

# Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer

A review and interpretation of published literature and recent/current research from the ESF 'Assessing the Impact of GM Plants' (AIGM) programme for the European Science Foundation and the European Environment Agency

Experts' corner

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# Executive summary

In 2000 the EEA established a special project for the European Parliament, on the dissemination of research results from technologies characterised by scientific complexity and uncertainty, such as GMOs and chemicals, and on the use of such results by the public and their representatives in their governance, including the use of the precautionary principle. This project is in support of the EEA duty, added to its regulation in 1999, to 'assist the Commission in the diffusion of information on the results of relevant environmental research'. In order to access European scientific expertise and to minimise duplication, the EEA established a partnership with the European Science Foundation to bring together relevant scientific evidence. This is the first report from the project. Other reports will summarise monitoring programmes and exposure data for some representative chemicals, and the use of consensus conferences and other methods for involving the public in complex scientific issues. The project will support the EEA in its work of helping to develop appropriate monitoring and data sources on the impacts of complex economic/environment interactions.

The European Science Foundation (ESF) had already established a research programme, 'Assessing the Impact of GM Plants' in 1999. This AIGM programme brings together researchers and other scientists from 10 European countries involved in assessing the environmental and agronomic impact of GM crops, including studies of gene flow and dispersal through pollen, hybridisation and gene introgression. The AIGM programme was invited by the ESF to produce a review of pollen mediated transgene flow based on recent research by participants in the AIGM programme as well as from published reports and papers. (The AIGM programme is briefly described in the appendix).

This report considers the significance of pollen-mediated gene flow from six major crop types that have been genetically modified and are close to commercial release in the European Union. Oilseed rape, sugar beet, potatoes, maize, wheat and barley are reviewed in detail using recent and current research findings to assess their potential environmental and agronomic impacts. There is also a short review on the current status of GM fruit crops in Europe. Each crop type considered has its own distinctive characteristics of pollen production, dispersal and potential outcrossing, giving varying levels of gene flow.

Oilseed rape can be described as a high-risk crop for crop-to-crop gene flow and from crop to wild relatives. At the farm scale low levels of gene flow will occur at long distances and thus complete genetic isolation will be difficult to maintain. This particularly applies to varieties and lines containing male sterile components, which will outcross with neighbouring fully fertile GM oilseed rape at higher frequencies and at greater distances than traditional varieties. Gene stacking in *B. napus* has been observed in crops and it is predicted that plants carrying multiple resistance genes will become common post-GM release and consequently GM volunteers may require different herbicide management. Oilseed rape is cross-compatible with a number of wild relatives and thus the likelihood of gene flow to these species is high.

Sugar beet can be described as medium to high risk for gene flow from crop to crop and from crop to wild relatives. Pollen from sugar beet has been recorded at distances of more than 1 km at relatively high frequencies. Cross-pollination in root crops is not usually considered an issue since the crop is harvested before flowering. However a small proportion of plants in a crop will bolt and transgene movement between crops may occur in this way. Hybridisation and introgression between cultivated beet and wild sea beet has been shown to occur.

Potatoes can be described as a low risk crop for gene flow from crop to crop and from crop to wild relatives. Cross-pollination between production crops is not usually considered an issue since the harvested tuber is not affected by incoming pollen. In true seed production areas, however, the likelihood of cross-pollination between adjacent crops leading to con-

tamination is higher. The risk of gene flow exists if volunteers are allowed to persist in a field from one crop to the next. Naturally occurring hybridisation and introgression between potato and its related wild species in Europe is unlikely.

Maize can be described as a medium to high-risk crop for gene flow from crop to crop. Evidence suggests that GM maize plants would cross-pollinate non-GM maize plants up to and beyond their recommended isolation distance of 200 m. There are no known wild relatives in Europe with which maize can hybridise.

Wheat can be described as a low risk crop for gene flow from crop to crop and from crop to wild relatives. Cross-pollination under field conditions normally involves less than 2 % of all florets so any outcrossing usually occurs with adjacent plants. Hybrids formed between wheat and several wild barley and grass species generally appear to be restricted to the first generation with little evidence for subsequent introgression due to sterility.

Barley can be described as a low risk crop for gene flow from crop to crop and from crop to wild relatives. Barley reproduces almost entirely by self-fertilisation, producing small amounts of pollen so that most outcrossing occurs between closely adjacent plants. There are no records of naturally occurring hybrids between barley and any wild relatives in Europe.

Some fruit crops, such as strawberry, apple, grapevine and plum have outcrossing and hybridisation tendencies which suggest that gene flow from GM crops to other crops and to wild relatives is likely to occur. For raspberry, blackberry and blackcurrant the likelihood of gene flow is less easy to predict, partly due to lack of available information.

At present none of these crops has pollen which can be completely contained. This means that the movement of seed and pollen will have to be measured and managed much more in the future. Management systems such as spatial and temporal isolation can be used to minimise direct gene flow between crops, and to minimise seed bank and volunteer populations. The use of isolation zones, crop barrier rows and other vegetation barriers between pollen source and recipient crops can reduce pollen dispersal, although changing weather and environmental conditions mean that some long distance pollen dispersal will occur. Biological containment measures are being developed that require research in order to determine whether plant reproduction can be controlled to inhibit gene flow through pollen and/or seed.

The possible implications of hybridisation and introgression between crops and wild plant species are so far unclear because it is difficult to predict how the genetically engineered genes will be expressed in a related wild species. The fitness of wild plant species containing introgressed genes from a GM crop will depend on many factors involving both the genes introgressed and the recipient ecosystem. While it is important to determine frequencies of hybridisation between crops and wild relatives, it is more important to determine whether genes will be introgressed into wild populations and establish at levels which will have a significant ecological impact.



# Project summary

## A. Results:

Different crop species have different rates of autogamy (self pollination) and outcrossing. In addition some crops have hybridising wild relatives while others do not. The characteristics of the main crop types are summarised as follows:

Crop	Frequency of gene flow from outcrossing	
	Crop to crop	To wild relatives
Oilseed rape	High	High
Sugar beet	Medium to high	Medium to high
Maize	Medium to high	No known Wild Relatives
Potatoes	Low	Low
Wheat	Low	Low
Barley	Low	Low
Fruits - strawberry, apples, grapevines and plums	Medium to high	Medium to high
Raspberries, blackberries, blackcurrant	Medium to high	Medium to high

Source: EEA/Sweet

## B. Implications:

The environmental and agronomic impact of gene flow depends on the specific trait/plant combination and the likelihood that gene transfer will occur. (Risk = Hazard/impact x frequency). For example: Environmental fitness genes in frequently outcrossing species present the highest risk; environmentally neutral genes in inbreeding species present the lowest risk.

## C. Recommendations:

- Gene transfer through cross pollination can be limited by effective biological and physical barriers. More research is needed to examine the options for these in the light of recommendations from the EU on thresholds for contamination of non-GM crops.
- Transgene introgression into wild species is often associated with hybridising ability. However research has shown that there are physiological barriers operating that inhibit adoption of genes in

wild species or populations. Research is needed on actual levels of gene transfer into wild populations from crops and factors involved in genes being adopted by wild populations.

- Both temporal as well as spatial gene flow also arises through seed persistence and dispersal. More information is needed on the role of seed banks and dispersed seed of GM crops on contamination of subsequent crops.
- Better management systems and stewardship schemes to minimise GM contamination and gene flow require good scientific information on both seed and pollen mediated gene flow.
- Future monitoring of experimental and commercial releases of GM crops must be based on good scientific knowledge of the behaviour and ecology of the GM crop and its wild relatives. Understanding gene flow and introgression is a key part of this requirement.

# Introduction

## 1.1. Aims and objectives of the report

This report considers the significance of pollen mediated gene flow from six major crop species commonly grown in Europe that have been genetically modified and are close to commercial release in the European Union (EU). Existing data including the most recent research on oilseed rape (*Brassica napus* ssp. *oleifera*), sugar beet (*Beta vulgaris* ssp. *vulgaris*), potatoes (*Solanum tuberosum*), maize (*Zea mays*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) has been compiled under individual crop sections to form a review and interpretation of the potential environmental and agronomic impacts of each crop. With this we aim to advise on appropriate measures to restrict gene flow or minimise the impact of transgenes moving from crop to crop and from crop to wild plant species. Each crop is reviewed with particular reference to the following points:

- i) reproductive biology and crop use;
- ii) genetic modification;
- iii) pollen dispersal;
- iv) gene flow: Crop to crop;
  - hybridisation and gene flow;
  - possible consequences of gene flow;
- v) definition and status as a weed plant;
- vi) gene flow: Crop to wild relative;
  - compatibility and distribution;
  - hybridisation and gene flow;
  - possible consequences of gene flow;
- vii) conclusion.

Information on current GM research involving the main fruit crops grown in Europe will also be given in the form of short reviews. Grapevine (*Vitis vinifera*), plum (*Prunus domestica* L.), apple (*Malus x domestica*), strawberry (*Fragaria x ananassa*), blackcurrant (*Ribes nigrum*), raspberry (*Rubus idaeus*) and blackberry (*Rubus fruticosus*) will be focused on to give evaluations of the risk and possible effects of pollen-mediated gene flow from these crops.

To conclude the report, future recommendations and considerations are discussed with regard to crop to crop and crop to wild relative gene flow, along with methods of minimising gene flow, such as developing physical and biological barriers.

## 1.2. Background

Genetic modification can potentially improve crop quality and productivity. The molecular techniques employed to do this essentially involve the insertion and integration of a short segment of DNA from a wide variety of novel genes from unrelated plants, microbes and animals into the genome of a plant. Genetic modification has the advantage of allowing the addition of a single character to breeding lines and varieties without the need for backcrossing to remove unwanted genetic linkages (DoE, 1994). Genetically modified (GM) crops were first released commercially in 1992. Their global area covered 11.0 million hectares in 1997 and had increased by 16.8 million hectares to 27.8 million hectares in 1998. Estimates in 2000 suggest that approximately 50 million hectares of GM crops are now grown. The five main GM crops grown in 1999 were, in order of the largest area, soybean (*Glycine max*), maize, cotton (*Gossypium hirsutum*), oilseed rape and potato, with herbicide tolerance and insect resistance the most utilised genetic traits.

In 1998 the first commercialised GM crop was grown in the European Union (EU). Estimates suggest that introductory quantities of insect resistant maize were grown primarily in Spain (20 000 hectares) and France (2 000 hectares). Other crops being developed for commercial application in the EU include sugar beet, oilseed rape (herbicide tolerance) and potatoes (modified starch) (Dale, 1999). There is no commercial growing of GM crops in several European countries including the UK. However certain imported products have been approved for food use: slow ripening tomatoes, soya that is resistant to a broad-spectrum herbicide (glyphosate), insect-resistant maize (Halford, unpublished), and herbicide tolerant rapeseed for oil.

Despite the potential benefits of GM crops, there is also concern over the possible environmental and agronomic impacts if the transgenes 'escape' and become established in natural or agricultural ecosystems. From an agronomic point of view, the transfer of novel genes from one crop to another could have a number of implications, including

depletions in the quality of conventional and organic crop seed leading to a change in their performance and marketability. Maize, for example, will cross-pollinate with other cultivated maize and sweetcorn (*Zea mays* ssp. *saccharata*), directly affecting the quality and acceptability of the marketed product. Concerns over the ecological impacts of GM crops lie with whether or not a crop has wild relatives and the ability to cross-pollinate them. If crops hybridise with wild relatives and gene introgression occurs wild populations could incorporate transgenes that change their behaviour and they could present an economic threat as weeds or an environmental threat as competitors in natural communities. Oilseed rape, grasses and several fruit crops have varying degrees of sexual compatibility with a number of wild relatives found in Europe, and introgression of novel crop genes into some of these relatives is likely. Other crops, for example maize, have no wild relatives with which they could potentially cross-pollinate in Europe.

### 1.3. Factors affecting pollen dispersal and cross-pollination

#### 1.3.1. Size of pollen source and sink

The extent of cross-pollination between fields of crops or between crops and wild plant populations is largely dependent on the scale of pollen emission and dispersal (Raybould & Gray, 1993). Klein *et al* (submitted) used models to estimate the dispersal patterns of maize pollen in various spatial designs. The cross-pollination rate from one field to another was shown to depend on the sizes of both fields. If pollen disperses from a small source area it may behave as a narrow and unpredictable diffusion cloud. Evidence indicates that most airborne pollen from small to moderate sized fields contributes to the local component in this way (Treu & Emberlin, 2000). A theoretical study by Crawford *et al* (1999) examined the effect of increasing pollen source size on resulting levels of cross-pollination. He concluded that a square 400 m<sup>2</sup> crop would emit 3/4 the amount of pollen that a 4 ha (40 000 m<sup>2</sup>) crop would emit, but suggested that the effectiveness of pollen dispersal would decline significantly in crop areas of less than 400 m<sup>2</sup>. Due to conclusions of this kind many believe that small-scale field trials have done little to remove uncertainty over the scale of pollen emission and dispersal likely to emanate from genetically modified crops. Throughout this study we place greater emphasis on field trials carried out on an

agricultural scale when drawing conclusions for potential cross-pollination.

#### 1.3.2. Pollination vectors

As well as being dispersed on the air current and by wind, pollen can be effectively distributed by insects. Pollen produced by some crops, for example oilseed rape, can be dispersed over considerable distances by both wind and insects. The weather can affect the behaviour of pollinating insects on the crop and the occurrence of airborne pollen movement so the amount of cross-pollination can vary significantly from crop to crop and day to day. The numbers and even species of natural pollinating insects can vary considerably in their contribution to successful pollination (Faegri *et al*, 1992). The bumblebee (*Bombus* sp.) is an example of a pollinator which moves only short distances between flowers so the majority of pollen is deposited in the immediate surroundings of the pollen source. By contrast, the foraging habits of the pollen beetle (*Meligethes aeneus*) mean that they emigrate from a crop in large numbers and often fly over long distances (Skogsmyr, 1994).

#### 1.3.3. Environmental factors

Pollen released on the airflow can settle by gravity, can be removed by precipitation, be absorbed into water droplets, or can impact onto surfaces including vegetation, buildings, soil and water bodies. The relative importance of these sinks and the impacts they might have will vary with factors such as the terminal velocities of the pollen grains, climate, local vegetation and topography (Treu & Emberlin, 2000).

##### 1.3.3.1. Weather

Pollen dispersal can be heavily influenced by the weather and changes in temperature, humidity and light, as well as wind and rain. For example, studies on pollen dispersal by Scott (1970) over several years revealed that the average concentration of oilseed rape pollen during one day of one year measured 1.4 % of that on the same day the following year. This was due to heavy rain and high humidity on the first day compared with sunshine and low humidity on that day a year later. Wind strength can also have an important role in distributing pollen grains significant distances within their viability periods.

##### 1.3.3.2. Local environment

Patterns of pollen dispersal can be heavily influenced by variable factors in the immediate local environment such as the nature of

the plant canopy, surrounding vegetation and topography. Wind velocity and airflow are affected by topography, potentially influencing pollen movement from a pollen source to receptor plants.

#### *1.3.3.3. Physical barriers*

Woods and hedges can serve as barriers to air flow, having dual effects of depleting some pollen from the air flow by impaction and filtering and also creating a sheltered zone in the lee. Dense stands of shrubs, herb covers and tree-sized vegetation with full foliage act as catchments for airborne particulates, including pollen (Treu & Emberlin, 2000). Jones & Brooks (1952) conducted experiments with tree barriers adjacent to a crop of maize. The results indicated that a single row of trees with underbush were effective in reducing the amount of outcrossing by 50 % in the plants situated immediately behind the barrier, but was much less effective at greater distances from the barrier. The authors concluded that the tree barrier was less effective in reducing outcrossing than an area of barrier crop occupying an area of equal size to the trees.

The effects of barrier crops and isolation zones on pollen movement are discussed in Section 10.4.

#### *1.3.4. Pollen viability and competitive ability*

Biological factors influencing successful pollination begin with the ability of the donor plant to produce viable pollen, and the length of time the pollen grain retains its potential for pollination. If the competitive ability of the pollen grain is poor its capacity to compete with fresher pollen produced in the vicinity of the receptor plant will be poor. Pollen viability can vary greatly between species but is also dependent on environmental variables such as temperature and humidity (Treu & Emberlin, 2000).

#### *1.3.5. Levels of outbreeding in the crop*

The amount of outbreeding in the crop is an important aspect to consider. Govindaraju (1988) demonstrated a significant positive correlation between outcrossing rates (largely determined by pollination mode) and gene flow variables, reflected in the different isolation requirements for various crops. Wheat, for example, is typically self-pollinated, with cross-pollination under field conditions usually involving less than 2 % of all florets (Wiese, 1991). Oilseed rape is known to be mainly self-fertilising and/or insect pollinated although pollen can

become airborne and travel several kilometres downwind. Floral morphology and pollen characteristics are also important as the morphology and terminal velocity of pollen grains influence dispersal patterns.

#### *1.3.6. Degree of synchrony in flowering times*

There must be some overlap in flowering times between the pollen donor and the receptor plant so that ripe pollen and receptive stigmas are produced at the same time, in which case a higher degree of cross-pollination might occur than if partial self-pollination had begun in one of the plants.

### 1.4. Hybridisation, gene flow and introgression

In its broad sense 'hybridisation' can be defined as the cross-breeding of genetically dissimilar individuals. Such individuals may differ by one or a few genes (the pure lines of plant geneticists), by several genes (e.g. hybrid maize) or be very different genetically (as in most hybridisations between members of different genera). Hybridisation is common within species but can also occur between species and occasionally with species in different genera. Hybridisation between different species can be described as 'interspecific' hybridisation or, where species belong to a different genus, 'intergeneric' hybridisation (DoE, 1994). The incidence of natural interspecific and intergeneric hybridisation varies substantially among plant genera and families.

Hybridisation is a frequent and important component of plant evolution and speciation, although the resulting  $F_1$  plants are often sterile and relatively few populations persist, except where the parents remain in contact or where they are able to spread vegetatively (Raybould & Gray, 1993). Table 1 (overleaf) demonstrates the many factors that determine the production and establishment of viable hybrids. The frequent occurrence of fertile hybrids increases the chances of introgression, the incorporation of alleles from one taxon to another, mediated through repeated backcrossing of hybrid individuals to one of the parents.

Gene flow can be defined as 'the incorporation of genes into the gene pool of one population from one or more populations' (Futuyma, 1998). Such gene movement is a major determinant of genetic structure in natural populations. Gene flow is strongly

influenced by the biology of the species and is likely to vary with different breeding systems, life histories and modes of pollination. Assuming sexual compatibility between a crop and wild relative, the entry and subsequent spread of a transgene into natural populations will be determined to some extent by pollen movement. Different crop species have different pollination mechanisms (insect and wind) and different seed dispersal patterns. Both may act as vectors for transgenes from crops, but the subsequent dispersal of the genes through pollen and seeds may be completely different (DoE, 1995), depending on the reproductive characteristics of the species.

Gene flow is measured in various ways. The most common direct method for plants is the observation of seed and pollen movement, which gives an estimate of potential gene flow (dispersal). Other methods use genetic markers to estimate actual gene flow. A simple method is to introduce or identify a plant in a population with a unique genetic marker (e.g. an isozyme allele) and to follow the appearance of the marker in the next generation (e.g. Latta *et al.*, 1998). Transgenes can act as convenient markers for tracking gene flow and the results of various studies of this kind are discussed in later chapters.

Throughout the report both the potential for gene flow between crops, and from crops to wild relatives will be discussed. The frequency and occurrence of genetic movement between different plants forms the basis of practical decisions about the isolation requirements of crops where varietal purity of the seed is essential. Some crops have sexually compatible relatives that are found as wild plants and arable weeds. Sugar beet, for example, can be accompanied by related wild beet, and there is well-documented evidence of gene transfer between the two (Boudry *et al.*, 1993).

### 1.5. Routes of transgene movement between species

A transgene can be regarded as having 'escaped' from the crop if:

(1a) The plant containing it persists after the crop, possibly becoming a weed of agricultural, especially arable, land.

