybrid derivative's ability to become a more effective weed.

Weeds are often the wild or feral relatives of crop plants. "Compatibility between crops and their weedy populations is relatively high, because the weedy populations are usually derived from volunteers of the crop species, or from offspring of hybrids between crops and their wild relatives" (p. 3 Lu, 2003). For some plants, such as sunflower, squash and radish, a different variety of the same species may be known as a weed. For others, wild and/or weedy relatives are distinct species, such as wheat (compatible with jointed goatgrass), sorghum (compatible with johnsongrass) and oilseed rape (compatible with field mustard) (Snow, 2002).

Crops growing where they may not be wanted are also weeds (*Box 3*). This can occur when volunteers of one variety appear in fields of another variety or species of crop, or when feral derivatives of crops with weedy characteristics compete with the variety under cultivation. Volunteer plants grow from seed or vegetative propagules left in fields post-harvest.

For example, volunteer growth is a problem in oilseed rape fields because the seed is very small and easily spilled. It can remain in the seed bank or be spread by harvest machinery and trucks, creating

feral populations. Some studies have reported that up to 3000 viable seeds per square meter are lost to the seed bank from oilseed rape fields each year. This amounts to an average of 20 times the amounts added to the field each seeding season (Gulden et al., 2003).

Volunteers have important costs too. "Volunteer wheat and barley, at 7 to 8 plants/m^2 (6 to 7/yd^2) can reduce canola yield by 10 to 13%" (Canola Council, 2007). In the UK, oilseed rape, sometimes alternating with peas, is grown in rotation with wheat and barley, serving as a "break crop" (Senior and Dale, 2002). Crop rotation is in part a weed management practice. Weeds that prosper with the cereals can be eliminated during the break using alternate herbicides. However, if break crops give rise to multiple herbicide-tolerant volunteers by acquiring tolerance transgenes by gene flow, or

***Box 3*: Crops as weeds**

Sequential cross pollination between varieties of *Brassica napus* (oilseed rape, canola) in Canada has resulted in spontaneous triple-resistant variants (Hall et al., 2000). By 2000, 80% of Canadian canola was transgenic (Senior and Dale, 2002). Herbicide-resistant oilseed rape (canola), for which four resistance transgenes are available, has been used commercially in Canada since 1995 and came to be 51% of the 5.2 million hectares of the crop grown there in 1998 (references in Hall et al., 2000), around the time the first multiple herbicide resistant hybrids, attributed to transgene flow, were detected.

Canola is both a crop plant and a weed. Its volunteers are considered as "among the 20 most common weeds in Alberta fields, occurring as a residual weed in 11.8 and 10.5% of all wheat and barley fields surveyed in Alberta in 1997, respectively" (p. 688-689 Hall et al., 2000). Volunteers can emerge for up to four years after the last planting (Legere, 2005). Interestingly, "a single triple-resistant individual was located more than 550 m from the putative pollen source 17 mo after seeding" (p. 694 Hall et al., 2000), a distance over 5 times the recommended buffer zone. Herbicide tolerance in *Brasscica napus* can significantly reduce options for weed control. Volunteer herbicide-tolerant crops are likely to be expensive to eradicate after harvest (Smyth et al., 2002).

weeds acquire herbicide tolerance from break crops, then management becomes more complex and expensive (Pretty, 2001).

Weeds can lower the nutritional value of the crop or introduce undesirable toxins or allergens whether they are natural or from GM plants. The concern would be for transgenes that might accentuate the characteristics of weediness should they flow into a wild or feral relative or conventional crop. Alternatively, a GM crop may acquire genes for weediness from other plant populations. Volunteers arising from the seed of past seasons can be both weeds and reservoirs of transgenes, creating new opportunities for gene flow.

A GM crop plant that has acquired traits from wild relatives by gene flow may become a weed if the new characteristics make it more independent of human management or more difficult to eliminate from seed stock or fields (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Maize, for example, appears to be more receptive to wild teosinte pollen than vice versa (Baltazar et al., 2005). "Gene flow between feral crop populations and transgenic crops may create weeds that bear adaptations derived from the feral plants—such as seed dormancy—that suffice to produce new invasive-plant hazards in an agroecosystem or beyond" (p.

69 Committee on Environmental Effects of Transgenic Plants, 2002). A plant that is adapted to a novel environment (e.g. through a drought tolerance transgene) or directly released from the effects of a pathogen (e.g. virus resistance transgene) or predator (e.g. insect resistance transgene) may also become a weed.

Traits that may influence persistence include seed dormancy and germination, tolerance toward environmental conditions, and growth and resilience (Rissler and Mellon, 1996). Important weeds, such as charlock (*Sinapis arvensis*) in the UK, have seeds that can persist in soil for up to 35 years (IOR-HDRA, 2007). This observation is important, because a hybrid of charlock and herbicide-tolerant oilseed rape was detected in a large UK study. While the seeds of the hybrid did not germinate, the pollen of the plant was not tested for the transgene (Daniels et al., 2005). Thus, transgene flow could potentially make this important weed herbicide-tolerant.

Weeds and invasive plants are a serious problem which transgene flow could make worse. Invasive plants and weeds are already important contributors to lost productivity and producer profits, especially when the costs of weed control are considered. "Invading plant species cause damage both economically and ecologically. For example, in the USA alone, environmental damage and crop losses totalled an estimated US$138 billion in 1999" (p. 10 Stokes, 2001). The cost of weeds has been estimated separately at US$20 billion in the United States (reported in Basu et al., 2004) and A$3-4 billion in Australia (Sinden et al., 2004). In Korea, 5-10% of rice yield is lost to weedy rice (Chen et al., 2004). The DuPont Company estimates that without some form of weed control "the average crop losses for U.S. corn, soybean and cotton growers would be approximately 65%, 74% and 94%, respectively" (DuPont, 2007).

"Worldwide, between 67 and 104 plant taxa are responsible for 90% of the economic damage caused by weeds, so most crop losses are caused by [a] few species" (p. 391 Basu et al., 2004). These, of course, would be species of concentrated concern should genes for herbicide tolerance transfer into them from GM crops. Such events could also drive up the costs of weed control as new and possibly less environmentally friendly herbicides (Reddy, 2001) might then be applied.

Genetic resources and germplasm

"Genetic resources for agriculture, however, present a unique and separate set of problems and characteristics which must be appreciated for valuing them, because of their wide distribution, their status as public goods, their daily use by individual farm households, and their association with less developed agriculture" (p. 140 Brush and Meng, 1998). Crop genetic resources include both plant germplasm and ecosystems necessary to maintain it. Most often, germplasm of agricultural interest is sourced from varieties used in breeding and commercial production but also wild relatives of existing crop species, landraces and weedy forms (Brush and Meng, 1998).

Natural mechanisms for maintaining genetic diversity are under threat as the impact of human population growth reduces the space and variety of ecosystems that have been natural reservoirs of germplasm. Simultaneously, large scale monocropping practices accelerate the movement towards a narrower genetic resource base. In recognition of the importance of maintaining a large genetic diversity especially in the world's most important food and materials crops, gene banks attempt to preserve genetic diversity by maintaining pure stocks of different types of crops and their wild relatives. This has been especially important in crop centers of origin. The essence of this issue is whether gene flow will undermine effective coexistence of GM and other plants.

In situ conservation
Due to space and time limitations, *ex situ* conservation cannot both accumulate all germplasm forever, nor allow the combinatorial experiences of evolution to continue to act upon and create new germplasm from existing wild plants. "*In situ* conservation does not directly provision genes for crop improvement but preserves evolutionary processes which will yield new germplasm in the future" (p. 141 Brush and Meng, 1998). Recognition of constraints has fueled renewed interest in open "banks" to complement *ex situ* conservation.

In situ conservation is potentially at risk from the adoption of any modern variety, genetically engineered or conventional, of cultivated plant, especially when the modern variety is produced at large scale. Transgene flow may be easier to manage when the gene or trait is both known and easy to detect. How many transgenes will fall into this category is unknown. Transgenes that must be managed using molecular detection can be predicted to be difficult to trace in real-time experiments conducted on landscape scales, considering how difficult it has proven to be keep some genetically modified seed separate from conventional even under routine surveillance of concentrated sources. As an indication of the difficulty this task posses for even industrialized countries, the reader is directed to the running debate on gene flow from GM maize to Mexican landraces (reviewed in Soleri et al., 2006) and the complexities of monitoring transgenes in general (Heinemann et al., 2004).

Ex situ conservation.
Gene flow is of particular concern for the management and maintenance of gene banks and other repositories of germplasm (*ex-situ* conservatories). First, gene banks ensure the genetic identity of accessions in order to preserve type varieties. Thus, gene banks are continually vigilant when it comes to ensuring that the genotypes of accessions are free from exotic genes, including transgenes. Second, gene banks distribute germplasm and are an important source for agriculture in developing countries.

Agronomic advantages

Some agronomic advantages and agricultural systems require particular management practices to remain effective. When gene flow specifically undermines the management necessary to retain a cultivar's value, then it has an effect on farmers' options.

The development of multi-herbicide tolerant variants of oil seed rape in Canada (*Box 3*) due to gene flow is an example of an option under threat: gene flow is reducing herbicide susceptibility. Another example is provided by gene flow to feral and wild relatives of Bt crops which may also undermine insect resistance, thus potentially affecting those who opt to use either Bt crops or Bt pesticides (*Box 4*).

The engineering of Bt crops is expected to have a limited time value because insects will evolve resistance when exposed to "Cry toxins". The rate and likelihood of resistance development varies with insect and the number and range of plants upon which they prefer to feed (Janmaat and Myers, 2005). Environmental variables need to be tightly controlled to avoid an outbreak of insects that have become resistant to *B. thuringinesis* Cry toxins, both for the sake of the farmers who choose transgenic crops and for those who use natural sources of *B. thuringiensis* as a pesticide (Chapman and Burke, 2006).

To extend the useful life of Bt crops, and to avoid creating a cost to farmers who use natural Bt pesticides, a variety of management techniques have been introduced along with the crops (EPA,

2006). An example of a highly managed—and in many countries successful so far—strategy is the refuge surrounding high dose Bt crops.

***Box 4*: Bt management failures**
Maintaining Bt susceptibility in insect pests may be at risk worldwide. The US Department of Agriculture found that 21% of American Bt corn farmers in 10 states violated refuge requirements (Bates et al., 2005).

A study by researchers at Cornell University in the US report on the effects poor management of Bt crops can have. Bt cotton varieties have a strong record of reducing the amount of pesticide added to crops (beyond that which the crop itself makes) - as much as a 71% reduction in China, on par with savings reported in India, Argentina, Mexico and South Africa (Wang et al., 2006). But the savings in China began to erode by 2000. By 2004, pesticide use on Bt fields about equaled the use on conventional crops. When the Bt insecticide made by the plant is added back, Bt cotton fields have higher pesticide levels compared to conventional crops.

Why the reversal in pesticide use? "Detailed information on pesticide expenditures reveals that, though Bt farmers saved 46% Bollworm pesticide relative to non-Bt farmers, they spend 40% more on pesticides designed to kill an emerging secondary pest" (p. 4 Wang et al., 2006). Unlike farmers of conventional cotton, it is concluded that secondary pests, those not susceptible to Bt, are not as easily detected or controlled in a Bt crop. Other pests, such as the bollworm itself, are natural biocontrols of the secondary pests. "The extra expenditure needed to control secondary pests nearly offsets the savings on primary pesticide frequently cited in the current literature" (p. 5 Wang et al., 2006).

The flow of Bt toxin genes, whether on purpose or by accident, also increases the number of insects of different types and susceptibilities exposed to the toxin. "The success of the high-dose/refuge strategy is based on the assumption that heterozygote mortality is high, but a Bt plant may deliver a dose that is highly toxic to one species but only moderately toxic to another" (p. 59 Bates et al., 2005). The reduction of one herbivorous predator may be enough for secondary pests to invade the niche, as non-eucalypt insect pests from Australia have done with Australian eucalypts now growing in New Zealand (Withers, 2001). Those secondary predators that are presently less susceptible to the toxin, or who have built up resistance due to spotty exposures resulting from gene flow in their previous niche, may adapt to the crop.

As was experienced with the broad exposure of both clinically relevant and environmental bacteria to antibiotics through the overuse of antibiotics (Levy, 1998), the larger the population and variety of insects exposed to the toxin, the greater the opportunity to select individuals with inherently less susceptibility (Bates et al., 2005). In some cases, antibiotic resistance by gene flow was a predictable outcome of creating environments where the concentration of antibiotics waxed and waned (Willms et al., 2006). Resistance is more likely to evolve under conditions where Bt toxin expression levels, and range of plants expressing any level, are not carefully managed.

Transgene flow is special to effects on insect resistance management. Flow of Bt toxin transgenes is likely to change the concentration of Bt toxins in populations of plants, lowering their strength and the effectiveness of high-dose refuge strategies (Bates et al., 2005). This is expected because, even in the primary GM plant which has undergone intense selection for uniform expression levels, Bt

toxin expression can still vary with tissue, time, season, rainfall, number of copies of the transgene and many other stochastic variables (Adrian et al., 2002, Dong and Li, 2007, Sharma et al., 2004). "Season-long differences in expression of *Cry1Ac* among cultivars can vary as much as twofold throughout the growing season. A more recent study conducted in India further showed that the resistant power to the targeted bollworm in Bt transgenic hybrid cotton remained only for 110 days, after which the crop can be exposed to bollworm attacks. The *Cry1Ac* level declined as the plant grew and was found to drop below its 'lethal' level of 1.9 [micrograms/gram] within 110 days after sowing. It seems to be a common phenomenon that the efficacy is relatively high in early growing season, but significantly declines during late season for most commercialized Bt cotton varieties" (p. 22 Dong and Li, 2007) and "[e]ither temporal or spatail variability in efficacy may increase the probability of surviving pests" (p. 23 Dong and Li, 2007).

Movement of the transgene to plants predated by insects of low susceptibility may increase the likelihood of those insects ultimately switching to the crop, replacing insects of high susceptibility.

For traits such as pesticides, this landscape heterogeneity may promote the evolution of resistance to Bt among damaging insect pests. Thus, even cross-fertilization without gene introgression may introduce enough regular, low dose exposure to Bt to undermine management strategies (Chilcutt and Tabashnik, 2004).

Insect-resistant plants are grown on approximately 12 million hectares globally, and adoption is growing (Bates et al., 2005, Marvier and Van Acker, 2005). Moreover, the range of plants being made into Bt crops is expanding. Presently it includes cotton, corn, tomatoes, eggplant, soybeans, canola, potato, apple, peanuts and broccoli (Bates et al., 2005). This undoubtedly extends the frequency at which particular insects, and the variety of insects that, will one day be exposed to Bt toxins. The impact of management failures could extend to multiple crops and countries in a short period of time.

Varieties of Bt crops with more than one Cry toxin may be the most effective way to slow the development of Bt-resistance in insects (Bates et al., 2005). However, combining different Bt toxins in a region through introduction of crops expressing single but different toxins may be the most effective way to encourage Bt-resistance in insects. Such introductions create landscape-level toxin mosaics (Bates et al., 2005) of exposure, maximizing the number and variety of exposed insects and the heterogeneity of toxin concentrations. The mosaic can be created also through time by rotation of crops with single but different Bt toxins.

Gene flow is another way to create a landscape mosaic. Residual plants expressing Bt toxins, whether they be volunteer or feral crops or wild relatives, could undermine even region-wide efforts to coordinate Bt cropping practices. Stacked varieties might not significantly improve the management of Bt resistance if they are introduced after the same toxins have been individually introduced through commercialized varieties or their toxin genes separate during transgene flow.

Unanticipated or unintended effects on agronomic traits

Crop-to-crop gene flow introduces a unique spectrum of risk issues through a phenomenon called stacking (or pyramiding). Stacking refers to two or more separate transgenic events combined within a single genome through breeding. Stacking events can happen intentionally or accidentally. Transgenes can "stack" in crops and in wild plants. Examples include crops with multiple herbicide resistances (Senior and Dale, 2002), or multiple insect toxins (Bates et al., 2005, Gould, 1998) or combinations of both (Pretty, 2001).

