Gene flow study: Implications for the release of genetically modified crops in Australia

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Foreword

Since the first commercial production of genetically modified (GM) crops in 1994, worldwide production has steadily increased. In 2001 an estimated 5.5 million farmers throughout the world grew 52.6 million hectares of GM crops. Although Australia has only two GM crops currently commercially cultivated, cotton and carnation, many more crop species are undergoing field trials and more commercial releases are expected over the next five years.

GM crops have great potential to enhance the productivity and sustainability of Australian agriculture, but their adoption raises several important issues, highlighted by the strong consumer aversion to GM food products in some countries. One of these issues is the potential impact of genes moving from GM crops into neighbouring crops, related weeds or native species.

This study responds to an urgent need to collate existing information on gene flow and its implications for the introduction of GM crops in Australia. It reviews Australian and international literature on gene flow by cross-pollination to inform public debate and policy development.

Dr Peter O’Brien
Executive Director
Executive Summary

What is gene flow?

Gene flow is the natural process of movement of genes between individual organisms. Among plants this occurs mainly by pollen from one plant successfully cross-pollinating a flower from another plant and producing viable seed, a process known as outcrossing. Gene flow already occurs from conventionally bred crops but there is heightened interest in gene flow issues because of the release of genetically modified (GM) crops and the potential for the introduced genes – transgenes – to flow into other plants or organisms.

This report discusses gene flow via outcrossing among crops or from crops into related plant species. Outcrossing results in a high degree of natural genetic variability in a population and plants have developed many mechanisms to promote gene flow. Gene flow between different species is much less common, becoming increasingly less likely between distantly related species. Hybrids are formed when two genetically different plants successfully cross. The likelihood of successful cross-pollination and formation of a hybrid plant between two related species depends initially on their proximity and synchronicity of flowering but then many biological barriers also must be overcome for successful hybrid formation. However, historically gene flow has played a key role in evolution of closely related plant species.

How is gene flow measured?

Some studies measure the dispersal of pollen grains. These studies only measure potential for gene flow as not all pollen grains that reach a certain distance will result in seed production. Other studies measure the actual gene flow, which is affected by many factors including pollen viability, receptivity of the plant to the pollen, the amount of pollen produced by the plant and the likelihood of hybrid plant formation.

There is a large variation in the level of gene flow detected by different studies of the same crop species. This variation is caused by differences in the way studies are conducted, including the method used to measure gene flow, and the shape and size of the fields, as well as environmental and regional factors. Small-scale trials cannot always accurately predict gene flow rates from larger fields. Environmental factors such as humidity, wind speed and direction can greatly influence pollen viability and dispersal. Regional factors such as topography and local insect populations can also significantly influence cross-pollination rates.

How will gene flow from GM crops impact on natural populations?

One concern is the potential for transgenes to move into wild ancestors and close relatives of modern crop plants. This issue must be addressed in the context that for some species gene flow from conventionally bred crops into
Implications of transgene flow into wild relatives of crop plants

Wild species is already occurring. Further, in some cases, this has been shown to impact negatively on the biodiversity of wild populations. There are opposing views on whether the flow of transgenes in general will have more potential for impact than from conventional crops. However, there is consensus that the consequences of GM crop/hybrid formation on natural populations will depend on the transgene, the trait encoded and its interaction with the environment. The release of GM maize in Mexico is a worldwide focus of this issue as Mexico the primary origin of domestication and biodiversity of maize. In Australia almost all agricultural crops are exotic species originating from other countries so this is less of an issue. However Australia contains native plants related to crop species and the extent to which transgenes can flow into these populations will need to be considered.

Will gene flow from GM crops result in new weed problems?

Another concern is that the gene flow from GM crops into other relatives could enhance the weediness of existing wild plant populations. If gene flow does occur, the consequences of this will depend on the nature of the transgene and the environment to which it flows. Transgenes allowing herbicide tolerance have been extensively discussed for their potential to flow to weedy populations making them more difficult to control. This concern also applies to conventionally bred herbicide tolerant canola and wheat varieties that are already growing in Australia. It is considered unlikely that herbicide tolerant genes would be stable in weed populations if herbicides were not used to control them. Tolerance to particular herbicides is less of a problem if other effective herbicides are available, however it may reduce weed management options.

If transgenes flow into weedy species, the impact will depend on the nature of the transgene and the weed’s environment

Studies looking at the potential for pest resistant genes to enhance plant survival give conflicting results, depending on whether or not the insects serve to limit these populations. Transgenes allowing improved drought, salt, temperature and disease tolerance, or allowing increased nutrient efficiency may enhance fitness in the wild and the gene flow issue is more significant in these cases. More research in this area may be required before decisions can be made on the release of some GM crops.

Can GM and non-GM cropping systems co-exist?

Gene flow is also an important factor governing the conditions under which GM and non-GM cropping systems can co-exist. This has important economic implications and is crucial to Australia’s ability to supply non-GM products into world markets. For instance, the organics industry is one sector of agriculture that has chosen not to utilise GM crops. For some crops gene flow between adjacent crops via cross-pollination will be the primary issue for ‘contamination’ from GM crops to neighbouring farms, while for other crops seed contamination after harvest will be more significant. Acceptable levels of GM contamination in organic and other crops need to be determined because, although most pollen in a given crop may not move far, complete isolation is difficult, if not impossible, to guarantee. Once reasonable thresholds are agreed to, systems can be designed to manage GM ‘contamination’ within these limits.

Gene flow will impact on the co-existence of GM and non-GM cropping systems

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In the future, GM crops will be developed that produce industrial or pharmaceutical products. These crops will require strict segregation from food crops to maintain varietal purity over the whole production chain, as even a low level of contamination of a food crop with an industrial crop would be unacceptable in most cases.

**Likelihood and potential impact of gene flow from some crops in Australia**

Although individual GM crop gene flow risk assessments were beyond the scope of this report, in order to exemplify some of the issues of gene flow, six crops were chosen as case studies for this report (Box A). These crops represent broad categories of gene flow likelihood and potential impact in Australia. Note, however, that for each crop the potential impact will vary with any given transgene and this also must be taken into consideration. Sixteen other crops were grouped into a broad categorisation of likelihood and potential impact of gene flow in the Australian context. This categorisation is based only on the degree of outcrossing and presence and significance of related weeds. It should be used only as a preliminary indication of gene flow risk, as the likelihood of hybrid formation was not taken into account, neither was the potential for gene flow into related crop species or native relatives. Before the likelihood and potential impact of gene flow can be adequately assessed, detailed studies need to be done on a case-by-case basis. In Australia such detailed risk assessments are required before the Gene Technology Regulator decides whether or not to approve releases of any GM crop.

**What can be done to limit gene flow?**

This report reviews agricultural methods for limiting gene flow from cross-pollination. For example, certified seeds must be grown under strict regulations in order to guarantee the seed purity. These regulations are very crop-specific and have different requirements depending on the biology of the species. To meet the requirements of non-GM markets, higher standards and scrutiny of seed lots for even rare contamination by GM material may be needed, as most GM plants will not be visually different from the GM varieties. Evidence of genetic testing in addition to visual identification of off-types would give more confidence in the capacity of these methods to ensure thresholds are not breached in non-GM crops. Agricultural methods will be useful for reducing gene flow between crops, but will not completely eliminate intercrop flow.

Scientists are also developing other methods for limiting gene flow. Technologies such as asexual seed production, closed flower pollination and blocking of pollen production have potential to reduce gene flow, but some limitations are noted. Chloroplast or plastid transformation technology has potential to reduce transgene flow, as chloroplasts are not usually transmitted through pollen, but to date workable systems have been reported for only a few crop species. Genetic Use Restriction Technologies, such as terminator technology, limit viable seed production and therefore have great potential for reducing gene flow, however other issues may limit their acceptability. Most of these technologies are in the very early stage of
the development process and not all would be applicable to every crop species. Some reports on gene flow suggest these as magic solutions to the gene flow issues, when in actual fact most are years away from field trial testing. If the risk of gene flow from some GM crops is considered too high for current release, developing technological methods suitable for use in agriculture should be a priority for future research.

**Conclusion**

Some gene flow from crops, whether conventionally bred or GM, is inevitable. The actual amount of gene flow from GM crops and its consequences will depend on the crop, the transgene, the trait encoded, the particular environment and the risk management practices adopted. Any consideration of applications for release of GM crops in Australia would need to consider all these factors on a case-by-case basis through a risk assessment process and risk management plan. This is required under the Gene Technology Act 2000.
## Box A Case studies of six crops for potential impact of gene flow in Australia

<table>
<thead>
<tr>
<th>Level of outcrossing</th>
<th>Low potential for impact on farm or local environment</th>
<th>Significant potential for impact on farm or local environment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High</strong></td>
<td>Maize is an outcrossing species, but the impact of gene flow from GM maize into the environment is limited by the lack of close weedy or wild relatives in Australia. Gene flow between maize crops would have implications for the coexistence of GM and non-GM crops.</td>
<td>Eucalypts have a high degree of outcrossing, large numbers of close relatives, and hybridisation between closely related species is also common. If transgenes have a greater potential to impact on the natural genetic resources than genes from conventionally bred trees, the release of GM eucalypts in Australia would need careful consideration and stringent measures to minimise gene flow. Release, if approved, may even depend on reproductive sterility to prevent gene flow.</td>
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<td><strong>Medium</strong></td>
<td>Cotton is predominantly self-pollinating but outcrossing occurs at varying rates depending on the presence of insect pollinators and environmental conditions. Most cotton pollen is distributed very close to the pollen source. The Australian native flora contains many relatives of cotton, but fertile hybrid formation between these relatives and cotton is very unlikely. Neither cotton nor its relatives are significant problem weeds in the Australian environment.</td>
<td>Canola is predominantly self-fertile but with some potential for outcrossing. Low maximum thresholds of cross-contamination by cross-pollination should be attainable with the introduction of appropriate crop management plans. The use of different herbicide tolerant canola varieties, GM or conventionally bred, may have implications for control of canola on-farm weeds if cross-pollination produces varieties tolerant to multiple herbicides. Canola has some close relatives that are weeds in the Australian environment and there is some potential for gene flow into these weedy species, however the frequency of hybrid formation between canola and the most significant problem weeds is very low. The potential impact of any gene flow will depend on the transgene, the trait that it encodes and the environment of the plant to which it flows.</td>
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<tr>
<td><strong>Low</strong></td>
<td>Wheat is primarily self-pollinating, producing small amounts of pollen that has a short viability period. Potential gene flow is minimal at distances greater than 1 m from the source plant and this is reflected by the absence of any separation distances required between wheat crops for certified seed production. Wheat is not a significant weed in Australia, and does not have any closely related weeds.</td>
<td>Oat crops are predominantly self-pollinating and require no separation distance between crops for certified seed production. However oat crops have weedy close relatives that are significant weeds in the Australian cropping system. Although one study showed a low level of gene flow between species, circumstances could be envisaged where such gene flow from GM crops could have a high impact on the farm environment. If a gene were introduced into cultivated oats that encoded for herbicide tolerance, gene flow could quickly limit the effectiveness of that herbicide for controlling weedy oats within the crop.</td>
</tr>
</tbody>
</table>
# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>iii</td>
</tr>
<tr>
<td>Executive Summary</td>
<td>iv</td>
</tr>
<tr>
<td>Contents</td>
<td>ix</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Background behind this study</td>
<td>1</td>
</tr>
<tr>
<td>Some definitions</td>
<td>1</td>
</tr>
<tr>
<td>Focus of this report – gene flow via cross-pollination</td>
<td>2</td>
</tr>
<tr>
<td>Do GM crops pose a new category of risk for gene flow?</td>
<td>2</td>
</tr>
<tr>
<td>Seed certification schemes as guidelines on gene flow</td>
<td>4</td>
</tr>
<tr>
<td>Difficulties in comparing gene flow studies</td>
<td>4</td>
</tr>
<tr>
<td>Modelling to assess gene flow</td>
<td>5</td>
</tr>
<tr>
<td>Volunteer or naturalised populations</td>
<td>5</td>
</tr>
<tr>
<td>Why is gene flow an issue?</td>
<td>6</td>
</tr>
<tr>
<td>Will gene flow from GM crops result in unacceptable levels of ‘genetic pollution’?</td>
<td>6</td>
</tr>
<tr>
<td>Can GM and non-GM cropping systems co-exist?</td>
<td>8</td>
</tr>
<tr>
<td>Will gene flow from industrial GM crops to food use crops compromise food safety?</td>
<td>11</td>
</tr>
<tr>
<td>Will gene flow have negative impact for farm or natural environments?</td>
<td>11</td>
</tr>
<tr>
<td>Major factors affecting likelihood of gene flow</td>
<td>15</td>
</tr>
<tr>
<td>Probability of pollen transfer</td>
<td>15</td>
</tr>
<tr>
<td>Factors affecting probability of cross-pollination</td>
<td>19</td>
</tr>
<tr>
<td>Factors affecting probability of hybrid formation</td>
<td>22</td>
</tr>
<tr>
<td>Factors affecting probability of hybrid survival</td>
<td>23</td>
</tr>
<tr>
<td>Further comments on hybrid formation</td>
<td>23</td>
</tr>
<tr>
<td>Case studies of gene flow from plants in Australia</td>
<td>25</td>
</tr>
<tr>
<td>Highly outcrossing plant species</td>
<td>26</td>
</tr>
<tr>
<td>Case study – Maize (Zea mays)</td>
<td>26</td>
</tr>
<tr>
<td>Case study – Eucalypt species</td>
<td>27</td>
</tr>
<tr>
<td>Intermediate outcrossing plant species</td>
<td>29</td>
</tr>
<tr>
<td>Case study – cotton (Gossypium hirsutum L.)</td>
<td>29</td>
</tr>
<tr>
<td>Case study – canola (Brassica napus)</td>
<td>31</td>
</tr>
<tr>
<td>Low outcrossing plant species</td>
<td>39</td>
</tr>
<tr>
<td>Case study – wheat (Triticum aestivum)</td>
<td>39</td>
</tr>
<tr>
<td>Case study – oats (Avena sativa)</td>
<td>39</td>
</tr>
<tr>
<td>Broad classification of likelihood of gene flow in Australia</td>
<td>41</td>
</tr>
<tr>
<td>How is impact of a weedy species measured?</td>
<td>43</td>
</tr>
<tr>
<td>Likelihood of hybrid formation is not taken into account in this classification</td>
<td>44</td>
</tr>
<tr>
<td>Some issues to be addressed to assess likelihood and potential impact of gene flow</td>
<td>45</td>
</tr>
<tr>
<td>Agricultural measures to limit gene flow</td>
<td>47</td>
</tr>
<tr>
<td>Temporal isolation</td>
<td>47</td>
</tr>
<tr>
<td>Barrier crops</td>
<td>47</td>
</tr>
<tr>
<td>Isolation zones</td>
<td>48</td>
</tr>
<tr>
<td>Field size</td>
<td>49</td>
</tr>
<tr>
<td>Crop rotation</td>
<td>49</td>
</tr>
<tr>
<td>Conclusion</td>
<td>49</td>
</tr>
</tbody>
</table>
Introduction

Background behind this study

The global area under cultivation of genetically modified (GM) crops has grown from their introduction in 1996 to 52.6 million hectares in 2001. Four principal countries grew 99% of the global GM crop area in 2001 - USA, Argentina, Canada and China (James 2001). Globally GM crops were mainly soybean, maize, cotton and canola along with tomatoes, squash, potatoes, chicory, sugar beet, papaya, rice and others.

In Australia, the Office of the Gene Technology Regulator (OGTR) regulates the release of GM organisms under the Gene Technology Act 2000. To date Australia has only two GM plant species approved for commercial release, cotton and carnation. These crops covered an estimated 0.2 million hectares under commercial cultivation in 2001. Currently seventeen other GM crops are approved for field trials and the OGTR has just received an application for the commercial release of GM canola.

More commercial releases of GM crops in Australia are expected over the next five years. GM technology has great potential benefits for the agricultural sector, but there are also risks as with any new technology that need to be addressed. This report presents the main issues surrounding gene flow via cross-pollination from GM crops. The approach taken was to survey scientific review articles and to summarise the information in a form that facilitates public debate and policy development.

Some definitions

Some of the more important terms are explained in this section, for further definitions refer to the Glossary at the end of this report. Gene flow is the movement of genes between individuals. Gene flow is both natural and widespread and occurs to some degree within and between populations of almost all plant species. Gene flow between individuals of different species is much less common, becoming increasingly less likely the more unrelated the plant species are. However gene flow occurs frequently enough to have played a key role in plant evolution through crossing of related species to form hybrids. More than 70% of plant species may have originated from hybrids between species (Ellstrand et al. 1999). In addition, plant breeders have taken advantage of the ability to inter-cross cultivated plants and their relatives to transfer new traits into crops throughout the history of agriculture. For example, at the present time the Australian wheat crop is protected from stem rust, one of the most damaging fungal diseases of our most economically important crop, principally by resistance genes transferred into wheat from closely related wild grasses.

One of the issues involving the release of genetically modified (GM) crops is the potential for the introduced genes, transgenes, to be transferred by gene flow into other plants or organisms. Vertical gene flow can be defined as the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms. Horizontal gene flow refers to movement of genetic material between individuals independent of normal reproductive mechanisms. For GM crops, vertical gene flow could occur via successful cross-pollination between the crop and neighbouring crops, related weeds or native plants. Some confusion is evident in the literature as some papers refer to vertical gene flow as between individuals of the same species, and horizontal as inter-specific or inter-generic gene flow. However because plants can cross with closely related plant species via pollen, which falls into the category of ‘conventional heredity mechanisms’, most papers focusing on the gene flow issue in plants refer to this as vertical transfer. This report will use this definition of vertical gene transfer and concentrate on this rather than horizontal gene transfer. Horizontal
gene transfer refers to rare occurrences of gene flow between plants and other organisms (for example soil bacteria), or gene flow between individual plants by mechanisms other than cross-pollination.

**Focus of this report – gene flow via cross-pollination**

Genes may spread amongst plant populations via pollen travel or seed movement. The relative significance of these two avenues of gene movement depends on the biology of the crop species, as well as farm and delivery practices. Potential sources of seed contamination include seeding and cultivation equipment, harvesting machinery, transport and storage facilities. In some cases seed movement can occur by birds, domesticated and wild animals or even by wind and water. In many cases the likelihood of successful cross-pollination is very low and so the potential for cross contamination from seed movement will be the more significant factor. These avenues will need to be managed by supply chain management practices, and discussion of such practices is beyond the scope of this paper. This report will mainly focus on gene flow via cross-pollination, rather than by seed movement. However it is important to recognize the significance of seed movement for the issue of gene flow from and to volunteer and naturalised populations as discussed in later sections of this report.

At this point it is important to point out the difference between ‘potential’ and ‘actual’ gene flow (Levin and Kerster 1974). **Potential gene flow** via pollination is often categorised by the dispersal of pollen grains, but not all pollen grains that reach a certain distance will result in seed of a fertile plant (Treu and Emberlin 2000). The **actual gene flow** is affected by many factors including the pollen viability, germination and fertilisation, pollen vectors, the receptivity of the recipient plant, and likelihood of hybrid plant formation (Williams 2001). Hybrids are formed when two genetically different plants successfully cross but there are many natural barriers to hybrid formation. The incorporation of a transgene into a new plant population (an introgression) will also depend on the fertility of the hybrid plants. Many studies also suggest that a selective advantage must be offered by the transgene for it to be stably maintained in the new population. The stable integration of a transgene into a new population will depend both on the nature of the transgene and the recipient population.

**Do GM crops pose a new category of risk for gene flow?**

It has been questioned whether GM plants present a new category of risk for gene flow over non-GM plants. This question may be separated into a few different components. First, is gene flow more likely from GM plants? In other words, in the case of plant-to-plant gene flow, are GM plants more likely to cross-pollinate? One experiment, with the model plant Arabidopsis, found that GM plants were more likely to outcross (Bergelson et al. 1998). However, this experiment, discussed in Box 1, was inconclusive as it raised a number of questions that will only be answered by well-designed experiments not yet undertaken.