(1b) The plant containing it persists in the disturbed habitats associated with agriculture

Factors determining the likelihood of hybrids, between crop plants and related species, becoming established in agricultural or natural habitats

Table 1

#### The production of viable hybrid seeds

1. Compatibility of the two parental genomes (mitotic and genetic stability)
2. Ability of the endosperm to support hybrid embryo development
3. Direction of the cross: one parent may support embryo and seed development better than the other
4. Number and viability of hybrid seeds

#### Establishment of hybrid plants from seeds in soil

5. Seed dormancy
6. Vigour of the hybrid plant
7. Direction of cross: maternal effects influencing seedling vigour
8. Nature of habitat: wild, semi-wild or agricultural
9. Nature of competition from other plants
10. Influence of pest, disease and animal predators

#### Ability of the hybrid to propagate vegetatively and sexually

11. Method of vegetative propagation
12. Persistence of vegetative propagules in agricultural habitats
13. Dissemination of vegetative propagules
14. Invasiveness of vegetative propagules in natural habitats
15. Sexual breeding system: cross-compatible, self-compatible, ability to cross to either parental species
16. Male and female fertility: meiotic stability and chromosome pairing
17. Seed number and viability
18. Seed dormancy
19. Nature of habitat: wild, semi-wild or agricultural
20. Nature of competition from other plants
21. Influence of pest, disease and animal predators

Source: Dale (1994)

or other human activities (e.g. headlands, roadsides, waste tips).

(1c) The plant containing it invades semi-natural habitats (e.g. saltmarshes, sand dunes, heathland, and woodland).

or

(2a) The transgene is transferred by pollination to another crop which persists in agricultural habitats.

(2b) As (2a), but the plant occupying disturbed habitats.

(2c) As (2a), but the plant invading semi-natural habitats.

or

(3a) The transgene is transferred by pollination to a wild related plant which (possibly by introgression) persists in agricultural habitats.

(3b) As (3a), but the plant occupying disturbed habitats.

(3c) As (3a), but the plant invading semi-natural habitats. (DoE, 1994)

The first route involves either vegetative persistence or transmission of the genetic modification in seed from generation to generation. Plants produced by (1b) or (1c) are referred to as feral plants throughout the report. With exception to the cross-pollination event in route 2, this route is akin to route 1 and may range from the movement of a transgene from a GM crop to a non-GM crop of the same variety, to movement between crop varieties and between related crop species. The modes of escape described in route 3 refer to the movement of transgenes from crops to wild species or native populations. Hybridisation with wild relatives may cause problems in a number of

ways: by genetic erosion (particularly in centres of diversity); through genetic pollution of natural gene pools (Gray & Raybould, 1998), and by weeds conferring a selective advantage, such as pest and disease resistance, leading to a change to the persistence or invasiveness of a species (Dale & Irwin, 1995). Ultimately the consequences of hybridisation between crop and wild species will depend on the nature of the transgene and the fertility of the hybrid and any of its progeny (McPartlan & Dale, 1994).

Though the emphasis of this report is on pollen mediated gene flow, it is important to recognise that this comprises of only one element of the movement of genes within and between populations. Seeds may be distributed in time through their dormancy mechanisms as well as in space. The importance of the latter was highlighted recently when an import of conventional rapeseed from Canada to Europe was found to contain traces of adventitious GM material which has not been approved for planting in Europe (Coghlan, 2000). Although detailing gene flow through seed dispersal is beyond the scope of this report it will be highlighted where, together with cross pollination, it is considered to play a significant part in the movement of transgenes.

## 2. Oilseed rape (*Brassica napus* ssp. *oleifera*) with reference to turnip rape (*Brassica rapa*)

*Brassica napus* ssp. *oleifera* belongs to the *Cruciferae* family and is a member of the genus *Brassica*. Three major species of *Brassica* are grown commercially in Europe, *Brassica napus* (e.g. oilseed rape, swede), *Brassica oleracea* (e.g. cabbage, cauliflower, sprouts) and *Brassica rapa* (e.g. turnip and turnip rape). Both *B. napus* (amphidiploid) and *B. rapa* (diploid) are grown as oilseed rape crops in Europe in both spring and winter forms.

### 2.1. Reproductive biology and crop use

*B. napus* has dark green foliage and well-branched stems. Branches originate in the axils of the highest leaves on the stem, and each terminates in an inflorescence of nectar bearing flowers. The inflorescence is an elongated raceme, the flowers of *B. napus* are yellow, densely clustered at the top with open flowers borne at or above the level of the terminal buds, and open upwards from the base of the raceme (Downey *et al.*, 1980). *B. rapa* has slightly smaller and darker yellow flowers than those of *B. napus*, and flowers standing well above the unopened younger buds. Lower leaves are bright green and sparingly toothed, generally with more hairs than *B. napus*. *B. napus* is mainly self-pollinating though it is estimated that outcrossing can occur at levels between 12-47 % (Becker *et al.*, 1992). Wild *B. rapa* is an obligate outcrosser due to the presence of self-incompatibility genes, whereas cultivated forms have variable levels of self-compatibility. Cross-pollination occurs primarily through physical contact with neighbouring plants, though pollen is also transferred over longer distances by wind and insects. The flowers of both crops produce nectar and have a colour and structure which makes them attractive to insects, particularly bees.

Commercial plantings of rapeseed were recorded in the Netherlands as early as the 16<sup>th</sup> century. Although used widely for edible oil production in Asia, only through breeding for improved oil quality and through the development of improved processing techniques has rapeseed oil become important in western nations. It is now the third most important oil-producing crop in the world, following soya and palm oil. Oilseed rape has

been developed as a multi-purpose crop. Industrial rape seed oil with high erucic acid is used as a fine lubricant and as a fuel oil substitute for diesel. By contrast rapeseed oil for human consumption has low erucic acid in its oil and low glucosinolate content in its meal protein. Rapeseed production has increased dramatically in Europe and Canada in recent years. Today 'double low' commercial varieties of both *B. napus* and *B. rapa* dominate the oilseed *Brassica* production area in developed countries (Anon, 1999a).

### 2.2. Genetic modification

*B. napus* ( $2n=38$ ) is an amphidiploid derivative of *B. oleracea* ( $2n=18$ ) and *B. rapa* ( $2n=20$ ) (See Figure 8, Triangle of U). Because oilseed rape is self-compatible producing hybrid seed has involved a number of different technical approaches. One particular transgenic method has achieved pollination control by the incorporation of a copied bacterial gene, which blocks pollen production to produce male sterile GM oilseed rape. Fertility can be restored using a second gene, a fertility restorer. The reversible male sterility ensures cross-pollination and increased hybrid vigour of the offspring. This  $F_1$  generation then has restored fertility, self-compatibility, and is highly productive in commercial cultivation.

The main GM traits used in commercial crops of genetically modified oilseed rape are for tolerance to broad-spectrum herbicides. Several lines have been transformed to include copies of bacterial genes that confer tolerance to herbicides such as glyphosate and glufosinate, allowing more effective weed control in the crop (Anon, 1999a). GM herbicide tolerant rape is now widely developed in Canada, forming approximately 73 % of the commercial crop.

Oilseed rape has also been transformed with genes for modifying the amount and type of oil produced. Two such oil modifications are the amplification of stearic acid (Knutzon *et al.*, 1992), a fatty acid found in conventional rapeseed at low levels, and the introduction of lauric acid, a fatty acid not normally found in rapeseed which has been introduced from the Californian bay plant

(*Umbellularia californica*) (Voelker *et al.*, 1992). These oil types are of use in the specialist food and detergent industries and are of considerable commercial significance (Booth *et al.*, 1996). Also, by inserting a gene from meadow foam, plants producing super high (66 %) erucic acid have been engineered.

### 2.3. Pollen dispersal

Winter oilseed rape flowers in April to early May in northern continental Europe, and in June to July in Scotland and Scandinavia. The majority of spring oilseed rape flowers at least one month later. Consequently it is widely believed through observation of the crop that insects have a more important role in cross-pollination of later flowering crops. Oilseed rape pollen grains are typical of insect pollination, being fairly heavy and sticky. Insects, particularly honeybees (*Apis mellifera*) and bumblebees (*Bombus sp.*) are believed to play a major role in the transfer of pollen over long distances. Observations of honeybee colonies (Ramsay *et al.*, 1999) determined that these insects switch from one forage type to another, and that bees carrying many viable oilseed rape pollen grains can be found emerging from a hive. Bees were found to pick up large numbers of loose pollen grains within the hives, so with most honeybee colonies foraging up to 2 km in all directions from a hive, some pollen transfer and fertilisation up to 4 km can be expected. Bees in a colony in Scotland have been reported to have flown to a crop 5 km away (Ramsay *et al.*, 1999), so theoretically there is potential for pollen to be transferred to distances of at least 10 km by the mixing of bees foraging in different directions from the same hive.

The significance of both wind and insects as vectors of oilseed rape pollen have been widely researched with contradictory results that are probably influenced by varying environmental and topographical conditions, and the differences in research methodology used. Timmons *et al.* (1995) constructed pollen profiles along a linear transect up to 2.5 km from fields of oilseed rape (10 ha and 3 ha). Measurements were taken using volumetric spore traps. Airborne pollen levels were found to decline with distance, although wide day-to-day fluctuations in airborne pollen densities suggested that oilseed rape pollen moves rapidly from the source and does not remain airborne for significant periods of time (Timmons *et al.*,

1995). In each year over a period of three years, the pollen concentrations detected at 360 m were 10–11 % of that recorded at the field margin. Low densities were consistently recorded at 1.5–2.5 km from the source, probably representing background levels (Timmons *et al.*, 1995). In a further development of the study bait plants were used to investigate whether the low levels of pollen detected at long distances were sufficient enough to effect significant levels of gene flow. Emasculated and de-petalled oilseed rape plants were placed at increasing distances from an oilseed rape field, and levels of seed set were recorded. Seeds produced on plants 2 km away from the source contained 38 chromosomes and appeared phenotypically normal for *B. napus*, suggesting that there were sufficient viable pollen grains at distances of at least 2 km to effect gene flow. However, although the removal of petals may reduce the attractiveness of plants to pollinating insects, the prevention of visiting insects to de-petalled plants is not guaranteed so this gene flow may not have been effected entirely by wind pollination.

Thompson *et al.* (1999) described the relative importance of wind and insect pollination as being difficult to examine in field conditions. In their study, existing field crops of 55ha were used as pollen donors to measure levels of pollen dispersal. DNA profiling techniques were utilised to determine the source of pollen on male sterile bait plants, situated at various distances from the source. At one of the bait sites, with a pollination rate of 33 %, the majority of the sample (>80 %) was shown to have been fertilised by pollen from the nearest crop 900 m away. Levels of cross-pollination were recorded at a maximum distance of 4 km from the nearest known source. The patterns of pollen dispersal recorded in this study suggest that insects played an important part in pollination. For example, high numbers of seed set per siliqua occurred at distant sites, despite an overall low frequency of pollination events. Also, the incidences of pollination events in the absence of high levels of airborne pollen imply that insects carried pollen over a range of distances (Thompson *et al.*, 1999).

Simpson *et al.* (1999) measured pollen dispersal from a large area (approx. 9ha) of winter oilseed rape using plots of male sterile bait plants and fully fertile plants. Six of each type of bait plant were positioned in linear plots at a range of distances (100 m, 200 m, 400 m) in directions north, south,



east and west from the pollen source. During the study it was observed that the incidence of pollinating insects was notably low at flowering time. Male sterile bait plants were pollinated and seed set in all plots. Numbers of herbicide tolerant seed detected decreased rapidly with distance from the pollen source (see Figures 1 and 2).

Male sterile bait plants essentially act as pollen detectors as they produce no pollen of their own, and represent 'worst case scenario' cross-pollination levels. Results from fully fertile bait plants show a much lower incidence of cross-pollination occurring, and represent the likely levels of gene flow that may occur from crops to isolated wild or feral rape plants.

The evidence given in various studies show that there is potential for oilseed rape pollen to be dispersed by wind and insects and remain viable over considerable distances. Oilseed rape pollen is thought to remain viable for between 24 hours and one week according to Mesquida & Renard (1982), who studied the different factors on in-vitro germination of oilseed rape. Under natural conditions Ranito-Lehtimäki (1995) reported a gradual decrease in pollen viability 'over 4-5 days'.

## 2.4. Gene flow: Crop to crop

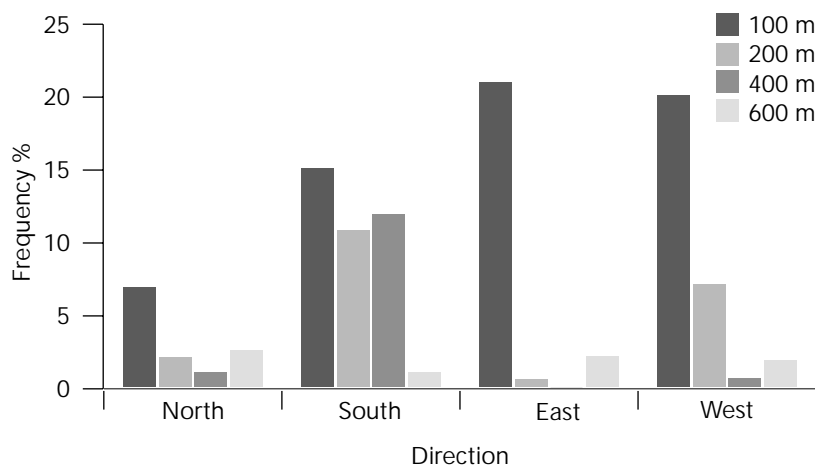
### 2.4.1. Within crop species gene flow

Recorded levels of cross-pollination in oilseed rape are variable, being dependant on factors such as experimental design, relative size of pollen donor and recipient, variety and type of oilseed rape studied, as well as site specific factors such as climatic conditions and topography. The data cited in this section is derived from studies which have predicted the levels of gene flow likely to occur under standard agricultural conditions. In Canadian studies of canola (spring oilseed rape) Downey (unpublished) measured outcrossing rates between adjacent commercial fields of conventional rape and herbicide tolerant rape. Samples were taken at various distances (0 to 100 m) from the closest edge of the conventional rape to the GM rape field. The levels of outcrossing between large (>16 ha) commercial fields of both *B. napus* and *B. rapa* were low (see Table 2).

Previous work by Stringham & Downey (1978, 1982) resulted in substantially higher levels of outcrossing where pollen flow from

Frequency of herbicide tolerant seed harvested from male sterile bait plants at varying distances and directions from GM herbicide tolerant oilseed rape

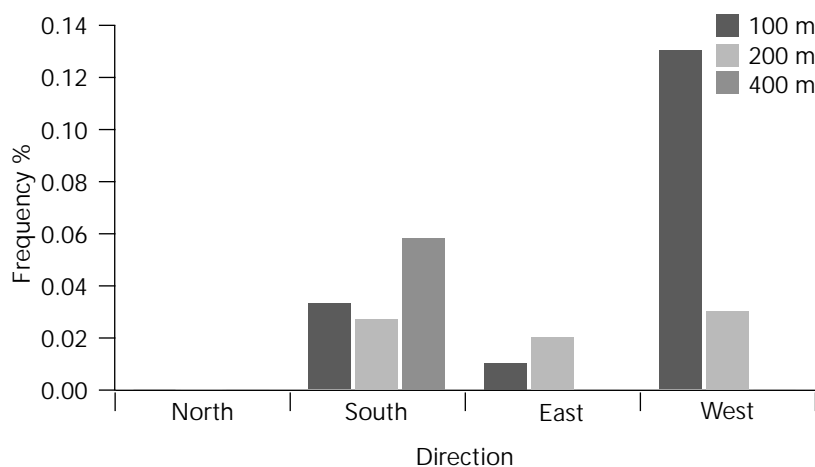
Figure 1



Source: Simpson (unpublished)

Frequency of herbicide tolerant seed harvested from fully fertile bait plants at varying distances and directions from GM herbicide tolerant oilseed rape

Figure 2



Source: Simpson (unpublished)

large commercial fields to small (46 m<sup>2</sup>) plots measured an average of 0.6 % at 366 m.

Norris *et al* (unpublished) studied gene flow between GM and conventional spring oilseed rape varieties at two large-scale sites in the UK. Both sites were planted with a 10ha block of Hyola 401 as the conventional variety adjacent to a 10 ha block of a glufosinate tolerant variety. Seed samples were taken at full pod development to test for gene flow along three transects. Each transect started at the boundary between the two crops, and seed was collected at various points going into the conventional variety up to distances of 250 m. Seed samples were

Table 2

Percent outcrossing between large fields (>16ha) of glyphosate tolerant and susceptible *B. rapa* and *B. napus* in Western Canada, 1998

Species & field No	Separation of fields (m)	Distance from field edge				Average
		0 m	33 m	66 m	100 m	
<i>B. rapa</i>		% of outcrossing				
1	1	0.7				0.70
2	4	0.2	0.4	0.3	0.2	0.28
3	170	0.3	(small 4 ha field)			0.30
4	250	0.0	0.1	0.0	0.0	0.02
5	600	0.0	0.0	0.0	0.1	0.02
<i>B. napus</i>		-	20 m	50 m	100 m	
1	1		1.5	0.4	0.1	0.60
2	1		0.1	0.0	0.4	0.16

Source: Downey, 1999

subjected to herbicide tolerance testing in order for gene flow to be detected. Results are shown in Figures 3 and 4.

Low levels of outcrossing occurred at both sites up to the furthest sampling point from the GM crop. Site A showed unusually high levels of gene flow at distances of 100 m and 200 m from the source, and although Norris *et al* (unpublished) did not have a conclusive explanation for this, there were several factors that may have been influential. One

is that the conventional variety Hyola 401 may have contained a low proportion of male sterile plants (<10%), and if so these individual plants would not have been subjected to normal levels of self-pollination competition. Therefore the male sterile plants would have been more receptive to airborne pollen thereby biasing cross-pollination frequencies. A second possible explanation is that seed used in the trial may have been contaminated with GM glyphosate and possibly glufosinate tolerance, as it was recently announced that batches of Hyola seed imported from Canada were contaminated. An alternative possibility for the unusual results observed at long distances from the GM pollen source at site A is the position of a copse situated in the centre of the trial field. Norris *et al* (unpublished) hypothesised that the copse may have altered the course of air currents, thereby distributing pollen in a different way. Although no explanation is conclusive, these results demonstrate the many unpredictable factors involved in the potential for gene flow between agricultural fields.

Simpson (unpublished data) established blocks of herbicide tolerant and conventional winter oilseed rape varieties in adjacent areas of approximately 0.8 ha in a 10 ha field. Three transects across each non-GM block (varieties Synergy and Apex) were sampled at various distances between 0 and 91.5 m from the edge of the GM glufosinate tolerant block. At full pod development the main raceme was removed from 20 plants within a 1m<sup>2</sup> quadrat at each sampling point, the seed extracted, sown, and the seedling samples subjected to a herbicide tolerance test. Results are shown in Figures 5 and 6.

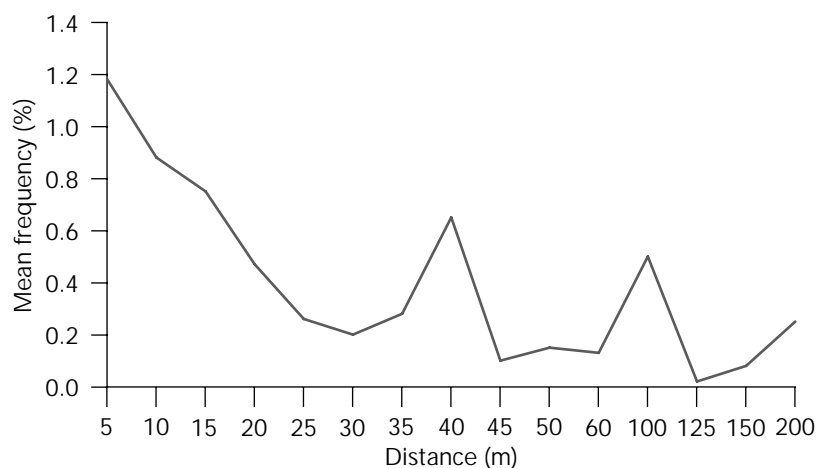
Levels of tolerance detected in seed samples from Synergy plots were considerably higher than in Apex. Simpson (unpublished) concluded that this was because Synergy is a varietal association containing 80% male sterile to 20% pollinator (Falcon), so that there was less competition from pollen produced by the Synergy plots. Apex is a normal open pollinating variety with high levels of self-fertility, reflecting the usual levels of pollen competition in conventional varieties. Both varieties are widely grown commercially. This experiment highlights the importance of oilseed rape variety in influencing cross-pollination levels between conventional and GM crops.