Varied expression
The US Environmental Protection Agency (EPA) has already begun approving Bt stacked varieties of corn which may be commercialized (EPA 730-F-05-001, 2006). In May 2005, EPA approved a hybrid between Monsanto's MON 810 and MON 863 varieties, called YieldGard Plus Corn, expressing the Cry3Bb1 and Cry1Ab proteins. A two-Bt toxin variety of cotton was approved in Australia and the US in 2002 (Bates et al., 2005). Expressing two different insect toxins simultaneously in a single plant may slow or halt the evolution of insects that are resistant, because resistance to two different toxins would have to evolve simultaneously (Bates et al., 2005, Gould, 1998). The probability of that happening has been optimistically estimated to be as low as one in a trillion (Gould, 1998). Probabilities vary depending on how refuges are used (see above) and the nature of resistance. The probability of an event must be interpreted also based on the size of an insect population and the rate of reproduction. Low probability rates may still result in a significant number of events in a large, rapidly reproducing population. Less optimistic models predict resistance could still emerge in only seven insect generations (Gould, 1998).

Insects with dual resistance will be able to forage on crops expressing either or both toxins, and on crops that are treated with Bt pesticide formulations with only either or both of these toxins. Approved stacked varieties can benefit from testing that assures that both toxins are being expressed at high levels, lowering the probability that insects will be exposed to sub-lethal concentrations of either toxin. However, as discussed above, unintentional gene flow could result in a mixture of expression levels, expression in some but not all tissues, and expression in some but not all plants that particular insect populations predate. Should that result in the selection of insects with multiple toxin tolerances, then gene flow causing the stacking of transgene may adversely effect pest management for GM, conventional and organic farmers.

Combinatorial effects
Combinations of genes can produce unanticipated or unintended effects in two ways. First, new gene combinations can have different effects on phenotype than are easily predicted from the actions of the genes studied in their source organisms. Second, the combinations can have different effects on the environment from those that are easily predicted from the actions of each phenotype studied in isolation.

Classic types of combinatorial effects are dominant/recessive relationships between alleles of genes at the same locus and epistasis between genes at different loci (Yin et al., 2004). These relationships cannot be revealed until the alleles or genes are brought together into the same genome, as would happen through gene flow.

Some combinations of different Cry toxins are, for example, synergistic and others are antagonistic (*Appendix 1*). The synergism or antagonism, moreover, may not be a universal quality. They sometimes are specific to only certain insects (reviewed in Sharma et al., 2004).

Consideration of combinatorial effects must begin at the level of the modified DNA and its transcriptional products (*Appendix 1)*. Stretches of sequence similarity between transgene events, for example that use a common promoter element or intron, may promote recombination (Butterfield et al., 2002, Yin et al., 2004) or gene silencing (Mello and Conte Jr., 2004).

Silencing occurs by the general pathways controlled by short double-stranded RNA (dsRNA) molecules (e.g., RNAi, PTGS, TGS, co-suppression) and are based on sequence matches between the dsRNA and the silenced genes. These reactions require that both events be present in the same cell and that they share some DNA sequence similarity, and, therefore, can only be seen when events, or sub-parts of transgenic events, are stacked.

It is a well-documented phenomenon that, for example, when two genes run by homologous promoters come to be together in the same plant cell, both genes may be silenced (De Schrijver et al., 2007). This can result in inadvertent silencing of agronomic traits. Interestingly, many commercial transgenes currently use the p35S promoter. "The cauliflower mosaic virus 35S (35S) promoter has been extensively used for the constitutive expression of transgenes in dicotyledonous plants. The repetitive use of the same promoter is known to induce transgene inactivation due to promoter homology" (p. 988 Bhullar et al., 2003). In an elegant study, Al-Kaff et al. showed directly that infection of susceptible plants with the cauliflower mosaic virus (the source of the 35S promoter) can cause silencing of a herbicide tolerance transgene with a 35S promoter (Al-Kaff et al., 2000).

Another potential effect on other members of the ecosystem is apparent from this observation. When the duplication of genetic elements causes silencing, generally both genetic elements are silenced. In the case of CaMV virus causing the silencing of a transgene, viral genes controlled by 35S will also be silenced. This could make weed plants with such transgenes resistant to the virus. An increase in the population of virus-resistant weeds could increase pressure on the virus to evolve a resistance to silencing, a trait that other viruses of plants are already known to possess (Baulcombe, 2004). In the meantime, the relative contribution of the virus to the biocontrol of cruciferous weeds may be reduced.

2. PLANT AND ANIMAL BIODIVERSITY

Gene flow can increase, decrease or have no effect on wild plant and animal diversity, depending on the gene, the transgenic plant and the ecosystem. Currently, there are no transgenes designed to increase diversity of either plants or animals and no specific evidence that the flow of transgenes has resulted in an increase in either plant or animal diversity, so the focus of this section will be on how transgene flow could reduce diversity.

Plants

Gene flow through crosses with wild relatives may reduce genetic diversity in two broad ways (Wolf et al., 2001). New genes may change the balance between varieties or species when flow results in a change in relative fitness (i.e. competitiveness) between intermediate types and plants of different species, resulting in an invasive plant, and changes in competitiveness between the intermediate and parental types. The latter results in hybrid vigor or outbreeding depression (see Chapter I.2). A wild species can be 'genetically assimilated' when crop genes replace wild genes (Andow and Zwahlen, 2006). Demographic swamping can result when hybrids are less fit. One outcome could be that these hybrids produce less pollen, thus making wild populations more susceptible to pollen from other sources (including transgenic crops), or hybrid seed may be sterile (Baltazar et al., 2005).

A popularly referenced observation of *Ellstrand et al.* also emphasizes the importance of gene flow between crops and wild relatives. They catalogued hybridization between 12 of the world's 13 most important human food crops with their wild relatives (Ellstrand et al., 1999). This poignant analysis underscores the need to understand the impacts of gene flow, because of global dependence on such a small number of crop species and the limited number of *in situ* and *ex situ* gene pools left to draw upon for crop improvement.

Both hybrid vigor and outbreeding depression are a general risk of agricultural practices, particularly large monocropping operations. However, if the transgene makes a direct contribution

to vigor or depression, then the risk is specific to GM crops. For example, under discussion is the introduction of so-called transmission mitigation genes (Gressel, 1999) with the specific purpose to reduce the fitness of hybrids (see Chapter IV). Through this and possibly other ways, "[r]elative to nontransgenic crops, a transgenic crop could pose a greater extinction risk to a wild relative or, more likely, alter its genetic diversity if it [is] permit[ed]…to be grown in closer proximity to the wild plants, thereby increasing interpopulation hybridization rates and detrimental gene flow" (p. 135 Committee on Environmental Effects of Transgenic Plants, 2002).

The implications for biodiversity extend to distant relatives as well because each relative is a potential bridge for the transgene to flow into more species than are considered relatives of the GMO (*Figure 4*) (Ellstrand, 2003a, Heinemann and Bungard, 2005).

Gene flow may accelerate the effects of agriculture on biodiversity because of the potential large-scale asymmetry in the recurrent introduction of transgenes that flow to wild populations or conventional crops derived from recycled seed relative to the flow back from these populations to GM crops (Groot et al., 2003, Haygood et al., 2003). Not only are large geographic areas covered in GM crops, but they are replenished frequently by human cultivation, leading to repetitive introductions of the exotic genes into an environment.

Hybrids between wild relatives and GM crops could also further threaten endangered or culturally-significant plants. "A recent review identified ten cases worldwide involving crops and their wild relatives for which there is evidence that extinction or genetic assimilation has occurred as a result of hybridization with a crop" (p. 246 Hails and Morley, 2005).

> *"In addition to its creative role, it is now known that hybridization can also have a destructive role as a factor in plant extinction. The Catalina Island mountain mahogany,* Cercocarpus traskiae*, is an illustration. This endemic is native to a single gully on an island off the coast of California (33°239 N, 118°259 W). It hybridizes with the more common and widespread species* Cercocarpus betuloides*. Since the discovery of the island endemic, the adult population size has plunged from more than 40 to 11. A few of these appeared to be hybrids. DNA and isozyme analysis revealed that almost half of the total reproductive population, five adults, are of hybrid origin, as well as several seedlings. Clearly, if future hybridization occurs, it will rapidly send the species to extinction" (p. 1165 Ellstrand, 2003a).*

Wild relatives can be important members of ecosystems, too (Gurian-Sherman, 2006). The protection of wild relatives is important for the maintenance of biodiversity or cultural practices.

Region-specific information is required to form an accurate characterization of wild and/or weedy relatives in and around the receiving environment(s), including where the relatives are situated in relation to the areas of GM cultivation (e.g., Armstrong et al., 2005, Serratos-Hernández et al., 2004). This analysis should extend to the possibility of a non-native weed relative being introduced into a hospitable environment where the GM crop will be grown. Common weedy relatives should be of primary concern, because they are expected to most rapidly assimilate and possibly introgress transgenes, and they may be "already adapted to man-made habitats and can be difficult to control" (p. 11 Jenczewski et al., 2003).

Regional analysis may take the form of a biogeographical assessment of wild relatives' distribution patterns and agro-biodiversity patterns (Groot et al., 2003) or more simple species inventories, compiled using existing databases or direct environmental surveying. The accuracy and specificity of the method should be appropriate to the nature of the GM crop and the scale of the release.

The proximity of wild populations to GM crops may also be influenced by agricultural practices in the region. Smaller-scale agriculture, using smaller fields, may bring GM crops into contact with wild relatives more often at the edges of plots. The smaller field sizes in Norway have been noted as having the potential to encourage more hybridization between varieties than the larger average sizes in the European Union (Torgersen et al., 1998). However, in a study of pollen transfer from transgenic canola grown in Australia, edge effects were not consistently demonstrated (Rieger et al., 2002).

Crop/wild gene flow may result in transgene reservoirs that go undetected, because it is unlikely that wild plants will be monitored and controlled in the same way as crops (Ellstrand, 2003a). Therefore, "a post-release monitoring strategy, which surveys those non-cultivated niches that have been populated with wild relatives of adjacent crops" (p. 40 Flannery et al., 2005) may be warranted.

Animals

Agriculture can profoundly affect wild biodiversity (Ammann, 2005). The effects range from loss and disruption of habitat, food supply and other resources for animals to the direct toxic effects of agricultural chemicals. Many GM crops are today engineered to resist insect pests. Transgenes not intended for pest-resistance can also produce plants that are toxic to insects and other animals as an unintended effect (Freese, 2002), notably future pharma and industrial crops (non-food, non-feed crops) will likely produce other compounds with deleterious effects on some animals.

While there are generic concerns about the effects of GM crops on non-target animals (Pilson and Prendeville, 2004), particularly those engineered to express toxins, what makes this an issue of special relevance to gene flow is that the range of exposed animals, and levels of toxin to which they are exposed, may be very different from that expected if the transgene were confined to the original crop.

The general consensus is that, while the currently approved GM crops have demonstrated no significant adverse effect on non-target species (Velkov et al., 2005), the potential for these and new crops to do so cannot be completely ruled out on present data (Andow and Hilbeck, 2004, Clark et al., 2005). Effects of GM crops on animal diversity have not been comprehensively measured. Select groups of crops and particular traits (*e.g.* herbicide tolerance and Bt), however, have been measured using indicator species and extrapolated from effects on specific non-target species that may be exposed to GM crops. Studies completed to date sometimes find non-target effects while others do not, or not at relevant predicted exposures (for reviews on invertebrate studies, see Clark *et al.*, 2005, Pilson and Prendeville, 2004, Velkov *et al.*, 2005). Overall effects of agriculture on biodiversity appear to be negative and some argue that agricultural practices, rather than Bt or herbicide tolerant crops per se, are the more important determinants of impacts on animal biodiversity (Ammann, 2005).

Moreover, most observations of effects have been short term (Lang et al., 2007). *Harwood et al.* recently followed the flow of Bt toxins through the food web and found disturbingly high concentrations in insect predators, such as spiders, which can over time react to the toxins (Harwood et al., 2005). Such long term and food chain studies are rare.

Of obvious concern is the effect on insects that are pollinators or other insect predators of insects (Clark et al., 2005). The latter may be affected by consuming the toxin in their prey or simply by

falling numbers of prey (Pilson and Prendeville, 2004). They are an especially important group since insect herbivory reduces crop yields by an estimated 30-40% (Pilson and Prendeville, 2004).

Unintended toxicity to some kinds of parasitic nematodes, such as hookworms (Cappello et al., 2006), might release vertebrate crop pests to consume more. Even at low densities, rabbits can consume up to 50% of the biomass of pasture lands (Williams et al., 1995). In New Zealand, vertebrate pests cause an estimated 25% of crop losses (Pimentel, 2002). Omnivorous vertebrates also predate native wildlife (e.g. Murphy et al., 1998). Mustelids and rodents annually kill about 95% of brown kiwi hatchlings, and stoats probably made the kakapo and kokako birds extinct in the South Island of New Zealand (DOC, 2006). The nematode burden also augments the effects of predation; reducing it increases the competitiveness of pests. Hares with low parasite loads are 2.4 times more likely to survive, mainly because they are less likely to be eaten (Murray et al., 1997).

3. HUMAN AND ANIMAL HEALTH

Gene flow to and from GM crop plants may introduce toxic, allergenic or anti-nutrient compounds into the food supply of humans, domestic and wild animals. The issues specific to gene flow are that transgene flow might result in the unintentional expression of undesirable compounds derived from transgenes in other plants, and in an unanticipated and different undesirable product because the transgene is expressed in a different plant. The flow of transgenes, for example genes for antibiotic resistance, to microorganisms could increase the occurrence of antibiotic resistant bacteria in the human gut (Netherwood et al., 2004).

The most common example of purposeful toxicity is the use of *cry* genes in Bt crops (de Maagd et al., 2005, Heinemann and Traavik, 2004, Heinemann and Traavik, 2005). Cry toxins are not the only options being considered for transgenic plants, with new toxins, for example from spiders and other kinds of bacteria, still to be tested (Bowen et al., 1998, Khan et al., 2006). Concerns that some Cry toxins might also cause allergic responses in people was the reason that Starlink corn, engineered to express Cry9c, was restricted for use only as an animal feed.

Known allergens have also been produced by transgenic crop plants. In 1996 researchers reported that a variety of soybean genetically engineered to increase its content of the sulfur-containing amino acids through the expression of a methionine-rich protein, 2S albumin, from the Brazil-nut gene for this protein, would be likely to invoke an allergic response in humans allergic to Brazil nuts (Nordlee et al., 1996). This landmark study was influential in shaping current novel food risk assessments where consideration is now given to the source of a transgene (Nestle, 1996). If the transgene comes from a source of a known food hazard, then additional testing is highly recommended.

Australian researchers (Prescott et al., 2005) cancelled plans for developing a pea expressing the α-amylase inhibitor-1 protein from the common bean because they found early evidence that the protein could invoke an immune response when expressed in peas.

New generation GM plants may also be derived from, or related to, plants used as human food (Ellstrand, 2003b), but instead of being used as food they will be used as bio-factories for chemical products that are not currently created by these kinds of plants, or any kind of plants (Moschini, 2006, UCSUSA, 2007). Such crops are known as non-food and non-food/non-feed, PMPs (for plant-made pharmaceuticals) and PMIPs (for plant-made industrial products).

Assuming that PMPs and PMIPs were effectively segregated from the human food supply (but see *Box 5*), then the specific effect of transgene flow could be to introduce the transgene into crops that were used as human food. On this possibility, the journal *Nature Biotechnology* said "we *should* be concerned about the presence of a potentially toxic substance in food plants. After all, is this really so different from a conventional pharmaceutical or biopharmaceutical manufacturer packaging its pills in candy wrappers or flour bags or storing its compounds or production batches untended outside the perimeter fence?" (Editor, 2004). That position was reaffirmed by the journal in 2007 (Editor, 2007). Non-food plants for PMP and PMIPs are in development (Williams, 2007).

***Box 5*: PMP management failures**

Early indicators of containment track records are not uniformly assuring (Marvier and Van Acker, 2005, Vermij, 2006). In 2002, ProdiGene was fined US$250,000 when its biopharmaceutical pig vaccine produced in corn was found in soybeans and corn grown for human consumption (Fox, 2003). The clean-up costs were estimated to be up to nearly US$4 million (USDA Office of Inspector General, 2005). During the writing of this report, Bayer CropScience of Monheim, Germany, acknowledged long-term contamination of the US rice supply with an unapproved research variety called Liberty Link, which was last knowingly planted in 2001 (Vermij, 2006). Furthermore, the Starlink (Marvier and Van Acker, 2005) and Bt10 experiences (Lee and Natesan, 2006), where non-food GM corn was subsequently found mixed with human food in the global food supply, illustrates a limited ability to maintain containment.