Other than the Bergelson experiment, the consensus of scientific opinion is that genetic modification *per se* will not increase the likelihood of gene flow via cross-pollination. However specific modifications, particularly those that affect the plant reproductive system may affect the ability of plants to outcross. It is easy to envisage that certain genetic modifications could affect pollen fertility levels, resulting in differences in the likelihood of hybrid formation, or even influence the relationship between plants and pollinators. For example **male sterility** is one genetic modification that affects the ability of a plant to cross-pollinate and be cross-pollinated. In some crops such plants are useful for hybrid plant production. Most genes would not be expected to influence the outcrossing potential but this would be considered as part of any risk assessments of GM crops.
Second, if gene flow does occur, what is different if it comes from a GM plant? One view is that GM technology represents a new category of risk because it enables the transfer of genes between species, and the modification and expression of genes within species beyond the extent and range that would naturally occur, or be possible with conventional plant breeding techniques. There are concerns because GM technology allows the transfer of genes into, often, totally unrelated species, with synthetic constructs. To date scientists generally cannot control where transgenes insert and this may lead to unexpected consequences. Some argue that transgenes are more likely to have impact on natural environments because they usually encode single traits with a selectable phenotype. In addition broad host range promoters (controlling switches) are often used which enable genes to be switched on in many different plant species.

However, GM enables a much more precise method of developing new plant varieties than conventional breeding by enabling single, well-characterised genes to be introduced. Even though in general transgenes cannot be precisely targeted to specific positions in the plant genome (with the exception of plastid transformation, see later section entitled – Technologies that reduce transgene flow) scientists can precisely determine where genes have inserted. GM plants undergo many controlled trials to assess for unexpected consequences due to the modification. Many scientists prescribe to the view that the GM process is simply a technologically advanced breeding tool that allows rapid and precise development of new varieties. Thus the process itself does not present a new category of risk over traditional breeding but the specific traits introduced by both approaches can pose unique risks (National Academy of Sciences 2002).

Box 1 - Do GM plants have a greater propensity to outcross?

The majority of scientists assume that the level of gene flow from GM crops is equivalent to the gene flow from non-GM crops. A brief study published in Nature has questioned this assumption (Bergelson et al. 1998). A small-scale experiment was designed to compare gene flow from GM herbicide tolerant plants with a mutant non-GM herbicide tolerant variety. The plant used was Arabidopsis thaliana, a highly self-fertilizing member of the Brassicaceae family often used as a model plant in modern plant genetics due to its small size and quick generation time. In this experiment herbicide tolerant mutant plants and two different GM herbicide tolerant lines were randomly planted in a field site of herbicide sensitive plants. Plants were allowed to flower and then seeds were collected and screened for herbicide tolerance. Progeny of these herbicide tolerant plants were then screened to determine whether the herbicide tolerance came from the GM or the non-GM plants. This study found that the GM Arabidopsis were roughly twenty times more likely to outcross than the ordinary mutant and thus concluded that genetic engineering could substantially increase the probability of transgene escape. However these researchers also admitted that the generality of their result is unclear, as the underlying genetic mechanism for the difference was not studied.

One obvious explanation for these results is that instead of an increased gene flow from the GM plants, the mutant herbicide tolerant plants may have had a decreased transmission of the herbicide tolerant trait. As the mutant allele was produced by a non-directed chemical mutagenesis it is possible that another mutation in this plant background is affecting its transmission through pollen. This hypothesis would be simple to check by using directed manual crosses to determine the transmission of the three alleles through the pollen. The transmission of other genetic markers could also be checked in these lines, and compared with the transmission in non-GM, non-mutant Arabidopsis plants. Until these checks are done the results of these experiments should be interpreted with caution.
Seed certification schemes as guidelines on gene flow

There is much discussion in the recent scientific literature about the likelihood and impact of gene flow from GM crops. Scientists have addressed this issue from many different standpoints, from the impact of GM crops on agricultural and natural ecosystems to the compatibilities of GM and non-GM cropping systems. Where the assumption holds that the rate of gene flow from GM crops is similar to that of their non-GM counterparts, there is also a wealth of much earlier literature that deals with gene flow/cross-pollination issues. The production of certified seeds is a sector of traditional agriculture where prevention of unwanted cross-pollination is of longstanding concern. Seed variety integrity requirements have driven controlled production schemes, quality assurance systems and strong regulation in this sector. This report will not attempt to track this early literature, but will rather discuss the application of knowledge gained from seed certification schemes to address the issue of gene flow from GM crops.

Difficulties in comparing gene flow studies

From an assessment of the scientific literature on gene flow, what quickly becomes obvious is the large variation between gene flow detected in different studies on the same crop plants. Field experiments that aim to measure the effect of separation distances on levels of cross-pollination give varying results because of the many factors that influence gene flow. These factors are discussed in more detail in later sections. Environmental factors can greatly influence pollen viability and dispersal, and influence hybrid formation and survival (discussed in more detail in the section entitled Factors affecting likelihood of cross-pollination). Regional factors determine the presence of related species and local insect populations that influence cross-pollination. Climatic factors such as wind speed and direction also significantly influence cross-pollination. Experimental design is also important including factors such as the shape of, and the absolute and relative sizes of, the pollen donor and acceptor plots.

Pollen flow can be measured in various ways such as; pollen traps, visual identification of off-types, direct molecular methods to detect genes or proteins, collection of seed and selection of plants with specific traits in the field, glasshouse or laboratory. Some advantages and disadvantages of each method are detailed in Table 1. These methods will differ greatly in their sensitivity and reproducibility. Field tests may underestimate the level of outcrossing as compared to more controlled conditions in the glasshouse but may be a closer estimation of the survival rate of these events. Cross pollination of a pollen producing or male fertile plant is less likely as the plant is more likely to be self-fertilised. Male sterile plants lack pollen competition so cross-pollination is more likely, therefore male sterile bait plants will overestimate actual gene flow into pollen producing plants.

Table 1 Methods of determining pollen flow (Feil and Schmid 2002)

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Capture of pollen by traps (for example, slides with sticky surfaces)</td>
<td>Simple to set up</td>
<td>Time consuming to count pollen, viability of pollen is not considered (measures potential not actual gene flow)</td>
</tr>
<tr>
<td>Use of male sterile bait plants</td>
<td>Small receptor crops suffice, viability of pollen is determined by the number of seed set</td>
<td>No pollen competition so will overestimate gene flow into fertile plants</td>
</tr>
<tr>
<td>Visual detection of off-types or hybrids</td>
<td>Measures the actual rate of outcrossing</td>
<td>Experimental design is crucial, large donor and receptor plots must be established.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Synchronous flowering must be assured in order not to underestimate pollen flow</td>
</tr>
<tr>
<td>Method</td>
<td>Advantages</td>
<td>Disadvantages</td>
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<tr>
<td>Collection of seed from receptor plants and testing of specific trait</td>
<td>Measures the actual rate of outcrossing</td>
<td>Experimental design is crucial, large donor and receptor plots must be established. Synchronous flowering must be assured in order not to underestimate pollen flow. Genes must be expressed to be measurable</td>
</tr>
<tr>
<td>(for example herbicide resistance)</td>
<td></td>
<td></td>
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<tr>
<td>Collection of seed from receptor plants and testing by direct molecular methods (for example Polymerase Chain Reaction)</td>
<td>Measures the actual rate of outcrossing, genes need not be expressed to be detected</td>
<td>Experimental design is crucial, large donor and receptor plots must be established. Laboratory work needed. Synchronous flowering must be assured in order not to underestimate pollen flow.</td>
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Variation in some or all of these components often makes direct comparisons between studies extremely difficult. Another factor for consideration is that studies of pollen distribution from an outcrossing plant generally show pollen declining rapidly as a function of distance. The significance of this for gene flow studies is that very large sample sizes are needed to detect low-probability events of long distance pollen dispersal and therefore variability is high from study to study. Some scientists advocate that dense sampling close to the pollen source is more accurate for determining gene flow (Assuncao and Jacobi 1996), whereas others recognise that it is the tail of the pollen dispersion curves that are of more interest and significance for gene flow and must be extensively sampled (Klein and Laredo 1999).

**Modelling to assess gene flow**

Some scientists are analysing the issue of gene flow from GM crops using model systems. Although models should not be substituted for well-designed experimental field trials, they can be useful and usually deal well with variability. Altering one input variable and studying the affect of this change can identify crucial control factors impacting on gene flow. This was shown in one study that modelled the population dynamics of volunteer canola within a field and showed that the proportion of seeds germinating from the seedbank each year is a critical factor (Pekrun et al. 1999). The results from models such as GENESYS and MAPOD are discussed later in this report.

**Volunteer or naturalised populations**

An important potential avenue for gene flow is through volunteer or naturalised populations of both cultivated and non-cultivated plants. Volunteer plants may arise from seed escape from previous crop harvests, either in the paddock or elsewhere on the farm or along the seed transport route. Volunteers may become established and lead to naturalised populations of crops existing outside of cultivation. Naturalised populations may grow along fences, roadsides, railway lines or in natural environments. These populations will have significance for gene flow by acting as pollen donors or acceptors with nearby crops or related weedy species. Gene flow via these plants could potentially occur across growing seasons, by plants emerging in fields where GM crops were grown previously, or by seed escapes during farming practices or seed transport. Some crops, because of various aspects of their seed biology, such as seed size and dormancy, are more likely than others for volunteer populations to be an issue. The significance of volunteers is discussed in more detail in later sections of this report.
Why is gene flow an issue?

Gene flow is a fundamental path in the evolution of plant species and is no doubt currently occurring between conventionally bred crop species and weeds or wild relatives. So why has gene flow become an issue of significance for the release of GM crops? Some concerns about the consequences of gene flow from GM crops that have been discussed in the scientific literature and the popular press include:

- Will gene flow from GM crops result in unacceptable levels of ‘genetic pollution’?
- Can GM and non-GM cropping systems co-exist?
- Will gene flow from industrial GM crops to food use crops compromise food safety?
- Will gene flow have negative impact for farm or natural environments?

These issues are discussed in the following sections.

Will gene flow from GM crops result in unacceptable levels of ‘genetic pollution’?

One reason why gene flow from GM crops is of concern arises from the potential for outcrossing with the wild ancestors and close relatives of modern crop plants. Postulated risks include that hybridisation of crops with wild populations could result in a fitness advantage of the hybrid and lead to extinction of the wild species through competition, or alter the fundamental nature of the wild species or its close relatives. Or genetic integrity could be reduced and the population’s ability to adapt to environmental change could be compromised (Daniels and Sheail 1999).

With these fears in mind, the potential for GM maize grown in Mexico to cross with local landraces has been the focus of much attention recently (See Box 2). This is obviously an issue in those countries or regions that are centres of diversity for the relatives of particular crop species. It is of less concern in Australia as most crops are exotic species imported from other countries (see Table 2). However, Australia is a centre of diversity for a range of economically important plants. One of the more obvious is Eucalyptus species, but there are others. Australia contains many native plants related to crop plants (see Table 3). In these instances, this issue is of great relevance to Australia.

Table 2: Centres of origin of some of the most economically important crops in Australia.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Gross Value $m</th>
<th>Origins of domestication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>5139</td>
<td>Southwest Asia (Diamond 1997)</td>
</tr>
<tr>
<td>Barley</td>
<td>1368</td>
<td>Southwest Asia (Diamond 1997)</td>
</tr>
<tr>
<td>Wine grapes</td>
<td>1235</td>
<td>Southwest Asia (Diamond 1997)</td>
</tr>
<tr>
<td>Cotton</td>
<td>1138</td>
<td>Mesoamerica (Diamond 1997)</td>
</tr>
<tr>
<td>Sugar cane</td>
<td>965</td>
<td>New Guinea (Diamond 1997)</td>
</tr>
<tr>
<td>Canola</td>
<td>633</td>
<td>Southwest Asia (Buzza 1991)</td>
</tr>
<tr>
<td>Sorghum</td>
<td>329</td>
<td>West Africa and Sahel (Diamond 1997)</td>
</tr>
<tr>
<td>Rice</td>
<td>272</td>
<td>China (Diamond 1997)</td>
</tr>
<tr>
<td>Lupins</td>
<td>202</td>
<td>Southwest Asia (Duke 1981)</td>
</tr>
<tr>
<td>Oats</td>
<td>173</td>
<td>Southwest Asia (Vavilov 1951)</td>
</tr>
<tr>
<td>Field peas</td>
<td>113</td>
<td>Southwest Asia (Diamond 1997)</td>
</tr>
</tbody>
</table>
Crop | Gross Value $m | Origins of domestication
--- | --- | ---
Chick peas | 78 | Southwest Asia (Diamond 1997)
Triticale | 75 | Developed by plant breeders (cross between wheat and rye) (Jessop and Wright 1991)
Tobacco | 49 | Central Andean including most of South America (Vavilov 1951)
Sunflower | 33 | Eastern United States (Diamond 1997)
Soybeans | 26 | China (Diamond 1997)

1 Taken from the December Quarter 2001 Australian Commodities (ABARE), from Table 23 Gross Value of Australian farm and fisheries production, 2001 - 2002 forecasts.

Table 3 Some crops with related Australian native species (Brown and Brubaker 2000).

<table>
<thead>
<tr>
<th>Field crops</th>
<th>Vegetables</th>
<th>Oils, fruit, nuts, spices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean</td>
<td>Celery</td>
<td>Banana</td>
</tr>
<tr>
<td>Cotton</td>
<td>Cucumber</td>
<td>Cinnamon</td>
</tr>
<tr>
<td>Flax</td>
<td>Potato</td>
<td>Citrus</td>
</tr>
<tr>
<td>Pigeon pea</td>
<td>Sweet potato</td>
<td>Eucalyptus</td>
</tr>
<tr>
<td>Rice</td>
<td>Tomato</td>
<td>Fig</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Watermelon</td>
<td>Macadamia</td>
</tr>
<tr>
<td>Soybean</td>
<td>Yam</td>
<td>Nutmeg</td>
</tr>
<tr>
<td>Tobacco</td>
<td></td>
<td>Olive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pepper</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raspberry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea tree</td>
</tr>
</tbody>
</table>

It is clear that agriculture has already had a profound effect on the evolution of many plant species (Daniels and Sheail 1999). There is evidence that at least 44 cultivated plant species hybridise with wild relatives around the world (Ellstrand 2002). In some cases, outcrossing between conventionally bred crops and wild relatives has had negative impacts on a wild species. For example, hybridisation between cultivated rice and a species of wild rice in Asia is blamed for the near extinction of the wild species (Ellstrand et al. 1999).

The question remains however, does GM gene flow enhance or alter this risk over traditionally bred species? Although this is largely unstudied to date, in most cases it would not be expected to increase this risk, and the consequences of hybrid formation will usually depend on the introduced trait (GM or not). The consequences of different traits on fitness are discussed in later sections of this report. Another viewpoint is that because GM allows the transfer of genes across evolutionary boundaries, it does present a novel category of risk. Interestingly the intense scrutiny that GM crops undergo in most countries before release compared with conventionally bred crops may allow increased understanding of these issues.

The concept of gene flow from agricultural crops into related wild species in itself being an argument against the release of genetically modified crops has to be balanced with the constant gene flow already evidenced in natural systems. Some plants have even evolved mechanisms to promote outcrossing among individuals of a species to ensure population diversity.
variability (see section entitled Plant mating systems). Although gene flow between species is much less common, in some cases outcrossing between closely related species does occur. Agricultural systems show evidence of gene flow and many conventional breeding programs cross related plants to introduce genetic variability into cultivated crops. Discussions of genetic pollution often enter religious, cultural and ethical realms; perhaps increased education and understanding of the science behind these issues will enhance dialogue and help resolve questions in this area.

**Can GM and non-GM cropping systems co-exist?**

One of the reasons that gene flow is an important consideration for the release of GM crops is the implications for the co-existence of GM and non-GM cropping systems. Organic agriculture is one example of a sector that has chosen to remain ‘GM-free’. What are the implications for a farmer if neighbouring crops are GM? The possibility of gene flow from GM crops contaminating non-GM crops is an important consideration for the agricultural industry. This is an issue about choice, in that significant numbers of consumers world-wide, want non GM foods. For example, in response to this market the supermarket chain Sainsburys (UK), with an annual turnover of £17.4 bn, states in its ‘Code of Conduct’: ‘In response to customer demand all Sainsbury's Supermarkets own-brand products have been manufactured without genetically modified (GM) ingredients since July 1999.’

Discussions of acceptable thresholds of genetically modified material in non-GM food products are ongoing world wide and are influenced by many factors such as the testing methods available, their sensitivity and reproducibility as well as the statistical significance of the sampling regimes. Although GMO use is prohibited in EU organic production, it does not directly prohibit the marketing of produce that has been accidentally contaminated (for example by cross-pollination)(Agriculture and Environment Biotechnology Commission 2001). The need for threshold levels that cannot be exceeded in food and feed products are currently being discussed. Different organic certification schemes in the UK have individual approaches to the GM issue. The Soil Association’s organic scheme undertakes a full risk inspection of individual properties if the organic crops are grown within established distances (1 km for beet, 3 km for maize and 6 km for rape) of GM crops, and threaten to decertify the farmer’s field if they feel the likelihood of GM contamination by cross-pollination is high. Organic Farmers and Growers, another UK organic certifying body, has specified a maximum presence of 0.1 parts per million (ppm) of quantifiable GM material in organic produce (Agriculture and Environment Biotechnology Commission 2001), even though this level is well below the limit of detection of current methods (Bock et al. 2002).

The National Standard for Organic and Bio-dynamic Produce in Australia (Organic Produce Advisory Committee 1998) specifies that ‘organic products or by-products are not compatible with the principles of organic agriculture and therefore are not permitted under this Standard where b) they are derived from genetic modification technology.’ Certified organic farmers must address potential risks of external contamination by employing various farm management techniques including the use of buffer zones and/or the withdrawal of land/product from certification (Organic Produce Export Committee 2002). The new draft standard also specifies that land previously used to crop GM plants must have a minimum five-year period before any agricultural produce can be labelled as ‘organic’. The introduction section of the draft National Standard for Organic and Bio-dynamic Produce in Australia (Organic Produce Export Committee 2002) also acknowledges that this standard cannot ensure organic products are GM free, due to the influence of other environmental factors that are beyond the control of the certified operator. Presumably these factors correspond to those affecting the likelihood of cross-pollination as discussed in later sections of this report.
Box 2 Mexican Maize Summary

Maize is a naturally outcrossing species and pollen can be transferred to other plants by wind, gravity and insects. Mexico is the primary centre of domestication and diversity of maize and most of the area planted to maize is planted to landrace varieties. For these reasons in 1998 the Mexican Government introduced a moratorium on the planting of genetically modified maize to prevent gene flow to local varieties and the ancestors of maize, teosinte.

Is there strong evidence for gene escape into local Mexican maize varieties?

In September 2001 a preliminary announcement was made by Mexican government researchers that maize grown locally in the mountains in Mexico (landraces) contained transgenes (introduced genes from GM crops). This was supported by data reported by two researchers at the University of California, D. Quist and I. Chapela, in a paper published in Nature in November (Quist and Chapela 2001). This paper was widely reported in the popular media and various groups use the study as a basis for claims that transgene spread could threaten food security. This issue sparked a call from the Mexican senate to its Department of Agriculture to stop all imports of US corn into Mexico, although the government has so far resisted these calls.

The controversy continued when researchers at the International Maize and Wheat Improvement Centre in Mexico (CIMMYT) could not detect this same DNA either in its extensive seedbank or field samples collected from Mexico. In February of this year an article written on behalf of the editorial board of the journal Transgenic Research claimed that the data presented by Quist and Chapela was technically flawed and based on scientific techniques prone to false positives (Christou 2002). Other teams of university-based researchers notified Nature of the methodological shortcomings of these experiments and in April of this year, Nature published several short communications detailing these problems (Kaplinsky et al. 2002; Metz and Futterer 2002), as well as a response to these criticisms by the original authors (Quist and Chapela 2002). Most significantly Nature added in a brief editorial note that the ‘evidence available is not sufficient to justify the publication of the original paper.’ Most recently the Mexican government has confirmed that their studies show the presence of transgenes in samples taken from sites in two states, Oaxaca and Puebla, but to date this has not been published.

However, significantly, all of the critics of the original Nature paper acknowledged that if GM maize was grown in close proximity to other maize varieties, gene flow through cross-pollination would be highly likely. This is because of the outcrossing biology of maize, as well as the traditional management practices of Mexican farmers. These include saving seed, using seed from other local or distant sources, deliberate mixing of seeds, multiple maize populations growing on the same landscape and the practice of ‘creolization’ – where farmers allow crossing between local and external varieties to improve their crops. Maize landraces are therefore not static but a very dynamic population due to the potential for outcrossing combined with the management practices of Mexican farmers. It is also important to realise that gene flow between maize and teosinte (ancestor of maize) is less probable than between maize varieties.