#### 2.4.2. Between crop species gene flow

In an experiment on hybridisation rates

Figure 3

Mean percentage cross-pollination frequencies between adjacent blocks of spring herbicide tolerant and non-tolerant oilseed rape at farm scale evaluation Site A



Source: Norris *et al* (unpublished)

between *B. rapa* and *B. napus* by Norris (pers. communication), a 24 m x 50 m plot of turnip rape (*B. rapa*) was situated adjacent to smaller plots of Apex and a glufosinate tolerant oilseed rape variety. Samples of 20 main racemes of oilseed rape were taken at three intervals along the edge of the plots closest to the turnip rape (5 m away). Results of hybrid identification showed a higher proportion of hybridisation with Apex as the maternal parent than with the glufosinate tolerant transgenic rape variety (LL1) as the maternal parent (see Table 3). Samples of turnip rape seed have also been taken to investigate the reciprocal hybridisation, though results are yet to be confirmed.

Crop plants classified within the species *Brassica oleracea* (cabbage, cauliflower, broccoli, brussel-sprout) are highly interfertile and are closely related to oilseed rape, so theoretically the potential for hybridisation exists.

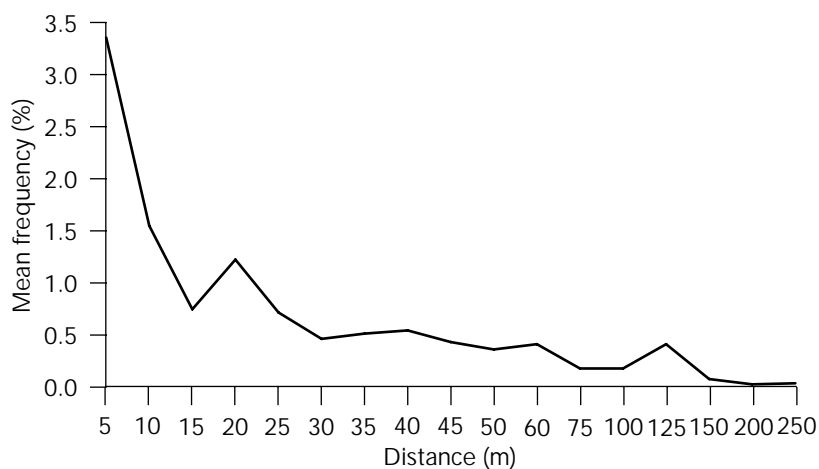
#### 2.4.3. Definition and status as a weed plant

*Brassica* crops produce large numbers of very small seed, which without proper management during harvesting and transportation procedures can result in severe volunteer problems in subsequent crops. Such volunteer problems are difficult to control, particularly in broad-leaved crops. Volunteer rape is a common and widespread weed in cereal rotations and on field margins, roadsides and soil dumps. Feral rape populations are less common; many die out quickly but others persist in fields and around agricultural land for at least 10 years. The crops and ferals are mainly *B. napus*, accompanied in certain areas by crops and ferals of *B. rapa*, and by wild *B. rapa* (see Section 2.5.2) (Squire *et al*, 1999). Studies have provided evidence that rape seeds can persist in the soil for at least five years (Lutman, 1993; Schlink, 1994, 1995) and very likely for ten years (Sauermann, 1993), although there is considerable genotypic variation in the development of secondary dormancy. Experiments by Pekrun *et al* (1997) clearly demonstrated that rape cultivars differ in their potential to persist.

Norris *et al* (1999) monitored the persistence of volunteers and feral populations at various sites in the UK following large-scale GM releases. These sites were planted with herbicide tolerant *B. napus* and *B. napus* with a seed oil modification for high lauric acid. Large numbers of oilseed rape seed persisted in the soil for up to three years after the GM release at some sites. Norris *et al*

Mean percentage cross-pollination frequencies between adjacent blocks of spring herbicide tolerant and non-tolerant oilseed rape at farm scale evaluation Site B

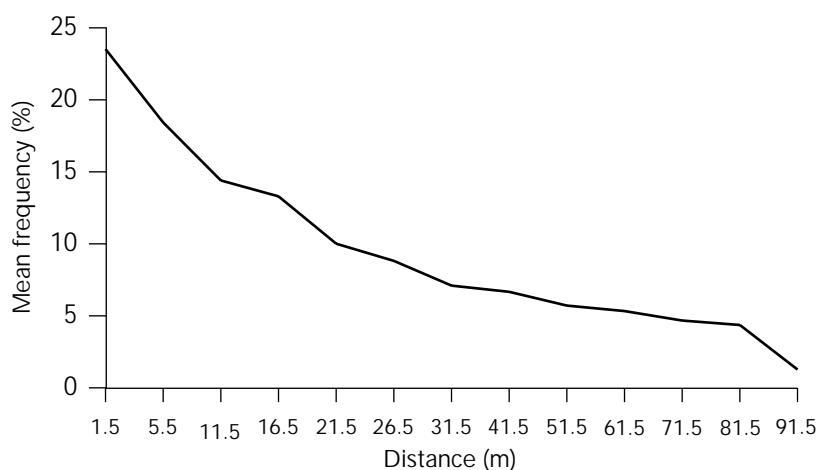
Figure 4



Source: Norris *et al* (unpublished)

Mean percentage glufosinate tolerant seed samples of Synergi at a range of distances from plots of glufosinate tolerant winter oilseed rape

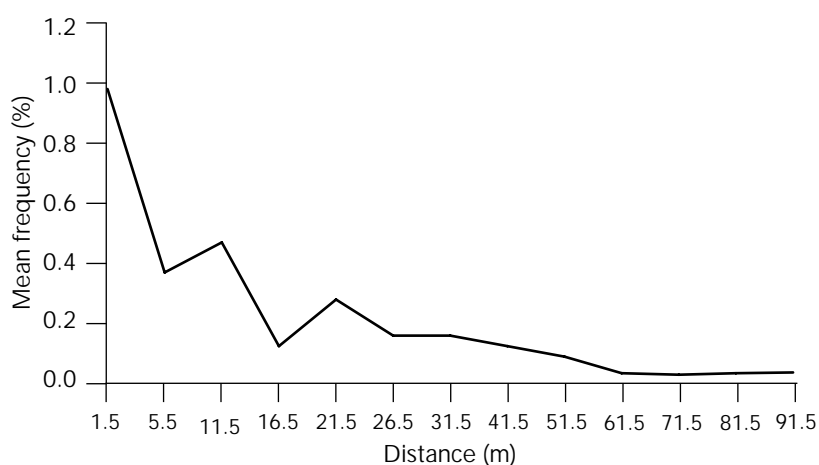
Figure 5



Source: Simpson (unpublished data)

Mean percentage glufosinate tolerant seed detected in seed samples of Apex at a range of distances from plots of glufosinate tolerant winter oilseed rape

Figure 6



Source: Simpson (unpublished data)

Numbers of <i>B. napus</i> x <i>B. rapa</i> hybrids found from oilseed rape seeds			
Oilseed rape variety	Number of seedlings screened	Number of hybrids found	% Hybridisation
Apex	4800	47	0.98
LL1	9000	24	0.27

Source: Norris (pers. communication)

(1999) and Simpson (in press) concluded that GM oilseed rape conferring these traits was not more weedy in an agricultural environment than conventionally bred oilseed rape. Furthermore, transgenic volunteers with these traits did not appear to be more invasive of habitats outside the crop than their non-transgenic counterparts. However, these observations may not relate to environments where volunteer populations are subject to different herbicide applications and post-harvest management. Also, where different transgenic oilseed rape varieties are cropped in adjacent fields volunteer management may become more difficult. Champolivier *et al* (1999) detected double resistant rape volunteers in a series of experiments in France, where three herbicide resistant rape

varieties were sown in adjacent fields at three sites. Post-harvest detection of double resistant plants was carried out using two different methods: by applying the herbicides on volunteers in the field, and by seed sampling followed by direct herbicide application on resulting seedlings. Results are shown in Figure 7.

Both methods gave similar results with respect to the rate of double resistance detected. Although the results varied depending on the variety, the average rate of double resistance estimated under the specific conditions set by Champolivier *et al* (1999) are as follows: 2 % at 1 m from the pollen source, 0.2 % at 20 m and <0.01 % at 65 m (see Figure 7).

Predictions currently being made by researchers are that oilseed rape plants modified with other transgenic traits yet to be approved for wider release in oilseed rape, such as pest or disease resistance, may be more persistent or invasive.

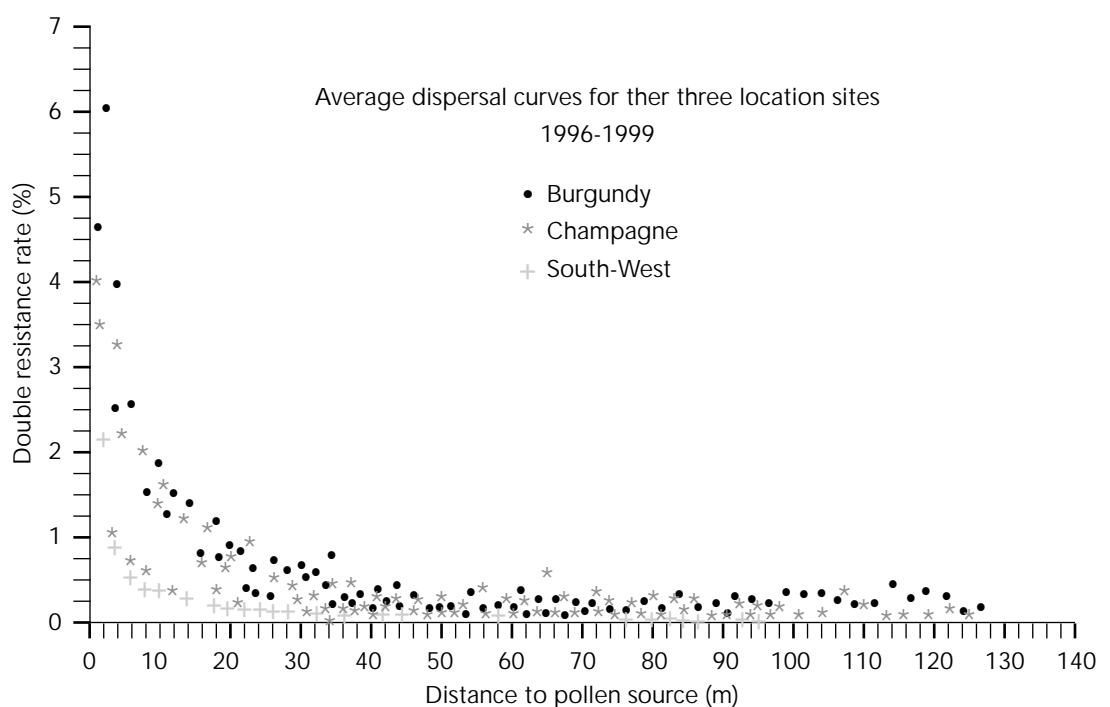
#### 2.4.4. Possible consequences of gene flow

Evidence from studies of cross-pollination of GM oilseed rape highlight the risks of gene transfer, and the production of multiple herbicide tolerant rape volunteers. Although the majority of volunteers would normally be killed by herbicide treatment and/or cultiva-

Figure 7

Percentage of double resistant oilseed rape volunteers detected after cropping three herbicide resistant oilseed rape varieties in adjacent fields

Source: Champolivier *et al*, 1999



tion, gene stacking in volunteer *B. napus* has already been observed in crops (Champolivier *et al*, 1999; Downey, 1999). Senior *et al* (in press) carried out research on stacked herbicide tolerance genes in oilseed rape. By stacking together glyphosate and glufosinate tolerance traits into both winter and spring lines of *B. napus*, the study allowed possible interactions between these transgenes in the presence of a variety of different herbicides to be observed. The authors reported that multiple herbicide tolerant oilseed rape did not alter in its susceptibility to other, unrelated herbicides, and no gene silencing was observed. Downey (unpublished) predicts that with the increasing use of cultivars carrying three or four different herbicide tolerant genes, plants carrying multiple resistance's will become common after commercial GM release. The risk then exists of volunteers becoming difficult to control with a range of herbicide treatments, especially if crops other than cereals are following oilseed rape in the rotation. It is important not to allow volunteers to discharge viable seed because of the large increase in the burden for following crops (Harding & Harris, 1994). The potential for gene exchange from GM volunteers to conventional crops is also increased, along with the possibility of crop contamination. In some feral populations studied by Thompson *et al* (unpublished), up to 50 % of the progeny of some individuals contained genetic material that came from other plants as a result of outcrossing in that season.

Colbach *et al* (1999) have developed a system to model the effects of cropping systems on gene flow from GM rape to rape volunteers in neighbour plots and following crops, the objective being to propose combinations of cropping systems to limit gene flow. Simulations demonstrate that with one year of rape cultivation a volunteer rape population would occur, then steadily decline over many years. Subsequent crops of GM rape would boost the volunteer population, allowing it to persist in the seed bank.

Downey (1999) highlighted that *Brassica* breeders have long known that inter-specific crossing among *B. napus*, *B. rapa* and *B. juncea* occurs naturally, as recently documented by Bing *et al* (1991). The importance of gene flow between crop species is subjective. Jørgenson & Anderson (1994) were successful in introgressing a herbicide tolerance gene from *B. napus* into *B. rapa* with apparently no adverse effect on the

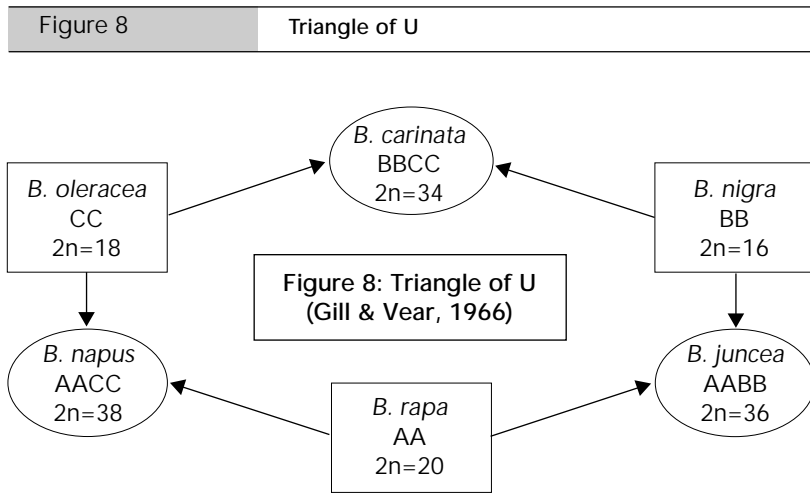
agronomic fitness of the recipient plants (Snow & Jørgenson, 1999). Downey (unpublished) regards gene flow among related *Brassica* oilseed species as a 'minor concern', on the premise that if transfer of a herbicide tolerant trait, for example, were to occur among these species, the agronomic and environmental consequences would be essentially nil since the presence of such genes do not make the recipient plants more competitive or invasive of cultivated or natural ecosystems in the absence of the specific herbicide (MacDonald, 1994; Belyk & MacDonald, 1994 & 1995 a, b, c). Similarly, results of monitoring by Simpson *et al* (1999) and Norris *et al* (in press) currently do not indicate that GM herbicide tolerant oilseed rape varieties are any more persistent or invasive than conventional types. Problems likely to occur are those related to crop contamination affecting quality and marketability of produce.

Crops classified within the species *B. oleracea* are grown for their vegetative product, the harvesting of which usually occurs prior to flowering. Seed production of these crops is usually in areas suitably isolated from oilseed rape to avoid contamination. The actual risk of hybridisation between these crops is therefore low.

## 2.5. Gene flow: Crop to wild relative

### 2.5.1. Compatibility and distribution

Oilseed rape is thought to have developed in the western Mediterranean from hybridisation's between *B. oleracea* and *B. rapa*. Probably the commonest yellow crucifer of road verges, disturbed land and waste places, it is widely reported to be both a persistent component of such habitats and also to be currently expanding its geographical distribution (DoE, 1994) due to increased growing of rape. A large number of relatives of oilseed rape exist throughout Europe, some of which are cultivated as crops and others that are known as weeds in farming systems and wild flowers outside cultivated areas. *Brassica* and related genera present a complex of species, many of which are partially or fully isolated by breeding barriers of various degrees. In considering species cross compatible with *B. napus* one must include not only several *Brassica* species, but also some species in related genera with which hybridisation may be possible (e.g. *Raphanus raphanistrum*, *Hirschfeldia incana*). Hybridisations should be considered in light of genomic relationships between three



Source: Gill and Vear, 1966

diploid basic species (*B. nigra*, *B. oleracea* and *B. rapa*), and three allotetraploids (*B. carinata*, *B. juncea* and *B. napus*) derived from them (DoE, 1994) through cross breeding in the course of evolution, as illustrated in the triangle of U (Gill & Vear, 1966), Figure 8, below.

### 2.5.2. Hybridisation and gene flow

Methods of ovule culture and embryo rescue, developed over several decades, have extended the range of species that can be sexually hybridised with oilseed rape. There are about 100 species sufficiently related to cultivated *Brassicaceae* to be capable of experimental hybridisation with them. Many of the new hybrid combinations made in this way would not occur naturally because of the barriers within the plant which prevent normal embryo or endosperm development.

Table 4 Relative ranking of species by their ability to form hybrid progeny when crossed with *B. napus* (scale 1-9)

Source: Scheffler and Dale, 1994

Species	Status and distribution	Hybridisation		Rank
		F2 progeny produced	Backcross progeny produced	
<i>B. rapa</i> / <i>B. campestris</i> (2n = 20) Wild turnip	Locally abundant on roadsides, arable fields, waste ground. Probably native in most of Europe.	Yes	Yes	1
<i>B. juncea</i> (2n = 36) Brown mustard Indian mustard	A casual of fields, roadsides, tips and cities. Introduced to Europe.	Yes	Yes	2
<i>B. oleracea</i> (2n = 18) Wild cabbage	Probably native on Mediterranean coasts from Spain to Greece. Widely introduced elsewhere, and naturalised on sea cliffs in France, UK and Germany.	Yes	Yes	3
<i>B. nigra</i> (2n = 16) Black mustard	Sea cliffs, roadsides, fields. Probably native through most of W. Europe to Turkey and C. Europe, southern Scandinavia.	? <sup>b</sup>	Yes	5
<i>H. incana</i> / <i>B. adpressa</i> (2n = 14) Hoary mustard	Common on waste ground, railways, sand dunes. Native around the Mediterranean to the Near East. Introduced to N. Europe.	No	Yes	6
<i>R. raphanistrum</i> (2n = 18) Wild radish, Runch	Casual of fields, gardens, docks etc. Probably a native of Europe.	No	Yes	6
<i>S. arvensis</i> (2n = 18) Charlock	A very common weed of fields, riverbanks, roadsides, waste ground. Probably native in Europe.	No	No	8

While these techniques provide an important method for transferring genes that would not otherwise be accessible, the ease with which hybrids are formed when *in vitro* methods are used is not indicative of the probability of similar hybrids occurring by cross-pollination under natural conditions. The hybrids formed can, however, provide valuable information on the chromosome pairing behaviour between weakly homologous genomes, and facilitate the evaluation of hybrids between *B. napus* and related species, to assess their potential to survive and persist in nature (Scheffler & Dale, 1994). The relative ranking of species by their ability to form hybrid progeny when crossed with *B. napus* is discussed by Scheffler & Dale (1994), and is summarised in Table 4.

Research can be outlined which to date has centred on a few of the most closely related species to determine the likelihood of gene flow.

#### Hybridisation of *Brassica napus* with -

##### *Brassica rapa ssp. sylvestris*/ *Brassica campestris* (Wild turnip)

Frequencies of hybridisation between oilseed rape and the wild *B. rapa* have been reported from numerous sources (Bing *et al.*, 1991; Jørgensen & Anderson, 1994; Scott & Wilkinson, 1998). Gene flow measurements by Scott & Wilkinson (1998) from crops of *B. napus* to *B. rapa* populations growing outside the field boundaries suggest that hybridisation frequencies are low (0.4-1.5 %). In addition they found that on average less than 2 % of all hybrid seedlings survived, so that unless the transgene conferred better survival characteristics, establishment of GM *B. napus* x *B. rapa* populations would be poor and introgression of the gene into *B. rapa* populations would be very slow (Sweet *et al.*, 1999). Hybridisation frequencies appear to be much higher where *B. rapa* occurs as a weed in *B. napus* crops. Jørgensen *et al.* (1996) reported frequencies of spontaneous interspecific hybridisation between *B. napus* and *B. rapa* from various experimental field designs in Denmark. The frequency of hybrids varied significantly with experimental design, as outlined in Table 5.