Recently, US regulators have not faired well in evaluations of their oversight of pharma crop field tests, possibly creating public liability. In a ruling in August 2006, the first of its kind on pharma crops, a US Federal Court found that the United Stated Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) illegally issued permits to Monsanto, Prodigene, Garst Seed and Hawaii Agricultural Research Center to plant corn and sugarcane engineered to produce experimental vaccines for HIV and Heptitis B. The pharmaceuticals were experimental and thus neither the companies involved nor USDA could know their impact on herbivores or further up the food chain. Thus, the court ruled that in issuing permits for these pharma crops between 2001 and 2003, the USDA "violated both the [Endangered Species Act and the National Environmental Policy Act] in issuing the four permits (p. 4 CFS v. USDA, 2006). In December 2005, the USDA Office of the Inspector General concluded that "at various stages of the field test process - from approval of applications [to field test GE plants including pharma plants] to inspection of fields - weaknesses in APHIS regulations and internal management controls increase the risk that regulated genetically engineered organisms (GEO) will inadvertently persist in the environment before they are deemed safe to grow without regulation" (p. i USDA Office of Inspector General, 2005).

A US Federal Court has ruled that the USDA failed in its oversight role in approving varieties of genetically modified alfalfa because it did not complete an Environmental Impact Statement. A key finding was the failure of USDA to properly assess the impact on conventional alfalfa from transgene flow (Geertson Seed Farms v. USDA, 2007). The Court said that "A federal action that eliminates a farmer's choice to grow non-genetically engineered crops, or a consumer's choice to eat non-genetically engineered food, is an undesirable consequence."

Even segregation will not prevent exposure of the plant to wild animals, who may also consume other plants that receive the transgene by gene flow. The outcome could eventually decrease animal diversity (Kirk, 2001). Unintended effects on wildlife require more research.

Non-food/non-feed crops

Industrial crops (*Table 2*) include those designed for biofuel application (McLaren, 2005) and phytoremediation (using plants to absorb or remove pollutants). Pharma crops (*Table 3*) produce pharmaceutical-grade chemicals, vaccines and other therapeutics (Borch and Rasmussen, 2005, Ma et al., 2005a, Ma et al., 2005b) or other highly active biological compounds including allergens (Obermeyer et al., 2004) hormones (Staub et al., 2000) and monoclonal antibiodies (Moschini, 2006). Development is not restricted to proteins. Some groups are developing plants that produce libraries of small RNA molecules for gene silencing (Zhou et al., 2004). This raises the possibility of fields of GM crops with the capacity to produce all RNA molecules necessary to silence all genes

Table 2: Industrial crops*

Crop	Product	Application
Arabidopsis thaliana	spider silk	various biomaterials
	β-ketoacyl-CoA thiolase acetoacetyl-CoA reductase PHB-polymerase/synthase	biodegradable plastics
Aspen	cytochrome P450	phytoremediation
Brassica	SMT sulfurylase	phytoremediation
	β-ketoacyl-CoA thiolase acetoacetyl-CoA reductase PHB-polymerase/synthase	biodegradable plastics
Cn/Maize	α-amylase	biofuel production
	laccase	paper and textile production
	β-ketoacyl-CoA thiolase acetoacetyl-CoA reductase PHB-polymerase/synthase	biodegradable plastics
Eastern Cottonwood	mercuric ion reductase, organomercury lyase glutamylcysteine synthetase	phytoremediation
Potato	spider silk	various biomaterials
Sugar beet	β-ketoacyl-CoA thiolase acetoacetyl-CoA reductase PHB-polymerase/synthase	biodegradable plastics
Tobacco	citrate synthase	phytoremediation
	spider silk	various biomaterials

Assembled from APHIS-USDA, 2007, FSANZ, 2007, Moschini, 2006, Scheller and Conrad, 2005)

in the human genome.

Biopharmaceuticals could include antibiotics, and thus environmental exposure to these agents would occur on a massive scale, particularly if transgenes were to flow to other crops and wild

Table 3: Pharma crops*

Crop	Product/therapeutic	Application
Arabidopsis	Human intrinsic factor	Vitamin B12 deficiency
Barley	Lactoferrin	treat infections
Corn/Maize	Lactoferrin	treat infections
	Trypsin	protease with various applications, including insulin production
	Aprotinin	protease inhibitor with various applications including wound care
	E. coli LT-B subunit protein	vaccine
	E. coli heat labile toxin	diarrhea
	Avicidin	colorectal cancer (withdrawn)
	Gastric lipase	supplement for cystic fibrosis patients, pancreatitis
Lettuce	Hepatitis B surface antigen	hepatitis B
Potato	Hepatitis B surface antigen	hepatitis B
	E. coli heat labile toxin	diarrhea
	Norwalk virus capsid protein	diarrhea
Rice	Gastric lipase	supplement for cystic fibrosis patients, pancreatitis
	Lysozyme	treat infections
Spinach	Rabies glycoprotein	rabies
Tobacco	CaroRx (antibody)	treat dental caries
	monoclonal antibody fragments	non-Hodgkin's lymphoma and infectious diseases
	Human serum albumin	expand blood volume
	Collagen	wound dressings

* (Assembled from Ma et al., 2005a, Ma et al., 2005b, Marvier and Van Acker, 2005)

plants. Recombinant peptide antibiotics have been produced in tobacco and demonstrated activity against fungal pathogens (Cary et al., 2000), parasitic plants (Hamamouch et al., 2005) and bacteria (Huang et al., 1997). Proteins with antibiotic properties are also being engineered into animals (e.g., Yazawa et al., 2006). While the use of antibiotic resistance genes in some commercially grown transgenic crops has always carried the contested theoretical risk of providing another avenue for antibiotic resistance to horizontally transfer to human pathogens (Heinemann and Traavik, 2004, Netherwood et al., 2004), that issue is completely different. The flow of transgenes that make antibiotics in plants to plants used as food for people and animals will directly expose bacteria that are ingested by humans and farm animals to antibiotics, and that would be a significant increase in the risk of spreading antibiotic resistance among the bacteria.

There are theoretical advantages to transgenic plants as sources of proteins with medical and research applications. The motivation to develop them is in part expectations of savings from economies of scale in production, and purity from undesirable contaminants (such as may co-purify from human or animal sources) (Crosby, 2003, Ma et al., 2003, Twyman et al., 2003). "It is estimated that recombinant proteins can be produced in plants at 2–10% of the cost of microbial fermentation systems and at 0.1% of the cost of mammalian cell cultures, although this depends on the product yield. For proteins that can be produced at high yields, the economic advantages of

plant production systems are clear. One bushel of maize producing recombinant avidin at 20% total soluble seed protein has the same total yield as one tonne of chicken eggs—the natural source of avidin—but at 0.5% of the cost" (p. 570 Twyman et al., 2003). The actual economic benefits for consumers are disputed (Freese, 2002, Kostandini et al., 2006). Even producers may have more modest returns than first forecast after costs of segregation and identity preservation are included (Kostandini et al., 2006, Moschini, 2006). Nevertheless, with biopharmaceuticals now being "approximately one in every four genuinely new pharmaceuticals coming on the market" (p. 553 Walsh, 2005) and sales estimated at €30 billion (Walsh, 2005), the drive to produce pharma and industrial crops is clear. Some have estimated that in time, molecular plant farming "could account for 10% of the corn acreage" (figure accredited to Zitner on p. 18 of Kirk, 2001).

Non-food crops

Two examples of non-food crops are Aventis Starlink corn and Monsanto's high lysine corn LY038. The former was engineered to express an insect toxin that was considered unusually resistant to digestion and thus had greater potential to invoke an allergic response in humans (Marvier and Van Acker, 2005). Therefore, it was approved for environmental release for cultivation only for animal feed. The latter was engineered with a gene from the bacterium *Corynebacterium glutamicum* that allows the amino acid lysine to accumulate in the seed. This crop has since been approved by Canada for use in human food, but the stated intent of the manufacturer is for use as animal feed (FSANZ, 2004).

Monsanto's LY038 corn line is a particularly good example of how gene flow might create novel hazards despite the source of the transgene not being a recognized source of food hazards. This corn line accumulates total lysine in the range of 3,500 to 5,300 parts per million (ppm), and free lysine is in the range of 1,000 to 2,500 ppm. Research hybrids with parents similar or identical to LY038 could have much higher levels of lysine and free lysine when crossed with high lysine lines that accumulate lysine by a different biochemical pathway (Huang et al., 2005). Such hybrids can accumulate 6,160 ppm total lysine and free lysine levels to 2,908 ppm (Huang et al., 2005). These concentrations of lysine and especially free lysine in hybrids were not expected by the researchers, who labelled the phenomenon a form of synergy between the different biochemical pathways. This example illustrates that the flow of genes between GM crops can produce combinatorial effects that are difficult to diagnose from knowledge of specific, single transgene, varieties.

Transgene flow resulting in the unintentional introduction of undesirable compounds

There are at least three historical examples where the products of non-food GM crops have been found mixed with food and feed crops. Prodigene's vaccine produced in corn is a non-food/non-feed example (*Box 5*). Bt10 (unapproved for either food or feed) and Starlink corn are non-food examples.

Bt10 corn is a research line that has similarities with an approved variety, Bt11. But Bt10 was not approved for release or human consumption by any regulatory authority in the world when, in early 2005, Syngenta admitted that Bt10 stocks had mixed with Bt11 stocks since 2001 (Herrera, 2005). Initially, Syngenta told US regulators that Bt10 was identical to Bt11, but later it was realized that Bt10 retained an antibiotic resistance gene that was not present in Bt11. Moreover, the transgenes were inserted into different chromosomes and there were additional small DNA sequence differences between constructs. Closer examination of Western blots also revealed some differences in protein profiles in the two varieties (TWN, 2006).

Starlink was approved for use only in animal feed. Nevertheless, in 2000 the *Cry* toxin from Starlink was detected in taco shells, sparking a recall of products that might have been contaminated with Starlink corn. After three years of intense efforts to recall the seed, the transgene was still present in detectable concentrations in the US food supply. A recent evaluation strongly suggested that the amounts of Starlink are underestimated and that there is no convincing data to prove that present levels of contamination are reducing further (Marvier and Van Acker, 2005).

These mixtures were either caused by mixing corn harvested in different fields or they arose from pollen flow and hybridization (most likely). Past containment failures illustrate the effects of gene flow, even if gene flow was not the cause of each example, and they illustrate the challenges in keeping non-food/non-feed segregated from the food supply.

Transgene flow resulting in an unanticipated and different undesirable product because the transgene is expressed in a different plant

Gene flow can create novel hazards in conjunction with post-translational modification. Post-translational modifications are alterations to the chemical structure of a polypeptide that are not controlled by the reactions involved in forming a polypeptide (*i.e.* translation). They can be additions of chemicals, for example sugars (called glycosylation) to the polypeptide or removal of amino acids from the polypeptide. In both cases, the modification is not specified in the DNA sequence for a gene.

In 2005 researchers reported that mice mounted an immune response to a variety of peas genetically engineered to express the α-amylase inhibitor-1 protein from the common bean even though the same protein produced in bean caused no reaction in mice (Prescott et al., 2005). Critically, the immunological reaction was not to the protein itself, but to its structure and glycosylation that were unique to its expression in the pea. Importantly, exposure to the immunoreactive form of the protein sensitized the mice to a variety of other proteins that would normally not be allergens. This study demonstrated unequivocally that the source of the gene alone is not a reliable guide as to the potential for the GM crop to have potent, and adverse, biological activity.

This example also reinforces an important point about potential gene flow hazards. A gene considered safe in one plant may cause adverse effects if it were to flow to another plant and be expressed, due to differences in post-translational modification. Even occasional flow without introgression could cause concern because rare escapes may still produce sizable local populations of hybrid plants that cause intermittent harms to humans or wild animals.

Proteins are more than just a sequence of amino acids. They often may also be modified by addition of different kinds of molecules to various amino acids. This is relevant to risk assessment because the modifications can alter protein structure and function, as well as change the potential for the protein to be a toxin or allergen. For considerations of gene flow risks, it is necessary to recognize that:

- there are many forms of post-translational modifications. Most are the addition of molecules to particular amino acids in a protein, but some modifications result from removing amino acids or re-folding a protein into an alternative three dimensional structure;
- the range of potential post-translational modifications varies by species, tissue and stage of development (Elbers et al., 2001, Gomord et al., 2005). This has medical and food relevance because, for example, proteins modified in plants can be immunogenic in humans and may cause cross-reactivity to similar epitopes that occur in proteins from animal sources (e.g., Prescott et al., 2005);

- the same protein can exist in hundreds to thousands of different isoforms in the same cell at the same time, but each form may not exist at the same concentration. Thus, detecting different forms can be very difficult (Elbers et al., 2001).

Humanization and horizontal gene transfer?

Production of pharmaceutical compounds may also require additional, complementary, changes to crops. For the production of protein-based pharmaceuticals, for instance, the plant-derived compound needs to not only be a particular protein, but might be a protein that has a particular glycosylated isoform, one that may not be produced naturally in plants (Gomord et al., 2005). While a human-specific pattern and type of glycosylation may be necessary for proper function of the protein as a therapeutic, addition of sugars specific to plant glycosylation can cause adverse effects (Gomord et al., 2005). In an attempt to reduce toxicity or allergenicity and increase efficacy, special "humanized" plants may be used (Ma et al., 2003). Such plants are engineered with the genes that direct the addition onto proteins of sugars found on proteins produced by humans.

Co-technologies such as humanization create additional risks. However effectively GM plants are segregated from other crop plants, and their transgenes are contained, they will remain the host of insects, fungi, bacteria and viruses. In the latter case, consideration should be given to humanization as a cause of changes in virus virulence characteristics and host ranges. Very little is known about the host ranges of viruses, beyond the fact that most viruses transfer to a larger number of types of organisms than the number of types in which they cause a recognized disease (Dabrowska et al., 2004, Gorski et al., 2006, Takeuchi et al., 1998, Woolhouse et al., 2001). An example of this was the finding that Ebola virus, a type of filovirus that causes fatal hemorrhagic disease in humans and primates, replicates in fruit bats but does not cause any apparent disease (Swanepoel et al., 1996).

Changes in the post-translational modification of coat proteins of viruses may be sufficient to alter host range. Differences in glycosylation explain the cell-type host range of influenza viruses (Romanova et al., 2003) and the virulence of plum pox virus in Arabidopsis (Scott et al.). This could be of importance because viruses transfer not just between different species of animals but also between plants and animals. Interestingly, "[i]nformal speculation has included the suggestion that filoviruses may be plant viruses, perhaps even involving transmission by arthropod vectors" (p. 321 Swanepoel et al., 1996). While this remains speculative, to date the possibility has not been excluded (Peterson et al., 2004).

The immediate consequences may not be disease, but an opportunity for recombination between viruses that normally would not have overlapping transfer ranges. For example, a DNA virus, that infects *animals*, evolved via recombination between a DNA virus, that infects *plants*, and an RNA virus, that infects *animals* (Gibbs and Weiller, 1999). (Another remarkable intermediate in this chain of events was the likely contribution of a special enzyme, called reverse transcriptase, from a third virus acting on the animal RNA virus to convert an RNA genome into DNA.) The plant virus must have been able to transfer to animals (but caused no obvious phenotype) in order to create the opportunity for this chain of events. Although still in the realm of speculation, plant viruses passing through humanized plants might have altered infectious ranges for both plants and animals.

Conclusion

Agriculture

The possible effects of gene flow that are specific to agriculture may include:

- development of new weeds;

- loss of genetic resources;
- loss of agricultural and commercial options; and
- unanticipated or unintended effects on agronomic traits.

Crops and their wild relatives, as well as other wild plants, can be weeds depending on whether or not they are desired where they grow. Wild plants could be converted into more effective weeds by the flow of genes from GM crops when those new genes make the wild plant more effective at growing where it is not desired. GM crops can become weeds by flow of genes to them from wild plants, particularly if those genes restore in the GM crops characteristics that reduce their dependency on human intervention for their survival. Historically grown GM crops may simply become considered weeds when they are no longer grown as crop species but nevertheless persist. There is no agreed upon criteria for predicting weediness, so case-by-case assessment is essential.