Significance of this gene flow?

The key question still to be answered is what would be the significance of gene flow from GM maize for the local maize varieties? Some argue that the transgenes will impact negatively on genetic integrity; others see transgenes as another avenue for introducing biodiversity into farmer’s crops and addressing significant problems such as insect pests. Research and discussions of the implications of gene flow are ongoing, but may be difficult to resolve.
Implications of gene flow for the release of GM crops in Australia

In other parts of the world, legal issues surrounding contamination of organic farms by neighbouring GM properties are currently being resolved in the courts. In January 2002, an organic organisation representing about 1,000 certified Canadian organic farmers initiated a lawsuit against agbiotech companies Monsanto Canada and Aventis Cropscience Canada. The basis of the lawsuit is the allegation that organic crops have been contaminated with GM herbicide-tolerant canola and organic farmers can no longer certify their canola crops as GM-free thereby losing important domestic and international market access (Bouchie 2002).

A UK research report on this issue (Eastham and Sweet 2002) acknowledged that neither pollen nor seed contamination can be completely prevented even by using seed certification procedures to limit gene flow between GM crops and organic or non GM crops. A recommendation from this report was that acceptable levels of GM contamination in organic crops need to be decided because complete genetic isolation cannot be guaranteed. Other researchers have also made this point as it is difficult for scientists to advise on realistic segregation and testing measures before acceptable thresholds are decided (Sweet 2002). This could be viewed in the same way that organic farms currently use practices to minimise (not eliminate) the possibilities of spray and fertiliser drift from neighbouring non-organic farms. The authors of this UK report also raise the question who determines the measures to minimize mixing of GM and non-GM crops – should isolation distances be the responsibility of the GM or organic farmer or both (Moyes and Dale 1999)? These are issues that also need to be dealt with in an Australian context.

A series of studies coordinated by the European Commission Joint Research Centre have examined this issue of co-existence of GM and non-GM crops. These studies focused on three crops, canola, maize and potato, and compared the significance of different avenues of cross-contamination in different farm types (Bock et al. 2002). Farming practices that could reduce cross-contamination were identified. One conclusion from these studies is that a 0.1% threshold limit of GM in non-GM crops would be extremely difficult to meet for any farm/crop combination considered. Threshold limits of 0.3% or 1% would be technically possible, but economically difficult especially for canola and maize. In addition co-existence of GM and non-GM crops on the same farm would be unrealistic. These issues need to be addressed in an Australian context as farm sizes and distances between farms will differ significantly between Europe and Australia.

In Australia the Department of Agriculture, Fisheries & Forestry - Australia (AFFA) allocated funding over 4 years from July 2000 under the National Biotechnology Strategy, to address segregated supply chain development and management to meet domestic and international market requirements for commodities and processed foods. The AFFA supply chain management for GM products project aims to enable individual producers and industry to make informed choices about whether to adopt GM crops and the need for systems of traceability, segregation or identity preservation. This project also aims to ensure that governments are able to appropriately support these systems in regard to GM products (including export verification where appropriate).

At a recent meeting of the Primary Industries Ministerial Council (PIMC) it was agreed that the management of the risk of gene flow from crop to crop should be handled by industry self-regulation supplemented by government monitoring. The Plant Industries Committee of the Primary Industries Standing Committee (PISC) is developing guidelines that support the establishment of industry stewardship programs to address the responsible management of GM crops. These programs will include on-farm, crop management plans that aim to limit gene flow from crop to crop and crop to weed.

The GM Canola Technical Working Group (GMCTWG) was formed in Western Australia (WA) in April 2000 to address issues surrounding the introduction of GM canola into WA. A report was published in September 2001 that discussed the practicality of segregating GM and
non-GM canola. The Eastern Zone Gene Technology Grains Committee (EZGTGC) was established in Eastern Australia to address similar supply chain issues necessary for introducing GM crops into Australia. Both committees are made up of scientists, growers (including organic growers), bulk handlers, processors, and representatives from life science companies, Commonwealth and State governments. One of the important goals of both groups is to develop action plans for canola that cover various stages of the supply chain and the evidence needed to support product verification as GM or non-GM.

In order to determine thresholds for unintended GM presence in non-GM material, the sensitivity and reproducibility of the testing methods need to be considered as well as the statistical significance of the tests. The only way to have total assurance of the purity of a sample seed lot is to sample 100% of seed, which is neither possible nor practical. Thus the design of the sampling procedure is also a very important factor that must be considered in determining threshold levels (Remund et al. 2001).

Market implications for the introduction of GM crops into Australia were considered beyond the scope of this report. These issues were discussed specifically for the grains industry in a recent Australian Bureau of Agricultural and Resource Economics (ABARE) report (Foster, 2001).

**Will gene flow from industrial GM crops to food use crops compromise food safety?**

The first generation of GM crops to reach the world market have mainly incorporated genes that aim to improve farm production, for example herbicide tolerance and insect resistance. Future generations of GM crops will incorporate genes that enable the plants to produce novel compounds, some specifically for industrial use. Researchers are developing GM crops that make industrial oils for the production of plastics, polymers, glues and other speciality chemicals. Gene technology may also allow much cheaper commercial production of biodegradable plastics in plants than the current bacterial production methods. Plants are also being developed to produce vaccines, pharmaceutical proteins and industrial enzymes for non-food uses (reviewed in (Kempken 2000; Green and Salisbury 2001).

The production of these crops will require strict segregation from food crops to maintain varietal purity, as even a low level of contamination of a food crop with an industrial or pharmaceutical crop would be unacceptable. Avoiding the use of cross-pollinating plants for these applications and strict isolation of these crops from related food crops on a regional level would greatly reduce the likelihood of this sort of contamination (Hill 1999). For some applications, depending on the scale of plantings required, complete isolation by growth in glasshouses would be possible. Suggestions of methods for physical and genetic containment and their feasibility are discussed elsewhere (Canadian Food Inspection Agency n.d.). A recently released document produced by the Biotechnology Industry Organisation in the US also discusses key elements of confinement procedures that would be required for pharmeceutical-producing crops (Biotechnology Industry Organization 2002).

**Will gene flow have negative impact for farm or natural environments?**

An oft-cited concern for the release of GM crops is the likelihood that the transgene will flow from the crop into related crops or weeds, increasing weedingness and causing problems in agricultural and natural environments.
Predicting likely invaders is difficult

Scientists cannot always successfully predict what species are likely to become weedy (Lonsdale 1994; Marvier 2001), and even the less likely invaders may have significant economic impact. Cultivated crops are usually so domesticated that their overall fitness, in other words their ability to survive and regenerate, outside cultivation is low (Marvier 2001). This is exemplified by the fact that, for all of the plant species naturalised in Australia between 1971 and 1995, only 7% were agricultural introductions (Groves and Hosking n.d.). Food crops are in general less likely to be successful invaders, but species used for pasture grasses have a great tendency to become weedy.

Are GM crops more likely to be invasive? The few studies that have examined the fitness advantage of GM crops compared to non-GM have either found no difference or reduced fitness amongst GM plants (Marvier 2001). One study grew canola, potato, maize and sugar beet in twelve different habitats and compared the invasiveness of GM crops to non-GM crops over a ten-year period. In no case were the GM plants (encoding herbicide tolerance and insect resistance traits) more invasive or persistent than the non-GM bred crops. (Crawley et al. 2001).

Significance of gene flow depends on whether transgenes offer weeds a selective advantage

One important factor impacting on the likelihood of a transgene to increase weediness of a crop or related weed is the potential for it to confer a selective advantage in agricultural or natural environments. This will depend on the nature of the transgene, the trait it encodes as well as the weed itself and its environment.

Insect resistance is one trait that is thought to confer a fitness advantage in the wild. However this is only potentially true for populations where insect pests serve to limit population numbers. In addition, the genes from *Bacillus thuringiensis* (Bt) commonly used to confer pest-protection in current GM crops provide protection against only a subset of one group of insect pests, Lepidoptera. Many other insect species can impact on a plant’s survival in natural environments and Bt will offer the plant no advantage for these pests. However a trait encoded by a transgene need not be the limiting factor, it still could influence survival and hence change population dynamics.

One study on insect resistant oilseed rape found increased fitness under insect selection pressure, but only in the absence of plant competition, showing the importance of other factors impacting on plant survival (Stewart et al. 1997). A recent Australian study compared the persistence of GM insect-resistant cotton with non-GM cotton and found no fitness advantage conferred by the transgene (Rowena Eastick, personal communication). Natural populations nearly always consist of a mixture of plant species so pest insects may only be a minimal part of the selection pressure on any one species (Dale 1994). For some crops, traditionally bred or naturally occurring insect resistant crop varieties have already been used and these have not been reported to lead to undesirable consequences (Duvick 1999; Hokanson et al. 1999). Some studies have shown that insect pests can limit plant population density especially in open, disturbed and potentially stressful growth conditions (for example agricultural fields and their margins). Increased resistance to pests in volunteers or weedy species could therefore alter the weed status of plants in such environments (Louda 1999).

Herbicide tolerance is another trait that is often quoted to be of concern for volunteer populations or gene flow into related weedy species. The impact of this gene flow will depend on the environment that the weed population inhabits, herbicide usage patterns and alternative weed management strategies. For example, if that particular herbicide were not normally used to control these weeds, there would be no selection pressure and most probably little impact. If the herbicide is used to control these weeds, then strong selection pressure would exist for
spread and persistence of this gene and alternative weed control strategies would be required. This is one reason why concerns have been raised with ‘gene stacking’, in this case referring to the deliberate or accidental incorporation of multiple genes encoding different herbicide tolerances in an individual plant. This has already been reported in canola crops in Canada (Hall et al. 2000; Orson 2002). This could have implications for controlling these crops as volunteer weeds in farm environments.

Effective crop management of herbicide tolerant crops would be needed to minimize the likelihood of ‘gene stacked’ volunteers causing problems (see section entitled Weediness potential of canola). This is not just an issue for GM plants as herbicide tolerant non-GM crops have also been produced, for example non-GM herbicide tolerant canola and wheat are already being commercially grown in Australia. Indeed the Canadian gene stacked canola consisted of two genetically modified herbicide tolerant traits combined with one conventionally bred herbicide tolerant trait (Hall et al. 2000). Crop management programs are needed to minimize the likelihood of gene stacking happening and the Plant Industries Committee of the Primary Industries Standing Committee (PISC) is currently considering this issue.

Genes that confer tolerance to environmental stresses such as salt, drought, temperature and heavy metals, and that offer disease protection, may offer significant selective advantages in agricultural and natural environments. In addition researchers are developing transgenes that enable crops to utilise nutrients more efficiently. These genes may alter the invasiveness of the crop species, and consequences of gene flow from crops containing these genes will need to be carefully assessed (Dale 1994). If such transgenes flow into related weedy species they could have the potential to become established in these populations and offer a fitness advantage if those stresses are limiting factors. This could change the distribution and invasiveness of the weeds and have impacts on farming and natural communities.

Another issue is the potential for gene flow from GM crops or volunteers into nearby naturalised populations of these plant species. These populations may be better adapted to these habitats and have different invasiveness potential to the original crop. Some transgenes may offer selective advantage to these populations and increase their weediness.

Potential for hybrid formation and introgression of transgenes into related weeds

Examples of domesticated conventionally bred crops that have, in the past, been shown to naturally hybridise with related wild species throughout the world are numerous, and in some cases this has led to enhanced weediness of the wild species (Ellstrand et al. 1999; Ellstrand and Schierenbeck 2000). Research in this area is ongoing: one study found that canola/wild radish hybrids have a higher seed dormancy than either parent and that may influence their ability to persist in natural environments (Adler et al. 1993). Seed dormancy will also delay hybrid detection and influence the time needed for eradication.

Hybrid formation does not necessarily lead to the next step, introgression - the successful integration of the novel gene in the chromosomes of the related species. Introgression may be relatively rare, but it is known to have occurred naturally. This requires repeated crossing of the hybrid to the new species (backcrossing) in order to get stable incorporation of the gene into this species. Early generations of hybrids between crops and wild or weedy species generally have lower fertility than the parents, but this can vary widely even with hybrids produced from the same cross-pollinated plants. Even if the hybrids do have reduced fertility, this may still allow for introgression of a transgene into a new species. After several generations backcrossed plants may be just as competitive and successful as the wild plants (National Research Council 2000). One study that looked at hybridisation between wild and cultivated radish found that even though hybrids had reduced fitness compared to the wild
species, crop genes were detectable in the wild population after three years (Snow et al. 2001).

The ability of the transgene to persist in new populations will depend on the nature of the transgene and the environment to which it flows (Ellstrand 2002). The frequency of hybrid formation, hybrid genotype and fertility, recipient population size and density, gene flow between populations, pollination vectors and genetic drift are all essential components of the impact of gene flow that need to be considered (Gliddon 1994; Raamsdonk and Schouten 1997).

**Inheritance pattern of the transgene is significant**

Another consideration is the inheritance pattern of the transgene (Ingram 2000a). If the transgene is **dominantly** inherited, and the GM crops are **homozygous** for this transgene, gene flow is an important issue for all outcrossing events. However if the GM crop is **heterozygous** for the transgene (and assuming that the transgene does not affect pollen viability) only half of the pollen will carry the transgene and only half of the outcrossing events will result in gene flow of the transgene. Another scenario is that the transgene is **recessive** and GM crops will be homozygous for this transgene to show a phenotype. In this situation (which seems rare in current GM applications) all pollen will carry the transgene but gene flow events may be more difficult to detect due to the recessiveness of the trait. Although not all trait inheritance is this simple, these points raise the issue that the inheritance pattern of the transgene is an important consideration of the potential impact of gene flow.
Major factors affecting likelihood of gene flow

Probability of pollen transfer

Plant mating systems

Plants may either be self-pollinated, cross-pollinated or a mixture of the two, sometimes referred to as a mixed mating system (Atwell et al. 1999). Self-pollination (or autogamy) as the name infers refers specifically to the transfer from pollen to stigma (or female floral organ) on the same plant. In some species, mechanisms have evolved to ensure high degrees of self-pollination such as homogamy where the male and female organ development is temporally synchronized, and cleistogamy where the flowers do not open so self-pollination is ensured. Cross-pollination (or allogamy) refers to the process of pollen from one plant being transferred to the stigma of another plant. Successful cross-pollination results in a high degree of genetic variability in the population so plants have evolved many mechanisms that promote outbreeding. Some of the more common mechanisms for allowing or ensuring outcrossing are summarised in Box 3.

Box 3 Examples of plant mechanisms for increasing outcrossing

Spatial or temporal separation of sexes

Monoecious – female and male organs in unisexual flowers on the same plant (for example maize).

Dioecious – unisexual flowers on different plants, that is male and female plants (for example asparagus).

Dichogamy – male and female organs of the flower mature at different times (for example avocado).

Sex expression

Sex expression (that is whether a plant has male or female flowers) is genetically and developmentally determined. The patterns of male and female flower development can be influenced by environmental factors such as light, temperature and day length, as well as plant growth regulators (for example cucumber and watermelon).

Self-incompatibility

Plants have also developed sophisticated genetic mechanisms such as self-incompatibility that prevent self-fertilization and thus promote outcrossing (many grasses and pasture legumes).

Male or female sterility

Various mechanisms lead to male or female sterility that can promote outcrossing. Plant breeders have used male sterility systems for decades to ensure outcrossing and facilitate hybrid production. Male sterile varieties that are used in hybrid seed production will have a much greater likelihood to be outcrossed by undesired sources due to the absence of pollen competition and consequently gene flow will need to be reduced by specific strategies such as separation distances.
Box 3 helps to reinforce the point that natural plant mating systems can be very complicated. This is one reason why risk assessments for the release of GM crops need to be done on a case-by-case basis. The degree to which a plant out-crosses depends primarily on its mating system so a good understanding of these systems is essential. It is not enough to simply group plants as outcrossing or not – the reproductive biology and production system of each crop needs to be well understood so as to assess the variations which contribute to the likelihood and impact of gene flow. For example a crop that is primarily inbreeding but with some insect-mediated cross-pollination, could have varying outcrossing rates depending on the insects present in a particular region. However a cleistogamous plant, with a closed flower, will have low outcrossing rates usually independent of the insect populations.

Agricultural domestication of plant species has also had an impact on the mating systems of plants (for example wild grapes are dioecious whereas cultivated species are hermaphrodite). The mating system of many plants, especially seed or fruit crops is an essential part of the agricultural process. Table 4 lists the mating systems of some GM crops undergoing field trials currently in Australia or overseas, grouped into general categories of high, intermediate and low outcrossing rates. Note that the degree of outcrossing can vary greatly among varieties of the same crops, from region to region and from season to season. In addition, the fact that a plant is classed as outcrossing does not indicate how far the pollen will travel. For most species outcrossing levels will be minimal at distances greater than ten metres of the source plant.

Table 4: Mating systems of some GM crops undergoing field trials in Australia or overseas.

<table>
<thead>
<tr>
<th>Crops</th>
<th>Mating system (Frankel and Galun 1977; Sindel 1997; Napier 2001)</th>
<th>Main pollination vectors (Frankel and Galun 1977)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High outcrossing potential ¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>Normally outbreeding, hermaphrodite, self-compatible or self-incompatible varieties</td>
<td>Bees</td>
</tr>
<tr>
<td>Maize</td>
<td>Normally outbreeding, monoecious</td>
<td>Wind, gravity</td>
</tr>
<tr>
<td>Papaya</td>
<td>Normally outbreeding, dioecious</td>
<td>Wind and insects</td>
</tr>
<tr>
<td>Pineapple</td>
<td>Normally outbreeding, hermaphrodite, self-incompatible</td>
<td>Birds</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>Normally outbreeding, hermaphrodite, self compatible or self-incompatible varieties</td>
<td></td>
</tr>
<tr>
<td>Sugar cane</td>
<td>Normally outbreeding ²</td>
<td>Wind</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Normally outbreeding, variable self incompatibility, 20-75% cross pollination</td>
<td>Bees</td>
</tr>
<tr>
<td>White clover</td>
<td>Normally outbreeding, hermaphrodite, mostly self-incompatible, &gt;90% cross pollinated</td>
<td>Bees</td>
</tr>
</tbody>
</table>

¹ Plants are classed simply in terms of their potential to outcross – with no reference to the presence of wild or weedy relatives.

² Note that sugar cane is normally propagated by stem cuttings.
<table>
<thead>
<tr>
<th>Crops</th>
<th>Mating system (Frankel and Galun 1977; Sindel 1997; Napier 2001)</th>
<th>Main pollination vectors (Frankel and Galun 1977)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intermediate outcrossing potential</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canola</td>
<td>Reported as either normally outbreeding or normally inbreeding (mixed mating system), hermaphrodite, &gt;10% cross pollination,</td>
<td>Wind, bees</td>
</tr>
<tr>
<td>Cotton</td>
<td>Mixed mating system, hermaphrodite, 5-40% cross pollination</td>
<td>Insects</td>
</tr>
<tr>
<td>Indian Mustard</td>
<td>Normally outbreeding, hermaphrodite, self-compatible or self-incompatible</td>
<td>Bees</td>
</tr>
<tr>
<td>Oilseed Poppy</td>
<td>Normally inbreeding, 0.5 – 33% cross pollination</td>
<td>Bees</td>
</tr>
<tr>
<td><strong>Low outcrossing potential</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>Normally inbreeding, hermaphrodite, many cultivars cleistogamic</td>
<td></td>
</tr>
<tr>
<td>Field Pea</td>
<td>Normally inbreeding, hermaphrodite, some cultivars up to 25% cross polination</td>
<td></td>
</tr>
<tr>
<td>Grapes</td>
<td>Normally inbreeding, hermaphrodite, some cultivars partly or fully self-incompatible</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>Normally inbreeding, hermaphrodite, partially cleistogamic, 1-6% cross pollination</td>
<td>Flies</td>
</tr>
<tr>
<td>Lupins</td>
<td>Normally inbreeding, hermaphrodite</td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>Normally inbreeding, some outcrossing, many cultivars produce non-functional pollen</td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>Normally inbreeding, some cultivars cleistogamic, varies climatically</td>
<td>Wind</td>
</tr>
<tr>
<td>Soybean</td>
<td>Normally inbreeding, hermaphrodite</td>
<td></td>
</tr>
<tr>
<td>Subterranean Clover</td>
<td>Normally inbreeding, hermaphrodite</td>
<td></td>
</tr>
<tr>
<td>Tobacco</td>
<td>Normally inbreeding, 2 –3% cross pollination, some reports higher</td>
<td>Birds, bees</td>
</tr>
<tr>
<td>Tomato</td>
<td>Normally inbreeding, hermaphrodite, protogynous 3, but flower shape facilitates self-pollination, less than 2% cross pollination, some reports higher</td>
<td>Solitary bees and thrips</td>
</tr>
<tr>
<td>Wheat</td>
<td>Normally inbreeding, hermaphrodite, largely cleistogamic, variable cross pollination up to 6%</td>
<td>Wind</td>
</tr>
</tbody>
</table>

3 Female develops before the male.
**Other factors affecting probability of pollen transfer**

Even plant species with the same mating system can have vastly different rates of outcrossing due to many other factors. These include the size of the pollen source, the distance the pollen can travel, how long the pollen remains viable, receptivity of the plant stigma to the pollen and the concentration of competing viable pollen. The following sections outline some issues influencing successful cross-pollination between the same species of plants. Later sections address outcrossing between related species and barriers to hybrid formation.