Single individuals of *B. rapa* in a field of winter oilseed rape produced an average of 265 hybrids/plant (93 % of seed set). These high proportions of hybrid seed set on *B. rapa* are due to the presence of self-incompatibility genes which make *B. rapa* an obligate

Average number of seeds per pod, seed germination and frequency of spontaneous hybrids between weedy, Danish <i>B. rapa</i> and <i>B. napus</i>			
	Seeds/pod	Seed germination (%)	Hybrids (%)
<b><i>B. rapa</i> ♀</b>			
1:1 mix of <i>B. rapa</i> and <i>B. napus</i>	16.3	55	13
Single <i>B. rapa</i> plants in <i>B. napus</i> field	5.1	74	93
Natural population of <i>B. rapa</i> in <i>B. napus</i> field	-	25	60
<b><i>B. napus</i> ♂</b>			
1:1 mix of <i>B. rapa</i> and <i>B. napus</i>	23.7	100	9

Source: Jørgensen *et al.*, 1996

outcrosser. As a result, when it is isolated from other sources of *B. rapa* pollen little or no seed is set by self-pollination (Anon, 1999a), allowing *B. napus* to pollinate the isolated *B. rapa* plants. The frequency of interspecific hybrid seeds harvested from *B. rapa* in a 1:1 mixture was 13 %, though the compatible intraspecific *B. rapa* pollen was available in large quantities, suggesting that compatibility with non-self *B. rapa* pollen is higher than with *B. napus* pollen. Although there appears to be no insuperable fertility or compatibility barriers to transgene introgression from *B. napus* to *B. rapa*, introgression will not occur if F1 or BC (backcross) generations do not survive in the field (Jørgensen *et al.*, 1996). Snow & Jørgensen (1999) produced crosses between glufosinate tolerant oilseed rape and wild *B. rapa* under glasshouse conditions. Not only was herbicide tolerance transmitted to the BC<sub>3</sub> generation of *B. rapa* at an average frequency of 50 %, but the BC<sub>3</sub> generation also had pollen fertility (88-95 %) and survival levels as high as those of pure *B. rapa* plants raised in the same glasshouse. These results suggest that transgenic herbicide tolerance is capable of introgressing into populations of *B. rapa* and persisting (Snow & Jørgensen, 1999). The rates of gene spread via introgression and gene flow will depend greatly on the selection pressures exerted on the gene of interest (Scott & Wilkinson, 1998; Sweet *et al.*, 1999; Snow & Jørgensen, 1999). A critical factor affecting introgression is also whether or not the original gene is located in oilseed rape on the A (*rapa*) or C (*oleracea*) genome (Anon, 1999a). Because *B. rapa* is AA and *B. napus* is AACC (see Figure 8, triangle

of U), transgenes on the C genome may eventually be lost due to unpaired chromosomes. However, crossing over between A and C genomes can occur, so it is possible that introgression can take place from the C genome into *B. rapa* (AA), but at reduced frequencies.

Hybrid combinations successfully created with *B. rapa* as a female parent may be relevant to gene flow considerations in terms of acting as genetic bridges to other related *Brassica* species (Anon, 1999a).

*Brassica oleracea* (Wild cabbage)

Despite the inter-fertility of *B. oleracea* and *B. napus*, researchers have found crosses difficult to obtain, especially those with *B. oleracea* as the female parent. Robbelen (1966) reported producing viable hybrid plants with diploid *B. oleracea* as the female parent. The pollen obtained during this study had reduced fertility and although F<sub>2</sub> progeny were produced the seeds were often shrivelled and germinated poorly (Scheffler & Dale, 1994). Homna & Summers (1976) reported one successful cross from hundreds of hand pollinations and Chiang *et al* (1977) obtained three crosses from 6148 pollinations. Introgression is possible in theory though there are no known reports of hybrids being produced by open pollination (Scheffler & Dale, 1994).

*Brassica juncea* (Brown mustard, Indian mustard)

Spontaneous hybridisation between oilseed rape and *B. juncea* was measured in field trials by Jørgensen *et al* (1996). In the progeny from plants of the self-compatible *B. juncea* that were surrounded by oilseed rape, 3 % of interspecific hybrids were identified. Bing *et al* (1991) reported the same frequency of spontaneous hybridisation between *B. napus* and *B. juncea*, also with oilseed rape as the male parent. Crosses are known to occur in both directions though the production of hybrids with *B. napus* as female have been less successful (Jørgensen *et al*, 1998). Pollen fertility was low (0.28 %), although the *B. napus* markers were all transferred to the first backcross generation with *B. juncea* (Jørgensen, 1999).

*Brassica nigra* (Black mustard)

Under controlled conditions set by Bing *et al* (1996) 100 pollinated stigmas produced only one interspecific hybrid in the cross *B. napus* x *B. nigra*, while 1000 stigmas were pollinated to produce one interspecific hybrid in the reciprocal cross. Seeds from *B. napus* and *B. nigra* were also planted in

the field following their harvest from a co-cultivation plot in an experiment by Bing *et al* (1996). No true hybrid was identified between these two species, and similarly in the reciprocal cross no hybrid was identified in glyphosate resistant tests from a co-cultivation experiment. The authors concluded that given the very low levels of hybridisation obtained under controlled conditions, gene escape from *B. napus* to *B. nigra* under open pollination conditions in the field seems unlikely.

*Sinapis arvensis* (Charlock, Wild mustard)

*S. arvensis* and *B. napus* are generally not considered to be sexually compatible because the majority of studies have found embryo rescue or ovule culture to be the only methods of successfully effecting hybridisation. In five years of studies of *S. arvensis* growing in and around GM rape crops in the UK, Sweet *et al* (1997) and Norris *et al* (unpublished data) failed to detect any occurrence of hybridisation in *S. arvensis* under natural conditions, despite the many locations studied and the different spatial distributions of the wild species.

In co-cultivation experiments by Bing *et al* (1996) a *S. arvensis* female failed to support the development of intergeneric hybrid seed in more than 7500 seeds examined, regardless of pollination direction. Lefol *et al* (1996a) detected successful hybridisations only when male sterile oilseed rape was used as the female. During this study, six hybrids were obtained from 50 000 flowers. Chevre *et al* (1996) obtained 0.18 hybrid seeds per 100 flowers under open pollination conditions, again using *S. arvensis* as the pollinator. Hybridisation was not detected with *B. napus* as the pollinator. Results of similar trials by Moyes *et al* (1999) with seed samples from populations of *S. arvensis* in the UK and France are still awaiting publication. Downey (1999) concludes that from the data available so far there appears to be general agreement that natural gene flow is not likely to occur between *B. napus* and *S. arvensis*.

*Raphanus raphanistrum* (Wild radish; Runch)

In an experiment by Darmency *et al* (1998) herbicide tolerant oilseed rape was mixed with *R. raphanistrum* at different densities. Hybridisation was detected at a rate of 1:625, wild radish: oilseed rape, interpreted as 0.2 % of the seeds derived from wild radish were intergeneric hybrids. In backcrossing the hybrids no stable integration of oilseed rape genetic material into the genome of



wild radish was observed. In a similar experiment by Chevre *et al* (2000) one hectare of Synergy glufosinate tolerant oilseed rape was sown and wild radish plants were transplanted at different densities within the field, in the border of the field and in the field margin, both in groups of plants and as isolated plants. Wild radish was harvested plant by plant and the seedlings obtained from the seeds were herbicide treated either under greenhouse or field conditions. They found that in experiments where *R. raphanistrum* was the female, a very low frequency of hybrid production was seen. Among the total of 189,420 seedlings treated, only one hybrid at  $2n=37$  (ACRrRr) conferred glufosinate tolerance. In a programme monitoring gene flow from GM oilseed rape crops in the UK, natural populations of *R. raphanistrum* growing near fields of herbicide tolerant oilseed rape were sampled over a period of five years. No evidence of hybridisation between *B. napus* and *R. raphanistrum* was observed during this period (Sweet & Shepperson, 1996; Norris, pers. communication).

Darmency *et al* (1998) obtained 45 intergeneric hybrids from each *B. napus* plant when male sterile oilseed rape functioned as the mother. Grown in mixtures with wild radish each hybrid produced less than one backcross plant, but fertility improved in subsequent backcross generations with the wild parent. However, no stable integration of *B. napus* genetic material into the genome of *R. raphanistrum* was observed (Jørgensen, 1999).

*Hirschfeldia incana/B. adpressa* (Hoary mustard)  
Spontaneous hybridisation is known to occur in this species, although where *H. incana* is the female, low frequencies of hybridisation have been observed. When herbicide resistant oilseed rape was mixed with *H. incana* at a density of 1:625, *H. incana*: *B. napus*, 1.5 % of the *H. incana* seeds germinated were found to be hybrids (Lefol *et al*, 1996b). However, where male sterile oilseed rape was used as the female parent in a 1:1 ratio, 70 % of the seeds were hybrids (Lefol *et al*, 1996b). Chevre *et al* (1999) concluded that the hybrids were as vigorous as, if not more competitive than the weed although fertility was low at 0.2 seeds per plant on average. Darmency and Fleury (1999) reported an average spontaneous hybridisation rate of 0.6 hybrids per plant when GM oilseed rape was the pollinator. However they reported that back crossing the interspecific hybrids to *H. incana* for 5 generations produced non

viable plants so that introgression of the transgene was not achieved.

### Hybridisation of *B. rapa* (Turnip rape) with -

*B. rapa ssp. sylvestris/ Brassica campestris*  
(Wild turnip)

GM herbicide tolerant turnip rape is widely grown in Canada, where Raney & Falk (1998) recorded pollen from a small (0.4ha) block of high erucic acid turnip rape at distances up to 260 m from the crop. The weedy form of *B. rapa* does not occur in Canada, but cultivation of GM turnip rape in northern Europe where populations of wild *B. rapa* exist is likely to result in gene flow between these individuals.

#### 2.5.3. Possible consequences of gene flow

The previous section has considered the potential frequency and occurrence of gene flow and interspecific hybridisation between *B. napus* and its wild relatives. Clearly, some introgression into wild populations will occur. Therefore it is also necessary to consider the impact of the transgene after dispersal and introgression into wild species or populations. Many crop-weed comparisons show that plants can evolve into invasive genotypes based on a few gene polymorphisms (Hoffman, 1990). Herbicide resistance in many cases can be achieved by a single gene (Schulz *et al*, 1990), though gene expression varies with genetic background, due to epistasis, linkage and pleiotropy. Therefore it can be difficult to predict how the genetically engineered gene(s) will be expressed in a related wild species (Tiedje *et al*, 1989). Harding & Harris (1994) consider a hypothetical possibility of the long-term creation of a herbicide tolerant, competent weed, for example by introgression into *S. arvensis* of herbicide tolerant genes. *S. arvensis* is already a serious weed in some oilseed rape crops where it can make the seed product unusable for processing (Knott, 1990). Some researchers believe that producing GM herbicide resistant crops will ultimately lead to an increase in herbicide use (Hoffman, 1990; Ellstrand & Hoffman, 1990; Williamson, 1991). Downey (1999) believes that if a herbicide tolerant gene were to escape into a weedy relative, that weed would be no more difficult to control than before the broad spectrum herbicide became available, and furthermore that the trait would not confer an advantage to the plant unless the specific herbicide was applied and normal competition eliminated. It has been suggested that the introgression of transgenes conferring enhanced fitness

characters such as pest or disease resistance into wild plants may make them more competitive or invasive in natural habitats. The fitness of wild relatives containing introgressed genes from oilseed rape will depend on both the genes introgressed and the recipient ecosystem (Jørgensen, 1999).

## 2.6. Conclusion

Although variable levels of outcrossing between oilseed rape crops have been reported some conclusions can be drawn. The bulk of cross-pollination has been shown to occur over very short distances, and successful pollination appears to decline exponentially with distance from the pollen source, the pollen only very occasionally traversing several hundred metres (Gray & Raybould, 1998). Conclusions drawn from the studies on pollen dispersal by Timmons *et al* (1995) and Thompson *et al* (1999) indicate that at the farm scale very low levels of gene flow will occur at long distances and thus complete genetic isolation will be difficult to maintain. Gene flow will also occur to and from volunteers and feral populations, acting as gene pools carrying over the contamination into subsequent rape crops. Models of cropping systems on a regional level by Colbach *et al* (1999) have shown that growing a GM rape crop in one field on a farm can lead to the development of GM rape seed banks and volunteer populations in other parts of the farm. Under current farm practices, local contamination between crops is inevitable, albeit at low and variable levels (Anon, 1999a). While pollen is important in the spatial dispersal of transgenes from oilseed rape, it has a short life-span and provides little temporal dispersal. Seed is also very important in spatial dispersal of transgenes as has been shown by studies of dispersal from farm to farm in harvesting machines (Sweet *et al*, 1997), along transport corridors (Crawley & Brown, 1995) and by reports of Canadian seed being introduced into Europe. Seed also provides temporal dispersal in that it has longevity of several years in the seedbank and thus can provide sources of GM plants for many years after a GM crop has been grown. Segregation of GM rape crops from non-GM therefore has to take account of whether GM crops were grown previously in a field and whether farm practices were likely to have moved seed between fields.

Management systems can be used to minimise GM rape seed spread on a farm and to

minimise seed bank and volunteer populations. Spatial separation and isolation of crops can be used to restrict cross-pollination in any one growing season. In his report Ingram (2000) proposed isolation distances of 100 m for conventional fully fertile varieties of oilseed rape. However it is now apparent that varieties and lines containing male sterile components will outcross with neighbouring fully fertile GM rape at higher frequencies and at greater distances. The contamination of certain seed crops of hybrid spring oilseed rape varieties in Canada occurred because effective isolation of male sterile parent lines was not achieved (Button, pers. communication). In addition it is clear from the results of work at NIAB (Simpson *et al*, 1999) that varietal associations such as Synergy will require considerably greater isolation distances from GM crops than conventional varieties. Ingram (2000) proposed 100 m isolation to achieve 1 % contamination and was not able to state a distance likely to give contamination levels below 0.5 % in varietal associations.

It is not possible to make simple statements to describe the precise limits of sexual barriers between *B. napus* and its related species, or the likelihood of hybrids forming and persisting in agricultural and natural habitats (Scheffler & Dale, 1994). Regardless of variation in sexual compatibility and selection, variation in gene flow among crop relative populations might cause some transgenes to spread and become common very quickly, whereas others might remain rare and be confined to areas very close to the source crop. Raybould & Clarke (1999) believe that because transgenes are inherited in a mendelian way, gene flow among non-transgenic natural populations is an 'appropriate biological model' (Gliddon, 1994) for predicting the movement of transgenes within and among populations of crop relatives. Research of this kind is being conducted in several European countries to determine how *B. napus* transgenes will behave in natural crucifer populations. Until this research has been carried out the risk assessment of gene flow must take into account the specific trait introduced (e.g. herbicide resistance, oil quality), the biology of the plant (self-pollination or cross-pollination, seed dormancy) and the agricultural context (cropping systems, spatial organisation of the crops) (Champolivier *et al*, 1999).

### 3. Sugar beet and fodder beet (*Beta vulgaris* ssp. *vulgaris*)

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) belongs to the Chenopodiaceae, a comparatively small and distinct family. Beets have been grown as vegetables for many years but it is only in the 20<sup>th</sup> century that sugar beet has become one of the most important crops of the cool temperate regions.

#### 3.1. Reproductive biology and crop use

Cultivated beet exists in diploid, triploid and tetraploid forms and is harvested for the conical shaped roots it produces at the end of the first growing season. The root crop is purely vegetative and therefore does not depend on a flowering and pollination period for its formation. Because commercial varieties of beet are biennial they do not usually flower within the harvesting regime for root production so that very little pollen and seed is produced. Sugar beet seed crops are grown in southern Europe to supply seed for the north European root crop. The biennial seed crop flowers in its second season, producing large amounts of pollen. By contrast, in the root crop only a small proportion of plants flower (bolt) in their first year (typically less than 1 % of the crop) and may set seed which is shed to the soil. These 'bolters' can arise due to early sowing and/or cold weather after sowing which cause vernalisation of some of the plants, or because of pollen contamination of the seed crop by annual beets. It is probably from this source, and from volunteer or 'ground-keeper' beets remaining after harvest, that the population of annual beets which constitute the 'weed beet' population arose (Hornsey & Arnold, 1979). Encouraged by changing cultural methods, notably increased scale and mechanisation (Longden, 1974), weed beet became a problem throughout several European countries, including Britain, in the late 1970's (DoE, 1994) (See Section 3.5).

#### 3.2. Genetic modification

Research to date on transgenic sugar and fodder beet has centred on developing herbicide and virus resistance. Beet is a broadleaved crop that establishes slowly and thus has poor tolerance to weed competi-

tion. It is also sensitive to many of the available herbicides, so that repeated low dose rates are often applied to give effective weed control. This is expensive, and poor weather conditions often further reduce the effectiveness of the herbicide treatment. Annual beet cannot be controlled by the selective herbicides used on beet crops and are removed by hoeing or pulling by hand or with glyphosate wicks. The development of herbicide tolerant sugar beet allows herbicides to be used to control weed beet efficiently for the first time and is therefore an attractive prospect for growers.

There are two important virus borne diseases in beet at present for which transgenic resistance is being developed. The first is rhizomania, a disease caused by beet necrotic yellow vein virus (BNYVV) which is soil borne. The second is virus yellows caused by two viruses, beet yellows virus and beet mild yellowing virus, which are spread by aphids. BNYVV resistance has been incorporated into beet varieties from wild beet sources using traditional breeding methods, and although this confers effective control there is interest in developing virus resistant sugar beet plants expressing the viral coat protein. Plants expressing coat protein genes offer a better alternative for the introduction of BNYVV resistance into new breeding lines, and active research is under way within seed companies.

Another possibility is to introduce insect resistance genes into sugar beet in order to control aphid vectors (DoE, 1994) of, for example, virus yellows. However research on transgenes for aphid control is at a very preliminary stage.

#### 3.3. Pollen dispersal

Sugar and fodder beet are both wind and insect pollinated although it is generally acknowledged that wind plays the most important role in its pollen dispersal. Work on pollination of sugar beet carried out in seed production fields where high densities of flowering plants occur, has concluded that wind is the main vector of pollen (Vigouroux *et al.*, 1999). Continuous monitoring by Scott (1970) of a pollen trap located 230 m east of

a sugar beet crop recorded 36 pollen grains/ $\text{m}^3$ , 0.85 % of the average of 4208 pollen grains/ $\text{m}^3$  caught in a trap in the centre of the crop. Both traps were operated at a height of 1.5m above ground, indicating that the potential for sugar beet pollen to become airborne, and remain airborne over considerable distances, is high. Appropriate atmospheric conditions combined with peak pollen release times (Scott, 1970 found an hourly concentration of 50000 grains/ $\text{m}^3$ ) can account for long distance dispersal. Reheul (1987) recorded pollen dispersal by wind over distances of more than 1000 m. These figures represent potential pollen dispersal and it must be considered that by the time of final deposition a lesser proportion will be viable. According to Scott (1970) beet pollen can remain viable for 50 days when stored cold and dry, but does not survive wetting by dew or usually remain viable for more than a day (Treu & Emberlin, 2000).

Vigouroux *et al.* (1999) found that some insects have a significant role in pollen transfer from sugar beet. The flowers are visited by a large range of insect species but in Britain rarely by bees (Bateman, 1947b). Tetraploid plants produce fewer and larger pollen grains than diploid and the pollen is less readily released from the anthers (Scott & Longden, 1970). Insect pollination is therefore probably more important in root than seed crops, where there is a lower density of pollen donors.

### 3.4. Gene flow: Crop to crop

#### 3.4.1. Hybridisation and gene flow

*Beta vulgaris* comprises several cultivated forms of *B. vulgaris* ssp. *vulgaris*, including leaf beets (var. *cicla*; spinach beet, seakale beet, swiss chard) and root beets (var. *esculenta*; beetroot, mangles, fodder beets and sugar beets). With the exception of the differences in ploidy between the normal diploids ( $2n=18$ ) and the artificially produced tetraploids all of the above varieties are highly interfertile and can be considered as potential source/recipients of beet pollen (Treu & Emberlin, 2000), although some varieties may be incompatible due to differences in flowering time (Bateman, 1947a).