Agriculture depends on continual access to genetic resources. What genes will be beneficial in the future cannot with accuracy be predicted now. So both *in situ* and *ex situ* collections of wild relatives of present day crops as well as different cultivars of crops are highly prized. The purity of accessions is specifically threatened by unintended gene flow.

Gene flow creates potential heterogeneity of traits in an environment. This heterogeneity may compromise management strategies such as the use of refuges surrounding high dose Bt plants. For traits such as pesticides, heterogeneity may promote the evolution of resistance to Bt among damaging insect pests. Thus, even cross-fertilization without gene introgression may introduce enough regular, low dose exposure to Bt to undermine management strategies (Chilcutt and Tabashnik, 2004).

Biodiversity
Gene flow may affect biodiversity if it compromises survival of populations of plants that are valued for not having a particular transgene. Plant biodiversity may be threatened by the escape and proliferation of a competitive accession with the transgene. Perhaps less well recognized is the loss of diversity through erosion. While this is a natural process, GM agriculture can be conducted on scales that significantly distort the normal impacts of erosion through gene flow. Not only are large areas covered in GM crops, but they are replenished frequently by human cultivation, leading to repetitive introductions of the exotic genes into an environment.

Animal biodiversity may be affected by the expression of compounds in plants that have a direct toxicity, allergenicity or anti-nutrient quality in consuming herbivores, or that concentrate as they move up the food chain (Harwood et al., 2005). Diversity may also be affected by the secondary loss of food sources due to the elimination of particular kinds of insects or other animals. Neither of the above effects is special to gene flow. However, the animals that are affected may be different from those expected due to the movement of the gene into different populations of plants or the plants into new environments.

Human and animal health
Crops are being modified to serve as “biofactories” for the production of pharmaceutical products and industrial chemicals, or to alter their nutritional value. Such crops may be considered non-food, because they have no history of safe use or because they are expected to be unsuitable as human food. They may also be considered non-food non-feed, if their use is to be further restricted.

Gene flow from such crops creates obvious hazards. These hazards may directly translate from the original transgenic crop. For example, a gene for the production of a vaccine protein may be

expressed in a non-GM crop through transgene flow, with the same spectrum of concerns surrounding the inclusion of either the original or the hybrid crop entering the human food supply. Alternatively, novel hazards might arise from gene flow. The expression of a protein in one food crop may be significantly different from its expression in another. This was illustrated using the example of a protein from beans with a history of safe use as human food being a potential allergen when produced in peas (Prescott et al., 2005).

III. LEGAL, SOCIAL AND ECONOMIC EFFECTS OF GENE FLOW

The legal implications of gene flow emerge predominantly as liability and intellectual property (IP) issues. Related economic effects will be discussed in terms of coexistence costs and market expectation issues. These effects are intertwined through international and national liability frameworks (Smyth and Kershen, 2006). IP issues also extend to concerns for sustaining a diversity of food production systems.

Transgene flow arises from a suite of circumstances. Conventional crops and wild plants currently co-exist in some environments where they grow side by side with GM crops. In addition, seed transport and international trade form a conduit for the flow on a global scale. Economic and legal implications of transgene flow only emerge in the context of the global, regional, national and local levels of GM and non-GM plant coexistence.

Agricultural production is becoming increasingly segregated to allow different products to be targeted at different markets. Techniques for detecting transgenes in agricultural goods are very sensitive, quantitatively increasing the ability to detect transgenic material in products, and creating a basis for quantifying the proportion of GM contribution to the product (Heinemann et al., 2004, Holst-Jensen et al., 2006). Some countries and regions have adopted quantitative thresholds of tolerance to "adventitious presence" of GM plant-derived content in non-GM products (Heinemann et al., 2004, Moschini, 2006). For PMPs and some PMIPs, many countries have "zero-tolerance" (Moschini, 2006).

Segregation of different products implies a high level of rigor because the goal is to satisfy legal and safety requirements (Smyth and Phillips, 2002). They may also be used for regulatory purposes, such as to avoid market rejection in jurisdictions sensitive to any detectable level of GMO content and, where possible, to maximize returns on the product through marketing its purity, but the latter is formally the role of identity preserving production and marketing (IPPM) systems (Smyth and Phillips, 2002).

Product differentiation began with the need to produce pure, certified seed varieties and differentiate between grades of grain, but has now extended to accommodate various differentiated marketing channels, such as organic, non-GM and GM production channels (Smyth and Phillips, 2002, Zepeda, 2006). Demand for segregation between and within these channels has led to the development of IPPM systems, based on supply chain agreements to retain access to markets with specific rules and thresholds (Smyth and Phillips, 2001, Smyth and Phillips, 2002).

Segregation and IPPM systems create market expectations that may from time-to-time offer premium returns on product, or at least offer preferred access or avoidance of rejection. Market expectations of purity may even exceed legislated requirements. To the extent that one form of agricultural production may erode these returns by reducing the marketability of a product, cases for compensation arise (Kershen, 2004, Smyth et al., 2002).

Traceability systems allow products to be tracked through the supply chain, for food safety, quality control measures, or recall (European Parliament, 2003, Smyth and Phillips, 2002). Traceability is either imposed by regulators as a post-market monitoring measure or by producer collectives as a consumer assurance for food safety. For the most part, the focus is on an accurate paper trail. The path of the product, from seed and planting to sale, should be recorded. Products are identified by unique codes, logged in national registries, or international services such as the Cartagena Protocol's Biosafety Clearing House.

Traceability is a prerequisite for a robust GMO labelling regime (Recitals 3 and 4 European Parliament, 2003).

> "*Traceability requirements for GMOs should facilitate both the withdrawal of products where unforeseen adverse effects on human health, animal health or the environment, including ecosystems, are established, and the targeting of monitoring to examine potential effects on, in particular, the environment. Traceability should also facilitate the implementation of risk management measures in accordance with the precautionary principle.*"

> "*Traceability requirements for food and feed produced from GMOs should be established to facilitate accurate labelling of such products, in accordance with the requirements of Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (6), so as to ensure that accurate information is available to operators and consumers to enable them to exercise their freedom of choice in an effective manner as well as to enable control and verification of labelling claims. Requirements for food and feed produced from GMOs should be similar in order to avoid discontinuity of information in cases of change in end use.*"

The capacity to monitor and detect GM material, for those who can afford it, may assist producers to comply with safety and legal requirements. However, gene flow, even at low frequencies, may also be detected in the product of those who wish to avoid the effects of GM production. By extension, they and the biotechnology producer may become liable for the GM content, where laws governing this form of product harm are applicable. The economic fall-out of the Starlink corn recall, estimated at US$1 billion to Aventis alone (Moschini, 2006), illustrates the magnitude of claims that can arise, especially from late detection. The economic ramifications of the LL601 rice escape are alleged to be as high as 10% of the US$1.9 billion rice market (CMHT, 2007).

Quantitative tolerance levels to GM plant material are restricted to those GM ingredients that can be identified and have some evidence of safety (Heinemann et al., 2004, Vermij, 2006). It can be difficult or impossible to make a positive identification at detectable levels of adventitious presence and there is evidence that some regulators may not have been uniformly vigilant about enforcing zero-tolerance in these cases.

1. LIABILITY

Liability may be considered from three perspectives: traditional dammage, environmental damage, and economic damage. Gene flow has the potentials to cause all three types of damage. Environmental damage resulting from transgene flow is described by, for example, harming endangered species of wildlife who may feed on intermediate types. Traditional damage concerns the effects of transgene flow that result in damage to human health or to property, *e.g.* where a product containing transgenes from a GM crop that has not been authorised cannot be marketed. Economic damage could arise if transgene flow were to result in direct or indirect economic losses, for example, by loss of reputation, certification, or market price received.

Depending on a country's domestic legislation various forms of damage transgene flow may cause may or may not be covered by the relevant liability provisions. While in some countries liability may be excluded if the GM product causing damage through gene flow has met legislated and any regulatory agency's imposed requirements, such a 'permit defence' does not exist in others. While some countries believe that transgene flow does not require any new or additional liability legislation, other countries have introduced or are currently introducing laws that specifically

address liability for transgene flow (Gerdung, 2006). For a recent comparative review of legal frameworks, see *Smyth* and *Kershen* (2006).

Particularly challenging is the case of "economic damage", where transgene flow does not have any impact on human health, the environment or property but results, for example, from the fact that products containing traces of transgenic crops can only be sold at a lower price. "Gene flow from GM plants creates liabilities in two ways. First, through conventional agricultural harvest practices, some seeds are left behind that germinate in the spring and, depending on the crop planted, may create a tolerance-level liability. [Seeds that are caught in harvesting equipment, or mingle during transport and storage create a similar risk.] The second is that pollen of a GM plant could fertilize a non-transgenic or 'conventional' plant and the resulting hybrid seed might possess the trait for that transgene" (p. 538 Smyth et al., 2002).

Coexistence issues are not merely concerned with the minimization of gene flow and maintenance of thresholds for GM adventitious presence. They can also cover related and cumulative effects of GM agriculture on other forms of cultivation. Lawsuits in the USA have alleged liability both on the grounds of property damage through pollen flow and loss of market access (Kershen, 2004). Organic and traditional agricultural systems rely on the maintenance of agro-biodiversity for suitable crop varieties, mix-cropping and crop rotation, all of which may be affected by transgene flow. Biodiversity in other plants and animals in the ecosystem may also be integral to pollination and non-chemical pest control (Altieri, 2005).

"The issue of co-existence refers to the ability of farmers to provide consumers with a choice between conventional, organic and GM products…Co-existence is concerned with the potential *economic loss* through the admixture of GM and non-GM crops which could lower their value, with identifying workable management measures to minimise admixture, and with the cost of these measures" (EU, 2007). A recent US District Court also found fault with the USDA for issuing permits that would threaten farmer and consumer choice through transgene flow (*Box 5*).

Concerns about the potential effects of GM agriculture on differential marketing channels focus on two major issues: organic and non-GM food certification, and the potential of novel organisms to influence the biodiversity that organic farmers in particular rely on for successful crops.

Organic and non-GM farmers may be affected by the release of GMOs in their region. Gene flow from GM to non-GM crops may prevent organic certification. Some government regulations allow the adventitious presence of GM material in organic and non-GM crops without requiring labelling. However, many private certification services can be expected to be less tolerant of contamination.

The potential economic damage from an inability to provide certified organic or non-GM food due to transgene flow is significant. The average "price premium paid for organic products within the EU is 20%–50%" and "the price premium for organic products in Japan varied between 100% and 200%" (p. 1203 Zepeda, 2006). The price premium paid in the USA could be 10-40% (Winter and Davis, 2006). The estimated price premium for organic canola oil is 100% (Smyth et al., 2002).

The effect of coexistence on differentiated market channels may be to blemish the reputation, and therefore the price premium, of entire source countries, possibly regions. For example, the "Canadian Department of Agriculture and Agri-Food stated in 2003 that: 'The production of GE canola is currently adversely affecting the value of non-GE canola in some markets. The EU is effectively closed to all Canadian commodity canola'. Since 1998, Canada's annual sales of canola to Europe have dwindled to about $1.9 million a year from $230 million, the Canadian Department of Foreign Affairs and Trade calculates" (p. 13 Terry, 2005).

The "market reality" can only be expected to assert itself more powerfully as PMP and PMIP production increases. "It is unclear at this point whether the possibility of deregulating certain pharmaceutical crops will be considered. In an increasingly globalized economy, however, relaxing the current implicit zero-tolerance level for PMPs and PMIPs in the U.S. food and feed system may not suffice anyway. As long as there is the possibility for shipments of commodities to be rejected by foreign markets, failure to meet the zero-tolerance standard could still have sizeable economic impacts" (p. 1190 Moschini, 2006).

The issue of liability and redress for damage resulting from the transboundary movements of living modified organisms was one of the themes on the agenda during the negotiation of the Cartagena Protocol on Biosafety. The negotiators were, however, unable to reach any consensus regarding the details of a liability regime under the Protocol. The matter was, nevertheless, considered both critical and urgent. As a result, an enabling clause to that effect was included in the final text of the Protocol. Accordingly, the first meeting of the Conference of the Parties serving as the meeting of the Parties to the Protocol (COP-MOP) established an Open-ended Ad Hoc Working Group of Legal and Technical Experts on Liability and Redress to fulfil the mandate under Article 27. The took place from 19-23 February 2007 in Montreal, Canada. At this meeting, the Working Group considered, among other things, a blueprint for a decision on international rules and procedures in the field of liability and redress for damage resulting from transboundary movements of living modified organisms (UNEP, 2007). UNEP/CBD/BS/WG-L&R/3/3.

2. INTELLECTUAL PROPERTY RIGHTS

One effect of gene flow can be disagreements over the ownership of a crop. The likely contestants in such disputes would be those who hold the transgene or process IP and those who seek out the transgenic products for planting and purposefully fail to acknowledge the IP, those who do not explicitly seek out the transgenic products but are found to be in possession of the product, those who actively avoid the transgenic products (e.g., certified organic producers, non-GM producers) but are found to be in possession of the product, and those with proprietary claims to other transgenes or cultivars in which two different products mix (stacked plants).

The key question underlying all of these scenarios is whether gene flow can be managed sufficiently to allow coexistence of GM crops, non-GM cultivated crops and other prized plants, and accessions in gene banks, to a standard of purity that is agreed to by all.

The legal exposure of those found in possession of transgenes that are protected as IP will be determined by their national patent laws. Civil contracts entered into between farmer and seed supplier may also create obligations.

Agriculture in a region can cover a wide variety of disparate farming techniques. Those originating from indigenous and local cultures are termed 'traditional agriculture'. These practices are fluid, constantly being adapted and developed by farmers. Traditional agriculture serves as a contrasting idea to industrial agriculture, which describes large-scale farming serving bulk markets with a dominant business ethos. The use of the term should not imply the superiority of one or the other approach, or that industrial agriculture does not have its own internal variation or cultural links.

Traditional agriculture is usually understood as involving smallholder farming households, with small plots and few external inputs, growing a number of crop varieties bred specifically for their conditions, perhaps using intercropping, crop rotation, or other techniques. Small-scale farming is often characterised by having "loose, incomplete links with the market" (p. 84 Kostov and Lingard,

2002), perhaps selling produce on an *ad hoc* basis. Farming is not seen as merely a business for those engaged in selling part of their harvest, but is tied to culture and community (Cleveland and Soleri, 2005).

The context-specific knowledge held by farmers of land and crop varieties is very important to sustainable agriculture in developing countries. Farmers may select and save seed each season according to growing conditions and culinary and economic requirements. By breeding their own varieties, farmers are able to adapt seed to their conditions and needs and expand crop diversity.

All agricultural practices, regardless of origin, may be affected by novel crops and potentially incompatible crop management practices. Not only is the *preservation* of agricultural practices of concern in the event of transgene flow from GM crops, but the continuation of some practices may compromise potential benefits from GM seed. For instance, seed saving and exchange may lower the dose of Bt in subsequent seasons, intensifying the development of pest resistance to the toxin. In this way, traditional agriculture may be both under pressure from, and in conflict with, GM crop farming.

Traditional agriculture is often associated with subsistence farming. Subsistence farming involves crop production and livestock rearing for the consumption of the household rather than for external markets. While providing for household use is the primary aim, supplementary market activities may be included.

Subsistence agriculture is widespread. To illustrate this using two very different parts of the world, just over half of Romanian and 77% of Bulgarian farms, for instance, do not sell produce (Kostov and Lingard, 2004). Approximately 84% of Solomon Islanders depend on subsistence farming (Evans, 2006).

While subsistence farming has been characterized as often unproductive, it can play an important cultural, economic and food security role in a society (Brüntrup and Heidhues, 2002). Some degree of self-sufficiency in agriculture can be vital in situations of economic uncertainty, transition or conflict (Bourke et al., 2006).

In many developing countries it is traditional practice to save seed from one season's crop for use as breeding material in a later season (Pretty, 2001). The practice is also common in developed countries. "20–30% of US soya farmers reuse seed, and many wheat farmers only return to the market to buy seed once every 4–5 years" (p. 250 Pretty, 2001). Seed saving can help to guarantee an adequate seed supply from year-to-year, allowing farmers to independently sustain their crops. Transgene flow can introduce transgenes into non-GM seed stocks that are saved and shared.