**Pollen source**

The size of the pollen source is an important determinant of the extent of cross-pollination and is governed by a range of factors such as plant density (number of plants per unit area), flower density (the number of flowers per plant) and plant distribution. The size of the receptor plot is also important when considering the proportion of cross-pollination events. Small-scale field trials are thought to be inadequate to assess gene flow from GM crops grown on a large agricultural scale (Eastham and Sweet 2002).

**Pollen travel**

Pollen dispersal generally follows a leptokurtic distribution, in other words most pollen is distributed close to the source and drops off rapidly with distance. This dispersal pattern is typical for both wind and insect dispersal, but the methods of pollen dispersal do influence the direction and spread of pollen migration. Vertical dispersion of pollen may also be important and can be influenced by many factors such as air turbulence, barriers, topography and convection currents. Vegetation barriers such as trees and bushes can also affect pollen dispersal and this has been reviewed elsewhere (Treu and Emberlin 2000).

Wind pollination is an obvious example where the direction and velocity is very important in determining the pollen dispersal. Environmental conditions have been shown to affect pollen travel for example it has been shown that some pollen bounces off dry surfaces and therefore travels further (Ingram 2000a). This reinforces the importance of regional studies. For example a Californian study showed a 10-fold difference in the degree of outcrossing within tomato varieties (Laverack and Turner 1995). Another study measured average canola pollen concentrations on one day of the year to be 1.4% of that measured on the same day the next year, probably due to varying weather conditions (Scott 1970).

The pattern of pollen distribution is also influenced by the mass, volume and surface structure of the pollen. These factors combine to determine the capacity of the pollen to remain airborne (Feil and Schmid 2002). Also the height of the plant influences the pattern of pollen deposition. In general the taller the plant the greater the distribution of pollen deposited away from the source plant (Feil and Schmid 2002).

Insects are the main biotic pollen dispersal agents, and many evolved concurrently with flowering plants to become specialised pollinators. Bees are the most important pollinators for cultivated plants and rely almost entirely on the pollen and nectar of flowers (Frankel and Galun 1977). Attractiveness of crops to pollinators as well as the numbers, types and pollen preferences of insects combined with the mating system of the crop in question are also important when considering the role of insects in cross-pollination (Ingram 2000b).

Vertebrate vectors such as birds, bats and small animals are rarely the primary pollinators of cultivated plants (though some tropical plants use primarily flying vertebrate vectors). Some agricultural pests may also act as involuntary biotic pollen vectors and may be important pollinators in some situations.
The presence of pollinators obviously varies between region and season, in addition pollinator species can also change over time because of accidental or deliberate introductions. For example, the accidental or illegal introduction of bumblebees into Australia, first reported in Tasmania in 1992, could potentially impact on plant-pollinator interactions. Another example of changing pollinators is leafcutter bees that are currently being imported into Australia to facilitate lucerne cross-pollination.

Another issue to be considered is that pest protected GM crops may change local pollinator populations due to reductions in spraying of chemical pesticides. Data from commercially grown insect-resistant cotton in Australia show that there is a 37-54% reduction in the amount of insecticide required on this cotton, depending on the environmental conditions during the growing season (The Cotton Research and Development Corporation). This may have significant impact on the local insect populations and potentially change the levels of cross-pollination.

Factors affecting probability of cross-pollination

After the pollen arrives at the receptor crop, there are then several other factors that influence potential cross-pollination. The amount of pollen produced by the receptor crop is an important factor. This is a significant issue because gene flow is often measured through monitoring of cross-pollination of male sterile plants. These plants will have no pollen competition and will overestimate gene flow that would occur to fertile plants that have competing pollen. This is significant for production of hybrid varieties too as the male sterile female parents will be far more likely to be cross-pollinated than fertile varieties of the same crop.

Synchronisation of the pollen arrival with the receptivity of the flower is also an important factor to consider (Ingram 2000b) and depends on the timing of flowering of both plants. Staggering the planting of GM and non-GM crops is being considered as one way of reducing the likelihood of gene flow between crop populations. However this approach could not always be relied on to eliminate gene flow as flowering times will vary from season to season and not all plants in a population flower synchronously.

Viability of the pollen varies between species and with environmental conditions. If the pollen’s arrival is delayed or affected by adverse conditions, local pollen may have viability and competitive advantages. Although this is not thought to be an important consideration in the UK (Ingram 2000b), under Australian conditions this may be a limiting factor for cross-pollination.

Crop to same crop cross-pollination likelihood

As mentioned in the introduction, crop-to-crop gene flow issues have historically been addressed for the production of certified seed. Currently, certified seeds must be grown under strict regulations in order to guarantee the seed purity and high germination rates. Regulations are very crop-specific and have different requirements for isolation distances, field history requirements and purity standards due to the biology of the species. In Australia, certified seed regulations can be obtained from the State governments, and access to some international regulations are detailed in Table 5. NSW and US Seed certification regulations for some crops of interest are detailed in Tables 6 and 7.

Significance of crop to crop cross-pollination - Nature of crop product

When considering crop-to-crop gene flow, the nature of the receptor crop is a very important consideration for determining consequences. If viable pollen from a GM plant is deposited on the flower of a compatible non-GM crop plant, and seeds are produced, these seeds will
potentially carry the transgene. However, the genetic composition of the vegetative parts of the maternal plant will remain unaltered and will not contain the transgene (Ingram 2000a). In other words, if the crop is a root or leaf crop, the genetic nature of the harvested material from this generation will still be non-GM. For root or leaf crops cross-pollination from GM crops is only significant for seed production plots, or if cross-pollinated seeds contaminate subsequent crops.

Table 5 Access to some international seed certification regulations and GM field trial guidelines

<table>
<thead>
<tr>
<th>Seed Regulations</th>
<th>Access</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canadian Seed Growers Association</td>
<td><a href="http://www.seedgrowers.ca/regulations/reg1.html">http://www.seedgrowers.ca/regulations/reg1.html</a></td>
</tr>
</tbody>
</table>

GM Field trial conditions

| Supply Chain Initiative on Modified Agricultural Crops (SCIMAC) guidelines (UK) | [http://www.ukasta.org.uk/scimac/contents.html](http://www.ukasta.org.uk/scimac/contents.html) |

Table 6: NSW Seed regulations for some crops of interest (Beverly Zurbo, personal communication) – based on the OECD Rules and Directives.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Separation distances in metres (Varietal purity %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-basic/basic seed</td>
</tr>
<tr>
<td>Barley and Wheat</td>
<td>0 (99.9)</td>
</tr>
<tr>
<td>Canola – fertile</td>
<td>200 (99.9)</td>
</tr>
<tr>
<td>Canola – hybrid</td>
<td>500 (99.9)</td>
</tr>
<tr>
<td>Cotton</td>
<td>8</td>
</tr>
<tr>
<td>Field pea</td>
<td>10 (99.7)</td>
</tr>
<tr>
<td>Lupins</td>
<td>100</td>
</tr>
<tr>
<td>Subterranean clover</td>
<td>3 (95)</td>
</tr>
<tr>
<td>White clover</td>
<td>100</td>
</tr>
</tbody>
</table>

4 Physical barrier to prevent mixing
5 Defined physical barrier for varieties with similar leaf types

Implications of gene flow for the release of GM crops in Australia
Table 7 US Seed regulations for some crops of interest (Association of Official Seed Certifying Agencies 2001)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Foundation (Maximum % of other varieties)</th>
<th>Registered (Maximum % of other varieties)</th>
<th>Certified (Maximum % of other varieties)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley and wheat</td>
<td>6 (0.05)</td>
<td>6 (0.1)</td>
<td>6 (0.2)</td>
</tr>
<tr>
<td>Canola - fertile</td>
<td>201</td>
<td>201</td>
<td>101</td>
</tr>
<tr>
<td>Canola – hybrid</td>
<td>402</td>
<td>402</td>
<td>101</td>
</tr>
<tr>
<td>Cotton (upland)</td>
<td>7 8 (0.03)</td>
<td>7 8 (0.5)</td>
<td>7 8 (0.1)</td>
</tr>
<tr>
<td>Field peas</td>
<td>0 (0.05)</td>
<td>0 (0.1)</td>
<td>0 (0.2)</td>
</tr>
<tr>
<td>Lupins – self pollinated</td>
<td>183</td>
<td>91</td>
<td>50</td>
</tr>
<tr>
<td>Lupins – cross pollinated, &gt; 5 acre field</td>
<td>274 (0.01)</td>
<td>137 (0.02)</td>
<td>50 (0.05)</td>
</tr>
<tr>
<td>White clover, &gt; 5 acre field</td>
<td>183</td>
<td>91</td>
<td>50</td>
</tr>
<tr>
<td>White clover, &lt; 5 acre field</td>
<td>302 (0.1)</td>
<td>151 (0.25)</td>
<td>50 (1.0)</td>
</tr>
</tbody>
</table>

Use of isolation distances from seed schemes for GM crops

Current isolation standards for crops are sufficient for maintaining varietal purity based mostly on visual identification of a range of botanical characters. For example in NSW canola crops grown for certified seed are visually inspected three times during their growth cycle – at the rosette stage, early flowering and late flowering/early seed set (Beverley Zurbo, NSW Agriculture, personal communication). Low levels of cross-contamination by neighbouring varieties are allowable as long as they do not exceed thresholds specified in certification standards. Out of season growth of a portion of harvested seeds is a common purity check of hybrid seeds. For some crops, biochemical assays such as isozymes have been used to check seed lot purity (Smith and Register 1998) and more modern methods such as image analysis, protein electrophoresis and DNA profiling are being gradually introduced for some crops (Cooke 1999). However, for the majority of crops, visual identification of varietal purity is still used. Higher standards and scrutiny of seed lots for even rare contamination by GM material to meet the requirements of non-GM markets may now be needed, as GM plants will not generally be visually different from the GM varieties. Sensitive methods for testing for GM material may result in market rejection based on very low levels of contamination. Note that sensitive methods can also lead to false positive tests due to inadvertent contamination during the testing procedure (Bock et al. 2002).

Thus, the current isolation standards for seed purity need to be carefully reviewed in order to confirm that they are adequate for the purpose of segregation of GM from non-GM produce.

6 Strip of ground adequate to prevent mechanical mixture, mowed, uncropped or another crop
7 Natural barrier or crop boundary
8 Minimum distance shall be at least 30 m if the cotton plants in the contaminating source differ by easily observable morphological characteristics from the field to be inspected. Isolation distances between upland and Egyptian types shall be at least 402 m, 402 m, and 183 m for Foundation, Registered and Certified classes, respectively.
9 Distance adequate to prevent mechanical mixture
With this in mind, the production of hybrid seed in maize was recently reviewed by seed producers in the United States (Burris n.d.). The basis of this review was that the seed production practices have remained basically unchanged for thirty years or more, even though detection methods for purity standards are far more sensitive. This study found that isolation distance is an effective way to reduce pollen contamination but that its effect can sometimes be reduced due to other factors. However, high purity seed could be produced even under the most difficult conditions. The findings of this review are discussed in more detail in later sections of this report (see section Agricultural measures to limit gene flow).

**Factors affecting probability of hybrid formation**

Hybrids are formed when two genetically different parent plants successfully cross. Of interest for this report are hybrids that may result from a cross between a GM crop (or in some cases a GM volunteer) and other species such as:

1. A related non-GM crop
2. A related weed
3. A related native species

Hybridisation is an important mechanism of plant evolution, however hybrid plants that result from crosses between different species are often sterile and rarely persist. However, as mentioned previously hybrids are an important route for introgression of genes between related plant species, through backcrossing to the parent species. Table 8 shows a series of barriers that must be crossed for successful gene flow to occur between two species. Some of these barriers will be discussed in more detail in the following sections.

**Distribution of weedy or wild relatives**

Spatial isolation is obviously a very significant barrier to hybrid formation. Knowledge of the distribution patterns of relatives of GM crops is an essential first step to assessing the likelihood and potential impact of gene flow. A study that considered 235 crops grown in Australia found that 22% of these had no weedy relatives here, 17% had no weedy relatives other than the crop itself (naturalised or volunteer plants) and 61% had more than one weedy relative growing in Australia (although not necessarily in the same region) (Sindel 1997). This study further grouped crops according to their likelihood of outcrossing with weedy relatives – shown in Table 9. In this case crops with a high potential for gene flow have either weedy relatives of the same species, or high numbers of weedy relatives and/or evidence of some reproductive compatibility with weedy relatives. However, Sindel (1997) stresses that this is a preliminary assessment and needs to be tested with more research into reproductive compatibility and hybrid formation. Sindel (1997) also identified limitations in the knowledge of regional weed distribution in Australia as often such data is reported on a whole state basis.

**Internal barriers to hybrid formation**

Internal barriers to hybrid formation are due to genetic or physiological incompatibilities between species. In interferfertile plants, pollen grains germinate, forming a long pollen tube that carries the sperm cells through to the egg cell located in the female structure (ovule) of the plant. Fusion of these sperm nuclei with the egg cell is known as fertilization. Hybrid plants may fail to form because of pollen failing to germinate, the pollen tube failing to reach the egg cell, or even failure of the sperm nuclei to combine with the egg (Ammann et al. n.d.).
Table 8: Barriers to gene flow between species (Levin 1978).

| Premating | Spatial   | Ecological and mechanical |
|           | Reproductive | Temporal divergence |
|           | Floral divergence |

| Postmating | Prezygotic 10 | Reproductive mode |
|           | Cross-incompatibility between pollen and pistil 11 |
|           | Postzygotic 12 | Cross-incompatibility in the seed 13 |
|           | Hybrid lethality or weakness |
|           | Hybrid floral isolation |
|           | Hybrid sterility |
|           | Hybrid breakdown |

Table 9: Potential of crops to outcross to weedy relatives in Australia, based on preliminary information only (Sindel 1997)

<table>
<thead>
<tr>
<th>High 14</th>
<th>Medium</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celery, carrot, parsnip, sunflower, lettuce, canola, cabbage, radish, silver beet, white clover, subterranean clover, vetch, onion, asparagus, linseed, oats, barley, rice, sorghum, capsicum, tobacco, potato</td>
<td>Safflower, watermelon, cucumber, lupin, lucerne, faba bean, mungbean, rye</td>
<td>Pumpkin, peanut, chickpea, soybean, lablab, bean, pea, cotton, sugarcane, triticale, wheat, maize, buckwheat, tomato</td>
</tr>
</tbody>
</table>

Factors affecting probability of hybrid survival

Some plant species can be crossed to produce hybrid zygotes, but these are non-viable because of various incompatibilities within the developing zygote (genome, genome-cytoplasm and embryo-endosperm). In some cases, crosses between two plants produce a viable hybrid plant, but this hybrid may not be able to produce fertile seed because of structural differences between the two parent genomes. In the cases where fertile hybrid plants are formed, the progeny of these plants can sometimes be weak or sterile (a process known as hybrid breakdown). Where partial fertility occurs, such as hybrids that are female fertile but pollen sterile, there is an ongoing possibility of further cross-pollination.

Further comments on hybrid formation

Plant breeders often use hybrids between species to introduce genetic variation or specific traits into crop species. Therefore sophisticated laboratory techniques have been developed to evade some of these natural barriers to hybridisation. It is important to recognise that hybrid

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10 Before fertilization.
11 This includes failure of pollen to germinate, abnormal pollen tube growth and pollen competition on the style.
12 After fertilization.
13 This may result in no or poorly developed endosperm, and/or malformed or aborted embryos.
14 High potential for gene flow here indicates that crops have either same species weedy relatives, or high numbers of weedy relatives and/or evidence of some reproductive compatibility with weedy relatives.
formation in a laboratory does not indicate that this process will readily occur in the field. Some examples of hybrids between *Brassica* species that have been successfully formed in artificial environments have been reviewed (Rieger et al. 1999). Caution must also be exercised when extrapolating from hand-pollination experiments under ideal conditions to what might occur in the field (Rieger et al. 1999). Note that the direction of the cross (which is the pollen parent and which is the female parent) can also greatly influence the extent and significance of hybrid formation.
Case studies of gene flow from plants in Australia

The likelihood and potential impact of gene flow via cross-pollination from GM crops depends on many factors specific to the crop and the environment of the proposed release. Before GM crops can be grown on Australian soil they must be thoroughly assessed on a case-by-case basis for each crop and crop/trait combination by the Office of the Gene Technology Regulator (OGTR). This report is not meant to prejudge such risk assessments, but to review factors affecting gene flow and highlight issues that should be considered in the assessment processes. In order to explore some of the issues involved in assessment of the likelihood and potential impact of gene flow, several crops were chosen as case studies for this report. These crops were selected on the basis of representing the following broad categories of gene flow likelihood and potential impact:

**High level of outcrossing** - Low potential for impact on the farm or natural environment

**High level of outcrossing** – Significant potential for impact on the farm or natural environment

**Medium level of outcrossing** – Low potential for impact on the farm or natural environment

**Medium level of outcrossing** – Significant potential for impact on the farm or natural environment

**Low level of outcrossing** – Low potential for impact on the farm or natural environment

**Low level of outcrossing** – Significant potential for impact on the farm or natural environment
Highly outcrossing plant species

Case study – Maize (Zea mays)

Mating system
Maize is a monoecious species, with separate male and female flowers on the same plant (Emberlin 1999). It is predominantly a wind pollinated species, with limited evidence of insect pollination (Eastham and Sweet 2002). Each plant produces large quantities of pollen, but the pollen grains themselves are large in size, limiting their dispersal compared to other wind-pollinated species. Maize pollen viability ranges from 3 hours – 9 days depending on the environmental conditions, with cool temperatures and high relative humidity allowing for longer survival times. Maize is predominantly cross-fertilising and in the field estimates are that at least 95% of the seed are produced by cross-pollination events (Treu and Emberlin 2000).

Gene flow studies
Studies examining the potential gene flow from maize, by assessing pollen dispersal, show variation due to the differences in experimental design and environmental conditions. Often such studies express pollen dispersal as a ‘half-distance’ that refers to the distance required to reduce pollen by 50%. Half-distances have been reported ranging from nearly 4 m up to nearly 50 m in one study (reviewed (Emberlin 1999; Burris n.d.), however data from most studies suggest that the vast majority of pollen remains within 25 – 50 m of the maize field (Eastham and Sweet 2002).

Actual gene flow as measured by percentage outcrossing has also been measured in many studies. Again these vary widely, but one study has estimated that as a rough guideline, if two maize plots are flowering synchronously and producing equal amounts of pollen, cross pollination would be in the order of 1.9% at 60 m, 1.1% at 200 m and less than 0.7% at 500 m (Emberlin 1999). Another study notes that outcrossing has been recorded at 800 m and that under suitable atmospheric conditions maize pollen has the potential to travel over much longer distances (Eastham and Sweet 2002).

Seed certification of maize
Seed certification regulations for maize grown in NSW require that crops be at least 200 m from any sources of contaminating pollen. In the US the Association of Official Seed Certifying Agencies (AOSCA) standards require a 201 m separation distance for certified seed production from standard varieties of maize, but for the production of hybrid maize seed 125 m can be used if the contaminating pollen is from maize with the same colour and texture. Less than 125 m can be used if the plots are surrounded by border rows of the pollen parent, and this can even reduce to zero separation distance if ten rows of the pollen parent are used around the seed plot (Association of Official Seed Certifying Agencies 2001). Recently hybrid maize seed production standards have been reviewed by the coordination of an industry wide study in the US. This study, which was conducted over a three year period, measured outcrossing percentages in hybrid seed production fields and found that although separation distances decreased cross-contamination, the effect was often overshadowed by other factors such as wind velocity and direction (Burris n.d.).