Jensen & Bogh (1941) recorded outcrossing levels between mangels (fodder beet) and sugar beet of 0.42 % at 400 m, with levels falling gradually to 0.11 % at 600 m and 0.12 % at 800 m, typical of the leptokurtic

pollen concentration distribution. Dark (1971) used a genetical trapping system with red beet as a point source pollinator in normal sugar beet crops. Eight red beet plants were planted at the centre of a sugar beet seed production crop. At harvest time seed samples were collected at 9m intervals in 10 directions radiating from the point source and the percentage of red seedlings produced was obtained. Overall Dark (1971) recorded comparatively little cross-pollination in the crop, the maximum measuring 5 % in the adjacent downwind plants, falling to 0.3 % at 90 m and 0.1 % at 180 m.

A second experiment by Dark (1971) consisted of a sugar beet seed crop with a 37m long single line of red beet stecklings planted upwind (for the mean seasonal wind direction) from the crop. The red beet plants simulated a wave of contaminant pollen into the receiving crop, which was divided into plots for sampling. Seed samples were harvested and the percentage contamination for each plot was calculated. The separate contamination figures for each plot showed that most of the red beet pollen was absorbed in the windward 4m strip of plots. Dark (1971) concluded that removing this 4m windward strip would remove 73 % of the contamination. Removing the whole perimeter row would further halve the contamination. (See Figure 9: Histogram to show % contamination of red beet pollen for each plot of sugar beet).

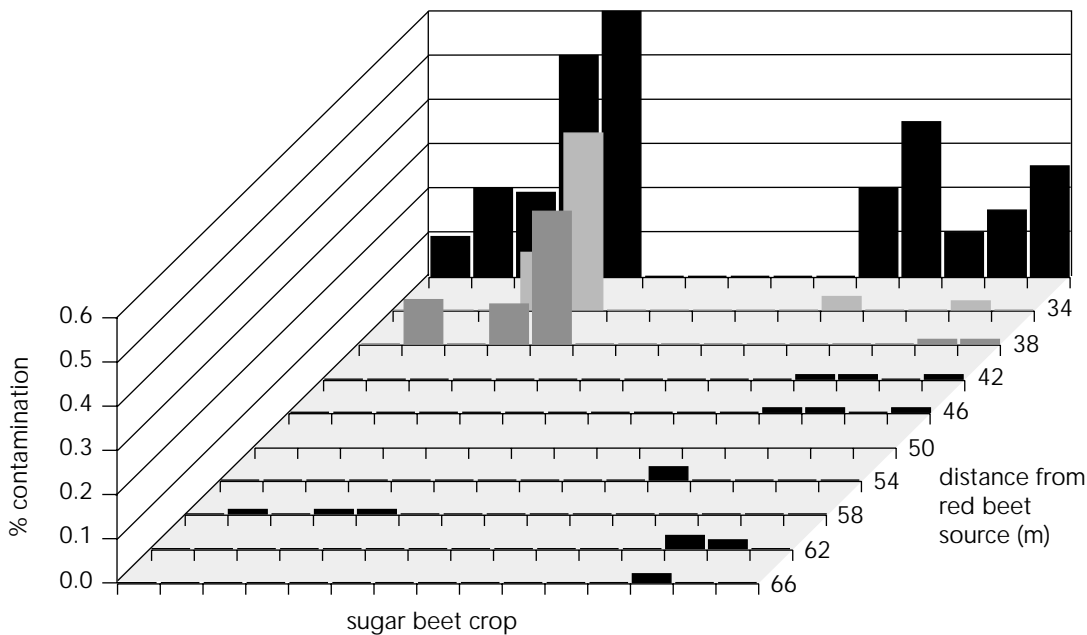
#### 3.4.2. Possible consequences of gene flow

Pollen flow between bolters in GM and conventional root crops would not result in harvested roots becoming genetically modified in the year of cultivation because the yield is vegetative. However the seed produced by these bolters could result in GM annual weed beet occurring in the next root crop. The risk of contamination then exists if the weed beet is inadvertently harvested and processed along with the surrounding non-GM crop. Moreover, with a product such as refined sugar, which contains neither nucleic acid nor protein from the source plant, there is no analytical way of detecting whether it is derived from a GM or non-GM crop, or if gene flow has occurred in the crop. The consumer's 'right to know' is therefore compromised and may be satisfied only by an elaborate and costly system of traceability (Squire *et al.*, 1999).

Further indirect risks of GM contamination in conventional root crops exist in the process of seed production. Because high

A graph reproduced from Dark (1971) showing the % contamination for each plot in a sugar beet plot from a row of flowering red beet

Figure 9



densities of flowering plants occur in seed production crops the risk of crop-to-crop gene flow is much higher. Thus pollination between crops or between the crop and GM weed beet could result in seed that is produced as non-GM becoming contaminated with GM seed and the resulting stock sown for root crops. Isolation distances of 1000 m exist for GM beet seed production to prevent this type of contamination although complete isolation cannot be guaranteed in the long term.

### 3.5. Definition and status as a weed plant

Many plant species can be found both as crop and weed types (Harlan, 1987). This situation can be due to the evolution of a cultivated type to a weed type, the selection of cultivated forms from weedy races (Pickersgill, 1981) or a change of habitat of a wild form closely related to a cultivated species (Keller, 1989). In all these cases, this phenomenon involves evolution from a cultivated or a wild type to a weed type (Boudry *et al*, 1993). With regard to beet Desplanque *et al* (1999) have defined these weed types on the basis of habitat as ruderal and weed beet. He described ruderal beet as being found on disturbed habitats such as waste ground and road verges in regions of inland France, although their origins are not entirely clear. Similar populations occur in

other sugar beet growing regions of Europe (Hornsey & Arnold, 1979).

Weed beet can be a serious problem in sugar beet fields. The main phenotypic difference between cultivated and weed beets is their annual nature and ability to flower in their first season whereas cultivated beets are biennial and do not normally flower until after a period of vernalisation. The vernalisation requirement is controlled by the one-locus, two-allele, B-gene. Weed beets have been shown to contain the dominant B-allele that gives them an annual life history, enabling them to reproduce without vernalisation in the disturbed cultivated environment.

Vigouroux (pers. comm.) measured maximum distance gene flow between bolters in a GM beet crop and naturally occurring weed beet. Levels of hybridisation were gained by collecting seed from the weed beet progeny found in set-aside near the crop. The seed was sown and the numbers of seedlings showing herbicide tolerance counted. These data are presented in Table 6.

In concurrence with Vigouroux's findings, Boudry *et al* (1999) found that consistent gene flow between cultivated beet and weed beets takes place in seed production areas, and that gene exchange still occurs in this way despite the great care of seed produc-

Table 6

Levels of hybridisation between bolters in a GM beet crop and naturally occurring weed beet

Years	Distance (m) from GM beet field to flowering weed beet	No. of flowering weed beet progeny in the field	% of hybrid seedlings with herbicide tolerance
1996	36	99	0.2
1997	27	20	5.9
1998	29	66	2.4

Source: Vigouroux (pers. comm.)

tion companies. This type of gene flow can be important for potential GM beet hybridisation and introgression into wild beet populations, and is discussed further in Section 3.6.

### 3.6. Gene flow: Crop to wild relative

#### 3.6.1. Compatibility and distribution

Wild sea beet (*Beta vulgaris* ssp. *maritima*) occurs naturally in the coastal areas of the eastern Mediterranean and has spread to coastal areas of western and central Europe. Sugar beet is a relatively recently developed crop, having origins from fodder beet dating back only 200 years (Treu & Emberlin, 2000). All modern sugar beet are thought to originate from a single cultivar and it has been variously suggested that spontaneous hybridisation with sea beet has contributed to the genetic diversity of sugar beet (Bock, 1986). Cultivated beets (sugar beet, fodder beet, red beet and Swiss chard: *Beta vulgaris* ssp. *vulgaris*) and weed beets are cross compatible with the wild sea beet and thus there is a high likelihood of gene flow. Aspects of the floral biology of the crop and its wild relative affect the likelihood of transfer of modified genetic material. Both cultivated beet and sea beet are obligately outcrossing species (Scott, 1970), a factor which enhances the possibility of gene flow.

#### 3.6.2. Hybridisation and gene flow

As cultivated beet is propagated from seed, separate areas for seed production are required and it is in these areas that the potential for hybridisation and introgression between crop, weed or wild beets is high (Treu & Emberlin, 2000). This may have occurred especially in south European seed production of triploid monogerm varieties, where the male-sterile diploids used as mother plants are particularly susceptible to

pollination by diploid wild plants rather than the intended tetraploid pollinators (Scott & Longden, 1970). The product of this hybridisation would be a diploid weed beet with a high tendency to behave as an annual and flower in the season it is sown, producing viable pollen and seeds. If the seed crop were genetically modified such contamination would result in the occurrence of GM weed beet in commercial crops.

A study by Bartsch *et al* (1999) examined how gene flow and introgression from cultivated sugar beet to sea beet has impacted the genetic diversity of affected wild populations. Allozymes were used to characterise the genetic variation within accessions of wild sea beet adjacent to the sugar beet seed production region of north-eastern Italy. The genetic relationship of these accessions and groups were then assessed. Substantial genetic evidence for gene flow from domesticated sugar beet seed production fields into nearby wild sea beet populations was identified. Two alleles that are common in cultivars, but otherwise typically rare in wild beets, were found in unusually high frequencies in the natural populations. The results of this study are supported with previous research by Bartsch & Schmidt (1997) which reported that these wild populations had a substantial number of individuals displaying morphological traits that are common in cultivars but typically rare in the wild subspecies (Bartsch *et al*, 1999).

In California, McFarlane (1975) traced a serious weed beet problem specifically to hybridisation between sugar beet and annual wild beet *Beta macrocarpa* during a year when flowering in the two species, normally at widely different times, was concurrent. Later work in the same area by Bartsch *et al* (1999) was undertaken in 1998 when a flowering time overlap was again identified. Introgression could be detected at a rate of 2 % of wild beet individuals (13 of 594 examined Californian plants) which were morphologically similar to *B. macrocarpa*, but had isozyme alleles specific to *B. vulgaris*. They concluded by stating that genetically modified cultivars grown in some areas of California would have a high probability of gene escape to wild relatives especially if their bolting properties were similar or higher than tendencies of the current cultivars planted in that area.

#### 3.6.3. Possible consequences of gene flow

The history of hybridisation and introgression between cultivated beet and wild

beet suggests that, when grown on a commercial scale, escape of transgenes from the crop is likely. Even without hybridisation the transgene may be able to persist in weed beets derived from bolters or volunteers which produce GM seeds and a future weed beet population. Hybridisation of these plants or of wild beet with the crop and selection under cultivation could produce annual weed beets containing the transgene. Thus transfer of the modified gene from crop to weed and ruderal beet is entirely plausible (DoE, 1994). Bartsch *et al* (1999) showed that transfer of the transgene from sugar beet (or other cultivated *B. vulgaris*) to sea beet is also possible as hybridisation between the two has occurred in the past.

The study cited earlier in this section by Bartsch *et al* (1999) indicated how the genetic variation of sea beet populations can be affected by gene flow from sugar beet. There were concerns that crop to wild species gene flow would cause a decrease in genetic variation in wild populations. However, they found, for most parameters, a slight increase in genetic variation in the Italian wild beet populations compared to their counterparts from elsewhere in the range of the wild subspecies. Bartsch *et al* (1999) suggested several reasons why gene flow from the crop had not lead to the erosion of genetic diversity in this particular system. First, although most crops examined have low genetic diversity compared to their wild relatives (Ladizinsky, 1985, Doebley, 1989), beet cultivars typically hold a level of genetic diversity roughly equivalent to that of their wild progenitor. In fact, allozyme diversity of sugar beet cultivars is substantially higher than that of the wild beet accessions. Thus, if evolution via gene flow from sugar beet continued to equilibrium, diversity would be expected to increase to the level of the crop.

Second, sea beets in Italy have received gene flow from many cultivars over the last century as new varieties have emerged (van Geyt *et al*, 1990). If each new cultivar contained less variation than the previous one, we might expect erosion in diversity of the wild populations receiving gene flow. But if the new cultivars were well-differentiated, we might see at least a temporary accumulation of different alleles in the wild populations. A further explanation is that in this particular system, sea beets received gene flow from both sugar beet and red beet. These different cultivars are genetically distinct, so a population receiving gene flow from two

well-differentiated sources would be expected to evolve more diversity than one receiving gene flow from a single source.

Overall this demonstrates that in the case of these populations of sugar beet and sea beet, a century of crop to wild species gene flow has had limited evolutionary effect on the wild populations. However, Bartsch *et al* (1999) consider that certain transgenes (e.g. those conferring biotic and abiotic stress tolerance) may be more likely to alter the fitness of hybrid or introgressed individuals than supposedly neutral alleles like allozymes, and therefore the introgression of transgenes into wild populations may change their niche relationships. Given that crop alleles apparently move with ease into populations of sea beets in north-eastern Italy, Bartsch *et al* (1999) recommends that populations should be monitored after seed production of GM beets begins in that region.

Raybould & Mogg (1999) investigated the genetic structure of populations of sea beet on the south-west coast of England. A high degree of substructure was found within populations, which is thought to be due to repeated, very localised extinction and recolonisation events (Levins, 1970). The high degree of substructure was found to have an impact on estimating gene flow between populations, and hence on the spread of transgenes on a wider scale. However, the results suggested that above the scale of the individual populations (say 1-20 km), the rate of migration of a transgene from one population to another will be inversely proportional to the distance between the populations. In other words, isolation by distance may be more important than founder effects in determining the population structure at this scale. They imply also that above a certain distance gene flow will be insufficient in itself to maintain the transgene in the recipient population because of potential loss through drift. However, predicting the rate of decline of gene flow with increasing distance at which the gene flow becomes negligible is difficult (Raybould & Mogg, 1999).

It is also important to consider what the agricultural consequences of the transfer of genetic traits, such as herbicide tolerance, into weed and wild beet populations might be. A glyphosate-resistant weed could presumably be easily removed from other rotational crops such as cereals by a selective herbicide, but could provide a serious sugar beet weed problem in herbicide tolerant

sugar beet crops. The weed could only be removed by growing a different herbicide tolerant beet or by mechanical forms of weed control.

The effects of the transfer of resistance to virus infections are more complex to model. Resistance or tolerance of rhizomania and virus yellows is known to occur in wild beet (*B. vulgaris sensu lato*) although there is no knowledge of its frequency in wild populations. The spread of virus resistance genes into the wild population could have beneficial effects on the crop by removing susceptible plants that provide a source of future infection (DoE, 1994). Alternatively, increasing the exposure of a virus to the resistance genes may enhance the possibility of the virus developing new strains that overcome the resistance. Henry *et al* (1995) consider that GM virus tolerance in weeds may increase weediness through increased vigour, and thus, for instance, the number of seeds it produces or its potential for overwintering successfully. However, it is unlikely that transgenic virus resistance would confer more weediness than conventional resistance and there is no evidence that these currently cause significant problems in agriculture (Henry *et al*, 1995). Cooper (1999) further discusses the ecological significance of virus resistance genes in wild plant populations.

### 3.7. Conclusion

The proportion of bolting plants in sugar beet root crops is typically less than 1 % and therefore the chances of widespread pollen mediated gene flow between root crops are minimal. The frequency of bolting could be further reduced through careful screening of GM varieties to ensure they are not susceptible to vernalisation, by later sowing, and by removal of bolters from the crop. Although seed quality might be considered an issue, it is generally rare for seed beet to be contaminated with bolters and weed beet. In 1991, 66 % of UK sugar beet seed producers effectively controlled bolted beets, mainly by hoeing as selective herbicides cannot be used (Longden, 1993). However there are a minority of beet crops in which a high number of bolters occur and farmers are often unable to provide the resources required for effective control, causing a perpetuation of the problem.

Cultivated beet is highly interfertile with a number of agricultural variants also classi-

fied within *Beta vulgaris ssp. vulgaris*, and is highly interfertile with weed, feral and wild sea beet.

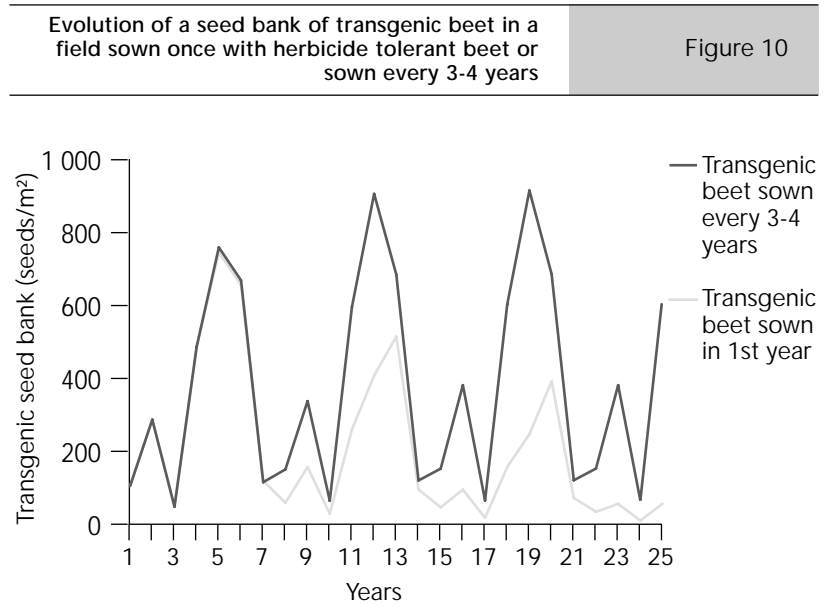
The seed production areas of Europe present a high risk for gene flow from cultivated beet to wild beet. Bartsch & Schmidt (1997) recommend that if the use of transgenic cultivars is inevitable in breeding districts, the use of transgenic male-sterile mother plants for seed production would minimize the gene flow to wild beet populations. In addressing the problem of gene flow between cultivated beet and weed beet, Boudry *et al* (1999) similarly concluded that the incorporation of the transgene for, e.g. herbicide resistance, into the diploid male sterile mother plants would be the most logical scenario because they produce little or no pollen. However, the authors point out that each contamination by wild pollen carrying the B-gene will directly lead to the formation of hybrids that contain both the B-gene and the resistance transgene. These hybrids are likely to form annual weed beet populations resistant to the herbicide in the sugar production areas, if the farmer fails to eliminate contaminating plants. Transgenic sugar beet can offer some agronomic benefits, but weed beet problems might be exacerbated when resistant genotypes containing the B-allele appear (Boudry *et al*, 1999).

GM beet seed of varieties containing fitness genes that could affect the ecology or genetic diversity of wild beet should be produced in selected areas away from the coast in southern Europe and in areas where inland wild beets are absent. Appropriate isolation management should be adopted with the transgenic cultivars to minimise and manage gene escape (Rubin, 1991). This management should be practised in both crop and seed production areas, especially as the latter are frequently neglected in present regulatory policies concerning transgenic crops (Boudry *et al*, 1999). Isolation management could incorporate the use of barrier crops to absorb pollen as demonstrated by Dark (1971) (see Figure 9), as well as extensive isolation distances between beet crops and wild relatives as shown in the work of Archimowitsch (1949) and Dark (1971).

In the case of beet it is also appropriate to emphasise the importance of indirect gene flow between populations of cultivated, weed and wild beet through seeds. A study by Delanoy *et al*. (2000) to model the effects of gene flow from transgenic herbicide tolerant



(HT) beet to weedy beets found that beet seed persists in the soil for a considerable length of time. Figure 10 illustrates this by plotting the evolution of a seed bank of transgenic beet in a field sown once with herbicide tolerant beet and a field sown every 3-4 years with herbicide tolerant beet. The graph shows that with a single cultivation of GM beet the seed bank begins to deplete over 15-20 years, but if, more realistically, the beet crop is rotated regularly the seed bank is maintained at a high level. Other types of cultivation (e.g. set-aside, barley) used in the four year rotation are also important in affecting the probability of the development of weedy beet populations. Further work is required to establish suitable cultivation types that can be used together to minimise the effects of the cropping system which may increase the possibility of gene flow from transgenic beet to weed beets.



Source: Delaney *et al*, 2000

## 4. Potato (*Solanum tuberosum*)

Potato (*Solanum tuberosum*) belongs to the order *Solanaceae*, which embraces a large number of plants chiefly found in tropical and sub-tropical regions. The crop was introduced into Spain from South America in around 1570 and is now cultivated world wide except for the lowland tropics (DoE, 1994).