Seed exchange or seed sharing involves a web of transactions and interaction among farmers in local communities and regions. It can include the purchase, borrowing, gift, exchange, and covert acquisition of seed (Basdstue et al., 2002). Seed exchange is a form of human assisted gene flow, potentially increasing the risk of dispersing GM seed to new farms and other environments and increasing the potential for gene flow through fertilization.

Depending on the relevant domestic legislation of a country, gene flow to and from transgenic crop plants may lead to restrictions regarding seed saving and sharing due to intellectual property rights granted for the crop, the transgenes or the process used for the modification of the plant. Without a farmers' exemption, allowing farmers to save seed after crop cycles, authorization from the patentee (usually involving the payment of royalties) would be required. Farmers who plant back and sell

seeds include intermediate types formed with GM crops may be beholden to the patent holder, whether or not their actions were intentional (Thomas, 2005).

3. CONCLUSION

The industrialization of the gene has benefited from the ability to use powerful enzymatic and chemical reactions to manipulate, describe and trace DNA (Heinemann, 2004). Some of these techniques, particularly the polymerase chain reaction (PCR), make it possible to identify DNA sequences protected as IP at profoundly low concentrations, in a way similar to their use in forensic police work.

Previously, plant traits that enjoyed IP protection were identified at a phenotypic level. That is, the trait could be observed with the eye or by monitoring the use of management practices that were unlikely to be used with other varieties of the same crop. Cross-fertilization in maize, for example, has traditionally been estimated by xenia, the effect of pollen on endosperm and embryo development (Devos et al., 2005).

As powerful as xenia is for observing cross-fertilization, and useful as it has been to develop and maintain individual lines, it pales in comparison to the ease and sensitivity of PCR which can, in theory, detect a single transgene in 10,000 genomes in a laboratory exercise taking no more than a few hours. At least from a legal liability point of view, “what is important for risk assessment of transgenic crops appears not to be the probability of gene flow itself but the traces of introduced gene(s) in subsequent generations in recipients” (p. 156 Yamamoto et al., 2006), especially as these traces do not have to produce noticeable phenotypes to make themselves noticed.

While these sensitivities may not be enough to guarantee that consignments of seeds are so dilute in GM material that there can be no threat to human health or the environment, or fall within thresholds limits of adventitious contamination (Heinemann et al., 2004), they are sufficient to dramatically increase the risk to farmers from adventitious contamination of their crops or seeds.

Transgenes are regulated under various national laws and international agreements, and through grower certifications. Some legal frameworks allow transgenes in non-GM crops, provided that they are below a quantitative threshold. It is difficult to ensure detection and to quantify transgenic material. As detection and quantification technologies improve, more trade disruptions can be expected as those with older technologies overlook contamination detected by importers with more advanced technology.

Transgene flow may also increase liabilities of non-GM producers and those GM producers that might face transgene flow from other GM varieties. This may have differentially adverse effects on societies with traditional, subsistence and seed savings cultures.

IV. MANAGING GENE FLOW

Theoretically, perceived and actual harms of gene flow may be mitigated or eliminated by containment and management strategies. Two containment strategies, or combinations of strategies, in addition to long standing segregation and identity preservation practices, have been proposed. Physical and biological containment strategies are discussed in the context of pollen and seed-mediated pathways described in Chapter I. There is no explicit strategy, physical or biological, to prevent horizontal gene transfer.

Will containment provide an adequate safety net to prevent predicted undesirable effects of gene flow? This can be more difficult to answer than it at first appears to be, and the answer is dependent on estimates of the harm being controlled by the containment strategy. For example, if the GM crop is designed to produce a potent human pharmaceutical, any escape of either the accession or the transgene may be deemed unacceptable. If the GM crop has an altered agronomic profile and is used exclusively in animal feed, containment might only need be at or below a legislated adventitious contamination level in human food.

"[I]t is essential to consider - from the very beginning of the process of developing a GEO [Genetically engineered organism] and its possible confinement - the risks and consequences of failure, the means of failure prevention (particularly by bioconfinement), and the potential for post failure remediation, to determine what, if any, bioconfinement measures to take" (p. 53 Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).

The strategies have different strengths and weaknesses. Two generic risks, however, should be considered.

1. Risk 1: failure to contain.

Due to environmental variation and human error, it is likely that no single containment strategy can be completely effective. It may be that no combination of containment strategies will achieve the level of containment necessary to avoid all harm, so the nature of the harm and the severity of consequences must be considered when evaluating a containment strategy.

2. Risk 2: failure to contain over time.

Containment strategies are new and very little testing has been done to verify their long-term effectiveness.

However, these strategies may restrict flow to below legislated threshold limits of transgenes outside of approved crops and products, and meet local or international requirements for safety depending on the transgene and the type of GM plant.

1. MONITORING CONTAINMENT

Monitoring is not a method for stopping gene flow, but if properly applied it may augment strategies designed to restrict flow and the area affected by flow to rates and sizes that are acceptable to set goals, such as legislated thresholds.

> *"The degree to which failed confinement can be monitored and managed depends on whether the GEOs* [genetically engineered organisms] *are easily detected, the scale at which they are released into the environment, and their subsequent population dynamics and the degree to which they can hybridize with related species" (p. 124 Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).*

There is considerable debate about the effectiveness of monitoring GM plants. “Monitoring transgenes has been proposed as a way to measure their possible environmental impacts and to serve as a warning system for deleterious effects. If gene flow were predictable, it might be easy to target when, where and how to monitor. Given the idiosyncratic nature of gene flow, and given that a tiny amount of gene flow is sufficient to establish an allele in a population, it will be difficult to create an effective monitoring programme” (p. 1167-1168 Ellstrand, 2003a). While monitoring is used worldwide, it is expensive and highly dependent on expensive technologies and sometimes dependent on access to proprietary information (references in Heinemann et al., 2004). Applying those technologies consistently between GM plants, laboratories and countries can also be problematic (Bonfini et al., 2001, Heinemann et al., 2004, Weighardt, 2006). This reduces the range of users who may successfully use monitoring as a management tool.

As argued by the US National Research Council, there are many compelling reasons for monitoring GM crops that are not specifically related to managing gene flow (Committee on Environmental Effects of Transgenic Plants, 2002). Adopting monitoring for these reasons will also augment capacity for managing gene flow.

Two significant challenges for the effective implementation of monitoring are encountered when gene flow to wild relatives is considered or when the transgene or the recipient may be slow to develop into a detectable population. Monitoring is difficult because of the stochastic nature of gene flow and the odd but occasional escape across unexpectedly large distances. In addition, wild populations are not routinely screened for gene flow. Thereby, hybrid populations may escape notice during the time when the population is either small enough to destroy or geographically localized. For some organisms (e.g., trees) and some genes (e.g., those with small contributions to fitness or those linked to fitness-enhancing genes), the lag-time until a detectable population emerges may be long. Meanwhile, the hybrids and descendant seed or vegetative material may have been extensively disseminated.

2. PHYSICAL CONTAINMENT

Physical containment strategies may be considered to ensure co-existence between GM plants and unmodified crop or wild plants. Physical barriers that might allow cultivation practices to coexist include manipulation of the spatial and temporal arrangement of crops and manipulation of reproductive structures.

Physical containment is best applied to control crop-to-crop gene flow, because separating crops from wild relatives may be difficult in some environments. For some crops, volunteers of the same or different species will always be present.

Spatial barriers

Spatial barriers involve the separation of fields by a zone of open land or other crops. Isolation distances are usually measured as open land and may be used to confine both pollen and seed. A crop zone is generally a pollen barrier.

Many studies have been conducted to provide a measure of the ideal isolation distance between GM and non-GM crops to maintain various contamination threshold levels. If the objective is to maintain gene flow below certain set thresholds, then the effectiveness must be measured on a crop-by-crop and landscape-specific basis.

The leptokurtic pattern of pollen and seed flow compromises isolation barriers. Still, some studies suggest that the use of such barriers may in general be effective at keeping the transgene content in neighboring crops, especially if the flow is dominated by wind, below some thresholds (*Appendix 2*).

Crop zones may be used instead of open land to limit pollen-mediate gene flow. Crop zones are a band of plants grown around source and/or recipient crops. This space allows crops to be isolated by distance, serve as pollen sink and offer the added protection of pollen production - conspecific plants release pollen to compete with incoming GM pollen and reduce air flow. Crop zones may therefore be more effective than other forms of separation (Devos et al., 2006). Pollen barriers can be used around both source and recipient crops, but barriers including pollen producing crops, hedges, trees and screens are usually recommended around recipient fields. This strategy is based on data showing that cross-fertilization rates are usually higher at crop edge rows than closer to the center of fields (*Appendix 2*).

Other physical and biological barriers, such as trees, or features of the terrain may be used to lower wind speed and create local eddies that may trap pollen and seeds. This can cause pollen and seeds to fall and cross-pollination to therefore be more concentrated around the barrier than would normally be expected (Henry et al., 2003).

For plants pollinated by insects, screen cages can be used around plants to prevent their access. This is impractical for general release, however, and will not prevent vertical wind-assisted pollen movement (Gurian-Sherman, 2006). For plants producing seeds that are transported by animals, fences and nets may inhibit seed-mediated flow, provided that access to seed is also restricted at other stages of the production and distribution chain.

Implementing spatial barriers
Few gene flow studies are available that take into account the nature of agriculture in practice; therefore, coexistence measures may overlook landscape scale effects. The ability to implement isolation distances will depend on the configuration of crop fields, terrain, and farm ownership.

Landscape-scale issues are well illustrated in two recent studies. *Messean et al.* analyzed the affect of introducing isolation distances in a farming region of Poitou-Charentes, finding that “isolation distances in practice affect a number of farmers that may not be able to freely choose the production type” (p. 37 Messean et al., 2006). The positions of their fields would make maintaining thresholds difficult regardless of segregation measures. Therefore, it is important that isolation distances are wide enough to reduce gene flow, but narrow enough to be workable for existing farms and farmer choice.

The studies by *Messean et al.* and *Squire et al.* agree that while the nature of regional agriculture may be significant in terms of background pollen, the proportion of GM to non-GM crops is not the sole criterion upon which to predict the effectiveness of coexistence strategies (but it may affect the feasibility of field configurations and isolation distances). Since large crop fields increased gene flow regionally (Messean et al., 2006, Squire et al., 1999), isolation distance and pollen barrier recommendations must consider the position of fields with reference to agriculture in the region, rather than using the common premise of a single pollen source. *Messean et al.* found that adventitious presence in an area with 10% of the crop being GM was sometimes lower than that when the 50% of the crop was GM, possibly because of the wider dispersal of GM fields. They concluded that “the distribution of GM fields in the landscape [is] what is important for coexistence rather than the global penetration of the GM technology in the region” (p. 34 Messean et al., 2006).

It may be possible for farmers to declare their region a GM crop-free zone or GM crop production zone on the basis of voluntary agreements, possibly supported by governments. This may be "the most effective and least costly measure to ensure co-existence" (p. 83 Devos et al., 2005).

While field placement with isolation distances may be possible for agricultural plots, this approach may be less useful for crop-to-wild gene flow if there is a lack of accurate information on the locations of inter-fertile wild relatives within pollinating distance (Flannery et al., 2005).

Limits to the effectiveness of spatial barriers
It is unlikely that even the strictest isolation distances will completely eliminate cross-pollination. *Rieger et al.* found canola pollen flow at up to 3km, even though most falls within 100m from the source (Rieger et al., 2002). Wind currents can also hinder the effectiveness of this strategy. Pollen can be carried to high altitudes by the wind and fall into a field a long distance away from the source without being challenged by the height of pollen barriers (Devos et al., 2005).

The merits of pollen barriers in different crop configurations are still uncertain. *Hokanson et al.* found that the use of large trap crops reduced long-distance pollen flow significantly. However, in demonstrating the importance of trap/pollen source ratios they used a crop configuration that is unrealistic in practice because they had borders that were larger than the transgenic fields (Hokanson et al., 1997). Lower ratios increased short and long-distance pollen flow.

They also found a significant anomaly in their measurements due to environmental conditions. While there was a consistent level of long-distance pollen movement to satellites within plots (0-4.7%), one satellite experienced high-magnitude gene flow of 38%, possibly explained by the location of ponds nearby, influencing the paths of pollinators (Hokanson et al., 1997). They concluded that while border rows may be effective for small trials, for commercial plantings they are "of dubious value" not only due to scale, but also to the fact that "environmental variation can result in substantial levels of isolated gene flow" (p. 1080 Hokanson et al., 1997).

The *Messean et al.* study (*Box 6*) also looked at non-GM buffer zones, concluding that they are only useful if GM and non-GM crops are close together. They used buffer zones of 9 and 18m around GM fields (intra-cluster), finding that it could be useful to maintain a 0.9% threshold, but will not always be enough. They found that with incoming wind currents to a non-GM field, an 18m buffer could not maintain cross-fertilization frequencies below 0.9%. They suggest that a buffer zone combined with a flowering time-lag (see below) could reduce GM adventitious presence to 0.5%. Flowering time-lags in intra-cluster arrangements could be expected to achieve 0.9% (30 degree-days), 0.4% (60 degree-days) and 0.1% (90 degree-days, with high seed purity and no mixing in machinery) (Messean et al., 2006).

There are very few studies dedicated to evaluating the long-term ability of spatial barriers to limit seed flow. In many ways, seed flow is a more complicated issue because seeds can have a dormancy of years, allowing their continual movement by vectors such as animals and human activity. Contaminated harvesting equipment and transport vehicles quickly transgress spatial barriers, undermining the ability of most landscapes to harbor effective zones. Land used for GM agriculture may be unsuitable for later non-GM cultivation due to volunteer growth, because volunteers within and around fields may hybridize with other related crops and wild relatives. Therefore, containment of transgenes depends on the proper disposal of plants and the seed bank after harvest by removing volunteers and stray seed.

***Box 6*: Landscape studies.**

Messean et al. conducted a landscape study measuring maize pollen flow using the MAPOD® model in two main farming scenarios (Messean et al., 2006). In the first, farmers in an area (or 'cluster') decided whether or not to grow GM maize as a group. In the second, farmers would grow their preference on fields within a cluster. Gene flow would therefore occur both inter- and intra-cluster. The study also looked at the effects of machinery use and cleaning.

Inter-cluster gene flow did not prevent achieving a 0.9% threshold. Isolation distances were sufficient to prevent adventitious presence over this target. The main variables beyond this would be seed purity and machinery cleaning, but high seed purity would maintain the threshold while allowing for typical lapses in machinery cleaning. The 0.1% threshold would only be achievable for inter-cluster arrangements with "almost pure seeds and no admixture due to machinery" (p. 34 Messean et al., 2006).

For intra-cluster gene flow measuring adjacent fields, 0.9% was not always an achievable threshold. The authors found "over 0.9% for some small non-GM fields adjacent to GM fields" (p. 34 Messean et al., 2006). Cleaning machinery can help, however. 0.1% thresholds could be achieved by some fields which had high seed purity and no mixing from machinery. Altogether, however, they concluded 0.1% "not achievable at landscape level with current practices in the intra-cluster case" (p. 34 Messean et al., 2006).

It is unrealistic to expect that all GM volunteer growth from some kinds of crops could be prevented. In fact, most cases of contamination with non-GM crops is likely to be associated with volunteers and stray seeds. Studies can measure the likelihood of persistent GM volunteer populations growing within or near non-GM fields and whether it would result in enough cross-pollination to push GM adventitious presence over thresholds (Flannery et al., 2005). Seed-mediated gene flow requires considerably more attention before firm conclusions can be drawn about maintaining thresholds.

Temporal isolation

Temporal isolation involves the use of delayed plantings and crop rotation. This reduces the possibility that GM and non-GM crops will fertilize one another because their pollen production and reception cycles are not synchronized. Temporal isolation also reduces the chances of seed-mediated gene flow because shared harvesting and transport equipment go back and forth between conventional and GM crops less often and cleaning may be more effective. Temporal isolation may also occur on the level of years, where break crops are introduced in areas that are in transition from GM to non-GM. This practice could reduce the frequency of volunteers in later years.

Staggering the sowing times of GM and non-GM crops may help to reduce gene flow by changing the times different plants flower. If crops do not have overlapping flowering periods, the chances of hybridization are greatly reduced. There was a reduction of 75% in cross-fertilization sowing crops no more than three weeks (reported in Devos et al., 2005).