Weediness potential of maize
Maize is included in the CSIRO Handbook of Australian Weeds (Lazarides et al. 1997) as naturalised in some environments but is not considered a problem weed (Groves et al. 2000; Groves et al. 2002).
Hybridisation with wild or weedy relatives

A relative of maize, *Zea mexicana* (teosinte) is naturalised in some environments in Queensland, but is not considered a problem weed (Groves et al. 2000). No other close relatives of maize are present in Australia, although two distant native relatives (in a different genus) grow in the Cape York Peninsula (Bryan Simon, personal communication).

Crop to crop gene flow

A model, *Matrix* based approach to *pollen* dispersal (MAPOD), was recently used to assess the effect of changing farming practices on cross-contamination levels of GM in non-GM maize feed production farms (Bock et al. 2002). Inputs into this model include field sizes, climatic data, pollen dispersal, cropping system and maize variety. Different farm types were studied and the main variables influencing contamination levels were found to be:

- Seed impurity at source
- Relative proportions of maize in the landscape
- Relative field size of both donor and acceptor crops
- Field pattern
- Grain harvesting process

These studies concluded that a 0.1% threshold of maximum cross-contamination could not be achieved for maize in Europe. A 1% threshold would be achievable, with some farm types needing to change practices to reach these levels (Bock et al. 2002). Another study concluded that an isolation distance of 200 m could maintain crop purity at 99% in most cases (Eastham and Sweet 2002).

Conclusion

Maize is a highly outcrossing, predominantly wind-pollinated species. Gene flow between maize crops would have implications for the co-existence of GM and non-GM crops. The adoption of appropriate agricultural methods to reduce gene flow such as separation distances should be able to limit contamination below certain thresholds. The impact of gene flow from GM maize into the environment is limited by the lack of weedy or wild relatives in the Australian context. Currently there are no field trials of GM maize in Australia and little research in this area. OGTR would assess any applications for field trials or commercial release of maize and include gene flow in its environmental risk assessment framework.

Case study – Eucalypt species

Mating system

*Eucalyptus* species have mixed mating systems with high levels of outcrossing (Frankel and Galun 1977) and usually cross pollination is mediated by pollinators such as birds, insects and mammals (Potts et al. 2001). The accessibility of the open Eucalypt flower means that the pollinators are usually not specific (Strauss 2001).

Gene flow studies

Studies of pollen dispersal in Eucalypt populations show that like other species most pollen is deposited close to the source, with rare long-distance dispersal. Percentages of hybrids formed at different separation distances between source populations vary greatly depending on the biology of the two species in question, overlap of flowering times, environmental conditions and insect populations, examples are shown in Table 10 (Potts et al. 2001). A hybrid has even
been reported at a distance of 6 km from a pollen source, but this is considered rare and needs to be confirmed.

**Table 10:** Reports of hybrid formation between eucalypts separated by distance. Adapted from (Potts et al. 2001).

<table>
<thead>
<tr>
<th>Distance between populations (metres)</th>
<th>Hybrid formation (%)</th>
<th>Eucalypt Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.8</td>
<td>Eucalyptus amygdalina x E. risdonii</td>
</tr>
<tr>
<td>30</td>
<td>47</td>
<td>E. ovata x E. nitens</td>
</tr>
<tr>
<td>50</td>
<td>1.3</td>
<td>E. amygdalina x E. risdonii</td>
</tr>
<tr>
<td>150</td>
<td>5-16</td>
<td>E. ovata x E. nitens</td>
</tr>
<tr>
<td>300</td>
<td>&lt;0.5 - 4</td>
<td>E. ovata x E. nitens</td>
</tr>
<tr>
<td>400</td>
<td>14.2</td>
<td>E. grandis x E. urophylla</td>
</tr>
<tr>
<td>500</td>
<td>0.1</td>
<td>E. amygdalina x E. risdonii</td>
</tr>
<tr>
<td>800</td>
<td>2.8</td>
<td>E. grandis x E. urophylla</td>
</tr>
</tbody>
</table>

**Hybridisation with wild or weedy relatives**

Eucalypts are grown world-wide but virtually all of the 800 or more taxa are native to Australia and surrounding islands (Potts et al. 2001), making this country a centre for biodiversity in a similar way that Mexico is the centre for biodiversity for maize.

Eucalypts are known to readily hybridise with other eucalypt species. The poor reproductive barriers between species have even led to taxonomic classification problems (Potts et al. 2001; Strauss 2001). Out of 883 taxa described by one classification, at least 14% were considered to have originated from hybrids. Gene flow between plantation eucalypts (non-GM) currently grown in Australia and native populations is an issue that to date has not been studied extensively, but researchers realise that it may be a priority considering the expected increases in area under cultivation. Gene flow may impact biodiversity, and not just the genetic integrity of the local eucalypt population. Hybrid formation may impact on herbivorous insect and pathogenic fungal populations, and may even result in evolutionary shifts of insect feeding behaviours by acting as ‘bridges’ between species (Potts et al. 2001; Strauss 2001). Factors including the likelihood of hybrid formation, distribution patterns of hybridisation, potential impacts of gene flow from GM eucalypts, and suggested methods to limit gene flow have been recently reviewed (Potts et al. 2001).

**Conclusion**

Eucalypts have a high degree of natural cross-pollination and hybrid formation between species is common. Gene flow is no doubt already occurring between forest plantations and native eucalypts. There are currently no GM eucalypt field trials in Australia, and if research reaches this stage the OGTR will assess the environmental risk of gene flow as part of its wider risk assessments. The different biology of forest trees compared to most agricultural species will pose different questions that need to be considered in this process (James et al. 1998). If it is considered that modified genes pose a greater potential for impact to the natural genetic resources than genes from conventionally bred trees, the release of GM eucalypts in Australia will need careful consideration and well studied measures to minimise gene flow. Release, if approved, may even depend on reproductive sterility to prevent gene flow via pollen (Potts et al. 2001).

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Note that the significance of a certain percentage of hybrid formation depends on the amount of seed produced per plant.
Intermediate outcrossing plant species

Case study – cotton (*Gossypium hirsutum* L.)

**Mating system**

Cotton is one of the two GM plant species that have to date been approved for commercial release in Australia, carnation being the other. Cotton is generally self-pollinating but if insects are present cross-pollination can occur (Oosterhuis and Jernstedt 1999). Wind pollination is considered to be insignificant due to the size and stickiness of the pollen (Llewellyn and Fitt 1996). Outcrossing rates will vary from region to region and season to season. Experiments assessing outcrossing rates report from less than 10% in some cases but up to 40% in others. However cotton pollen dispersal studies suggest that outcrossing is highest close to the pollen source and drops off rapidly with distance.

**Gene flow studies**

An Australian study on transgene movement from cotton in field trials has been reported where the researchers measured outcrossing in non-GM cotton surrounding the GM plot in two different seasons (Llewellyn and Fitt 1996). In the first season 0.15% of seed collected from plants in the first metre surrounding the plot contained the transgene. This figure dropped off to less than 0.08% at 4 m distance. The second trial found 0.4% at 1 m and 0.03% at 16 m. These values were much lower than that found by a similar study in the US where the rates were measured at up to 5.7% in the first buffer metre and drop off to 0.7% at 22 m (Umbeck et al. 1991). An older study looking at gene flow from conventional cotton found that most gene flow occurred within 3.8 m of the source and detected none beyond 30.5 m, reviewed in (Levin and Kerster 1974). Varying rates of outcrossing can be attributed to a combination of factors such as differences in experimental design, environmental conditions and changing pollinator insect populations due to regional differences or differing pesticide applications. In cotton areas with few pollinators the cross-pollination rate is usually lower than in areas with few cotton flowers and many pollinator vectors (reviewed in (Levin and Kerster 1974).

**Seed certification of cotton**

Separation distances required for seed certification are shown in Tables 6 and 7. In NSW an 8 m separation distance is specified for pre-basic seed and basic seed, and 4 m for certified seed. Interestingly in the US no distance is specified but a natural barrier or crop boundary should be used for upland cotton seed production. Upland cotton is the *Gossypium hirsutum* L. species that makes up the majority of cotton grown in the world and the species grown in Australia. However in the US seed data table there is a footnote that qualifies this isolation distance. Minimum isolation distance should be at least 30 m if the cotton plants in the contamination source differ by easily observable morphological characteristics from the field to be inspected. This is interesting as it seems to support other claims that currently used isolation distances for most crops have been set mostly on visual identification of off-types – which can of course overestimate the level of genetic purity (Ingram 2000a).

A further footnote in the US regulations specify that isolation distance between upland and Egyptian types shall be at least 402 m, 402 m, and 183 m for foundation, registered, and certified classes, respectively. Egyptian cotton, *G. barbadense*, otherwise known as Extra-long staple or Pima cotton, is grown for its longer, finer fibres and used for specialised applications. Egyptian and upland cotton are interfertile and crossing by pollination must be avoided to preserve the purity of the seed as quality of the Egyptian cotton will be reduced if flow occurs.
Although this issue is not directly relevant to the Australian context as Egyptian cotton is only rarely grown here, this shows that careful analysis of the seed separation distances are required for each crop if these data are to be used in the context of separation of GM from non-GM crops.

A US study is currently collecting cross pollination field data from cotton growing in California in order to quantify the isolation distances needed to meet specific target maximum thresholds of GM (Thomas et al. 2001).

**Weediness potential of cotton**

Cotton is included in the CSIRO Handbook of Australian Weeds (Lazarides et al. 1997) but is classified as only a minor weed problem of natural environments in only a few locations in New South Wales (NSW), Queensland and Western Australia (Groves et al. 2000). Sporadic populations of cotton can be found in isolated patches in coastal areas and along roadsides and streams in northern Australia.

Cotton genetically modified to contain insect resistance is currently being commercially grown in NSW and southern Queensland. The pest protection that this genetic modification offers may allow expansion of the cotton industry into areas of northern Australia such as the Kimberley region. Conventional cotton used to be grown in this area but in the 1970s the insect pressure was so high that farmers ceased growing cotton. If the pest-protected GM cotton enables production of cotton again in these areas there are some concerns about the weediness potential of these varieties in this region, or the risk of gene flow increasing the fitness of current naturalised cotton populations. High insect pressure in this region may lead to strong selection for fitness advantages of naturalised cotton containing insect resistance genes.

A recent Australian study to begin to address this issue has compared the weediness risk of GM insecticide expressing cotton to conventional cotton (Rowena Eastick, personal communication). This study concluded that the GM cotton had similar seed dispersal, germination and survival rates to conventional cotton. The presence of the transgene did not result in a competitive advantage for the cotton in natural habitats. This is probably due to other factors more important for invasiveness of volunteers than pest protection such as drought, nutrition and competition. In addition the Bt proteins expressed by these plants only offer protection against a subset of Lepidoptera insects, and many other insects that attack cotton would not be affected.

Another related issue yet to be addressed is whether gene flow from GM cotton into already naturalised populations of cotton has the potential for impact. Naturalised cotton is more likely to be better adapted to altered habitats than the crop variety, and introgression of transgenes into these populations may change their invasiveness.

**Hybrid formation with wild or weedy relatives**

Although cultivated cotton originated in the Americas, the Australian native flora was reported to consist of 18 diploid species that are distant relatives (Brown et al. 1997). However more recent data on the classification has revised this to 17 species as *G. nandewarense*, which was originally thought of as a separate species, appears to be a segregate of *G. sturtianum* (Curt Brubaker, personal communication). These relatives differ from cultivated cotton in their genetics, physical appearance and cytology but some assessment of the likelihood of gene flow into these species is still needed.

An initial barrier to gene flow is geographic isolation of the crop from wild relatives. In Australia cotton is grown mainly in central and northwestern NSW and central and southern Queensland. Wild *Gossypium* species growing within these regions are *G. sturtianum* and *G. australae*. Cotton is also being grown in field trials in the Kimberley region of WA, in
southwestern NSW, in Katherine in the Northern Territory and in the Fitzroy River area of
Northern Queensland. *G. australe* and *G. rotundifolium* ranges overlap with some of these
areas and *G. bickii* may overlap in Northern Queensland.

Contact between cultivated cotton and the wild *Gossypium* species in these regions is thought
to be sporadic and limited but expansion of cotton acreage may increase this (Brown et al.
1997). Data on flowering times and pollination biology of most of the wild *Gossypium* is
very limited, and so a conservative approach for risk assessment is to assume that the flowering
times with cultivated cotton overlap and that the species share common pollinators (Brown et
al. 1997).

Detailed biological information is often limited in native plants so it is also difficult to assess
the significance of other possible prezygotic barriers such as autogamy, and competitive
disadvantage of foreign pollen on the style. However even if plant ranges did overlap and
cross-pollination was successful, post zygotic barriers are likely to prevent fertile hybrid
establishment (Brown et al. 1997). For example, *G. sturtianum* crosses most easily with
cultivated cotton (though this was still 1/100 the success rate of an intraspecific cross) but the
resulting hybrids were totally infertile. It has been concluded that the likelihood of transgene
escape from cotton into native species is negligible (Brown et al. 1997).

**Weediness of wild relatives**

*G. sturtianum* and *G. thurberi* are included in the CSIRO Handbook of Australian Weeds
(Lazarides et al. 1997) and *G. australe* can colonise disturbed habitats such as roadsides,
however these species are only considered minor problems in a few locations in some states
(Groves et al. 2000).

**Conclusion**

Cotton is naturally self-pollinating with varying outcrossing rates depending on the presence
of insect vectors and environmental conditions. Most pollen is distributed very close to the
pollen source, and this is reflected in the small separation distances required between crops
for certified seed production. The Australian native flora contains many relatives of cotton,
but fertile hybrid formation between these relatives and cotton is very unlikely. Neither cotton
nor its relatives are considered significant problem weeds in the Australian environment.

**Case study – canola (Brassica napus)**

**Mating system**

Canola has a mixed mating system. It is predominantly self-fertile with the potential for about
30% outcrossing; measured from 12 – 47% in field trials (reviewed in Rieger et al. 1999).
Other studies show the outcrossing rate to vary from 5 – 55% (reviewed in Ingram 2000a).
Male sterile plants are sometimes used for the production of hybrid seed and these are
exceptions to these rates as they must be cross-fertilised to produce seed. More complicated
hybrid mating systems are also being used where the commercial crop contains a mixture of
male sterile and male fertile canola (Ingram 2000a).

Wind pollination and insect pollination (especially honeybees) both appear to be significant
factors in the potential for canola to cross-pollinate. The relative importance for these two
vectors is unclear and varies regionally and even seasonally (Ingram 2000a; Treu and
Emberlin 2000). Honeybees are known to be significant pollinators of canola in the United
Kingdom and there is evidence to suggest that they also play a role in long-distance transport
of pollen. A study on canola pollen transport by honeybees in the UK studied the foraging
behaviour of a bee colony located 600m from a GM canola trial. They found that honeybees
leaving the hive carry many viable pollen grains from many different pollen sources and so may have the ability to disperse GM pollen to any compatible species in the area (Ramsay et al. 1999).

**Gene flow studies**

Gene flow from GM canola has captured the interest of the scientific community and most recent studies of gene flow concentrate on this crop. Studies show great variation in levels of gene flow due to experimental design differences, plant varieties used, ways of measuring gene flow (for example the use of male sterile bait plants), local environment conditions, local insect populations and field size (as discussed in previous sections). Outcrossing rates in canola have recently been extensively reviewed (Salisbury 2002a), so rather than replicate that work in this report, some of the individual studies will be discussed.

Studies show that most pollen travels less than 10 m from the source (Salisbury 2000) but pollen can be transported further either by wind or insect vectors. A study on individual plants showed that around 50% of pollen fell within 3 m of a plant and the remaining 50% fell at larger distances with a probability that decreased very slowly with distance (Lavigne et al. 1998). Studies measuring canola pollen counts at varying distances from source sites as a percentage of pollen at the field margin have found very different results. For example, pollen counts measured at 100 m from the source range from 2% up to 69% of the pollen at the field margin (reviewed in (Treu and Emberlin 2000). Pollen can be found to disperse over much greater distances as well and one study found canola pollen 3 km from the field plot (Thompson et al. 1999). Other examples of long distance pollen dispersal are reviewed elsewhere (Treu and Emberlin 2000), but these authors also point out that pollen counts do not assess pollen viability or represent pollination or gene flow events. Canola pollen has been known to remain viable for up to one week but this would probably occur only under optimal environmental conditions (Treu and Emberlin 2000).

Numerous studies have addressed the potential for gene flow from canola plants in the field. One standard method is to use male sterile canola plants as pollen traps to measure gene flow. Using such methods, a 5% pollination rate was detected at distances of up to 4 km from the pollen source (Thompson et al. 1999), in another study 0.8% pollination was found at 2.5 km (Timmons et al. 1995). However it is important to realise that the use of male sterile plants tends to overestimate the gene flow expected to pollen producing plants due to the absence of pollen competition. These data would be applicable to male sterile female parents used in hybrid seed production, and also to some extent to some varieties that contain a mix of fertile and sterile plants. A series of studies in the UK showed that Synergy, a variety with a mix of fertile and sterile plants, had a consistently higher outcrossing frequency than other varieties due to the proportion of male sterile plants (Simpson et al. 1999).

Some studies have measured outcrossing from GM plots into surrounding or nearby plots of non-GM canola and these have also showed varying rates of outcrossing. One study showed 1.5% outcrossing in canola 1 m from a small plot of GM plants planted amongst 1.1 ha of non-GM canola, decreasing to 0.4% at 3 m, 0.02% at 12 m and 0.003% at 47 m (Scheffler et al. 1993). Another study by the same research team used a different experimental design where outcrossing was measured in small plots at either 200 or 400 m separation from the GM plot. This study found 0.0156% outcrossing at 200m and 0.0038% at 400 m distance from the source plot (Scheffler et al. 1995). The higher gene flow rates in this experiment were thought to be due the small plot sizes with large separation distances requiring insects to forage further afield to obtain a full-pollen load (Rieger et al. 1999).
A Canadian study looked at outcrossing between GM canola crops resistant to two different herbicides at eleven different field sites (Beckie et al. 2001). This study found outcrossing rates to drop off rapidly at 50 m distance to less than 0.2%, but this level of outcrossing changed little between 50 and 400 m. Outcrossing at 800 m was measured at 0.07%.

Comparisons between outcrossing rates at similar distances from small field trials and large field trials found that in the vast majority of cases, rates were much higher for the large field trials (Salisbury 2002a). This emphasizes the general point that caution should be used in extrapolating from small-scale experiments to large-scale effects for ecological processes.

A recently published large-scale study looked at outcrossing from non-GM herbicide tolerant canola into neighbouring canola crops in the Australian environment (Rieger et al. 2002). This study utilised the fact that canola resistant to a certain group of herbicides (bred by conventional methods rather than GM) were commercially grown for the first time in Australia in 2000. Seeds were collected from sixty-three canola fields located 0 – 5 km away from herbicide tolerant crops in different regions throughout Australia. These seeds were then planted and screened with the herbicide to detect cross-pollination events. Even though large numbers of seeds were collected from each site, no outcrossing was detected in 23 of the fields, and the highest frequency of outcrossing detected per whole field basis was 0.07%. Gene flow was variable, in some cases no measurable gene flow was found in plots just across the fence from the source plot, while in other cases low levels were detected up to 3 km away.

**Separation distances between canola crops**

Canola is an example of a seed crop that can already require separation in the production process. High erucic acid crops (or HEAR crops) are subject to a separation distance from other crops of 50 m in the UK. This is required to meet the EU limit of 2% erucic acid in low erucic acid crops. However the level of checking on the produce is low (Ingram 2000a). High erucic acid crops typically produce about 50% erucic acid whereas low ones contain close to 0%. A hybrid would not fully express the erucic acid content of a homozygous individual so the crossing level has to be at least 4% before the 2% threshold of erucic acid would be reached. Field trial experiments that analysed the cross-contamination levels between high and low erucic acid crops found random fluctuations in erucic acid levels presumably caused by insect activity affecting cross-pollination (Bilsborrow et al. 1998).