### 4.1. Reproductive biology and crop use

Potato is a perennial grown annually from vegetative tubers, known as seed tubers or seed potatoes, which can persist in the soil when the plant dies back each autumn. Under European conditions the tubers persist poorly in cold wet soils and plants rapidly become infected with a range of fungal and viral diseases, which is why the crop is grown as an annual. Plants raised from seed tubers show the same features as those raised from seed with the exception that there is no tap-root, the progeny tubers are much larger, and the yield is greater. Before the tuber can produce new plants it must undergo a period of rest, and since the planting of tubers of any variety merely extends the life of that variety from year to year, it is almost impossible to raise new varieties in this way (Paterson, 1925).

The seedlings of potatoes grown from true seed develop branch shoots that grow into the soil in a horizontal direction forming underground stems or runners. The latter, which are usually short, bear rudimentary leaves called 'scale leaves', in the axils of which originate branch runners. After the formation of internodes both the branch and the main runners become swollen to form tubers. Tubers from seedling plants are usually very small, but through vegetative propagation for four or five years they gradually attain their normal size (Paterson, 1925). When the plant has attained its full size, flowers may be produced. The production of true seed is practised in breeding programmes and requires skilled manipulation of variables, such as degree and duration of flowering, to be successful (Treu & Emberlin, 2000). Potato cultivars grown in the UK are tetraploid ( $2n=48$ ) although the breeding programme is carried out largely at the diploid level where dihaploids derived

from anther culture and protoplast fusion can be combined to produce tetraploids with combinations of desirable traits (DoE, 1994). Successful flower initiation and development is frequently influenced by environmental fluctuations in, for example, light intensity and temperature. In the context of true seed production, the degree to which flowering occurs, the duration of flowering and the response of flowering behaviour to environmental conditions are greatly influenced by cultivar. Flower development does not ensure fruit set, and pollen sterility is frequently encountered (Anon, 1996) under field conditions in parts of Europe.

Potatoes are predominantly used for direct human consumption today. They are grown extensively as an important part of the diet in many geographical areas. A significant proportion of potatoes grown are also used for animal feed and for starch production (van Aken, 1999). The EU is currently the largest producer of potatoes in the world before China and Russia.

### 4.2. Genetic modification

As with other members of the order *Solanaceae*, such as tomato (*Lycopersicon esculentum*) and tobacco (*Nicotiana tabacum*), the species has proved to be amenable to transformation and regeneration. The advent of technological means to insert foreign genes into potato has increased the options for variety development. There are two main advantages, the first being that it has been possible to add traits to popular varieties. The fact that it is virtually impossible to recover parental phenotype in sexual breeding of potato lends great appeal to this feature of gene insertion. A second benefit is the enlargement of genetic variation beyond that which is available within *Solanum* species. While traditional breeding relied upon crossing and backcrossing to introduce new traits to the breeding pool, gene technology introduces one or a few genes from a range of different organisms that are expressed dominantly to achieve a phenotype. (Brown, 1995).

Targets for genetic manipulation in the potato include; fungal resistance; bacterial

resistance; insect and nematode resistance; herbicide resistance; altered starch content; stress resistance and anti-bruise genes.

#### 4.3. Pollen dispersal

The extent of pollen dispersal in potato is related to the species of insect pollinator concerned, weather conditions and the fertility of the cultivar (Treu & Emberlin, 2000). Potato produces no nectar so honeybees are not usually attracted to the flowers. Research has shown bumblebees to be effective pollinators though evidence suggests that they visit plants at the edges of plots more often than the centre of plots in some species (Free & Butler, 1959).

McPartlan & Dale (1994) tested for evidence of bee activity during field experiments to measure outcrossing levels. They recorded the highest levels of berry formation at the edge of the central plot, compared with the centre, suggesting that bumblebee activity was a contributing factor to pollination. The bumblebee is an example of a pollinator which moves only short distances between flowers so the majority of pollen is deposited in the immediate surroundings of the pollen source (Skogsmyr, 1994).

Wind is considered a more important vector than insects in effecting pollination. Potato is mainly self-pollinating, with estimates of the rates of cross-pollination under field conditions ranging from 0 to about 20 % (Plaisted, 1980), although studies suggest that there is generally limited potential for cross-pollination. Results of a field trial experiment by Tynan *et al* (1990) showed minimal dispersal of transgenic pollen beyond the immediate vicinity of the potato field trial. These results are displayed in Table 7.

In a study by Skogsmyr (1994) carried out in New Zealand, potato pollen was transmitted both over larger distances (of up to 1000 m) and to a higher degree than was demonstrated by Tynan *et al* (1990). The pollen beetle was thought to be attributable for this. The foraging habits of these beetles mean that they emigrate from a patch in large numbers and often fly over large distances (Skogsmyr, 1994). This research was scrutinised by Conner and Dale (1996) who concluded that there had been a large number of false positive results giving the impression of high levels of gene dispersal. They concluded that 20 m was an adequate isolation distance from other potatoes for

Pollen dispersal from a field trial of chlorsulfuron-resistant potatoes

Table 7

Distance from trial (m)	Number of seedlings screened	Number of seedlings resistant	Proportion of seedlings resistant (%)
Within trial	4 476	51	1.14
0.0-1.5	12 946	4	0.03
1.5-3.0	16 716	9	0.05
3.0-4.5	11 209	6	0.05
4.5-6.0	15 212	0	0.00
9.0-10	822	0	0.00

Source: Tynan *et al*, 1990

experimental containment of transgenic potatoes with novel constructs.

It would seem that pollen dispersal in potato is generally restricted (Dale *et al*, 1992; Harding & Harris, 1994) although results from the study by Skogsmyr (1994) show that under certain circumstances pollen dispersal can be very high as well as occurring over long distances.

#### 4.4. Gene flow: Crop to crop

##### 4.4.1. Hybridisation and gene flow

An examination of cross-pollination levels from herbicide tolerant GM plants by Tynan *et al* (1990) showed that frequency of transgenic seedlings among the progeny of non-GM potato plants growing within the trial was about 1 %. Out of a total 10 000 non-GM potato seedlings growing within 4.5 m of the GM trial, five contained the modified gene. There was no evidence of cross-pollination beyond this distance (see Table 7). McPartlan & Dale (1994) carried out a similar field experiment using the variety 'Desiree' transformed for herbicide tolerance. A central 20 m x 20 m plot of the transgenic potato plants was established, with non-transgenic sub-plots planted in four directions from the central plot at 10 m and 20 m. The frequencies of herbicide tolerant seedlings obtained from the non-transgenic potato plants are recorded in Table 8.

A potential indirect risk of gene flow lies in the fact that while usually vegetatively propagated in agricultural practice, many potato cultivars can produce true potato seed

Table 8

Frequencies of cross-pollination between GM potatoes and non-GM potatoes at various distances

Distance between GM/non-GM plants (m)	Frequencies of cross-pollination (%)	Total No. of seedlings screened
Plants touching	24	1 000
3	2	3 000
10	0.017	11 000
20	0	12 000

Source: McPartlan and Dale, 1994

(TPS). The cultivation of such potato varieties (e.g. Maris Piper, Desiree) can therefore add large quantities of TPS to the soil which can survive in field conditions for at least eight years, contaminating subsequent potato crops. The problem of volunteer potato plants has increased due to this in many areas (Lawson, 1983).

Within the family *Solanaceae*, potatoes have a number of relatives which are crop species, the closest being tomato, as well as tobacco, sweet pepper (*Capsicum annuum*) and petunia (*Petunia hybrida*). There is no evidence to suggest that intergeneric hybridisation can occur between potato and its related crop species (Treu & Emberlin, 2000).

#### 4.4.2. Possible consequences of gene flow

Cross-pollination between fields of potatoes may be less significant than in some other GM crops as the potato tuber is not affected by the fertilisation of the plant with foreign pollen. Furthermore, the crop is usually sown with seed tubers rather than true seed (Treu & Emberlin, 2000). However, many cultivars can produce TPS and seed producing areas do exist, though not on a large scale. TPS survival may result in a GM volunteer plant being harvested along with a non-GM potato crop, which could have implications for crop quality and seed purity.

#### 4.5. Definition and status as a weed plant

Volunteer tubers and plants seemingly fail to survive outside agricultural environments. Volunteers are known to occur near animal feed lots, on waste ground and on tips as a result of the handling and transportation process, though there is no evidence that potato plants will proliferate and become established as weeds (Anon, 1996). The

restriction to such habitats in Europe would seem consistent with the findings of Evenhuis & Zadoks (1991), who assert that this is the case because *S. tuberosum* demonstrates limited competitive abilities. *S. tuberosum* is not a primary colonizer in unmanaged ecosystems, and seedlings do not tend to compete successfully against plants of a similar type for space (Anon, 1996). The extent of existing feral potatoes and probable sparsity of these individuals, the infrequency of seed production and high percentage of self-pollination probably mean that feral plants present little or no risk of acting as either a GM pollen source or recipient. However, research on the subject should continue to ensure new varieties do not lead to an increase in feralisation (Treu & Emberlin, 2000).

#### 4.6. Gene flow: Crop to wild relative

##### 4.6.1. Compatibility and distribution

While there are eight cultivated potato species world wide, there are approximately 200 wild species, ranging in distribution from south-western US to the southern South American countries of Argentina and Chile (Frederick *et al*, 1995). Other than potato, there are around 13 species within the genus *Solanum* found in various parts of Europe. Most of these species are introduced casuals, although some, including *S. dulcamara* (woody nightshade) and *S. nigrum* (deadly nightshade) are native and common.

##### 4.6.2. Hybridisation and gene flow

McPartlan & Dale (1994) monitored frequencies of cross-pollination from a central 20 m x 20 m plot of GM herbicide tolerant potato plants to two weed species that were planted at a distance of 20 m from the corners of the central plot. The two solanaceous weed species (*S. nigrum* and *S. dulcamara*) were initially sown in a glasshouse and transplanted to the field to ensure synchronous flowering with the transgenic potatoes. Seeds harvested from the plants closest to the GM potato plants were screened for herbicide resistance, collected from a total of 77 *S. nigrum* plants and 63 *S. dulcamara* plants. None of the 8148 *S. nigrum* and 1102 *S. dulcamara* seedlings grown on in the glasshouse were found to contain the herbicide tolerance gene.

Experiments on crop to wild plant gene flow in the Netherlands under the EEC Biotechnology Action Programme involved over

2000 hand pollinations between potato and *S. nigrum* and over 500 hand pollinations between potato and *S. dulcamara*. No berries were formed from hybridisations with *S. dulcamara* and seedless berries were formed from hybridisations with *S. nigrum*. Embryo rescue was carried out for the cross with *S. nigrum* and no hybrid plants were obtained from c. 15000 embryos cultured later than 24 days after pollination. Two male sterile hybrids were produced after culturing c. 9000 embryos at 10-23 days after pollination. It is important to note that early excision and culture of embryos is a very artificial method of obtaining hybrids, and that in these hybridisation studies no seeds were set when berries were left to mature naturally (Dale *et al.*, 1992). Eijlander & Stiekema (1994) successfully hybridised potato with *S. nigrum* using embryo rescue techniques with *S. nigrum* as the pollen recipient. The resulting progeny were all sterile which lead to authors to conclude that 'potato is a naturally contained species in Europe'.

#### 4.6.3. Possible consequences of gene flow

Naturally occurring cross-pollination and subsequent gene flow between potato and its related wild species in Europe is unlikely. Without the help of sophisticated embryo-rescue techniques no viable hybrids between cultivated potato and its European related wild species have been obtained. Also, it is likely, given the breeding barriers known within the genus, that even if cross-pollination were successful, strong post-zygotic barriers would prevent the formation of a viable hybrid (DoE, 1994).

#### 4.7. Conclusion

The extent of pollen dispersal undoubtedly varies with cultivar, climatic conditions during flowering and presence and frequency of pollination vectors. The majority of field studies have detected pollen at a maximum distance of 20 m from the source with the exception of one study that recorded outcrossing levels of 31 % at 1000 m. The pollen beetle, thought to be attributable for the high levels of cross-pollination in this study, is a common insect in Europe. Further research should be undertaken to produce a more complete evaluation of the role of the pollen beetle in cross-pollination in the wider context.

In Europe pollen dispersal from a GM potato field would be unlikely to affect the receiving crop directly as the harvested

product is not affected by the process of fertilisation and seed production. Also, the crop is planted with seed tubers rather than true seed so a GM contaminant would not be transmitted to progeny crops. However in less developed areas of the world TPS has a considerable number of benefits and as such has been utilised in commercial potato production (Askew, 1993) so that cross-pollination could lead to contamination of subsequent crops. In addition varieties prone to producing fertile berries would be exposed to contaminating GM pollen, providing a source for GM volunteers. Volunteer potatoes appear to occur in virtually all crops to a greater or lesser extent on all farms where potatoes are grown in the rotation (Askew, 1993). The risk of a GM potato plant being integrated with a conventional crop could arise if volunteer tubers and plants are allowed to persist. In crop production systems, volunteer tubers and plants are usually removed with the production practices that are normally used for potatoes and the crops that succeed potatoes in the rotation though this can be difficult to achieve, especially in areas of TPS production. In recent years, the combination of reduced herbicide rates throughout the rotation due to declining arable margins, a succession of mild winters and the use of vigorous potato varieties has increased the numbers of volunteer potatoes.

Feral plants present little or no risk of acting as either a GM pollen source or recipient, though research on the subject should continue to ensure new varieties do not lead to an increase in feralisation (Treu & Emberlin, 2000). Data shows that natural gene flow from potato to its wild relatives *S. nigrum* and *S. dulcamara* is highly unlikely.

## 5. Maize (*Zea mays*)

Cultivated maize (*Zea mays*) is presumed to have derived from teosinte (*Z. mexicana*), a native of Central America, and was introduced into Europe in the sixteenth century. Maize is cultivated worldwide and represents a staple food for a significant proportion of the world's population (Anon, 1994b), as well as being grown as a livestock fodder crop in parts of Europe and elsewhere.

### 5.1. Reproductive biology and crop use

Maize (often referred to as corn in North America and elsewhere) is a tall, monoecious annual grass with overlapping sheaths and broad conspicuously distichous blades. The functional staminate flowers are borne in male tassels located terminally on the stems, and the female cobs are borne in the axils of the middle leaves. Pollen is produced entirely in the staminate inflorescence, and is released from the tassels in large quantities. It has been estimated that for each ovule developing into a kernel an individual plant delivers from 9000 (Bonnert, 1947) to 50000 (Weatherwax, 1955) pollen grains, often over a period of 2-14 days, though more usually over a shorter period of 5-8 days. Assuming an average ear of maize grows approximately 500 kernels, a plant will yield between 4.5 and 25 million pollen grains (Paterniani & Stort, 1974). The pollen grains are large (90-125 µm), and when compared with other anemophilous species have a high terminal velocity and therefore a higher comparative deposition. Maize is considered to be a protandrous species although there is usually some overlap of pollen shedding and silk emergence on the same plant that can account for up to 5 % self-pollination.

Maize is cultivated and utilized in several ways. Forage maize is harvested as a whole crop before seed ripening and either fed direct or in the form of silage to livestock. Grain maize is grown for its dry seed which is processed into a range of animal and human feeds. Sweetcorn is harvested when the cobs are unripe so that the seeds still contain mobilised sugars and the grains are consumed whole.

Breeder or foundation seed is produced from self-pollinated seed after several gen-

erations of inbreeding. A high degree of self-pollination is assured by planting in blocks that are isolated by distances of at least 200 m from other contaminating sources of pollen. Hybrid seed production is accomplished by inter-planting rows of the male and female inbred parents (e.g., one row of pollinator to four female rows). Self-pollination of the female parent is prevented through detasseling prior to pollen shed or by the use of male sterile females (Anon., 1994b). Hybrid seed production requires isolation similar to that for foundation seed.

### 5.2. Genetic modification

The use of the *Agrobacterium*-mediated transformation system to genetically modify maize has not been as easy as it has for other crops. The most successful procedure to deliver gene constructs into maize cells is particle bombardment (biolistics) of maize tissue cultures, from which fertile plants can be regenerated. Several constructs containing selectable markers expressing resistance to antibiotics, tolerance to glufosinate, glyphosate and bialaphos herbicides have been introduced into maize (Harding & Harris, 1994). The development of GM maize expressing toxins from the soil bacterium *Bacillus thuringiensis* (Bt) for managing pest insect populations represents a significant change in pest management practices. However, this technology suffered a setback following a recent report by Losey *et al* (1999) which raised concerns over the potential detrimental effects to larvae of the monarch butterfly (*Danaus plexippus*) following ingestion of Bt corn pollen in Canada. At present the widespread use of Bt maize has not been affected. Preliminary data do not provide evidence for a strong phenological overlap between monarch larval stages and peak pollen shed (Sears & Stanley-Horn, 2000) although research is ongoing. In 1998 introductory quantities of Bt maize were grown in Spain and France.

### 5.3. Pollen dispersal

Maize is primarily wind pollinated although there is evidence to suggest that bees and other insects collect pollen from maize

(Bateman, 1947a). Emberlin *et al* (1999) recorded observations of the collection of maize pollen by bees from the tassels at the top of the plants. They concluded that insects have a very small role in cross-pollination of maize presumably because there is no incentive for pollen collecting insects to visit the female flowers mid-way up the stem (Treu & Emberlin, 2000). Published data on the length of time that maize pollen remains viable under natural conditions varies from about 24 hours through to several days. This viability time could be reduced to a few hours in exceptionally hot, dry weather, or extended up to nine days in cooler, humid conditions (Emberlin, 1999).

Sears & Stanley-Horn (2000) examined the distance, direction and density of Bt maize pollen dispersal at several field sites in Ontario, Canada. Results showed that regardless of the direction from the field, most of the pollen fell within 5m of the field's edge. This data is consistent with the findings of other experiments (Pleasants *et al*, 1999). In seven of the nine fields for which pollen counts were completed, an average of nearly 90 % of the pollen caught on sticky plates was collected within 5 m of the field margins. Table 9 shows the cumulative percentage of maize pollen deposition at distances up to 100 m. This data indicates that 98 % of pollen remained within a 25-50 m radius of most of the maize fields.

Klein *et al* (submitted) performed an experiment in 1998 to measure hybridisation using seed colour markers near Montargis, France in a grain production field. A central plot measuring 20 m x 20 m was sown with maize plants producing blue seed, and was surrounded by an area 120 m x 120 m sown with yellow maize (hybrid variety Adonis). A total of 101 rows were sampled with increasing distance from the central plot in order to estimate the dispersal function, and 31 ears on each row were sampled and the number of blue grains on each ear counted. According to Klein *et al* (submitted) the pollen dispersal patterns in these experiments were very much dependent on experimental design (sizes, shapes, and positions of the marked and non-marked maize patches). They therefore used these data to model the effect of the size and shape of the marked source on pollen dispersal. The estimated individual dispersal function was used to predict the dispersal pattern from a source of varying shape and size into a 200 m x 200 m field. Figure 11 (overleaf) shows the marked source represented in black measur-

Cumulative percentage of pollen deposition of various distances from seven Bt maize fields in Ontario							Table 9	
Cumulative percentage pollen deposition (%)								
Field	0 m	1 m	5 m	10 m	25 m	50 m	100 m	
1	46	75	91	97	99	100	100	
2	43	70	88	94	96	97	100	
3	46	73	88	95	98	99	100	
4	47	81	92	96	98	99	100	
5	35	69	88	93	98	99	100	
6	41	76	90	97	98	99	100	
7	38	72	84	96	98	99	100	
Overall	43	74	89	95	98	99	100	

Source: Sears and Stanley-Horn, 2000

ing (top) 20 m x 20 m, 40 m x 20 m, 100 m x 20 m (bottom) 40 m x 40 m, 100 m x 100 m, 200 m x 200 m. It is clear from this figure that the pollen flow and cross-pollination frequency from one field to another depends on the sizes of both fields in a way that is not simple (at least, it is not a linear effect). We can also see that the distance at which a given rate of cross-pollination is reached (say 1 % for instance) depends on the sizes of the fields. The same modelling process was used to apply varying wind speeds and directions to the flowering maize crop to predict how pollen dispersal patterns would change. Both parameters were shown to influence pollen movement. Experiments which monitor the movement of pollen (Paterniani & Stort, 1974; Sears & Stanley-Horn, 2000) or record levels of crossing (Messean, 1999) have shown that pollination can be highly directionally-orientated with a much higher incidence downwind of the emitting crop. According to Jones & Brooks (1950) one year (1948) during their three years of experiments in the US showed particularly low pollen dispersal due to rainy weather and low wind velocity during much of the pollination season.