Crop rotation could be used to minimize contact with non-GM crops if neighboring farmers are able to closely coordinate. This may not be possible in places where monocropping is widespread (Devos et al., 2005). This approach may also be unsuitable for crops in some environments. For

example, maize may be damaged by frost if planted early, and delaying sowing may compromise crop yield (Devos et al., 2005).

Prevention of flowering

Pollen may be contained in small scale fields by preventing individual plants from flowering, or by covering flowers (Gurian-Sherman, 2006). For example, detasseling involves removing the pollen-producing tassel from maize plants. It is usually done by hand due to variation in plant height, but can also be mechanised. Detasseling is an effective way to breed maize varieties without allowing gene escape, but would be difficult to maintain in commercial agriculture (Luna et al., 2001).

3. BIOCONTAINMENT

A variety of biological strategies have been devised to augment or replace physical containment strategies (for a list, see *Appendix 3*). Biological approaches to containing transgenes are of two forms: natural means and the use of additional transgenes. Natural means include exploiting existing breeding barriers, such as alloploidy, and introducing transgenes into plants that outcross only rarely, if at all. It may also be possible to contain transgenes by integrating them into the genomes of subcellular organelles, the chloroplasts and mitochondria, in species in which those are strictly maternally inherited.

Natural barriers to transgene introgression are expected to fail at some frequency, because most natural barriers to hybridization fail at some frequency. Alloploidy, for example, can be maintained by a shift to apomixes until the chromosome incompatibility is overcome and the hybrids regain their sexual fertility. Meanwhile, a large asexual population may have established, effectively accomplishing escape of the transgene.

Transgenes are also proposed as tools for transgene containment. Transgenes that reduce the fitness or sterilize an intermediate type might not stop flow, but could significantly inhibit introgression. Transgene-based transgene-containment strategies are highly controversial because they are similar, if not identical, to GURTs (Genetic Use Restriction Technologies). Since they do not specifically stop fertilization, they can potentially create harms, such as causing sporadic crop losses, in their localities from time to time.

Some transgenes might be controlled at the level of expression (T-GURTs). Unless specifically activated, the gene is not expressed. Again, this type of construct does not prevent gene flow but could significantly reduce the chances, or rate, of introgression when it is dependent upon selection. This strategy would be vulnerable to chance mutations that uncouple the gene from the expected regulation, or failure to appreciate environmental variables that might substitute for the human-controlled activation signal.

GURTs are extremely new approaches and have not benefited from significant testing. Should the GURT construct be transmitted to other crop plants or wild relatives, then there is the chance of significant periodic environmental disruption (*Figure 5*). This disruption may go unnoticed for significant periods of time if the recipient species has a long lifetime and long reproductive cycle, as is the case for some trees.

***Figure 5*: Consequences of GURT failure.**

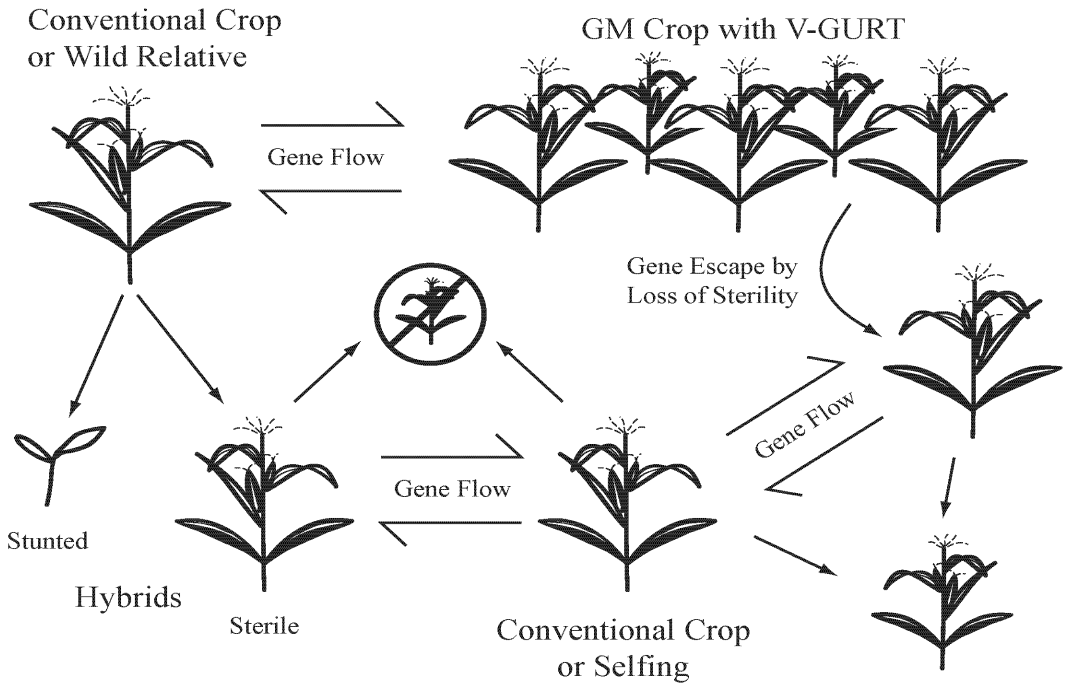

Figure 5: GURTs are designed to prevent the propagation of GM plants, whether they are the offspring of commercial varieties purchased from the manufacturer or hybrids that form by the bi-directional flow of pollen between GURT varieties and other plants (other GURT, conventional or GM varieties). Right: Recombination and mutation may be sufficient to cause GURT failure at frequencies that do not prevent escape of transgenes by cross-fertilization. Left: Mutations in regulatory DNA regions (e.g. promoters) or inhibitors (e.g. barstar) may cause constitutive expression of sterility-induced GURTs or prevent induction of fertility-induced GURTs. Depending on the sterility factor, stage of development and tissue regulation, constitutive expression could result in hybrids with grossly different growth and yield characteristics (stunted, far left) or regional crop failures (central left).

Biocontainment using transgenes holds more promise for managing crop-to-wild gene flow than the other strategies. However, this strategy also has not benefited from significant testing. Transgene-based transgene containment is a complex issue and is currently hypothetical. There are few or no natural analogs from which an experience of this approach can be extrapolated. For any strategy that affects fitness or fertility, the forces of natural selection will be formidable long-term antagonists of success (see *Appendix 4*).

Some containment techniques limit both pollen and seed/propagule-medated gene flow, while others are specialist for one of the two pathways.

Preventing pollen-mediated flow

Sub-cellular confinement
Plants carry their genes in three sub-cellular compartments, the nucleus, chloroplast and mitochondria. Some plants pass along the chloroplast (and also the mitochondria) only through the egg (maternally) and not through pollen. Hence, introducing transgenes into the chloroplast is ostensibly a method for keeping the transgene out of pollen (Daniell et al., 2005).

Many recombinant proteins are engineered to accumulate in the chloroplast. For example, the recombinant cDHDPS protein in Monsanto's corn line LY038 was engineered to accumulate in the chloroplast where its substrate and product is normally made (INBI, 2006). Chloroplasts maintain their own genomes (separate from the chromosomes of the nucleus), and thus could be transformed with these transgenes directly (Daniell et al., 2005).

However, chloroplasts are not strictly maternally inherited in all plant species (Dong and Wagner, 1994, Testolin and Cipriani, 1997). Even in species with a strong maternal bias, some escapes through the male line are possible (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004, Haygood et al., 2004, Medgyesy et al., 1986), just as has been shown true for mitochondria (Yu and Russell, 1994).

Moreover, gene transfer from the chloroplast to the nucleus can happen at high frequencies (Huang et al., 2003). While this has been dismissed by some (notably the Committee on the Biological Confinement of Genetically Engineered Organisms, 2004) as a viable route to escape, the evidence for dismissing is weak. The chance of transfer is as a high as 0.00006, an improbability that is easily overcome at the scale of commercial agriculture. Engineering expression for the chloroplast may reduce the frequency of expression in the nucleus, but it does not prevent transgene escape. Expression barriers are never absolute in any case. Thus, transgenes initially confined to the chloroplast genome may escape via the nucleus. Recent models indicate that containment strategies based on chloroplast transformation are likely to fail (Haygood et al., 2004).

A combination system for transgene containment has recently been proposed. In this system, a transgene is inserted into the chloroplast genome. In addition, the transgene is split into two halves that must be assembled through a process called protein splicing (inteins) after translation of each separate mRNA. Using natural inteins, analogous to the introns of RNA, different polypeptides can be spliced together (Gogarten et al., 2002, Yang et al., 2003). Unless the two genes remain together, and the polypeptides remain in the same location after translation, the protein cannot be assembled (Khan et al., 2005).

Protein splicing is a recent discovery. It is closely associated with genes that are frequently mobile, transmitted by horizontal gene transfer. While this fact alone does not mean that the use of inteins will result in greater transfers of transgenes, little is known about the possible unanticipated effects of trans-splicing. Specialist advice should be sought if trans-splicing is being considered as a containment strategy.

Apomixes
Plants that produce seeds without outcrossing or selfing reproduce by apomixes, or clonal reproduction. The genome of progeny is identical to the parent's genome. These plants may not produce pollen.

Plants that reproduce by apomixes may be unreceptive to pollen from others, but they are not necessarily male sterile and may still produce viable pollen. Moreover, there is considerable cause for concern about the consequences of plant escape because apomictic plants are often able to displace sexually reproducing varieties (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).

Male sterility
For transgenes that are inserted into the nuclear genome, male sterility may prevent pollen-mediated gene flow. Infertile pollen production is a characteristic of some plants. Genic male sterility arises from mutations in certain genes found in the nucleus. Cytoplasmic male sterility (CMS) is caused by an incompatibility between the mitochondrial and nuclear genomes (*Box 7* and Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Such an incompatibility could be introduced to restrict pollen fertility of GM crops. Male sterility can also be created by genetic engineering (Chase, 2006, Sandhu et al., 2007).

B*ox 7*: dsRNA-mediated sterility

Some RNA molecules are regulatory in the sense that they control the expression of other genes. As discussed in Chapter I, the RNA molecules of this type are called, variously, dsRNA (Denli and Hannon, 2003), short/small interfering RNAs (siRNAs), repeat-associated short interfering RNAs (rasiRNAs), microRNAs (miRNAs) (Meister and Tuschl, 2004) and short-hairpin (sh)RNA, and cause the phenomenon known by the names RNA silencing, RNA interference (RNAi), inhibitory RNA, quelling, MSUD, co-suppression and post-transcriptional gene silencing (PTGS) (Chandler and Vaucheret, 2001). dsRNA is rapidly being adopted as a genetic modification technology, making more widely available the tools necessary to construct and deliver vectors of dsRNA.

Sandhu et al. (2007) devised a means to use dsRNA to invoke CMS in tobacco and tomato plants that were producing viable pollen. The strategy was to use dsRNA expressed from a transgene in the nucleus to silence a gene called *Msh1*, found necessary to prevent changes in the mitochondrial genome that cause CMS. It could take several generations before the effect was bred true, but once invoked, CMS was stable in offspring created in crosses between the CMS plant and normal pollen. Apparently male sterile plants could create rare offspring from their pollen and most of these were also infertile.

This particular approach holds promise for introducing CMS to increase the efficiency of crossing self-pollinating plants. However, the effect could be irreversible and does not require the continued presence of the inducing transgene. Therefore, the products of this gene technology could not be traced using the transgene as a tag.

Transgene excision.
Transgenes could be removed before seed or pollen is made. The transgenes could be flanked by DNA sequences that were the target of a specific recombinase, such as Cre. Recombinases are enzymes that make sequence-specific breaks in double-stranded DNA, the structure of the molecule in chromosomes, and make reciprocal exchanges of DNA (Visser et al., 2001). Recombinases are isolated from different sources. Perhaps the best recognized recombinase is Cre, which was first sourced from the P1 virus that infects bacteria. This enzyme is illustrative of the essential features of recombinases used to remove genes from transgenic plants.

Cre cleaves DNA at a sequence called *loxP*. If a gene is flanked by two copies of *loxP*, Cre will break the molecule at each end of the gene, liberating the intervening DNA, and reseal the chromosome (which completes the DNA exchange reaction). Cre and other recombinases and their site-specific processing sequences are increasingly popular tools for removing transgenes from commercial transgenic plants (Konig, 2003). Antibiotic resistance genes, used to select the initial transformed plant cell, can be removed in the crop development phase where antibiotic selection is no longer required[2]. The most common method is to cross the primary recombinant plant line with a GM line expressing the Cre recombinase, and then segregating offspring to select those that are free of the *cre* and the target genes. However, viral vectors of *cre* are also being tested (e.g., Jia et al., 2006).

One test of excision as a containment strategy was conducted in tobacco and *Arabidopsis* using the *cre* gene under the control of a promoter that was selectively active in the microspore during pollen development (Mlynarova et al., 2006). However, if a recombinase is used to excise a transgene, the reaction must be working at an unexpectedly high efficiency to be effective (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004, Hara-Kaonga et al., 2006). In the example of a *cre* gene activated during pollen development discussed above, transgene excision was measured in samples ranging from 100-17,000 seeds. The transgene was detected in 0.027% of the seed (Mlynarova et al., 2006). This frequency appears low, but would not be at the scale of commercial agriculture. For example at this failure rate, 3-10 recombinant hybrids would be expected among the tens of thousands of canola-weed hybrids that are estimated to be produced annually in the UK (Marvier and Van Acker, 2005). It would amount to nearly 3,000 volunteers/hectare among the estimated 10 million seeds lost during harvesting (IOR-HDRA, 2006).

Moreover, the entire transgene will not be removed by excision. Processing leaves an intact target (e.g., *loxP*) sequence in the chromosome (Mlynarova et al., 2006). Processing of target sites does not entirely reverse the effects of the original insertion nor leave the site with the same risk spectrum as before the insertion of the transgene. The residual target sequence may make the chromosome vulnerable to double strand breaks should, by chance, the plant be crossed with a recombinase-containing (e.g., *cre*) line. In a study of tobacco plants, single *loxP* sites on different chromosomes mediated recombination between the chromosomes precisely at *loxP* in the presence of the Cre recombinase (Qin et al., 1994).

Preventing seed/propagule-mediated flow

Asexual plants
Asexual plants, for example bananas and seedless grapes, that produce neither pollen nor seed should produce no intermediate types. Nevertheless, they are propagated vegetatively and therefore the potential for propagule-mediated flow will depend on how independent propagules are of human assistance.

Infertility
For other plants, it may be possible to rely on their tendency to produce sterile offspring. The ability to form hybrids is not evenly distributed among plants (Ellstrand et al., 1996). Interspecific hybrids frequently have reduced fertility; however, they are rarely fully sterile (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Hybridization even at low frequencies can have profound impacts for plants over time or for plants which may be propagated purposefully

[2] See, for example, US Patent Application 0050132437.

or accidentally by human means. “A single, partially fertile, hybrid individual can suffice as progenitor of a new species. Several species or even genera may evolve from the resulting hybrid lineage. At any given time, only a tiny fraction of hybrids may give rise to new lineages. Still, that tiny fraction, summed over thousands of generations, can make a considerable impact in evolutionary time” (p. 5093 Ellstrand et al., 1996). At the scale of commercial agriculture, evolutionary time may not be as long as is sometimes implied (Heinemann and Traavik, 2004).

Hybrid phenotypes and fitness are also highly environment-specific (Arnold and Hodges, 1995). Thus, if hybridization is used as a containment strategy, it must be extensively tested in multiple environments over several seasons (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).

Sterile hybrids often derive from differences in ploidy, meaning different number of sets of chromosomes. The number of chromosomes in a species is usually called “n”. For example, humans have 23 chromosomes, or n=23. The cells of our body have 46 chromosomes (diploid, 2n), or two copies of each chromosome (one copy from each of our parents). Sterility can arise when individual chromosomes (aneuploidy) or entire sets of chromosomes (polyploidy) are present in higher numbers (e.g., 3n, or triploid). In humans, triploidy is lethal. But it is not lethal in plants, and is common among cultivated varieties (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).

Triploidy can result from fusion between a normal (1n) gamete and an abnormal (2n) gamete. Alternatively, it can result from hybridization between a tetraploid species (gamete 2n) and a diploid species (gamete 1n). Such hybrids often have reduced fertility. The odd ploidy number can be maintained through apomixes or vegetative propagation.

Unfortunately, hybrids, even if they have reduced fertility, may have enhanced abilities to spread by vegetative propagation (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Moreover, ploidy differences can be overcome. Bioconfinement characteristics must be measured under a variety of environmental conditions and over several growing seasons before drawing conclusions about effectiveness.