Most of the studies mentioned to date have measured outcrossing frequencies at different distances from the pollen source. The next question is how to use this information to assess the level of outcrossing in the whole field. The general assumption is that most of the cross-pollination events in the receptor plot will occur in the closest 10 m to the source (Ingram 2000a) and will drop exponentially with increasing distance from the field, known as the edge effect. Calculations estimating the level of outcrossing therefore need to take into account not only the size of the receptor field but also the proportion of the field that is closest to the source plot, in other words the relative orientation of the field. Table 11 shows results from experiments in the UK on the effect of separation distances in limiting gene flow to look at the effect of field size and orientation on outcrossing levels of whole fields (Ingram 2000a).

From this data a 1.5 m separation distance should limit outcrossing to 1% or less on a whole field basis. This gives approximately four times the safety margin for 5 ha fields and just under three times the safety level for 2 ha fields. To limit outcrossing to 0.5% or less on a whole field basis should be achievable with an 11.5 m separation distance, which gives a approximately 2.5 times the safety margin for 5 ha fields and just nearly three times the safety level for 10 ha fields (Ingram 2000a).
Table 11: Effect of field size, alignment and separation distance on the percentage outcrossing of whole fields (Ingram 2000a).

<table>
<thead>
<tr>
<th>Field area</th>
<th>Crop separation 1.5m</th>
<th>Receptor side adjacent to contaminating crop</th>
<th>Crop separation 11.5m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long</td>
<td>Short</td>
<td>Long</td>
</tr>
<tr>
<td>2ha</td>
<td>0.36</td>
<td>0.18</td>
<td>0.24</td>
</tr>
<tr>
<td>5ha</td>
<td>0.27</td>
<td>0.16</td>
<td>0.19</td>
</tr>
<tr>
<td>10ha</td>
<td>0.23</td>
<td>0.15</td>
<td>0.17</td>
</tr>
</tbody>
</table>

However it is important to realise that these figures just take into account cross-pollination rather than other means of contamination like seed contamination. Also the figures are based on one set of data and outcrossing rates may vary greatly from region to region and season to season. In addition, where there are multiple emitting plots surrounding the receptor crops, the level of outcrossing will be increased and this threshold may not be possible with smaller fields. In a comment on Ingram’s report by the National Pollen Research Unit it is asked why these recommended isolation distances are so much smaller than those required for certified seed purity (National Pollen Research Unit 2000).

Interestingly the recent Australian study of gene flow from herbicide tolerant canola crops to nearby fields did not show obvious edge effects (Rieger et al. 2002). Samples taken from the edge of the fields closest to the pollen source did not always show higher gene flow than samples from the middle of the field or the edge furthest from the herbicide tolerant fields. This indicates that pollen did not follow the expected leptokurtic distribution, and was much more variably distributed. This was thought to be due to the multiple pollinating agents of canola (wind and insects) as well as to the large size of the pollen source. This study emphasises the need for commercial scale studies, rather than extrapolating from small-scale field trials.

The required isolation distances to meet threshold levels of contaminants in canola have been reviewed recently (Salisbury 2002a). Crops were recommended to be separated by at least 1.5 – 30 m for a less than 1% threshold of seed purity, by 10 – 120 m for a less than 0.5% threshold of seed purity, and by 100 – 400 m for a less than 0.1% threshold of seed purity (Salisbury 2002a). Seed certification distances for NSW and the US are shown in Tables 6 and 7.

When the gene flow rates from the Australian canola study were converted to a whole paddock basis the levels ranged from zero to 0.07%, indicating that low attainable maximum threshold levels are attainable in the Australian environment (Rieger et al. 2002). However it is important to realise that these values relate only to gene flow via cross-pollination, other steps in the supply chain will need to be addressed in order to adequately segregate GM from non-GM canola. The two working groups mentioned previously, the GM Canola Technical Working Group (GMCTWG) and the Eastern Zone Technology Grains Committee (EZGTGC), are addressing supply chain issues necessary for introducing GM canola into Australia.

**Hybrid varieties**

In the UK hybrid varieties with varying percentages of male sterile plants are available. Ingram suggests that a 100 m separation distance would probably be sufficient to limit the seed contamination by cross-pollination into Synergy to less than 1% (Ingram 2000a).
However different varieties will have different ratios of male sterile plants and this will have a strong influence on the number of cross-pollination events, and the point at which a variety requires a greater separation distance is not clear. In NSW the separation distance for 0.5-1% seed purity in hybrids is set at 300m, for 0.1% the distance is 500m.

**Other crops**

Other *Brassica* crops related to canola are grown in Australia. Gene flow into *B. napus* vegetables such as swedes, rutabaga and Siberian kale is possible, as is gene flow into *B. rapa* vegetables such as turnip, Chinese cabbage and pak choi (Salisbury 2000; Salisbury 2002c). Gene flow into *B. juncea* condiment crops is also possible. Appropriate methods to limit gene flow from GM canola into these crops would be needed (such as separation distances) if non GM status were required for marketing reasons. However some of these crops are harvested prior to flowering so gene flow would not be a concern in most cases. Gene flow from canola into *B. oleracea* vegetables such as cauliflower, brussel sprouts, broccoli and several kale species is far less likely (Salisbury 2000; Salisbury 2002c).

**Weediness potential of canola**

Although canola occasionally escapes from cultivation it is not considered a major weed in managed or natural ecosystems in Australia (Rieger et al. 1999; Salisbury 2000). Volunteer populations of GM (especially herbicide tolerant) canola however do have implications for gene flow into weeds. On farm or roadside volunteers also have the potential to contaminate adjacent crops. Although most canola seed germinates within two years of dispersal, in some cases secondary dormancy can develop and volunteers can emerge four years later (Salisbury 2002b).

In 1998 a field of canola in Canada was identified as containing canola volunteers with multiple herbicide resistance (Hall et al. 2000). These volunteers were thought to have originated via consecutive outcrossing between three different herbicide tolerant crops (two GM and one conventionally bred) grown in close proximity, and then selected by subsequent herbicide treatments. Concerns are that such ‘gene-stacked’ volunteers may be more difficult to control in farm environments or non-cropped areas (Orson 2002). This is an issue for herbicide tolerant crops in general, as herbicide tolerant crops can be produced by means other than via transgenics and such herbicide tolerant canola and wheat crops are currently being commercially grown in Australia.

Research on outcrossing between large commercial fields of canola show variable outcrossing rates, but even in cases of very low outcrossing rates, the large number of canola flowers and seed produced could result in a substantial number of outcrossed seed per hectare. For example it has been estimated that there are 1 billion fertilisation events in 1 hectare of oilseed rape. Suggested strategies to limit the impact of gene stacked herbicide tolerant volunteers have included the following (Thomas 2000):

- The use of other herbicides such as 2,4-D for pre-seeding weed control
- Tillage and seed control of seed banks
- Rotation of herbicide use
- Crop sequence management
- Use tillage immediately prior to seeding to control volunteers
- Isolate crops with different herbicide tolerances
- Rotate canola with other crops as control of a multiple herbicide tolerant crop within a canola crop may be difficult
• Scout fields, fence lines and verges for volunteers which are not controlled by herbicide usage

A recent report specifically discusses the significance and management of canola volunteers in an Australian context. Similar management practices are detailed (Salisbury 2002b).

At a recent meeting of the Primary Industries Ministerial Council (PIMC) it was agreed that the GM crop management issues of this type should be handled by industry self-regulation supplemented by government monitoring. The Plant Industries Committee of the Primary Industries Standing Committee (PISC) is developing guidelines that support the establishment of industry stewardship programs to address the responsible management of GM crops. Strategies such as those mentioned above will no doubt be included in these crop management plans.

**Hybrid formation with wild or weedy relatives**

Canola belongs to the *Brassicaceae* family that consists of over 3000 species in 370 genera, and 160 of these species are present in Australia (Rieger et al. 1999). Studies on the likelihood of successful hybridisation between canola and weedy species have been recently reviewed and are summarised in Appendix 1 (Salisbury 2002c). From the crops where field hybrids have been reported, the significance of this hybrid formation will depend on many factors, including the significance of the weed in question in an Australian context.

The significance of weeds is often difficult to determine and varies between regions and over time. For the purposes of this report significance of weeds was determined from two databases categorising non-native naturalised plant species in the different states of Australia according to their impact on natural and agricultural environments (Groves et al. 2000; Groves et al. 2002). *Brassica rapa* ssp. *rapa* (turnip) is mentioned as a minor problem of natural environments in three or less locations in WA, and a major agricultural weed in this state. Another subspecies *Brassica rapa* ssp. *sylvestris* is listed as a minor problem of natural and agricultural environments in some states and a major problem in a few natural environments in Tasmania. *Brassica juncea* (Indian mustard) is mentioned as a minor weed problem in a few natural environments in some states, and a major agricultural weed problem in some. *Raphanus raphanistrum* (wild radish) is listed as a noxious weed of natural and agricultural environments in parts of NSW, and a problem weed in most other states. *Hirschfeldia incana* (Buchan weed) is listed as a weed problem of natural and agricultural environments in most states. *Sinapis arvensis* (charlock) is listed as a problem weed of natural and agricultural environments in most states.

The significance of wild radish as a weed has prompted field studies on the likelihood of hybrid formation. These studies have been reviewed previously (Rieger et al. 1999), and some more recent results are shown in Table 12. Note that one of these studies was carried out in Australia and showed much lower frequencies of hybrid formation than the other studies, indicating the importance of regional studies. Some studies show that the direction of the cross bears significance for the likelihood of hybrid formation, probably due to species differences in biological barriers to hybridisation (Rieger et al. 2001). Although in one case this directional difference was thought to be due to the proportion of male sterile plants in the canola population reducing pollen competition (Chevre et al. 2000). Significantly there have also been reports of variability in the wild radish populations (Thalmann et al. 2001) that can affect the strength of barriers to hybrid formation (Gueritaine and Darmency 2001).

A recent study on hybrid formation between canola and another problem weed, charlock, found very low rates of hybrid formation from hand pollination experiments when canola was used as the female parent (from undetectable to 0.0049% of the total seed set). The reciprocal cross with charlock as the female parent gave only one hybrid out of 1127 hand pollinations (a rate of 0.0015% of the potential seed set). A field experiment failed to detect a hybrid
amongst 16,000 seed collected from charlock plants flowering amongst canola plants. These authors confirmed the results of other earlier experiments that the rate of hybrid formation is very low and the potential for gene flow from canola into charlock is minimal (Moyes et al. 2002).

**Table 12:** Some results of field study detection of hybrid formation between canola and wild radish.

<table>
<thead>
<tr>
<th>Canola parent</th>
<th>Wild radish parent</th>
<th>Details</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male parent</td>
<td>Female parent</td>
<td>No hybrids detected out of 754 plants screened (approximately 3,000 seeds per plant)</td>
<td>(Thalmann et al. 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 hybrids obtained in the field with reduced fertility</td>
<td>(Darmency et al. 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 hybrid detected out of 189,084 seeds screened</td>
<td>(Chevre et al. 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No hybrids out of 25,000 seeds screened</td>
<td>(Rieger et al. 2001)</td>
</tr>
<tr>
<td>Female parent</td>
<td>Male parent</td>
<td>Frequency of hybrids ranged from $2 \times 10^{-5}$ to $5 \times 10^{-4}$</td>
<td>(Chevre et al. 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 hybrids were detected amongst 52 million seeds</td>
<td>(Rieger et al. 2001)</td>
</tr>
</tbody>
</table>

A group in the UK have used satellite sensor images to identify regions where gene flow was most likely to occur, based on the distribution patterns of canola crops near waterways and cliff-tops (potential *Brassica* weed habitats). Once these regions were identified the sites were visited and screened for hybrids that allowed large areas to be covered. This particular study detected one *B. napus/B. rapa* hybrid in an area of over 16,000 square kilometres from 505 plants screened (Wilkinson et al. 2000). If distribution patterns of weeds are accurately known, isolation distances between canola crops and these weeds may be a viable way to reduce potential for gene flow (Wilkinson et al. 2000). However *Brassica* weeds are widespread in Australia and are a problem weed within canola crops suggesting that geographic isolation would be difficult to achieve.

**GENESYS model to assess gene flow**

A demographic and genetic model, GENESYS, which analyses the effects of crop succession and crop management at a regional level on the gene flow from GM canola to volunteer populations is being developed (Colbach et al. 2001a; Colbach et al. 2001b). This model inputs a combination of farm management variables such as crop succession, stubble breaking, soil tillage, sowing date and density, herbicide usage, cutting, harvesting, pollination and seed production rates to simulate gene flow. Although it is recognised as a starting point and not as a replacement for actual field studies, it does predict the importance of seed banks, border management and volunteer control for limiting gene flow in the farm environment. A biological modelling approach was also part of the proposed methodology for a current Australian study (Smith et al. 2000).

GENESYS was also used in a recent study commissioned by the European Commission to examine the co-existence of GM and non-GM cropping systems (Bock et al. 2002). Adopting this model to different farm types identified the following important factors influencing contamination between fields:

- Rotation of crops
- Degree of isolation of field
• Initial seed purity

• Crop variety – for example crops with male sterile varieties are more susceptible to cross-contamination from other fields

• Degree of clustering of fields in the landscape

• Co-operation between neighbours

These studies concluded that contamination levels below 0.3% would be difficult to achieve employing current practices. Lower levels could be achieved in most situations with changes to farming practices (Bock et al. 2002).

**Conclusion**

Canola is predominantly self-fertile but with some potential for outcrossing. Although most canola pollen is deposited close to the source plant, some long-distance pollen travel is likely to occur at low levels. This has implications for the co-existence of GM canola and non-GM canola or related crops. Studies indicate that low maximum thresholds of cross-contamination due to cross-pollination should be attainable with the introduction of appropriate crop management plans.

The use of different herbicide tolerant canola varieties, GM or conventionally bred, may have implications for control of canola volunteers if cross-pollination produces varieties tolerant to multiple herbicides. Farm management strategies should also be used to minimise the occurrence of stacked volunteers.

Canola has some related weeds in the Australian environment and there is some potential for gene flow into these weedy species, however the frequency of hybrid formation between canola and the most significant weeds is very low. The potential impact if gene flow does occur will depend on the transgene and the trait that it encodes.
Low outcrossing plant species

Case study – wheat (*Triticum aestivum*)

*Mating system*

Wheat is largely cleistogamic, meaning that the pollen is shed when the flower is still closed (Frankel and Galun 1977), and therefore primarily self-pollinating (Eastham and Sweet 2002). Pollen is produced in limited amounts and has a short viability period estimates vary from 15 mins up to five hours, depending on the environmental conditions (Treu and Emberlin 2000). Wheat is wind pollinated and the contribution of insects to cross-pollination is considered to be minimal.

*Gene flow studies*

Gene flow as measured by cross-pollination of male sterile bait plants was found in one study to be on average below 10% at 1 m and none detected at 20 m from the source plot (referenced in (Eastham and Sweet 2002). Due to the lack of competing pollen in the bait plants, outcrossing frequencies would presumably be lower in male fertile wheat populations.

*Seed certification*

The NSW certified seed specifications require no separation distance between wheat crops grown for the various seed standards, but simply a physical barrier such as a fence to prevent seed mixing during harvest.

*Weediness potential*

Wheat is included in the CSIRO Handbook of Australian Weeds (Lazarides et al. 1997) as naturalised in some agricultural environments but is not considered a problem weed in most states and recorded as a minor problem in a few natural environments in Tasmania (Groves et al. 2000).

*Hybridisation with wild or weedy relatives*

From a limited literature search no reports of wild or weedy relatives of wheat have been noted in Australia, in contrast to the US where jointed goatgrass, a relative of wheat, is a major agricultural weed.

*Conclusion*

Wheat is primarily self-pollinating, producing small amounts of pollen that has a short viability period. Gene flow is minimal at distances greater than 1 m from the source plant and this is reflected by the absence of any separation distances required between crops for certified seed production. Wheat is not a significant weed problem in Australia, and does not have any closely related problem weeds. For this limited study GM wheat would have a low potential impact of gene flow, but a detailed risk assessment would be needed before field trial or commercial release.

Case study – oats (*Avena sativa*)

*Mating system*

Oat crops are mainly self-fertilising (Hartmann and Kester 1975), but some outcrossing can occur (Bowring et al. 1978).
Seed certification
The NSW certified seed specifications for cultivated oats are the same as for wheat and require no separation distance between oat crops grown for the various seed standards, but simply a physical barrier such as a fence to prevent seed mixing during harvest.

Weediness potential of oats
Domesticated oats are included in the CSIRO Handbook of Australian Weeds (Lazarides et al. 1997) as an occasional weed, but only considered a minor problem in a few locations in most states (Groves et al. 2000).

Hybridisation with wild or weedy relatives
There are several related oat species that are problem weeds in Australia. Avena species were identified as the second-most significant weeds in a survey of winter crop production across Australia (Jones et al. 2000), and with an annual cost to the Australian wheat industry estimated at $80 million (Nugent et al. n.d.). Two species are closely related to domesticated oats, wild oats (Avena fatua) and sterile oats (Avena sterilis ssp. ludoviciana also known as A. ludoviciana) and may be readily crossed with oat crops to produce fertile hybrids. In fact an oat/wild oat hybrid is identified as a minor problem weed in a few locations in Queensland (Groves et al. 2000). A more distantly related third weed species bearded oat (A. barbata) is also found in Australia. These species are listed as problem agricultural weeds in most states of Australia (Groves et al. 2002).

An Australian study measured the gene flow between oats and wild oats in a field situation where a few oat plants were present in populations of wild oats, and also where wild oats were occasional weed contaminants of cultivated oats. The study assessed outcrossing by the number of hybrid seeds formed, which varied between 0 and 0.93% (Burdon et al. 1992). Although this level of outcrossing is low, there is significant potential for gene flow into wild oats because of their widespread occurrence.

Conclusion
Similar to wheat, oat crops are predominantly self-fertilised and require no separation distance between crops for certified seed production. However cultivated oats have weedy close relatives that are significant weeds in the Australian cropping system. Although the outcrossing measured in one study showed a very low level of gene flow between species, given that the plants were intermingled, circumstances could be envisaged where such gene flow from GM crops could have a high impact on the farm or natural environment. For example, if a gene were introduced into cultivated oats that encoded for herbicide tolerance, gene flow would potentially limit the effectiveness of this herbicide for controlling weedy oats within the crop.
Broad classification of likelihood of gene flow in Australia

These case studies exemplify some of the issues that impact on the significance of gene flow for an individual crop species. These crops were chosen as representing six broad categories of likelihood and potential impact of gene flow. Further categorisation of other crops is demonstrated in Figure 1 using information on the mating systems to estimate the likelihood of pollen flow, and the presence of weedy relatives from the same genera in Australia to estimate the likelihood that pollen from the crop will reach a suitable target. It should be stressed that these classifications are simplifications based on potential impact and do not necessarily represent actual risk of gene flow as the likelihood of hybrid formation, or the probability of the survival of those hybrids, is not taken into account. The potential for gene flow into related crop species is also not taken into account.

Crops can be placed in these broad categories based on reports in the scientific literature both in Australia and overseas and this broad categorization can highlight some of the major issues that will be important for particular crops. For this report, crops were scored firstly as high, medium or low outcrossing as shown in Table 4. Note that such groupings are only a first indication as outcrossing rates can vary significantly between varieties of the same crop or in different environments, especially in species with mixed mating systems. In addition the biology of the crop species needs to be understood so as to assess the variations that contribute to the likelihood and impact of gene flow.

Next, two Australian weeds databases were checked to see if the crop itself or any relatives of the same genus were listed. These databases cover naturalised, non-native flora of Australia categorised as weeds in natural (Groves et al. 2000) or agricultural environments (Groves et al. 2002). The significance of these weeds was then noted according to the categorisation in these databases, that is whether the species were identified as minor or major problems in any of the states of Australia (Table 13). All crops were then grouped into categories as shown in Figure 1. Note that this information does not take into account any native flora that may be related to these crops, only weedy relatives. For example, in addition to the *Gossypium* species that are related to cotton, Australia also has native species that are in the same genera as soybean, rice, sorghum, mung bean, tobacco (Lawrence 1995) and others (shown in Table 3).