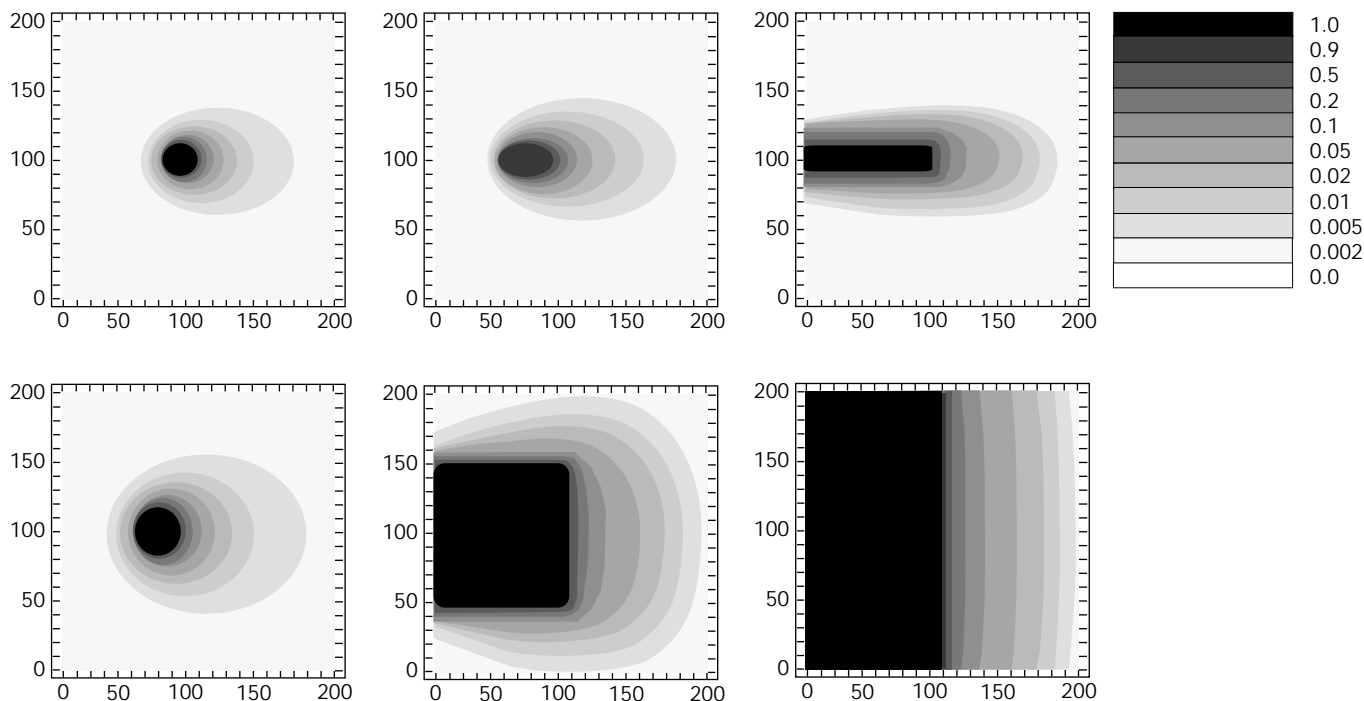
#### 5.4. Gene flow: Crop to crop

##### 5.4.1. Hybridisation & gene flow

Maize only interbreeds with other members of the genus *Zea*. There is no evidence for hybridisation between maize and other European crop species. There are, however, numerous varieties of maize grown and there is no evidence to suggest that any current variety is not interfertile with an-

Figure 11

A model to predict the dispersal pattern of maize pollen from a source of varying shape and size into a 200 m x 200 m field. The marked source is represented in black measuring (top) 20 m x 20 m, 40 m x 20 m, 100 m x 20 m (bottom) 40 m x 40 m, 100 m x 100 m, 200 m x 200 m



Source: Klein *et al* (submitted)

other variety (Treu & Emberlin, 2000). For example, sweetcorn varieties cross freely with maize. A substantial amount of research has been undertaken to determine the dispersal pattern of maize pollen (outlined in Section 5.3) and the levels of crop to crop cross-pollination. Jones & Brooks (1950) measured the percentage of outcrossing between large blocks of emitter and receptor crops over a period of three years at a maximum distance of 500 m. The mean hybridisation directly adjacent to the crop measured 25.4 %, falling to 1.6 % at 200 m and 0.2 % at 500 m. A similar study by Salamov (1940) reported mean hybridisation figures of 3.3 % at 10 m from the pollen source, 0.5 % at 200 m, 0.8 % at 600 m and 0.2 % at 800 m. The levels of outcrossing in immediate proximity to the pollen source are lower than those recorded by Jones & Brooks (1950). This is probably due to Salamov's trap being situated in the direction opposite to the prevailing wind (Treu & Emberlin, 2000). Bateman (1947b) found in an experiment that used 3 m<sup>2</sup> of source plants to pollinate isolated plants, that the level of cross-pollination dropped from 40 % at 2.5 m to approximately 1 % at 20 m, with some difference in the pollination levels in the two directions assessed. Messean (1999) reported a figure of 1 % cross-pollination at a separation distance of 25–40 m and concluded that

wind direction had an impact on the levels of gene flow recorded.

Simpson (unpublished data) measured gene flow from a 36 m x 12 m plot of glufosinate tolerant maize to an adjacent barrier crop of conventional maize in 1999. Samples were taken at various distances along three transects starting at 1 m from the GM maize plot up to 51 m away. Results show a steep decline in cross-pollination levels to below 1 % at 18 m from the pollen source (See Figure 12).

Jones & Brooks (1952) experimented with barriers to cross-pollination, and found that a single row of trees and underbush reduced outcrossing by 50 % immediately behind the barrier. However it appeared that the reduction was considerably less than if the space had been filled with intervening crop, presumably because the trees did not provide any competing pollen. When open ground or low growing barrier crops exist to isolate maize crops, it appears that the first few maize rows intercept a high proportion of the cross-pollination and then it decreases exponentially with distance.

#### 5.4.2. Possible consequences of gene flow

The levels of cross-pollination recorded between different maize varieties up to 800 m



show it is possible that pollen released from GM maize plants would cross-pollinate non-GM maize plants up to and beyond their recommended isolation distances. Although the studies considered have only investigated dispersal to distances up to 800 m, it is estimated that small quantities of pollen are likely to travel much further under suitable atmospheric conditions (Treu & Emberlin, 2000). Although different maize varieties are not considered in the cross-pollination experiments covered in the previous section, different varietal characteristics such as flowering times could have a major effect on cross-pollination levels. Crops for hybrid seed production containing a high proportion of male sterile plants are particularly vulnerable to cross-pollination. Such crops should be surrounded by effective barriers of male pollinators. The minimum separation distance in the EU is currently 200 m for all categories of seed production and this is deemed sufficient to maintain inbred lines at 99.9 % purity (Ingram, 2000).

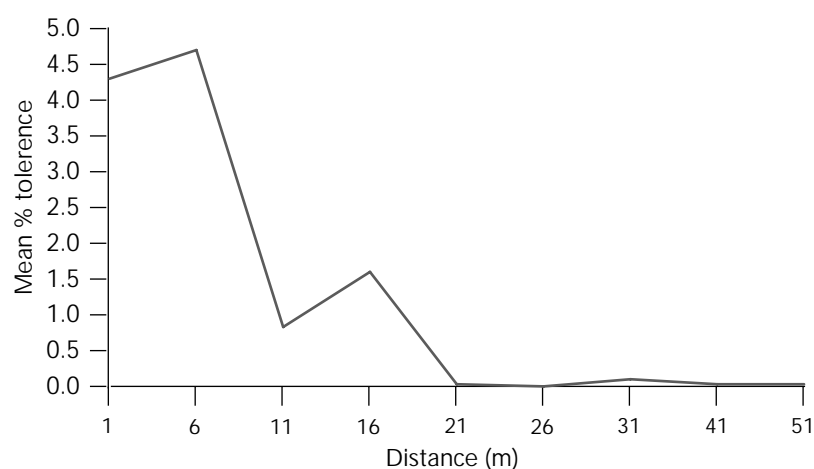
Treu & Emberlin (2000) describe GM maize as presenting a 'medium to high level risk' for cross-pollination with other maize crops due to the ability of the pollen to spread on the airflow. Pollination of a non-GM maize variety by GM pollen would affect the composition of the grain, but would not affect the composition of the stover and leaves. The authors also highlight that GM maize presents a medium to high risk for the inclusion of pollen in honey.

### 5.5. Definition and status as a weed plant

Maize plants occasionally grow in uncultivated fields and by roadsides or occur as volunteers in cultivated crops in the year following cultivation of a maize crop due to spilt grain, although maize is incapable of sustained reproduction outside of cultivation and is non-invasive of natural habitats (Anon, 1994b). There are no indications of maize ever having become a weed in the US where it is the most widely cultivated cereal. During its domestication from teosinte, maize lost the ability to survive in the wild. Seeds remain on the cob after ripening so that they do not disperse (Doebley *et al*, 1990) and any seeds that are dispersed in the harvesting process fail to survive more than one year in the soil due poor dormancy. Maize requires warm conditions for growth and does not tolerate prolonged cold and frost. It therefore has poor survival characteristics in much of Europe.

Mean percentage glufosinate tolerant seed detected in seed samples taken from non-tolerant maize at a range of distances from an adjacent plot of glufosinate tolerant maize

Figure 12



Source: Simpson (unpublished data)

### 5.6. Gene flow: Crop to wild relative

#### 5.6.1. Compatibility & distribution

Maize and other species and subspecies of teosinte are sexually compatible and can produce fertile hybrids (Wilkes, 1977). Related *Zea* species are geographically restricted and occur only in Mexico and Guatemala. The closest known relative of *Zea* is *Tripsacum*, three relatives of which occur in the US. Only one, *Tripsacum dactyloides*, has a distribution that includes the northern US maize belt (Gould, 1968). Teosinte is not indigenous to Europe and there are no relatives in Western Europe with which it or maize can hybridise (Goodman, 1976).

#### 5.6.2. Hybridisation and gene flow

Prior to 1980 there is evidence for repeated introgression between maize and teosintes, especially *Zea mays* ssp. *mexicana*, although a recent re-evaluation using gene mapping techniques has contradicted this premise (Smith *et al*, 1985). There is no evidence for hybridisation between maize and any wild European plant species.

#### 5.6.3. Possible consequences of gene flow

Maize is considered by many as an inherently safe transgenic crop under European conditions as far as weediness or direct environmental impact is concerned (Harding & Harris, 1994). As long as introduced genes do not enhance the weediness of the crop and do not effect non-target organisms in the environment (like pollinating and other beneficial insects or soil organisms) genetic

modification of maize does not appear to have adverse ecological effects (Kapteijns, 1993).

A potential problem with GM maize has been highlighted in North America where considerable research has been undertaken on the possible detrimental effects of Bt maize pollen on biodiversity. These effects are believed to be caused by the toxicity of the pollen itself without a cross-pollination event occurring and the details are therefore beyond the scope of this report. The impacts of Bt genes in maize are currently being evaluated to identify possible risks to biodiversity prior to Bt maize being widely commercialised in Europe.

### 5.7. Conclusion

In Europe the risk of pollen mediated gene flow in maize is restricted to cross-pollination between crops. Contamination of a conventional maize crop with GM maize may affect the market acceptability of the harvested crop due to reduced quality. Cross-pollination of sweetcorn by maize produces grain with less sweetness (Ingram, 2000). Further problems may be encountered in maintaining genetic purity in seed crops.

Maize pollen has been shown, by the action of wind, to cross with other cultivars of maize at up to 800 m away. It is estimated that small quantities of pollen are likely to travel much further under suitable atmospheric conditions. Evidence suggests that the extent of gene flow between GM and non-GM maize crops is mainly dependent on the scale of pollen release and dispersal (e.g. Klein *et al*, submitted), and on the distances between source and recipient populations. Recom-

mended separation distances required to maintain cross-pollination within fields of 2 ha or more, as outlined by Ingram (2000), are 200 m to maintain 99 % grain purity and 300 m to maintain 99.5 % grain purity. The potential impact of pollen increases notably with the size and number of fields planted (Treu & Emberlin, 2000). Jones & Brooks (1950) found that the percentage of outcrossing within a field was related to the depth of the field in the direction of the source of contamination. It would seem that 'depth of the field' is of greater importance than total acreage in reducing contamination. The authors also observed that the percentage of outcrosses occurring in successive rows at different distances of isolation indicates that the first five rows adjacent to the source of contamination function as a barrier to the dispersal of contaminating pollen. Additional border rows serve only to dilute the contaminating pollen (Jones & Brooks, 1950). Overall the percentage of cross breeding with other maize crops in the vicinity will depend on factors such as separation distance, local barriers to pollen movement and local climate and topography. If introgression between GM and non-GM varieties were to occur, the probability of a volunteer weed problem occurring is low because its reproductive capacity is limited due to characteristics such as its inability to shed seed naturally. It seems unlikely that any shed maize seed would remain viable for prolonged periods under European conditions due to lack of dormancy and the inability of seed to survive low temperatures.

In Europe there are no known relatives with which maize can hybridise. Therefore there appears to be no risk of gene flow from GM maize to wild plants.

## 6. Wheat (*Triticum aestivum*)

Wheat is a genus of the family Graminae (Poaceae) commonly known as the grass family. There are three main cultivated wheat crops known as common wheat, durum wheat and bread wheat. Common wheat (*Triticum aestivum*) is economically the most important of the three.

### 6.1. Reproductive biology and crop use

Wheat is a mid-tall annual or winter annual grass with flat leaf blades and a terminal spike. Spikelets are born on a main axis, or rachis, and are separated by short internodes. Each spikelet is a condensed reproductive shoot consisting of two subtending sterile bracts or glumes. The glumes enclose up to six florets which are born on a short axis. Each floret when fertilised can give rise to a kernel, which is attached at its embryo end and bears a brush of persistent hairlike epidermal cells at its terminus. Wheat is typically self-pollinated (via anthers within each enclosed floret), and any outcrossing that does occur is facilitated by wind pollen dispersal. de Vries (1971) reported that the duration of time that wheat florets remain open ranged from 8–60 minutes depending on genotype and environmental conditions. Once the anthers dehisce, 5–7 % of the pollen is shed on the stigma, 9–12 % remains in the anther, and the remainder is dispersed (Anon, 1999b). Cross-pollination under field conditions normally involves less than 2 % of all florets (Wiese, 1991), though in general, outcrossing rates in any species which is primarily selfing may be up to 10 % or higher, where the rate varies between populations, genotypes and with different environmental conditions (Jain, 1975).

Modern *Triticum* species fall into three natural groups based on chromosome number: diploids ( $n=7$ ), tetraploids ( $n=14$ ), and hexaploids ( $n=21$ ). Each group of seven chromosome pairs (genome) was apparently contributed to modern wheat by different ancestral parents. Natural outcrossing between wheat-like grasses is presumed to have initiated the modern polyploid wheats; *T. aestivum* L. and *T. compactum* Host (club wheat) are hexaploid, and *T. durum* Desf. (durum wheat) is diploid. These three species account for about 90 % of the cultivated crop

(Wiese, 1991). *T. aestivum* is a staple for about 40 % of the world's population, and the plant and seed are also used in industrial products and as feed for livestock. Wheat straw is used as fuel, animal bedding, and organic matter for soil. Wheat germ oil is used as a food supplement. Wheat grain and bran are important livestock feeds, and young wheat plants serve as livestock forage (Wiese, 1991).

### 6.2. Genetic modification

Wheat has been the subject of intense research in order for a reliable system for producing genetically modified plants to be established. Hess *et al* (1990) reported the successful transfer of kanamycin resistance of bacterial origin into wheat via pipetting *Agrobacterium tumefaciens* suspension into wheat spikelets. However, this experiment has not been repeated and has not been addressed in reviews of cereal transformation, suggesting that there may be problems with the technique (DoE, 1994). Of the other various methods used for the transformation of cereals, the successful production of fertile transgenic wheat plants have been obtained by the direct delivery of DNA into protoplasts by osmotic or electrical shock, or into intact cells by high velocity microprojectile bombardment. Vasil *et al* (1992) produced herbicide resistant transgenic wheat plants by microprojectile bombardment of embryonic callus. Mendelian segregation and functional activity of the introduced bar gene in  $R_1$  and  $R_2$  plants was demonstrated (Vasil *et al*, 1992). Other work on wheat has concentrated on transformation and regeneration of protoplasts. The direct gene transfer using polyethylene glycol (PEG) relies on the treatment of embryogenic protoplasts with PEG in the presence of foreign DNA. Recently, methods for protoplast isolation and for plant regeneration from suspension culture in wheat have been developed and are expected to obtain transgenic wheat plants in the near future (Pogna *et al*, 1994).

Suggestions for traits that might be improved by genetic modification include resistance to fungal diseases such as powdery mildew, yellow rust, take all and *Septoria* infections. Insect resistance is also an impor-

tant trait being considered, especially to aphids and shoot flies. Yield related characters include manipulation of the life cycle (by photoperiod and vernalisation genes), improvement in photosynthetic and water use efficiency and prevention of lodging through altering plant height. Another major interest for wheat breeders is the manipulation of grain quality, and in particular improving the baking characteristics of the flour (DoE, 1994).

### 6.3. Pollen dispersal

de Vries (1971) describes wheat pollen as being relatively heavy, a characteristic associated with the high ploidy level of wheat. Evidence would suggest that as wheat pollen is produced in relatively small amounts, has characteristics favourable for high self-pollination rates and has a limited viability period, outcrossing levels are likely to be low (Treu & Emberlin, 2000). However, in some cases strong winds can not only disperse pollen widely but can also increase the amount released by agitation of the anthers (Goss, 1968). de Vries (1974) established a trial to measure cross-pollination distances in wheat, using a 6 x 6 m central plot of spring wheat as the pollinator. Male sterile bait plants were positioned at various distances of 0.45 m up to 30 m from the pollen source in rows of 10 plants in directions north, south, east and west. It is important to note that the use of male sterile plants removes the high levels of self-pollination that would normally occur, so the results only represent pollen dispersal levels in wheat, rather than potential cross-pollination. Results from this experiment showed that after a distance of 1m from the pollen

source the average seed set was below 10 % and no cross-pollination events were recorded beyond 20 m from the source. Important differences in wind direction were established (see Figure 13). Higher cross-pollination frequencies were noted to the north and east of the pollen source, differences that were attributed by de Vries (1974) to the prevailing south-west wind.

Insects are known to visit the flowers of anemophilous species (Bateman, 1947b) though it seems unlikely that insects would visit wheat flowers in significant numbers, as they have no nectaries and produce relatively small quantities of pollen. Also, the prevalence of self-pollination means that any visiting insects are unlikely to contribute significantly to cross-pollination levels (Treu & Emberlin, 2000). Unfortunately there are no records of any specific studies on the potential of long distance wheat pollen dispersal.

Pollen of the Gramineae has a limited viability period. Firbas (1922) was able to determine the longevity of wheat pollen to be much shorter than that of rye pollen. Kul'bij (1959a; 1959b) found that wheat pollen remained viable after storing for five hours in a dessicator containing CaCl<sub>2</sub> at a temperature of 2–4°C, but that under field conditions the pollen lost its fertilising capacity after 15–20 minutes, in the anthers gathered for pollination. According to Watkins & Curtis (1967) pollen viability is prolonged by cool temperatures and high relative humidity levels.

### 6.4. Gene flow: Crop to crop

#### 6.4.1. Hybridisation and gene flow

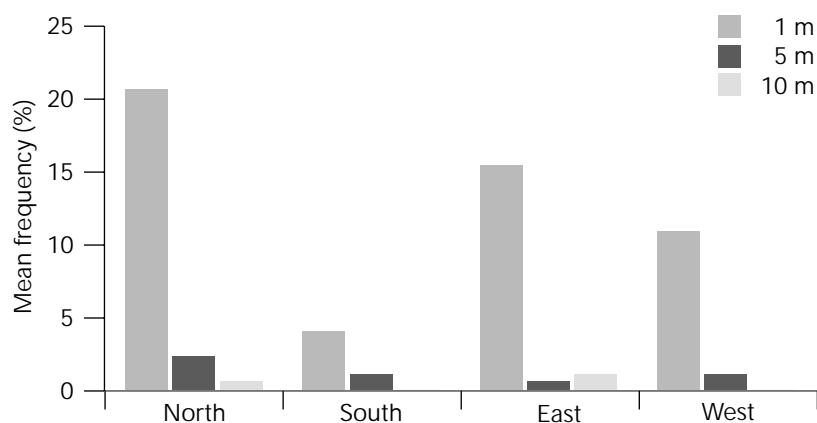
Wheat can be crossed with wild barley (*Hordeum*) species (see Section 6.6.2), although there is little or no evidence that cultivated wheat x barley hybrids exist naturally, and if they did they would most likely be sterile (Harding & Harris, 1994). An artificial hybrid crop, *Triticale* has been produced between wheat and rye, and is now commonly grown. There are no reports of a naturally occurring hybrid between wheat and rye (Treu & Emberlin, 2000).