Dioecy

A degree of containment may be achieved by growing only one sex of plant in a particular region. Unisexual GM plants, for example, may be prevented from crossing by isolating them from plants of the opposite sex. Thousands of plant species are sexually differentiated (dioecy) such that a particular plant makes only male or only female flowers (Vyskot and Hobza, 2004). Examples of plants with defined unisexuality are gingko, avocado and asparagus. Some plants have three defined sexes. For example, papaya (paw paw) can be male, female or hermaphroditic (Deputy et al., 2002).

Dioecy cannot be relied upon as a containment strategy, though, because the phenotype is “leaky”. In other words, male plants can still produce some seed at low levels, frequencies high enough to become relevant at commercial agricultural scales. This method is also extremely vulnerable to human error.

Tissue confinement

For plants with separate grafted rootstock and scion, the transgene could be separated from sexual tissues by using transgenic rootstock (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). However, transgenic rootstock can spontaneously generate flowers. Moreover, modifications based on dsRNA (*Box 7*) can be systemically transmitted through grafts (Palauqui et al., 1997, Vaucheret et al., 2001).

4. CONCLUSION

Managing transgene flow is considered by some to be the same challenge as managing the flow of any exotic or other gene that is considered to be a threat to human health, the environment, productivity or IP. In terms of the physical and biological strategies for managing gene flow, this view is probably correct. In terms of the types of harms that might result from the failure of management, however, this view is not generally agreed. In part, it cannot be true for some genes, such as those that produce potent human pharmaceutical agents, dsRNA with the ability to silence human genes, or allergens - the genes which would not be present in the relatively small number of significant human food crops either ever, or because of long term human selection against such plants.

For other transgenes, it might be possible to achieve legal, cultural and safety goals through managing their escapes. Two management strategies were reviewed. They were physical and biological containment. A third, abstinence from the release outside of contained laboratories, is implied.

The key conclusion is that no single, and possibly no combination, of containment strategies will be completely effective. The US National Research Council therefore recommends that, when the anticipated harms that might arise from the escape of a transgene are considered acceptable but undesirable, an integrated confinement system (ICS) be used (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). This concept makes use of a combination of complementary containment systems from those described above. This recommendation is sensible, but it does not address the regulator community's need to assess how effective a combination of strategies might be when the effectiveness of each individual system remains uncertain at the scale of commercial release.

V. IS CO-EXISTENCE SUSTAINABLE?

Can co-existence be maintained, or will transgene flow come to threaten the biological independence of non-modified plants and agriculture that does not use GM crops?

Most regulatory systems define GM plants at least in part by the "event" that they carry. Commercial developers do as well, securing their IP through a description of the modification. This "event view" is capable of distinguishing between two transgenes that confer the same trait by functions that are seemingly identical. For example, different *cry* alleles (same gene) in two different events made by two different manufacturers can be handled independently by both regulators and patent offices. Co-existence of these two different varieties, as legal, biological and commercial entities of GM plant could also be threatened by transgene flow.

The main objective of this study was to propose a typology characterizing transgene flow according to the effects it may have, as the basis for an objective consideration of gene flow and possible ways to deal with the phenomenon. The study describes the responses that governments and/or stakeholder have made, or which could be envisioned, in order to address some or all of these effects, and discusses possible ways in which national governments and the international community could address transgene flow. In this section, the most important scientific and policy gaps are identified.

It is difficult to extract universal truths about the actual extent or rate of gene flow from existing studies. What is a true quantitative measure in one environment may be different in another environment. Therefore, a definitive scientific study on gene flow is unlikely to replace the need for case-by-case assessment.

Nevertheless, studies on local scales do contribute to a global understanding of gene flow and thus have great value. Their value would be improved if there were consensus in experimental design, including agreement as to what variables, at a minimum, would be measured in all future studies and how they would be measured to maximize comparability between studies. Transgenes that create clear or anticipated environment harms require considerably more testing on their effects on wild animals, particularly endangered and key indicator species. A relevant and uniform research methodology for testing unintended and unanticipated effects of transgenes in GM crops and hybrids should be developed.

Some common ground approach also has been advocated by Andow and Hilbeck who proposed a promising new model for assessing the potential for transgenic plants to be unintentionally harmful to some insects or animals, which they call the 'ecological method' of non-target risk assessment (Andow and Hilbeck, 2004). They proposed six criteria for ecologically realistic experiments.

1. "Use the same foods in laboratory tests that are used by the test species in their relevant habitat. If a transgene product is used, it should be identical to what is produced in the transgenic plant."
2. "Verify that the food offered to the species actually contained the administered material at the intended concentration or dose throughout the investigation."
3. "Verify that all life stages of the species are exposed appropriately to the transgene product and actually contact the product in relevant ways."
4. "Either use intact plants (or plant parts) in the experimental system, verifying that the plant parts contain the transgene product, or use the transgene product at concentrations or doses much higher than normally expressed in the plant, which incorporates an uncertainty factor into the experimental design."
5. "Use a proper scientific control."

6. "Screen sufficient numbers of individuals and perform sufficient replication" (p. 645 Andow and Hilbeck, 2004).

Their methodology would overcome some significant inconsistencies between existing studies (the latter also discussed by Clark et al., 2005). Adoption of their recommendations would help to achieve comparability between studies and establish a sound base for ecological risk assessment.

While there is always the danger that more research will add more knowledge without resolving issues of uncertainty, it is also possible to underestimate the contribution that basic research in biology makes to problem solving. A sustained global commitment to untargeted, "curiosity-driven" research is essential to ongoing and informed evaluation of the impacts of agriculture in general and transgene flow in particular.

Transgenes that create clear or anticipated harms to humans, animals or non-target species are regulated in an inconsistent fashion. Among the countries listed as some of the largest producers of GM plants, and with the largest research efforts in developing non-food and/or non-feed crops, the track records for containing such plants is blemished.

International concern about pharma and industrial plants is justified, because past containment failures indicate how these experimental and commercial crops could escape and be globally distributed in short times.

Transgene flow may create legal liabilities and has already been the subject of large and acrimonious disputes. IP and liability disputes have the potential to significantly interfere with non-GM agriculture and the ability of public, not-for-profit, institutions such as Future Harvest Centres, to introduce potentially valuable GM crops into countries that suffer from low food security.

Resolving these issues may require an unprecedented level of organization of the agricultural landscape. In many ways, GM agriculture is segregated on the level of the nation state, with some countries (e.g. New Zealand) choosing to so far not grow GM crops commercially. The Cartagena Protocol on Biosafety, TRIPs and the Sanitary and Phytosanitary Agreement (SPS), among other international agreements, are the mechanisms for describing the global landscape with respect to GMOs.

Transgene flow simultaneously raises the legal risk profile of GM and non-GM farmers. Non-GM farmers may be accused of patent infringement if transgenes are found in their possession. GM farmers may also be accused of patent infringement if they are found in possession of a different transgene, for example, because of the growth of volunteers on land used to grow a different variety of the same kind of crop.

Heterogeneity of liability frameworks also creates new exposures for both GM and non-GM farmers. In some countries, the GM farmer or biotechnology company may be liable for claims of harm to those who trade on a GM-free status. In other countries, adventitious contamination is a cost borne by those claiming to be GM-free.

While these problems are expected to grow rapidly in developing countries, they are already significant in developed countries.

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GLOSSARY

ALLERGEN	A molecule that invokes an immune response associated with allergies. Can be life-threatening.
ALLERGENIC	Having the properties associated with causing allergies.
BIOLOGICAL INVASION	A change in natural communities and ecosystems due to disruption by the distribution of quantity of an exotic species.
BT CROPS	Plants engineered to produce protein insect toxins (pesticides) sourced from the chromosome or infectious agents within the soil bacterium *Bacillus thuringiensis*.
CARTAGENA PROTOCOL ON BIOSAFETY	International treaty on the transboundary movements of living modified organisms.
DEMOGRAPHIC SWAMPING	The decrease or extinction of wild populations as a result of lower fitness when acquiring crop genes.
GENE FLOW	Movement of genes into a new genome or environment.
GENETIC ASSIMILATION	Crop genes replacing wild genes (potentially reducing genetic diversity of wild populations).
HORIZONTAL GENE TRANSFER	Introduction of genes into organisms by processes which are independent of organism reproduction; infectious.
HERBICIDE TOLERANCE (HT)	Plants made herbicide tolerant (or resistant) using genetic engineering. Note that it is also referred to as herbicide resistance. The commercially predominant resistances are to glyphosate and glufosinate ammonium.
HYBRID VIGOR/HETEROSIS	Observed increase in vitality and/or fertility of hybrids.
HYBRIDIZATION/CROSS-FERTILIZATION	Crosses between individuals that belong to different varieties, subspecies or species. Hybridization is sometimes reserved for crosses between species while the term outcrossing is sometimes used to emphasize crosses between genetically similar individuals.
IDENTITY PRESERVATION	Coordination of agricultural commodities through the supply chain to maintain their purity, mainly to capture price premiums.
INDUSTRIAL PLANTS	Plants created by recombinant DNA techniques (see also PMIPs) that make compounds with the following combination of properties: new to that plant, not common in food or feed, and may be for purposes

	other than food or feed.
INTROGRESSION	Depending on who uses the term, its meaning can vary from inclusion of a gene into a species or population, to the extent that the gene is found in nearly all individuals of the latter population, to stability of gene in one or more backcrosses between hybrids and parental types.
IN VITRO	"In a test tube." A non-living system usually for use in laboratory experiments or industrial processes.
IN VIVO	Conducting an experiment under natural conditions, such as with molecules inside a living organisms.
INTELLECTUAL PROPERTY RIGHTS	Rights granted to persons or entities over intellectual property which they can claim is unique to them. Patents are legal instruments establishing certain intellectual property rights.
INVASION	See Biological Invasion.
IPR	See Intellectual Property Rights
ITPGRFA	International Treaty for Plant Genetic Resources for Food and Agriculture
LEPTOKURTIC	A type of symmetrical statistical distribution with a greater proportion of values close to the mean, than in a normal distribution.
LINKAGE	When genes are inherited together, or the degree to which they are inherited together.
MATERIAL TRANSFER AGREEMENT	These are legal agreements governing the transfer and use of tangible research materials between organizations or individuals.
MOLECULAR PLANT PHARMING	Production of therapeutics in recombinant (GM) plants (see also PMPs). The term makes reference to conventional farming of crop plants.
MONOCOTYLEDONOUS	Belonging to the subclass of flowering plants called *Monocotyledonae,* which are characterized by their embryos containing only one cotyledon, or seed leaf.
OUTBREEDING DEPRESSION	The reduction in fitness of progeny from crosses between individuals from different populations, compared to the progeny of crosses of individuals from the same population.
OUTCROSSING	Mating or crossing between members of different populations (i.e., each mating individual is mating 'outside' its own population).
PHYTOPLASMA	A type of prokaryote which lacks a cell wall. Many phytoplasmas

	cause disease in plants.
PHYTOREMEDIATION	The use of plants to remove or neutralise toxic or otherwise unwanted environmental contaminants.
PLASMODESMATA	The plural of *plasmodesma*, which are small channels linking the cytoplasm of plant cells, bridging the cellular walls between plant cells.
PLOIDY	The number of copies of a genome present in an individual.
PMP	Plant-made pharmaceutical.
PMIP	Plant-made industrial product.
POST-TRANSLATIONAL MODIFICATION	The modification of polypeptides after they have been synthesized. This often takes the form of the addition of molecular groups to particular amino acids within the polypeptide, often with effects on the three-dimensional structure of the protein.
PROPAGULE	A structure (as a cutting, a seed, or a spore) that propagates a plant.
PROPAGULE PRESSURE	Possibly the most important mechanism for invasion. It is a measure of the size and frequencies of introductions of members of an exotic species.
REFUGE STRATEGY	A management strategy used to reduce the probability of the evolution of pest resistance (usually to Bt toxin). It assumes that resistance is recessive and that heterozygotes for the resistance allele are sufficiently sensitive to In this strategy, a “refuge” crop of non-Bt plants capable of supporting insects (due to a lack of toxin) are located near the Bt crop. The rare resistant insect emerging from the Bt crop will most likely mate with a sensitive member of the refuge crop insect population, producing sensitive progeny which are then eliminated by the BT crop.
REGIOSPECIFICITY	A property of enzymes, referring to the ability to preferentially catalyse the breaking or formation of certain chemical bonds in a substrate, over others in that same substrate.
SEGREGATION	Separated processing and handling channels, mainly for food safety.
SPS	Agreement on the Application of Sanitary and Phytosanitary Measures.
STACKING	The accumulation or presence of multiple modified traits in one organism as a result of the crossing of individuals with component modified traits.
STOCHASTIC	Random or generated by random processes.

SYNTENY	The order of genes, as on a chromosome.
TORT	Refers to civil legal actions, arising independently of contract.
TRACEABILITY	Records and testing to track products through the supply chain.
UPOV	Union International pour la Protection des Obtentions Végétales – International Convention for the Protection of New Varieties of Plants.
VOLUNTEER PLANTS	Crop plant which persist for several seasons without being deliberately replanted.
WESTERN BLOT	Also known as an 'immunoblot'. A molecular biological method which uses antibodies to detect and quantify a protein in a sample.
WTO	World Trade Organisation

Appendix 1

Combinatorial effects

Combinatorial effects are emergent properties that are qualitatively or quantitatively more powerful that would be predicted from the knowledge of the characteristics of the genes or gene products measured separately. These effects can be useful or counter productive, depending on the effect and the application. Gene flow, can create unanticipated combinations of transgenes, or transgenes and endogenous genes, generating unintentional combinatorial effects.

Synergy and antagonism.
Regev et al. (1996) tested the efficacy of recombinant Cry1c and endochitinase (from the bacterium *Serratia marcescens*) on larvae of *Spodoptera littoralis*, both singularly and then in combination. Nearly seven times the concentration of Cry1c was necessary for maximum effect alone than when used in combination with endochitinase. Based on this observation, the authors suggested that the effect was synergistic, a term usually reserved for an effect that is much larger than would be predicted from the effect on the larvae of each toxin independently (Regev et al., 1996).

Meanwhile, Lee et al. (1996) found that mixtures of Cry1Aa and Cry1Ab were antagonistic when simultaneously applied to the gypsy mouth *Lymantria dispar*. This was surprising because Cry1Aa and Cry1Ab were more potent alone than Cry1Ac; Cry1Ab being 4 times more toxic by weight than Cry1Ac. The combination of Cry1Aa and Cry1Ac was up to seven times more toxic, and three times less Cry1Ac was needed in this combination to be as toxic as Cry1Ab alone (Lee et al., 1996).

Interestingly, the combination of Cry1Aa and Cry1Ab was not antagonistic when used against cotton bollworm *Helicoverpa armigera* (Chakrabarti et al., 1998). Chakrabarti et al. (1998) also found a 26-fold synergism with the combination of Cry1Ac and Cry1F.

An unexplained synergy also may be created by a novel combination of genes. This has been seen in varieties of maize engineered to achieve high lysine levels through two different biochemical mechanisms (Huang et al., 2005). The synergistic effect was revealed because in stacked varieties the levels of free lysine and lysine derivatives were higher than would be expected from an analysis of the modifications kept separate in different varieties.

Synthetic phenotypes.
Unrelated genes sometimes produce a novel or synthetic phenotype when brought together (Heinemann et al., 2000). As has been well documented in bacterial genetics, some combinations of genes produce a phenotype that is entirely dependent on both genes and different from the phenotype of either gene alone. For example, the combination of the alleles *rpoB*87 (conferring rifampicin resistance) and *gyrA*87 (conferring nalidixic acid resistance) provide protection against a third antimicrobial agent, the completely unrelated DNA-damaging agent mitomycin-C (Heinemann et al., 2000).

Synthetic phenotypes are also well known in plants. For example, in the study of *ZWI* gene mutations in Arabidopsis, it was revealed that a combination of particular alleles *zwi-3* and *suz1* caused a male sterile phenotype (Krishnakumar and Oppenheimer, 1999). Only this combination of alleles of the same gene caused that phenotype. More surprising, the *ZWI* gene was being studied because of its role in determining cell wall structure, and *suz1* has no observable phenotype when separated from *zwi-3*.