Table 13: Presence and significance of weedy relatives of some crops in Australia

<table>
<thead>
<tr>
<th>Crop</th>
<th>Categorisation of crop or weedy relatives as weeds in natural ecosystems (Groves et al. 2000)</th>
<th>Categorisation of crop or weedy relatives as weeds in agricultural ecosystems (Groves et al. 2002)</th>
<th>Significance of weeds (see Figure 1 below)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>Minor problem in two states</td>
<td>Not rated as agricultural weeds</td>
<td>Low. Box I</td>
</tr>
<tr>
<td>Maize</td>
<td>Naturalised in one state but not considered a problem</td>
<td>Not rated as agricultural weeds</td>
<td>Low. Box I</td>
</tr>
</tbody>
</table>

\[16\] Note that this categorisation makes no attempt to comment on the likelihood of hybrid formation, or introgression or the presence of wild relatives that are not weedy.\\[17\] Either not a problem or not known to occur in agricultural areas.
<table>
<thead>
<tr>
<th>Crop</th>
<th>Categorisation of crop or weedy relatives as weeds in natural ecosystems (Groves et al. 2000)</th>
<th>Categorisation of crop or weedy relatives as weeds in agricultural ecosystems (Groves et al. 2002)</th>
<th>Significance of weeds (see Figure 1 below)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papaya</td>
<td>Naturalised in one state but not considered a problem</td>
<td>Not rated as agricultural weeds</td>
<td>Low. Box I</td>
</tr>
<tr>
<td>Pineapple</td>
<td>Naturalised in one state but not considered a problem</td>
<td>Not rated as agricultural weeds</td>
<td>Low. Box I</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>Naturalised in four states, a major problem in a few locations in one state</td>
<td>Not rated as agricultural weeds</td>
<td>Medium. Box F</td>
</tr>
<tr>
<td>Sugar cane</td>
<td>Minor problem in one state</td>
<td>Not rated as agricultural weeds</td>
<td>Low. Box I</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Minor problem in most states</td>
<td>Minor problem requiring control in some states.</td>
<td>Medium. Box F</td>
</tr>
<tr>
<td>White clover</td>
<td>Minor problems in most states, major problem in some.</td>
<td>Minor problem requiring control in some states and a major problem in others.</td>
<td>High. Box C</td>
</tr>
<tr>
<td>Canola</td>
<td>Some relatives problem weeds in most states, one listed as a noxious weed in one state</td>
<td>Major problems in some states</td>
<td>High. Box B</td>
</tr>
<tr>
<td>Cotton</td>
<td>Minor problem in some states</td>
<td>Not rated as agricultural weeds</td>
<td>Low. Box H</td>
</tr>
<tr>
<td>Indian Mustard</td>
<td>Some relatives problem weeds in most states, one listed as a noxious weed in one state</td>
<td>Major problems in some states</td>
<td>High. Box B</td>
</tr>
<tr>
<td>Oilseed poppy</td>
<td>Minor problem in most states, a major problem in some areas</td>
<td>Minor problem in some states, control not considered necessary. One relative major problem in one state.</td>
<td>High. Box B</td>
</tr>
<tr>
<td>Barley</td>
<td>Some relatives major problem in some states</td>
<td>Major problems in some states</td>
<td>High. Box A</td>
</tr>
<tr>
<td>Field pea</td>
<td>Naturalised in one state but not considered a problem</td>
<td>Not rated as agricultural weeds</td>
<td>Low. Box G</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Minor problem in most states</td>
<td>Minor and major problems in some states</td>
<td>Medium. Box D</td>
</tr>
<tr>
<td>Lupins</td>
<td>Major problems in some states</td>
<td>Minor problems in some states, major problem in one state.</td>
<td>High. Box A</td>
</tr>
<tr>
<td>Oats</td>
<td>Major problems in some states</td>
<td>Major problems in most states</td>
<td>High. Box A</td>
</tr>
<tr>
<td>Potato</td>
<td>Major problems in some states</td>
<td>Major problems in some states</td>
<td>High. Box A</td>
</tr>
<tr>
<td>Crop</td>
<td>Categorisation of crop or weedy relatives as weeds in natural ecosystems (Groves et al. 2000)</td>
<td>Categorisation of crop or weedy relatives as weeds in agricultural ecosystems (Groves et al. 2002)</td>
<td>Significance of weeds (see Figure 1 below)</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Soybean</td>
<td>Naturalised in one state not considered a problem</td>
<td>Not rated as agricultural weeds</td>
<td>Low. Box G</td>
</tr>
<tr>
<td>Subterranean clover</td>
<td>Major problem in some states</td>
<td>Minor problem requiring control in some states and a major problem in others.</td>
<td>High. Box A</td>
</tr>
<tr>
<td>Wheat</td>
<td>Minor problem in one state</td>
<td>Minor problem in one state requiring control in following crops.</td>
<td>Low. Box G</td>
</tr>
</tbody>
</table>

**How is impact of a weedy species measured?**

Determination of the potential impact of weeds on the agricultural or natural environment is not a trivial matter. Success of an invader depends on complex interactions between chance, genotype and environment. As mentioned previously scientists cannot always successfully predict which species are likely to develop into significant weeds (Lonsdale 1994; Marvier 2001). Naturalised species that are currently considered only minor problems may in the future develop into major weed problems. In addition there can be a significant time lag between introduction of a new species and an ecological invasion (Kowarik 1995).

Even with the most obvious historical examples of biological invasions, there is still disagreement about the magnitude of impact. Scientists have recently suggested a framework for clarifying the different components of overall impact that may be useful in this context. According to this concept, the overall impact can be defined as the product of the range size of a species, its average abundance per unit area across that range, and the effect per individual of the invader (Parker et al. 1999). Although beyond the scope of this report, it could be useful to use such a framework to assist in the categorisation of risk potential of gene flow from crops into related weeds in the Australian context. Extent of range of the crop species as well as the related weed species should be incorporated into the risk assessment process. For example even though barley and potato are both categorised in Box A, the different areas under cultivation of these two crops influences the potential impact of gene flow. In Australia, in 2000, 2.6 million hectares of barley were grown, whereas for the same period only 36,829 hectares were under cultivation with potato (Australian Bureau of Statistics 2001). Areas under cultivation of other crops in Australia in 2000 are shown in the Appendix, Table A2.
Figure 1: Broad categorization of crop species for further risk assessment based on weediness potential of relatives from the same genera in Australia (note that the likelihood of hybrid formation is not taken into account).

Likelihood of hybrid formation is not taken into account in this classification

It should be emphasized that these data do not take into account the likelihood of hybrid formation and stable gene expression in the new population. For some crops, the likelihood of cross-pollinations with related species resulting in fertile hybrids, and subsequent frequency of transgene introgression is very low. Extensive literature searches and consultation with scientific experts would be needed for a thorough risk assessment, and this was considered beyond the scope of this report.

A paper discussed earlier (Sindel 1997) also grouped crop plants in categories of gene flow risk in the Australian environment based on the presence of weedy relatives (shown in Table 9). The categorisation method used in the current report differs slightly in that the significance of weedy relatives is taken into account. For this reason some crops are grouped differently. These differences emphasize the need for detailed, case-by-case analyses to adequately determine the likelihood and potential impact of gene flow from GM crops grown in the Australian environment.

This data should not be taken out of context, and simply gives initial clues as to which crops may need closer scrutiny. Before the likelihood and potential impact of gene flow can be adequately assessed, detailed studies need to be done on a crop-by-crop basis. A good understanding of the biology and cropping system of the plant in question is essential. The transgene itself and the phenotype of the modified plant may also impact on the likelihood and the potential impact of gene flow.
Some issues to be addressed to assess likelihood and potential impact of gene flow

As already stated, full risk assessments are beyond the scope of this report, however it is clear that there is a standard set of issues or questions that need to be answered on a crop by crop basis to assess the likelihood and potential impact of gene flow and its implications. The following issues could be used to form the basis of such an assessment.

**Issues to be addressed about the GM crop**

1. Mating system
2. Pollen source
3. Pollen dispersal
4. Pollen viability
5. Range size

**Issues to be addressed about the recipient crop, wild or weedy plant**

1. Proximity of GM to receptor plant
2. Nature of crop – leaf, fruit or seed crop?
3. Synchronisation of flowering
4. Amount of competing pollen
5. Significance of volunteers
   - Likelihood of volunteers
   - Persistence of volunteers
6. Potential for seed dormancy
7. Significance of weed

**Potential for hybrid formation**

1. Premating barriers
   - **Sympatry** (spatial proximity)
   - Pollen vector compatibility
2. Postmating barriers
   - Pollen-pistil compatibility
   - Seed compatibility
   - Hybrid viability
   - Hybrid fertility
   - Next generation viability (backcross or F2)
Issues to be addressed about the transgene (or trait encoded by the transgene)

(1) Spread and persistence of gene in wild or weedy population. Selective advantage conferred by the transgene?

(2) Dominance of transgene

(3) Nature of trait encoded by the transgene - potential for economic or environmental harm

For some crops and crop/trait combinations methods will be needed to limit or prevent gene flow. These methods are discussed in the following sections.
Agricultural measures to limit gene flow

Previous sections of this report have discussed the factors affecting the likelihood of gene flow between related plants. The potential for gene flow depends on the basic biology of the crops and related species and interactions of both with the surrounding environment, such as wind and insect pollinators. The extent of crop-to-crop gene flow can also be greatly influenced by agricultural practices. The following sections detail agricultural methods reported to limit gene flow, most of which have been used previously for the production of high purity certified seed.

Temporal isolation

Maintaining crops at different stages of development and ensuring they are not flowering at the same time can limit cross-pollination between crops. This process is sometimes practised on perennial grasses to maintain varietal purity of seed (Richards 2001). In India, seed crops of maize have been grown in the off-season when no grain crops are in the vicinity to ensure genetic isolation (Thompson 1979).

The utility of this strategy for limiting gene flow in the context of GM crops will vary between crops and depend on factors such as the length of the growing season and the length and synchronicity of the flowering period.

Barrier crops

One method to limit gene flow is to physically limit the spread of pollen by growing tall plant species between crops. Seed production plots in Europe are sometimes surrounded by hemp or sunflower species that due to their height and sticky leaf surface serve to reduce pollen flow (Richards 2001).

The effectiveness of these strategies has been discussed in the early seed production literature (reviewed in Levin and Kerster 1974). One study examined the effect of different width rows of maize to limit outcrossing between cotton crops. The maize barrier, which was approximately three metres tall when the cotton flowered, did reduce cross-pollination significantly, and wider rows gave further reductions. In contrast another study showed that a sorghum barrier was no more effective than open space for preventing cross-pollination between cotton varieties. This difference was probably due to the fact that the maize barrier was much taller (Levin and Kerster 1974).

A study (cited in Levin and Kerster 1974) looking at the effect of hemp and sorghum barriers in isolating pollen from sugar beet, a high outcrossing species, found that a 12 m wide plant screen was effective in reducing cross-pollination, equivalent to 200 m of open space (down to 0.7%). A more recent study assessing a hemp stripe containment strategy for preventing wind-pollinated gene flow from GM sugar beet plants detected significant cross-pollination more than 200 m behind the hemp containment. Male sterile bait plants were used in this experiment that would therefore overestimate the gene flow but even so it shows that such approaches would only serve to limit gene flow rather than eliminate it (Saeglitz et al. 2000).

Studies of wind velocities around vegetation barriers show that sometimes changing wind patterns may actually reduce the zone of protection from cross-pollination because of rapid down mixing of air. The extent to which this occurs depends on the weight of the pollen grains and the permeability of the barrier crop. Often studies show that cross-pollination
directly beyond the barrier are reduced, but at greater distances from the barrier no effect is seen (Levin and Kerster 1974).

Instead of providing a physical barrier, some crops can be planted around GM plants that limit pollen flow by several other mechanisms. Barrier crops of different species can be planted which function to reduce cross-pollination by increasing the distance that GM pollen has to travel to cross with other plants, and attracting pollinators so they are less likely to travel to other crops (Ingram 2000a; Williams 2001). One study on pollen flow between sweet clover plots showed that plantings of canola acted as a pollen vector competitor and reduced gene flow from 6% to only 0.2%. Another extension of this could be the planting of an attractive related crop which flowers at the same time and has the ability to attract pollinators thus minimising gene movement to other crops where gene flow would be of concern (Rieger et al. 1999).

Barrier crops of the same species have been shown to be much more effective barriers as they combine the effect of increasing distance with the act of attracting pollinators and providing additional local pollen which competes with escaping pollen. The effectiveness of such a ‘trap’ crop would rely on the foraging behaviour of the insect. For example if a bee foraged on the GM crop, to prevent any pollen escape it would need to visit the trap crop and deposit all of this GM pollen there before returning to the hive (Williams 2001).

A study on the efficacy of border rows on gene flow in cucumber fields found that the ratio of border plants to pollen source plants is very important. In small plots border rows may be useful to reduce pollen transfer, however in large-scale plantings the increased numbers of source plants compared to border rows increased the frequency of long-distance pollen movement and reduced the efficacy of the border row (Hokanson et al. 1997).

Another option is to place a barrier crop around the crop that one wishes to protect from cross-pollination. This would also serve to increase the distance that the GM pollen has to travel, again introduce competing pollen, and also serve as a physical barrier to decrease wind and insect pollination. Insects may also be more likely to forage to barrier plants than other crops (Ingram 2000a). This approach is obviously only designed to limit crop to crop gene flow (rather than crop to weed or wild relative).

Interestingly, there may be limitations in the use of the same or related species barrier crops. One issue to be addressed would be contamination of crops by pollen from the barrier crop. For example, the use of barrier crops was undesirable when GM cotton crops were grown to increase seed stocks for commercial release because of the potential for contaminating seed from the buffers. For these plots other methods such as isolation zones were required (Danny Llewellyn, personal communication).

Other issues specific to the trait encoded by the transgene may limit the application of non-GM barrier crops for limiting gene flow. For example, planting herbicide sensitive barrier crops around herbicide tolerant GM crops would not be useful as the trap plants would be affected by the herbicide.

**Isolation zones**

Isolation zones are commonly used to reduce gene flow between crop populations for both wind-pollinated and insect-pollinated crops (Bateman 1947). This is the basis of the seed certification regulations and successful production of certified seeds. Very few studies, however, have effectively compared the efficacy of barrier crops versus isolation zones for reducing gene flow among populations. One study with canola found that buffer zones were more effective at reducing gene flow than small isolation zones (less than 8 m) (See canola...
case study for more details). Computer modelling and some trials suggest that large isolation
gaps or separation distances may actually encourage gene flow by affecting insect foraging
behaviour (Rieger et al. 1999). The conclusion from another canola study was that
outcrossing rates at 30 m into a border crop area were comparable to outcrossing rates for a
200 m isolation zone, in other words that border crops were far more effective (Staniland et
al. 2000). However this conclusion came from comparing different experiments performed by
different researchers in different environments so this result could simply be attributed to
natural variation in outcrossing rates.

Whilst the efficacy of isolation zones for reducing gene flow needs to be established on a crop
by crop basis and even to some extent a regional basis, experience with separation distances
required for certified seed production can be used as initial guidance. Isolation distances serve
to reduce cross-pollination between crops to low levels that are suitable for seed production
but it is important to realise that they will not completely eliminate gene flow as these
distances are considerably shorter than the maximum flight ranges of insect vectors such as
bees. One researcher has suggested that to eliminate any gene flow via insect pollination the
isolation distances would need to exceed twice the maximum foraging distances of the
particular insect vector (Williams 2001).

Field size

Seed field size is often considered an important factor for high quality seed production in
some crops. A review of pollen contamination in hybrid seed production of maize in the US
found that fields with less than 100 acres had consistently higher pollen contamination than
larger fields. This study also found a relationship between field size and isolation distance.
Smaller fields (less than 100 acres) with less than 125 m isolation distances had more pollen
cross-contamination than those with greater isolation distances, however larger fields showed
little advantage in increasing the isolation distances (Burris n.d.).

Crop rotation

Growing seed crops for certification not only requires separation between crops but also field
history is important. For example seed certification regulations for the growth of canola and
mustard basic seed in NSW require that the land not have grown either species for the
previous five years. Similar standards could be used to prevent presence of GM plants in non-
GM crops, although there would be costs in terms of restrictions on a farmer’s cropping
options.

Conclusion

Agricultural methods could potentially be used to reduce gene flow by cross-pollination.
Suitability of each method needs to be established for each GM crop, and in some cases
requirements will vary between regions due to different climatic factors and pollinator
populations. Although agricultural measures have been shown to be effective for reducing
gene flow in the past, for example in the production of pure seed, significantly these methods
will not completely eliminate gene flow.
Using new technologies to limit gene flow

The preceding section discussed ways that gene flow can be limited by minimizing pollen spread once plants get to the farmer’s paddock. Choices can obviously be made before plants get to this stage by, for example, avoiding transgene incorporation into open pollinated, competitive and prolific species, or limiting growth of GM crops in areas with lots of wild relatives or land races (see section entitled Will gene flow from GM crops result in unacceptable levels of ‘genetic pollution’?). A UK report suggests options to minimise the likelihood and potential for impact of gene flow by: choosing recipient plants wisely, using flowering time differences between varieties to genetically isolate GM plants, and using varieties that are unattractive to pollinators (Advisory Committee on Releases to the Environment 2000). All these factors should be considered at an early stage in GM crop development.

Limiting the dispersal of transgenes in the farm and natural environment has been largely the focus of this report. Farming practices can be used to minimize outcrossing. However for some crop/introduced gene combinations, for example where the likelihood of outcrossing is great and the potential consequences of gene escape are negative, or where high genetic integrity of related species are required, other mechanisms may be needed to reduce gene flow. Biotechnologists are also developing mechanisms to either reduce gene flow or to limit its impact. In early transformation experiments unnecessary sequences were included but more modern transformation vectors are being streamlined to minimize unnecessary genetic material. This has the advantages of reducing the chances of adverse effects, as well as simplifying analysis and environmental impact assessments (Advisory Committee on Releases to the Environment 2000).

Many approaches to minimize gene expression (the extent to which a gene is switched on) are also being examined. Most transgenes in crops currently being trialed have the genes permanently switched on – but systems can also be developed to have genes switched on by chemical or environmental cues or to be turned on only in the tissue where they are required. For example, one of the currently debated topics is the potential effect of pollen from GM insecticide-producing plants on non-target insects. If a GM crop were engineered to only express the insecticide in the leaf tissue of the plant, only insects that ingested the leaves would be affected (presumably the pest species). However, it is important to realise that in this scenario the transgene itself would still be present in all of the tissues of the plant, including the pollen. Such approaches would therefore not limit the flow of the gene, but rather limit the potential impact of the gene flow.

Scientists are currently developing biotechnological methods that may be useful for reducing cross-pollination from GM plants. These include apomixis (production of seeds without meiosis), cleistogamy (unopened flowers), hybridisation barriers, blocking floral development and male sterility, non-germinating seed (including terminator technology) and plastid (or chloroplast) transformation. Some of these approaches will be discussed in more detail below.

Technologies that reduce pollen transfer

Apomixis, or the production of seeds asexually, has been suggested as one way to reduce gene flow from genetically modified crops via cross-pollination. This technology has much wider applications to agriculture and is the focus of many worldwide research programs because of its potential benefits for hybrid seed production (Grimanelli et al. 2001). Apomixis occurs naturally in many plant species, but very few of agricultural importance. Scientists are trying many approaches to introduce apomixis into agricultural crops, ranging from crossing...
crops with wild apomictic relatives, attempting to isolate the genes controlling apomixis, and by artificially engineering the components of the process through genetic modification of sexually reproducing plants (Spillane et al. 2001). Although work is very advanced in some of these areas, most scientists agree that a workable apomictic system is many years away from commercial release. In addition some apomicts still produce viable pollen so in these cases the application for the reduction of gene flow would be limited.

Complete cleistogamy refers to flowers that remain closed during pollination (Takahashi et al. 2001) and could be one way to reduce gene flow from genetically modified plants (Advisory Committee on Releases to the Environment 2000). Cleistogamy occurs to different degrees in many species of flowering plants, including agricultural crops such as wheat and soybean. Scientists are currently working on identifying and isolating genes controlling this process and even when they do, engineering of this process in a naturally cross-pollinating plant would require many years of development.

The ability to block functional pollen production (male sterility) has applications for the production of hybrid seed and scientists have been working in this area for many years. Some genetically engineered male sterile crops are commercially released worldwide, for example the US has male sterile maize, chicory and canola (Animal and Plant Health Inspection Service 2001). Genetically modified male sterile canola crops have also been grown in Australian field trials (Office of the Gene Technology Regulator). Male sterility has often been suggested as a useful mechanism for prevention of gene flow from GM crops. For some plant species this is undoubtedly true, male sterility would be useful for limiting gene flow in tree species such as eucalypts and may have added advantages of enhancing biomass production (James et al. 1998). However male sterility may have limited use for other crops especially some seed crops because effective pollination is needed to trigger the seed or fruit production. Depending on the male sterility system used, it is also often difficult to guarantee complete prevention of pollen production.

Technologies that reduce transgene flow

Plastids are small structures contained within plant cells that develop into chloroplasts – the light-harnessing energy batteries of the plant. Chloroplasts contain DNA and foreign genes can be inserted into this small genome with much more precision than into the rest of the plant DNA. Chloroplasts are usually inherited only from the mother plant and therefore minimise gene flow through pollination. Chloroplast transformation has other advantages (reviewed in Bock 2001; Daniell, Khan et al. 2002) but it is important to realise that it is not the magic solution for totally eliminating gene flow as inheritance through the pollen can also occur in some plant species (Cummins 1998; Stewart and Prakash 1998). Plastid transformation is being used by many researchers worldwide to introduce genes into tobacco plants, but so far this technology has been limited to tobacco and tomato because of obstacles in the regeneration of most crop plants from tissue culture (Bock 2001; Daniell 2002). Some progress has been made towards plastid transformation in rice and potato, but several obstacles need to be overcome before this technology is routine for crop plants. Some scientists have suggested that plastid transformation may also have increased potential for horizontal gene transfer, although this has not yet been demonstrated (Nielsen, Elsas et al. 2001).

Crops can have multiple genomes derived from different wild sources. One way to reduce the likelihood of gene flow might be to release only GM lines where the transgene is inserted in a genome incompatible with the weedy relatives. Introgression of the transgene may still occur but the likelihood will be greatly reduced (Gressel 1999). One idea for limiting ‘escape’ of the transgene in canola (AACC) into B. rapa (AA) is to only release plants where the transgene is located in the C genome (which is not common to both species) and therefore reduce the
likelihood of stable introgression (Wilkinson, Davenport et al. 2000). Another example is D genome of wheat which is compatible with the D genome of the related weed *Aegilops cylindrica* (bearded goat grass) allowing transgene spread, although it must be noted that wheat has a limited potential for outcrossing even with wheat plants growing in close proximity (Seefeldt, Zemetra et al. 1998).

Another very young technology that could potentially limit gene flow involves manipulations of the pollen coat to develop an effective ‘lock and key’ strategy to prevent pollen germination on non-target stigmas (Robert and Gleddie 1999). The idea is to develop a hybridisation barrier that would function to limit gene flow.

**Technologies to reduce hybrid survival**

‘Terminator technology’ is a widely used phrase that refers to a technology protection system developed in the US describing a method of modifying plants so that the seed produced by the parent plant is sterile. This and other similar technologies have been grouped under the collective term **genetic use restriction technologies** (GURTs) and are the focus of much international debate because of their implications for farmer saved seed (Food and Agriculture Organisation of the United Nations 2001). These issues are beyond the scope of this report save to mention that GURT could be useful for reducing the impact of gene flow on the environment as plants that inherit the transgenes would not produce viable seed.

Researchers in Finland have suggested another molecular technique called recoverable block of function (RBF) to limit gene flow, which is similar in concept to terminator technology (Kuvshinov et al. 2001). In this approach, the GM plant contains a blocking construct that prevents plant reproduction alongside another gene construct that bypasses this block under certain conditions not usually present during the normal life cycle of the plant. To demonstrate proof of concept these researchers produced GM tobacco plants which produced viable seed only when treated with two 40°C heat shock treatments during seed set and seed germination.

It is important to realise that genes are not inherited in isolation. If transgenes flow from GM crops into other plants, genes located nearby on the genome may affect the fitness of hybrid plants. A strategy known as transgenic mitigation suggests taking advantage of this fact by using tandem constructs of the desired trait with another gene. The accompanying gene will encode a trait that is neutral or positive for crops but deleterious for typical weeds. The idea is that because of the strong competition that exists among weed populations, harmful traits will not persist in the wild, therefore the transgene will likely also not persist. Suggestions for the tandem traits include genes affecting seed dormancy, seed ripening and shattering and dwarfing (Gressel 1999).

Two researchers have recently suggested another more complicated approach of combining an inducible system with excision technology to create non-GM crops from GM ones (Keenan and Stemmer 2002). Such a system could be used to excise transgenes selectively from pollen, thereby reducing the potential for transgene flow.

**Early stage of development**

It is important to emphasize at this point that most of these applications are in the very early stage of development (see Table 14). Some reports on gene flow suggest that these methods are just around the corner, magic solutions to the gene flow issues, but most are years off even the field trial stage of testing. Even if some methods have the potential to limit gene flow, they may raise other issues that limit their acceptance for agricultural systems (for example, the issues with the terminator technology and farmer saved seeds.) If the likelihood and
The potential impact of gene flow from some GM crops is seen as too high for current release, methods suitable for use in agriculture from Table 14 may need to be identified as priorities for future research direction.

**Table 14: Stage of development of technologies to reduce gene flow**

<table>
<thead>
<tr>
<th>Technology</th>
<th>Stage of development</th>
<th>Other comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apomixis</td>
<td>Early stages of research</td>
<td>Some apomicts produce pollen</td>
</tr>
<tr>
<td>Cleistogamy</td>
<td>Early stages of research</td>
<td>May be useful for some species that already show degrees of cleistogamy</td>
</tr>
<tr>
<td>Hybridisation barriers</td>
<td>Early stages of research</td>
<td>Very species specific</td>
</tr>
<tr>
<td>GURT technologies</td>
<td>Some technologies ready for field trial applications</td>
<td>Ongoing discussions of impact on agricultural practices (farmer saved seed)</td>
</tr>
<tr>
<td>Male sterility</td>
<td>Commercially released worldwide in maize, canola and chicory. Needs development for other crops.</td>
<td>Of limited use for some crops where high fertility is needed to produce high yield</td>
</tr>
<tr>
<td>Plastid transformation</td>
<td>Early stage of research for all but a couple of important agricultural crops</td>
<td>Some plastid gene transfer through the pollen in some plants</td>
</tr>
</tbody>
</table>
Conclusions

Gene flow is a natural phenomenon and occurs to a greater or lesser degrees in all cultivated plants. Gene flow between species is less common but still an important mechanism of plant evolution. This study has just looked at gene flow through cross-pollination that is just one mechanism of gene transfer. Gene flow among populations through seed movement and subsequent cross-pollinations will be more significant for some crops.

Gene flow rates reported by different studies on the same crops differ widely due to the complex array of variables influencing outcrossing rates. Small-scale field trials cannot always accurately predict gene flow rates in larger scale releases. For these reasons it is important to ensure experiments to assess gene flow are well-designed and conducted in different locations, mimicking as far as possible farming conditions.

Gene flow from GM crops to neighbouring crops may impact on the certification of non-GM or ‘GM free’ produce. This will depend on maximum thresholds allowable and the sensitivities of the testing and sampling methods used. Decisions on agricultural measures needed to limit gene flow will depend on the establishment and agreement of these thresholds. Once agreement is reached on threshold levels, guidelines for limitation of crop-to-crop gene flow can be based on current seed certification standards that specify separation distances and previous field use to maximise varietal purity. However existing guidelines may need to be reviewed in crops where visual identification of off-types has been traditionally used to establish varietal purity as this technique may underestimate the levels of gene flow. It is also important to realise that pollen contamination of neighbouring crops cannot be completely eliminated even by using seed certification procedures to limit gene flow.

The next generation of GM crops, plants with industrial or pharmaceutical applications will need to be strictly segregated from related food crops in order to eliminate risk of gene flow. The application of new technologies to reduce gene flow in these situations need to be evaluated to give guidance for future research priorities.

For crops that have native relatives in Australia, the potential for gene flow into these relatives needs to be assessed in more detail. Consequences of gene flow from GM crops into closely related native species will depend on the transgene and the environment to which it flows. Risk from this occurring needs to be viewed against the background risk of gene flow from conventional crops. Many scientists hold the view that gene flow from GM crops does not represent a new category of risk, rather what is significant is the trait encoded by the transgene.

Gene flow from GM crops to weeds could potentially result in new weed problems, but the likelihood of this happening depends on many factors including the distribution of close, significant relatives. The significance of transgene flow into weedy populations needs to be assessed on a case-by-case basis taking into account many factors, including the nature of the transgene and its interaction with its new environment. Genes that confer selective advantages in agricultural and natural environments will need to be carefully considered for the impact of gene flow. Accurate information on distribution patterns of weeds in Australia would assist in thorough assessments, as well as information on the likelihood of hybrid formation.
Acknowledgements

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Glossary

Definitions adapted from several different sources (Usher 1996; King and Stansfield 1997; Allaby 1998; Zaid et al. 1999)

**actual gene flow** Incidence of fertilisation and establishment of reproductive individuals

**allele** One of a pair, or series, of variant forms of a gene that occur at a given locus in a chromosome. In a normal diploid cell there are two alleles of any one gene (one from each parent), which occupy the same relative position (locus) on homologous chromosomes. Within a population there may be more than two alleles of a gene.

**allogamy** Cross-fertilisation, opposite of autogamy.

**apomixis** The asexual production of diploid offspring without the fusion of gametes. The embryo develops by mitotic division of the maternal or paternal gamete, or, in the case of plants, by mitotic division of a diploid cell of the ovule.

**autogamy** Self-fertilization, pollination of a flower by its own pollen, opposite of allogamy.

**barrier crop** A crop planted around a crop that serves to limit pollen flow into surrounding crops. Can be the same or different species. Also sometimes called a pollen trap or buffer crop.

**biodiversity** The variety of species (species diversity) or other taxa of animals, micro-organisms and plants in a natural community or habitat, or of communities in a particular environment (ecological diversity), or of genetic variation in a species (genetic diversity). The maintenance of a high level of biodiversity is important for the stability of ecosystems.

**chloroplast** Specialized cytoplasmic organelle that contains chlorophyll. They are the site of solar energy transfer and important reactions of starch or sugar synthesis. Chloroplasts have their own DNA and are inherited cytoplasmically, independent of nuclear genes.

**cleistogamy** Having flowers that do not open, and therefore, the pollination and fertilization of an unopened flower.

**cross-pollination** Fertilization of a plant from a plant with a different genetic makeup.

**cytology** The study of the structure and function of cells.

**cytoplasm** The living material of the cell, exclusive of the nucleus, consisting of a complex protein matrix or gel. The part of the cell in that essential membranes and cellular organelles (mitochondria, plastids, etc.) reside.

**dichogamy** Male and female organs of a flower mature at different times.

**dioecious** Male and female flowers on separate individual plants.

**dominant** Describing an allele whose effect with respect to a particular trait is the same in heterozygotes as in homozygotes. The opposite is *recessive*.

**electrophoresis** A technique that separates charged molecules - such as DNA, RNA or protein - on the basis of relative migration in an appropriate matrix (such as agarose gel or polyacrylamide gel) subjected to an electric field.
embryo An immature organism in the early stages of development. In plants, it is the structure that develops as result of the fertilization of an egg cell, or without fertilization.

endosperm Nutritive tissue that develops in the embryo sac of most angiosperms. It usually forms after the fertilization of the two fused primary endosperm nuclei of the embryo sac with one of the two male gamete nuclei.

erkyme A protein produced in living cells, which, even in very low concentration, catalyses specific chemical reactions but is not used up in the reaction.

fertilization Fusion of sperm nuclei from pollen with the egg cell to produce a zygote.

fitness The number of offspring left by an individual, often compared with the average of the population or with some other standard, such as the number of offspring left by a particular genotype. Depends both on survivorship and fecundity.

gene Conceptually, the unit of heredity transmitted from generation to generation during sexual or asexual reproduction. More generally, the term is used in relation to the transmission and inheritance of particular identifiable traits. Since the molecular revolution, it is now known that a gene is a segment of nucleic acid that encodes peptide or RNA.

gene flow The spread of genes from one breeding population to another (usually) related population.

genome 1. The entire complement of genetic material (genes + non-coding sequences) present in each cell of an organism, or in a virus or organelle. 2. A complete set of chromosomes (hence of genes) inherited as a (haploid) unit from one parent.

genus (pl: genera) A somewhat arbitrary group of closely related species, where perceived relationship is typically based on physical resemblance.

herbicide Any substance that is toxic to plants; usually applied to agrochemicals intended to kill specific unwanted plants, such as weeds.

hermaphrodite A plant whose flowers contain both stamen and carpels.

heterozygote (adj: heterozygous) An individual that has different alleles at the same locus in its two homologous chromosomes.

homogamy Where anthers and stigmas are mature at the same time.

homologous chromosomes Chromosomes that occur in pairs and are generally similar in size and shape: one comes from the male parent and the other from the female. Such chromosomes contain the same linear array of genes.
**homozygote**: (adj: homozygous) An individual that has two copies of the same allele at a particular locus in its two homologous chromosomes.

**horizontal gene flow** Movement of genetic material between individuals independent of normal heredity mechanisms.

**hybrid** The offspring of two parents that are genetically different. A cross between two genetically unlike individuals.

**hybridisation** Interbreeding of species, races, varieties and so on, among plants; a process of forming a hybrid by cross pollination of plants.

**introgression** The introduction of new gene(s) into a population by crossing between two populations, followed by repeated backcrossing to that population while retaining the new gene(s).

**isolation distance** Distance maintained between fields of crop plants to help minimize cross-fertilisation, normally used for certified seed production.

**isozyme** A variant of a particular enzyme. In general, all the isozymes of a particular enzyme have the same function and sometimes the same activity, but they differ in amino-acid sequence. With the help of isozyme analysis, based on electrophoresis techniques that can separate the different variants, related species or cultivars can be distinguished.

**landraces** Traditional crop varieties developed and maintained agriculturally.

**leptokurtic** Applied to a distribution where a few points occur far from the origin, but most are very close to it.

**locus** (pl: loci) A site on a chromosome.

**male sterility** The condition in which functional pollen is not produced.

**meiosis** The special cell division process by which the chromosome number of a reproductive cell becomes reduced to half (n) the diploid (2n) or somatic number. Meiosis results in the formation of spores in plants. It is an important source of variability through exchange of genetic material.

**monoeocious** Denoting plant species that have separate male and female flowers on the same plant (e.g., maize).

**mutation** A sudden, heritable change appearing in an individual as the result of a change in the structure of a gene (= gene mutation); changes in the structure of chromosomes (= chromosome mutation); or in the number of chromosomes (= genome mutation).

**naturalised plant** A cultivated plant capable of maintaining a population outside of cultivation.

**off-type** A plant of the same species as the rest of the crop but showing characters that do not conform to the variety description.

**pathogen** An organism that causes a disease in another organism.

**phenotype** The visible appearance or set of traits of an organism resulting from the combined action of genotype and environment.
plastid A cytoplasmic body found in the cells of plants and some protozoa. Chloroplastids, for example, produce chlorophyll that is involved in photosynthesis.

pollen germination Process whereby a pollen tube emerges from a pollen grain and carries the sperm nuclei through the female tissue, prior to fertilization of the ovule.

polymerase chain reaction (PCR) A procedure that amplifies a particular DNA sequence. It can be used to detect the existence of a defined sequence in a DNA sample.

potential gene flow Refers to the deposition of pollen and seeds from a source as a function of distance (in contrast to actual gene flow).

promoter A nucleotide sequence of DNA to which RNA polymerase binds and initiates transcription. It usually lies upstream of (5´ to) a coding sequence. A promoter sequence aligns the RNA polymerase so that transcription will initiate at a specific site.

recessive Describing an allele whose effect with respect to a particular trait is not evident in heterozygotes. Opposite to dominant.

selection pressure The intensity of selection acting on a population of organisms or cells in culture. Its effectiveness is measured in terms of differential survival and reproduction, and consequently in change in the frequency of alleles in a population.

selection Differential survival and reproduction phenotypes.

self-fertilization The process by which pollen of a given plant fertilizes the ovules of the same plant. Plants fertilized in this way are said to have been selfed.

self-incompatibility In plants, the inability of the pollen to fertilize ovules (female gametes) of the same plant. Opposite of self-compatibility where the plant can be self-fertilised.

separation distance = isolation distance

species A class of potentially interbreeding individuals that are reproductively isolated from other such groups having many characteristics in common. A somewhat arbitrary and sometimes blurred classification; but still quite useful in many situations.

stigma Receptive portion of the style, to which pollen adheres.

style Slender column of tissue that arises from the top of the ovary and through which the pollen tube grows.

sympatry Refers to different species or populations of the same species that live in the same geographic area.

terminator A name given to a seed-suicide mechanism developed in plants to make impossible the use of a second generation of seed by a farmer.

trait Any detectable phenotype or character of an organism.

transformation The uptake and establishment of DNA in a cell or organism, in this case a plant, in which the introduced DNA often changes the phenotype of the recipient organism.

transgene A gene from one genome that has been incorporated into the genome of another organism. Often refers to a gene that has been introduced into a multicellular organism.
**variety** A naturally occurring subdivision of a species, with distinct morphological characters and given a Latin name according to the rules of the International Code of Nomenclature. A taxonomic variety is known by the first validly published name applied to it so that nomenclature tends to be stable.

**vertical gene flow** transfer of genetic information from an individual organism to its offspring by conventional heredity mechanisms.

**volunteer** A domesticated plant that has resulted from natural propagation, as opposed to having been deliberately planted by humans. Volunteers may occur on farm or roadsides and are not necessarily naturalised.

**zygote** A diploid cell formed by the fusion of two haploid gametes during fertilization in eukaryotic organisms with sexual reproduction. It is the first cell of the new individual.
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### Appendix

**Table A1:** Potential gene flow between canola and weedy members of the *Brassica* family (adapted from Salisbury 2002c).

<table>
<thead>
<tr>
<th>Weed</th>
<th><em>Brassica juncea</em></th>
<th><em>Raphanus raphanistrum</em></th>
<th><em>Brassica fruticulosa</em></th>
<th><em>Brassica nigra</em></th>
<th><em>Brassica tournefortii</em></th>
<th><em>Diploptaxis muralis</em></th>
<th><em>Diploptaxis tenuifolia</em></th>
<th><em>Rapistrum rugosum</em></th>
<th><em>Brassica oxyrrhina</em></th>
<th><em>Diploptaxis tenuisiliqua</em></th>
<th><em>Conringia orientalis</em></th>
<th><em>Carrichtera annua</em></th>
<th><em>Capsella bursapastoris</em></th>
<th><em>Cardaria draba</em></th>
<th><em>Lepidium sp.</em></th>
<th><em>Myagrum perfoliatum</em></th>
<th><em>Sisymbrium orientale</em></th>
<th><em>Sisymbrium irio</em></th>
<th><em>Sisymbrium erysimoides</em></th>
<th><em>Sisymbrium officinale</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glasshouse hand hybrids</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Field hybrids</td>
<td>Yes</td>
<td>Yes</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
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<tr>
<td>Gene introgression</td>
<td>Yes</td>
<td>Not reported</td>
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<tr>
<td>Decreasing sexual compatibility</td>
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</tbody>
</table>

Implications of gene flow for the release of GM crops in Australia

<table>
<thead>
<tr>
<th>Crop</th>
<th>Area under cultivation (in hectares)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>2.6 million</td>
</tr>
<tr>
<td>Canola</td>
<td>1.9 million</td>
</tr>
<tr>
<td>Cotton</td>
<td>698,000</td>
</tr>
<tr>
<td>Field peas</td>
<td>496,000</td>
</tr>
<tr>
<td>Lettuce</td>
<td>5,170</td>
</tr>
<tr>
<td>Lupins</td>
<td>1.3 million</td>
</tr>
<tr>
<td>Maize</td>
<td>406,000</td>
</tr>
<tr>
<td>Oats</td>
<td>584,000</td>
</tr>
<tr>
<td>Papaya</td>
<td>355</td>
</tr>
<tr>
<td>Pineapple</td>
<td>2817</td>
</tr>
<tr>
<td>Potato</td>
<td>36,829</td>
</tr>
<tr>
<td>Sugar cane</td>
<td>428,000</td>
</tr>
<tr>
<td>Wheat</td>
<td>12.2 million</td>
</tr>
</tbody>
</table>