The synthetic phenotype and synergy examples provide two lessons. First, they reconfirm the level of uncertainty about assigning biochemical roles to genes and their products, based on extrapolation from highly limited numbers of contextual observations. Second, they show that unexpected combinatorial effects can occur as a result of gene flow when genes from different organisms are combined.

Silencing.
While silencing that occurs by the general pathways controlled by short double-stranded RNA (dsRNA) molecules (e.g., RNAi, PTGS, TGS, co-suppression) are targeted to sequence matches between the dsRNA and the silenced genes, there are often effects on non-target genes as well. The number of genes simultaneously silenced by a single dsRNA (including the two targets) can number in the hundreds (Jackson et al., 2003, Jackson et al., 2006, Jackson and Linsley, 2004), and a variety of dsRNAs with no sequence similarity can silence the same genes (Semizarov et al., 2003).

Silencing is also epigenetic and infectiously transmitted between plants by grafting (Vaucheret et al., 2001, Yoo et al., 2004). This means that once established the effect persists across generations and potentially infects all tissues within a plant. The instigating event is the initial combination of transgenes or genetic elements with similar DNA sequences, but the silencing effect then may persist even in hybrids that retain a single copy of the gene.

Not all genes that are silenced remain so, nor are all plants grafted with tissues from silenced plants capable of acquiring the silenced phenotype. The science of infectious dsRNA is still very young, leaving gaps in understanding how the molecules are transmitted and maintained, and in how the phenotype is regulated or reversed. This makes predicting the effects of gene flow particularly problematic.

Appendix 2

Evalutaion of isolation distances for containment of pollen

Pollen-mediated flow of transgenes between maize can be maintained at or below a 0.9% threshold by using isolation distances of up to 200m (reviewed by Devos et al., 2005). Luna *et al.* studied isolation distance by growing maize plots at different distances from a pollen source. The maximum cross-pollination distance recorded was 200m, with no dispersal recorded at 300m (Luna et al., 2001).

Optimistically, a 200m isolation distance is at the high end of suggested isolation distances for the maintenance of this threshold in maize. Henry et al. concluded that an isolation distance of 24.4m may be adequate for the 0.9% threshold (with 80m for a 0.3% threshold, and 200m for organic farming) (Henry et al., 2003). A Spanish study which used a combination of xenia and quantitative PCR to determine cross-fertilization frequencies found that a threshold of 0.9% adventitious contamination could be achieved by a separation distance of 20m and that a buffer of non-GM plants was more effective a barrier for pollen than distance alone (Pla et al., 2006).

An Australian study measured crop-to-crop pollen flow between GM and non-GM canola by testing seeds from 63 fields near GM herbicide-resistant canola fields, and exposing the planted seed to herbicide. They found that "[w]hen individual samples were pooled within fields, resistance was evident in 63% of these fields, although only a few had more than 0.03% resistance" leading to the conclusion that "even adjacent commercial canola fields in Australia will have much less than 1% gene flow" (p. 2387 Rieger et al., 2002).
However, a key problem for adventitious presence in canola cultivation will be the growth of volunteers. As discussed in Chapter II, volunteers and the seed bank can serve as bridges in time to maintain and amplify cross-fertilization frequencies.

The maintenance of a 0.1% GM contamination threshold for crops with leptokurtic pollen dispersal is much more difficult because small amounts of pollen can travel over long distances (the 'tail-end' of the pollen flow). Existing isolation distances may not take the 'tail' into account (Flannery et al., 2005). While only a small proportion of total pollen is likely to spread over long distances, this pollen "may still be translated into relevant out-crossing amounts at considerable distances from a source" (p. 81 Devos et al., 2005). There is no consensus that small amounts would not be sufficient to establish hybrid populations (Burris, 2003, Devos et al., 2005).

When crops are separated by open land, it may be important to minimize GM contamination before harvest by removing egde rows that face GM crops(10-20m) to. Rows facing a GM crop are likely to catch the most pollen from the pollen source if low barrier crops or land is used to separate plots (Devos et al., 2005). Henry et al. recorded hybridization with GM-facing plants (on the edges of plots) 650m away from the pollen source using a 142m isolation distance (Henry et al., 2003). The removal of these plants may bring cross-fertilization levels down significantly; however, it may impose costs on farmers.

However, in a gene flow study of canola, Rieger et al. did not detect a reliable edge effect. They found that "[i]n fields where the front edge was less than 100 m from the herbicide-resistant field, similar frequencies of resistance were found at all three collection points within the field. Overall, some fields did show a decline in resistant individuals with distance, but the majority of fields, particularly those further from the source field, were more variable" (p. 2387 Rieger et al., 2002).

The shape and density of fields may have an effect on pollen dispersal and hybridization. Studies have found that if the recipient field is elongated and the longer side faces the pollen source, cross-fertilization can double. This rise can be avoided if the field is made deeper so that more plants are further away from the source (Devos et al., 2005).

Variation in local conditions, wind currents and pollinator behaviour make it difficult for conclusions to be drawn about gene flow without studies for the specific crop in the receiving environment. In the absence of specific studies, the extrapolation of data to new cases should be done with care.

Appendix 3

Plant biocontainment methods

What to Contain	Method	Major Limitations
pollen and seeds	Sterile triploids or interspecific hybrids	Few triploid or sterile hybrid cases apply or are effective
	Use only male or female plants and propagate vegetatively	Not feasible if same species or compatible relatives could cross-pollinate with unisexual plants; unisexuality may not be absolute
	V-GURTs (e.g. terminator)	Still in early development, not reliably tested
pollen only	Male sterility	Only available for some species and may not be reliably stable; some transgenic methods in early development
	Maternal inheritance (e.g. transform chloroplast and not nuclear genome)	Not feasible for plants with paternal inheritance of chloroplast DNA (most gymnosperms); DNA can cycle from chloroplast to nucleus; not an absolute barrier
	Cleistogamy (closed, self-pollinating flowers)	Still in early development, not reliably tested; no known research examples
	Apomixis (asexual seed production)	Still in early development, not reliably tested;
vegetative propagules	V-GURTs (with inducible promoters to kill vegetative tissue)	
traits (not transgenes)	T-GURTs	Still in early development, not reliably tested; does not prevent spread of transgene and reactivation in other genomes
	Tissue- and organ-specific expression of transgene (e.g. controlled promoters)	Still in early development, not reliably tested; extremely difficult to get no expression from a promoter
transgenes	Only introduce into rootstocks (pollen and seed transgene-free)	Still in early development, not reliably tested; only applicable to grafted scions of certain woody species such as grapes and fruit trees
	Excise transgenes before reproduction (e.g. cre/lox systems)	Still in early development, not reliably tested; speculative
	Repressible seed lethality	Still in early development, not reliably tested
	Cross-incompatibility	Still in early development, not

		reliably tested; speculative
	Chromosome location in allopolyploids	Still in early development, not reliably tested; speculative; not reliable (Stewart Jr. et al., 2003b)
	Linkage to fitness-reducing gene (similar to V-GURT, but not directly lethal)	Fitness reduction must be special to wild relatives only; genes may separate by recombination; similar risks as V-GURTs
plant	Attenuated survival ability (e.g. auxotrophy) outside of human cultivation (i.e. domestication)	Under development; also fails to confine genes; genes may separate by recombination; similar risks as V-GURTs

(adapted from Table 3-1 of Committee on the Biological Confinement of Genetically Engineered Organisms, 2004)

Appendix 4

Evaluation of transgene-based sterility systems for containment

Genetic use restricting techniques or GURTs are transgene-based systems of induced sterility.

> *Researchers have proposed various transgenic methods by which sterility can be gained or lost by design. One type of reversible sterility blocks gene flow through pollen and seeds, thereby, for example, preserving a seed company's ownership of transgenic germplasm. With this method, transgenes that confer desirable traits are linked to transgenes that cause sterility, and the two are inherited together. Because this strategy restricts access to fertile plants, it is known as variety genetic use restriction technology (V-GURT). Trait genetic use restriction technologies (T-GURTs) induce transgenic traits in fertile plants by means of a specific stimulus, such as a chemical spray* (p. 72 Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).

In addition to potentially protecting the property of the developer, GURTs can function as gene containment strategies and can be used to accelerate the development of heterosis in self-pollinating crops for which there is no natural male sterility (e.g. Sandhu et al., 2007, Sodhi et al., 2006).

> *"Transgenic male sterility could allow for hybrid seed production to be introduced to crops for which natural genic or cytoplasmic systems do not exist. This could be a boon for productivity because hybrid seed crops often exhibit heterosis (hybrid vigor). Nuclear male sterility has been engineered in several species, including tobacco, rice, maize, alfalfa and Brassica, by using the Bacillus amyloliquefaciens barnase gene, which encodes a secreted ribonuclease that is cytotoxic…Jagannath and colleagues (2002) developed a transgenic line in Indian oilseed mustard (Brassica juncea) that was male-sterile by the action of the barnase gene but that was restored to fertility in the presence (expression) of a barstar gene, thus permitting both bioconfinement and the option for heterosis breeding"* (p. 77-78 Committee on the Biological Confinement of Genetically Engineered Organisms, 2004).

A recent innovation has been to transform the chloroplast to carry a gene that renders pollen unviable through degeneration of critical tissues involved in pollen development. By harboring the male sterility factor in chloroplasts, male sterility is inherited maternally in some plants. Male sterile plants still produce viable seed (which again produces male sterile plants).

Sterility-induced V-GURTs for seed work by exposing seed to an external chemical (e.g., the antibiotic tetracycline) or physical (e.g., temperature) stimulus that activates expression of genes that will make the next generation of seeds unviable without interfering with seed formation. Conversely, fertility-induced V-GURTs can be designed that will inactivate the seed unless they are exposed to an external stimulus.

An example of the sterility-induced V-GURT approach is as follows (Daniell, 2002). A gene that encodes a cytotoxin is inserted into the genome under the control of a promoter that is normally activated in late stage seed development. The gene remains inactive without a second event, the removal of an intervening sequence of DNA. This DNA is removed by the activation of an enzyme that specifically excises the intervening DNA and repairs the chromosome, e.g., the Cre recombinase (see Chapter V), and restores the gene for the cytotoxin. The recombinase is only produced in plants that have been induced by the external stimulus. In this scenario, sterility requires an active process that is unlikely to be replicated in nature, at least as long as the components continue to function as intended. For instance, low level uninduced expression of *cre* may in time accumulate enough recombinase in cells to produce unintended sterility.

Current sterility-induced GURTs rely on the sterility-causing factor not being expressed unless induced. To remain fertile, the GM plant—and any organism that has received the GURT by horizontal gene transfer or hybridization—must not encounter the inducer in the natural environment. If the inducer is a temperature shock or UV radiation, then there is the chance of sporadic induction in the field. Moreover, single point mutations may be enough to raise the basal level of expression of the sterility factor and/or cause it to be expressed in other tissues or during other times of development (FAO, 2001, Moore et al., 2005).

An example of the fertility-induced V-GURT approach is the "recoverable block of function" (RBF). This system is based upon the expression of a gene that renders the seed infertile unless its function is prevented. The system as first described involved the protein barnase (the fertility "blocker") which is expressed early in germination (Kuvshinov et al., 2001). Barnase is a cytotoxic ribonuclease and barstar is the barnase inhibitor (through direct binding to barnase). Only if barstar is actively induced will the seed germinate.

The "seed lethal" (SL) system is not strictly a fertility-induced V-GURT but it works by similar principles. SL uses a combination of a seed lethality gene linked to a commercial transgene on one chromosome, and a repressor of lethality gene on another chromosome (preferably the homologous chromosome) (Schernthaner et al., 2003). Selfing or crosses with similar GM plants produce viable seeds. Cross-fertilization or hybridization will frequently create unviable seed because the independent assortment of chromosomes during meiosis will often lead to the seed lethality-transgene pair being separated from the repressor.

Current fertility-induced GURTs are based on a toxin-antidote model (e.g. RBF, SL) where sterility is the outcome unless it is prevented by active induction of an antidote to the sterility factor (e.g., Kuvshinov et al., 2001, Schernthaner et al., 2003). Of the fertility-induced systems that have been described, all require two separate genes (e.g., barnase and barstar). These genes must remain together in the same genome, and their regulation must not be altered by mutation. Normal recombination, especially during meiosis, can uncouple the two genes which could result in inheritance of only the sterility factor through breeding, hybridization or horizontal gene transfer. It is also worth noting that in the SL system, there is no containment for the repressor transgene.

T-GURTs are strategies that restrict the expression of the transgene (FAO, 2001, Visser et al., 2001). Without an external stimulus, the transgene would not be expressed (Committee on the Biological Confinement of Genetically Engineered Organisms, 2004). Examples that have been proposed involve the use of chemicals that can be sprayed onto plants while in the field, in preparation for applying a herbicide to which the transgene confers resistance.

T-GURTS do not prevent gene flow. They rely on the presumption that the transgene is unlikely to introgress if it does not confer a selective advantage (see Chapter II for a discussion on selection), or that if it does spread, it will have no impact. It is difficult to know all the ways a transgene might become useful to other organisms or in novel environments (Heinemann et al., 2000, Heinemann and Billington, 2004, Heinemann and Roughan, 2000, Heinemann and Traavik, 2004), and whether those properties alone predict its likelihood of spreading (Heinemann and Bungard, 2005, Heinemann and Silby, 2003). For example, a gene found in *Arapidopsis* makes this and recombinant plants resistant to the antibiotic kanamycin even though it is unlikely that the gene evolved to make plants resistant to this antibiotic in nature (Rommens). There is no evidence that T-GURTs would be an effective transgene containment strategy.

There is currently no reason to expect that GURTs will be a fully effective solution to gene flow. First, recombination and basal mutation rates are expected to be high enough to compromise

GURTs as a containment strategy on the scales of commercial agriculture, at least using current approaches (Kuvshinov et al., 2001).

Second, sterility does not prevent horizontal gene transfer. The use of sterility methods for transgene containment is limited to preventing the normal ability of plants to breed successfully. It should be noted that the use of GURTs produces additional risks. Work in bacteria on analogous systems, called post-segregational killing systems, demonstrates that combinations of genes that antagonize one another, as sterility and inhibitor-of-sterility do, can become extensively transmitted by horizontal gene transfer (Cooper and Heinemann, 2000, Cooper and Heinemann, 2005, Heinemann and Bungard, 2005).

Failures in GURT control through mutation or recombination may be effective enough to cause sporadic crop loss—potentially compromising the seed-saving efforts of neighboring farmers. Even the threat of this can influence post-commercialization behaviour of either farmers or seed suppliers.

The specific issue of GURT failure has been the focus of a number of investigations from a Finnish group. They have now conducted small laboratory scale experiments in which up to three separate copies of the sterility gene have been inserted into the same tobacco genome (Kuvshinov et al., 2005). This strategy is meant to overcome chance mutational inactivation of the sterility factor. Moreover, the sterility factor is closely linked to the transgene (either adjacent to it, copies flanking it, or inserted within an intron of it (Kuvshinov et al., 2004)), further reducing the chance of separation by recombination.

During the course of their work, upwards of 30% of the primary transformants were rejected because they exhibited morphological phenotypes consistent with excess expression of the sterility factor (barnase) (Kuvshinov et al., 2005). Such observations should be taken seriously when considering the risks associated with using GURTs. The systems are designed to stop gene flow using toxins. Therefore, any number of combinations of systems failures, including subtle changes in the balance (stoicheometry) between sterility factor and inhibitor, could result in undesirable consequences either immediately (due to expression of the toxin at the wrong time or in the wrong tissues during development of the correct plant) or in the next season (due to expression of the toxin at the appropriate stage of development but in the wrong plant).

When considering GURTs, the probability of two different V-GURT varieties cross-hybridizing should also be considered. Duplications of the same transgene in a single genome can induce post-transcriptional gene silencing (PTGS, see Chapter III) (Baulcombe, 2004, Denli and Hannon, 2003). PTGS might act on the sterility factor in the hybrid. Sterility might be maintained or lost in subsequent crosses, leading to unpredictable and stochastic crop losses.

V-GURTs and T-GURTs are designed to delay or prevent the spread of fertile hybrid offspring that inherit both the commercial agronomic trait and the second, containment, transgene construct. They are not designed to prevent gene flow per se. Hybrids either are less competitive in their normal environment because of the mitigation gene(s), or are sterile because of the sterilization construct. Farmers may replant seed from GM hybrid varieties and find themselves in difficult financial situations as their crops give a disappointing harvest (Shand, 2002).

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