#### 6.4.2. Possible consequences of gene flow

The reproductive biology of wheat and lack of evidence for natural outcrossing to date show that wheat has little potential for hybridisation under field conditions with any related crop species currently grown in Europe.

Figure 13

Mean percentage seed set on male sterile wheat plants at various distances and directions from the pollen source



Source: de Vries, 1974

## 6.5. Definition and status as a weed plant

During the domestication of modern wheat, key traits were modified that benefited early farmers but eliminated the ability of the resulting wheat races to survive in the wild. For example, plants with heads that do not shatter are easier to harvest, but prevent the plants from efficiently distributing seed. Wheat is commonly seen as a relic in fields and waste ground and on roadsides and tips. Such plants are most likely to be the result of seed spillage and are not usually persistent. Similarly, wheat plants can also grow as volunteers in a cultivated field following a wheat crop. These plants are usually eliminated with cultivation management or the use of herbicides (Anon, 1999b), although seed may survive in the seed bank and grow in subsequent crops, causing contamination if GM and non-GM wheat is grown in the same rotation.

## 6.6. Gene flow: Crop to wild relative

### 6.6.1. Compatibility and distribution

Wheat is probably derived from a wild form of diploid einkorn (*Triticum monococcum* sensu lato) in the Near East. There are many examples of successful cross-breeding within the genome linkage of *T. aestivum*, accounted for by the specific genetic structure of the genus *Triticum*. There are 27 wild *Triticum* species that are distributed in the Mediterranean area and Southwest Asia, the centre of the distribution being Turkey, Syria, Iraq and Iran, which contain nearly 20 of the wild species (van Aken, 1999).

### 6.6.2. Hybridisation and gene flow

Spontaneous intermediates between cultivated wheats and their wild relatives occur frequently on margins of wheat fields when wild *T. turgidum* subspecies or certain species of *Aegilops* are present (Ladizinsky, 1992). Genes from several wild species have been introduced into bread wheat by introgression (DoE, 1994). Many spontaneous hybrids and backcrossed progeny have been found in Greece, Turkey and Israel involving both bread and durum wheat.

With regard to *T. aestivum*, Raybould & Gray (1993) reported successful hybridisation with several *Hordeum* (barley) species, and hybrids have also been formed by embryo rescue with *Elytrigia* spp. (couch grasses) and *Leymus arenarius* (Lyme grass). The probability of gene flow from wheat crops to these three wild species in Europe is consid-

ered minimal by the authors. As well as using embryo rescue techniques, some species of *Elytrigia* have only been found to cross-pollinate with wheat under controlled greenhouse conditions and notably there are no reliable reports (Anon, 1999b) of the very common *E. repens* (common couch grass) hybridising with wheat (Treu & Emberlin, 2000). Ellstrand *et al* (1999) confirm that although breeders have produced fertile hybrids between wheat and its wild relatives, 'all natural hybrids . . . are highly sterile, although seeds may occasionally be found' (Popova, 1923; van Slageren, 1994). This hybrid sterility may explain why hybridisation generally appears to be restricted to the first cross with little evidence for subsequent introgression (Ellstrand *et al*, 1999).

*Aegilops cylindrica* (jointed goatgrass) is a major weed in the wheat producing areas of the western US. The shared D genome between wheat and *A. cylindrica* allows hybrids between these species to be produced in the field, including the transfer of herbicide resistance genes which has been reported in two recent studies (Seefeldt *et al*, 1999; Mallory-Smith *et al*, 1999). In glass-house experiments by Mallory-Smith *et al* (1996) viable seed was produced and the resulting interspecific hybrids showed a low level of female fertility (2 %) that allowed for backcrossing to occur between the hybrid and either *A. cylindrica* or wheat (Zemetra *et al*, 1998). At present, *A. cylindrica* is recorded as a rare birdseed/wool alien (Stace, 1997) in the UK and other parts of Europe.

### 6.6.3. Possible consequences of gene flow

Evidence suggests that wheat has limited potential for outcrossing with wild relatives. The wild relatives with which wheat has been known to cross are confined to field margins or disturbed places and never seem to form substantial populations or become invasive of other habitats. This is likely to be the case for any hybrid (DoE, 1994).

## 6.7. Conclusion

Wheat can be described as a low risk crop for gene flow from genetically modified varieties to other crops and to wild species. Wheat has limited potential for outcrossing even with related plants growing in close proximity, and reports of hybridisations that have occurred support little evidence for subsequent introgression.

## 7. Barley (*Hordeum vulgare*)

Barley (*Hordeum vulgare* L.) is a small-grain cereal which belongs to the grass family poaceae, tribe Triticeae. Most cultivated barleys have either a winter or spring growth habit, and exist as either two-rowed or six-rowed types. The two forms have been named individually as *Hordeum distichon* (two-rowed) and *Hordeum vulgare* (six-rowed). Stace (1991) considers that on biological grounds the two types are better amalgamated as *Hordeum vulgare*, as we have done here.

### 7.1. Reproductive biology and crop use

Cultivated barleys are annuals and some wild barleys exist as perennials. A single barley plant usually produces three to six stems, which vary in length from 10 cm to more than 150 cm in the tallest types. Single leaves arise at each node of the stem and are borne alternately on opposite sides. At the top of each stem the head is made up of spikelets attached to the node of the rachis. Each spikelet has one flower, consisting of two glumes and a floret. In two-rowed barleys, only the central floret is fertile, while in six-rowed barleys, all three florets are fertile. Each floret, when fertilised can give rise to a kernel. The number of kernels that develop per head varies from 25 to 60 in six-rowed types and from 15 to 30 in two-rowed types. Barley is typically self-pollinated, and any outcrossing that does occur is facilitated by wind.

Cultivated barley is one of the major cereal crops in the world, ranking fourth behind wheat, maize and rice. In recent years the crop has been produced on 91 million hectares world wide (Mathre, 1997). Its principal use is in animal feed as a whole plant for fodder or silage, or threshed and fed crushed or rolled. The second major use of barley, and the most valuable crops are those used for malting in beer and whiskey production which require rapid and uniform germination (DoE, 1994). Barley is also widely used in processed foods.

### 7.2. Genetic modification

The development of techniques for the genetic modification of barley has, in gen-

eral, been slower than for other cereals. Transformation is restricted to a limited number of genotypes and occurs at low frequencies (Harwood *et al*, 2000). However, there have been a number of reports of successful barley transformation systems, the majority of these utilising particle bombardment as the method of DNA delivery and immature embryos as the target tissue (Wan & Lemaux, 1994; Jensen *et al*, 1996). Recently, *Agrobacterium*-mediated transformation of barley has also been reported (Tingay *et al*, 1997), again using immature embryos as the target tissue. Microspores have also been successfully used as transformation targets in barley (Yao *et al*, 1997) and direct DNA transfer to protoplasts has yielded transgenic barley plants (Funatsuki *et al*, 1995). Harwood *et al* (2000) bombarded a spring barley variety with three different particle delivery systems in order to monitor transient and stable transformation of the plants. In addition, a range of techniques for the preparation of the DNA coated gold particles was examined. The authors found that although barley transformation was achievable for certain genotypes, it was still inefficient and requires a greater understanding of the transformation process in order for improvements to be made (Harwood *et al*, 2000).

Generally speaking, traits being developed for wheat are for the most part applicable to barley, especially with respect to yield and disease resistance (DoE, 1994). The modification of starch composition is of interest to barley processors. In the case of malting barley, particularly valued targets are high hydrolytic enzyme content, low total nitrogen, high starch content as well as other more specific aims (Mannonen *et al*, 1994). High lysine content might also be a target for genetic modification in barley as attempts to breed high lysine varieties from high lysine mutants have been largely unsuccessful.

### 7.3. Pollen dispersal

Cultivated barley reproduces almost entirely by self-fertilisation (~99%). Observations of outcrossing rates within natural stands of wild barley and in cultivated barley have

demonstrated that pollen dispersal rates between and within populations of wild and cultivated barley appear to differ very little. The pollen production per spike of common barley varieties is roughly 10 % of that produced in a spike of rye (Price, unpublished), and the proportion of pollen grains released outside the floret varies considerably between varieties. StØlen (1981) carried out research on the floral characteristics of barley, and found great variation in length as well as width of anthers in different barley varieties. Previous work in wheat (Beri & Anand, 1971) and rice (Oka & Morishima, 1967) has shown that close correlation between size of anthers and pollen load exists, and in some cases the pollen load may be too limited to guarantee cross-pollination.

Studies concerning the dispersal patterns of airborne barley pollen have shown that it is not possible in practice to confirm the true origin of individual captured pollen grains with reliable genetic and molecular analyses. Therefore, the spatial origin of the pollen grain usually remains obscure. Furthermore, pollen from several open-pollinating grass species is very difficult to discriminate reliably from barley pollen grains on a slide. Also, it is not easy to reliably measure the vitality of barley pollen, so half-dead pollen grains will often be mistaken for vigorous grains. Due to these difficulties pollen dispersal is usually measured by cross-pollination rates rather than with pollen traps.

It is clear from experimental work that the bulk of outcrosses amongst the species take place between closely adjacent plants. Support for this conclusion has come from a number of studies designed to obtain precise quantitative estimates of the amounts of pollen migration that occur in barley. Wagner & Allard (1991) measured outcrossing rates among adjacent barley plants in various planting patterns. The seeds of pollen donor and pollen recipient varieties were mixed in 50:50 proportions and sown at a high seed rate in a block of rows so that many plants in the same row were in physical contact with each other. The average rate of outcrossing observed was 0.84 % (range 0.31 % to 1.89 %). In another experiment physical contact between plants was almost eliminated by spacing plants 30 cm apart within and between rows. Under this planting plan pollen migration fell to 0.23 %.

The main finding of the study by Wagner & Allard (1991) is that pollen dispersal can

occur in potentially significant amounts up to distances of at least 60 m. However, a cross-pollination event such as this is rare in barley and the authors suggest that a crop isolation distance of 1m would be effective maintaining contamination below acceptable levels for most materials. Only if very low levels of contamination are acceptable does the isolation distance need to be increased to up to 60 m.

Wagner & Allard (1991) concluded from their experiments that distance was the single most important factor affecting pollen dispersal rates in barley. There were indications that prevailing wind direction influenced pollen dispersal as more migration occurred downwind than upwind, but differences were small and statistically nonsignificant. Differences in pollen dispersal rates in arid locations and moist locations and in years when conditions were dry during flowering were also small. StØlen (1981) found wind and air movement to be a major factor affecting pollen movement, the amount of pollen recorded being low on days when air movement was low. Barley pollen is lighter than, for example, maize pollen, and has the potential to be carried somewhat further by wind.

#### 7.4. Gene flow: Crop to crop

##### *7.4.1. Hybridisation and gene flow*

Hybridisation can occur between barley cultivars. Tammissola (1998) arranged experimental plots to measure gene flow in barley in Finland. A central 255 m<sup>2</sup> block of transgenic donor barley was surrounded in directions north, south, east and west with recipient plots of various male sterile cultivars at 1, 2, 3, 6, 12, 25 and 50 m distances from the border of the central plot. Cross-pollination frequencies were generally low. On average one seed per head (less than 2 %) was obtained in male-sterile recipient plots at 1m distance from the donor plot in 1996, and less than 1/2 seeds (less than 1 %) in 1997.

Wheat x barley crosses are reported to be hampered by extremely low crossability (Molnar-Lang & Sutka, 1994). Barley is not thought to hybridise with wheat under natural conditions (Taketa *et al.*, 1998), and there is no evidence that wheat x barley hybrids exist naturally. Experimental data suggests that if such plants were produced, they would most likely be sterile (Harding & Harris, 1994). Thomas & Pickering (1979)

obtained barley x winter rye hybrids by embryo culture though all plants were sterile.

#### **7.4.2. Possible consequences of gene flow**

The reproductive biology of barley and lack of evidence for substantial natural outcrossing to date show that barley has little potential for hybridisation under field conditions with any related crop species currently grown in Europe. Hybridisation can occur between cultivars, though generally at low levels and over short distances.

### 7.5. Definition and status as a weed plant

Barley occurs on roadsides, waste ground and tips. Such plants are most likely to be the result of seed spillage and are not usually persistent.

Barley plants can grow as volunteers in cultivated fields following a barley crop. Such plants are usually eliminated with cultivation management or the use of herbicides. Tammissola (1998) monitored the occurrence of transgenic barley volunteers during subsequent years of a transgene flow trial. A total of four barley volunteers were found and the presence of the transgene was confirmed. Due to the persistence of barley seed in subsequent crops, GM and non-GM barley should not be grown in the same rotation.

### 7.6. Gene flow: Crop to wild relative

#### **7.6.1. Compatibility and distribution**

Barley was domesticated over 10000 years ago. Two-rowed barleys are believed to be derived from a wild species similar to *Hordeum spontaneum*, which occurs widely in Syria and Turkey (DoE, 1994). Six-rowed barleys are believed to have come from *Hordeum ischnatherium*. There are about 25 *Hordeum* species distributed throughout the temperate regions of the world. Some wild *Hordeum* species present in Europe are listed in Table 10, overleaf. Several other species from America and Asia have also been recorded as casuals in Europe.

#### **7.6.2. Hybridisation and gene flow**

Hybridisation barriers in the genus *Hordeum* are strong, and only occasionally have hybrids been produced without the aid of embryo culture. Von Bothmer *et al* (1983) carried out an extensive survey of hybridisation between wild and cultivated barley,

where *H. vulgare* was intercrossed with 26 wild *Hordeum* species representing 37 cytotypes. Seed set was obtained from all crosses, though adult plants (hybrids or haploids) resulted from only 15 species. Crosses were found to be more successful when *H. vulgare* was used as the pollen donor rather than the female parent.

In a cross with the hexaploid *H. murinum* two plants were obtained with *H. vulgare* as the male parent. The progeny were haploids or dihaploids of *H. murinum*, and were weak, had a slow growth rate and both died before anthesis. A total of 20 tetraploid *H. jubatum* hybrids from three F<sub>1</sub> families were obtained, again all with *H. vulgare* as the male parent. The hybrids developed into vigorous perennials and were smaller than is usual, morphologically resembling *H. jubatum*. In the cross with *H. secalinum* three F<sub>1</sub> families with 11 hybrid plants were raised, all with *H. vulgare* as the male parent. The progeny resembled *H. secalinum* but were smaller. They were perennial and slow growing. In a cross with *H. marinum* three families, all with *H. vulgare* as the male parent, were produced. All plants were haploids of *H. marinum*, they were annual and morphologically typical of this species but smaller and more slender (Von Bothmer *et al*, 1983). None of the hybrids produced were fertile.

Von Bothmer *et al* (1983) attempted crosses between some wild *Hordeum* species and tetraploid lines of *H. vulgare*. This cytotype did not function efficiently, and out of the 93 crossing attempts only three resulted in plants, one with *H. marinum* and two with *H. procerum*.

Tammissola (1998) studied the occurrence of wild barley species in Finland. Altogether eight species were recorded, all of which are ephemerals and apparently unable of forming permanent populations. Plants were mainly recorded in coastal areas considerable distances from barley fields. Hybridisation attempts by forced crosses between *H. vulgare* and *H. jubatum* proved unsuccessful.

Barley can be made to hybridise with wheat (Sharma & Gill, 1983) and rye (Thomas & Pickering, 1979) but recovery of plants usually requires embryo rescue and the hybrids are sterile (DoE, 1994).

#### **7.6.3. Possible consequences of gene flow**

Barley has been made to hybridise with some wild relatives and cultivated cereals but there are no records of natural hybrids occurring



Some wild relatives of *H. vulgare* and their distribution in Europe

Table 10

Species	Distribution
1. <i>H. geniculatum</i> (Mediterranean barley)	Waste ground and rubbish dumps, rarely as a weed in cultivated areas. Mediterranean region.
2. <i>H. jubatum</i> L. (Foxtail barley)	Alien from birdseed, grass seed and garden outcast; casual in waste places. Scattered casual in Europe.
3. <i>H. marinum</i> (Sea barley)	Salt marshes, sea walls and waste ground. Distributed along coasts of UK, Western Europe and Mediterranean.
4. <i>H. murinum</i> L. (Wall barley)	Waste areas and disturbed ground, roadsides and margins of cultivated land. Throughout Europe.
ssp. <i>Murinum</i>	A weed of waste and rough ground. Throughout Europe.
ssp. <i>Leporinum</i>	Mediterranean region, occasionally introduced into the UK.
ssp. <i>Glaucum</i>	Eastern Mediterranean region, occasionally introduced into the UK.
5. <i>H. nodosum</i> L.	Abundant throughout Europe.
6. <i>H. pubiflorum</i> (Antarctic barley)	Wool alien on tips, waste ground and in fields in UK, possibly other parts of Europe.
7. <i>H. pusillum</i> (Little barley)	Frequent wool-alien on tips and waste ground and in fields. Scattered in UK.
8. <i>H. secalinum</i> (Meadow barley)	Lowland coastal and inland meadows on moist, heavy soils. Throughout Western and Southern Europe.
9. <i>H. hystrix</i>	Throughout Europe
10. <i>H. bulbosum</i> L.	Throughout Europe
11. <i>H. bogdanii</i>	Wet meadows. S. E. Russia and W. Kazakhstan.
12. <i>H. brevisubulatum</i>	Saline meadows. S. E. Russia and W. Kazakhstan.
13. <i>Hordelymus europaeus</i> (Wood barley)	Woods and shady places. Scattered through Europe from Sweden southwards.

Source: Stace, 1991 and Tutin *et al*, 1980

in the wild. The risk of transgenes from cultivated barley introgressing into wild relatives of barley is very slight, given the high self-fertility of barley and the strong hybridisation barriers between *Hordeum* species. Similarly, establishment of substantial invasive feral populations seems unlikely (DoE, 1994).

### 7.7. Conclusion

Barley can be described as low risk for crossing of transgenes from genetically modified varieties. Barley has limited potential for outcrossing even with plants in close proximity. There is currently no evidence of naturally occurring interfertility with wild or crop relatives in Europe.

## 8. Fruit crops

### 8.1. Strawberries (*Fragaria x ananassa*)

#### 8.1.1. Crop use and distribution

Strawberry (*Fragaria x ananassa*) is one of the most important soft fruits, being grown extensively in most temperate and in some subtropical countries. World production, mostly accounted for by Europe and North America, exceeds 1.5 million tonnes. Yields of 10-15 t/ha are now obtained from seasonal fruiting varieties outdoors and higher yields are attained in glasshouses. Many strawberries are eaten fresh, and they are also processed for canning, for jams and preserves, for freezing and for flavouring drinks and confectionary.

Strawberry plants are perennial herbs with short, woody stems or stocks and rosettes of leaves. All species and most cultivated varieties are seasonal and produce a sequence of inflorescences and stolons or runners. General propagation is from the plants formed on runners, and each variety is a clone. The berry is a false fruit, an enlarged fleshy receptacle, growth of which is stimulated by the development of many small true fruits (achenes). Strawberry flowers are hermaphroditic and self-compatible to a certain extent. The degree of self-compatibility can be highly variable among different cultivars. In commercial greenhouse cultivation of strawberries the European honeybee has been used as a pollinator (Free, 1968, Abe, 1971). Pollen viability is genetically controlled but varies with air temperature during flowering. Only highly viable pollen retains suitability for pollination and fertilisation at unusually high or low temperatures (Zebrowska, 1997).

*Fragaria ananassa* is octoploid ( $2n=8x=56$ ) and is derived from crosses between two American octoploids, *F. virginiana* and *F. chiloensis*. These species were introduced separately into Europe where they hybridised and were selected and re-crossed to give some of the current modern varieties (de Rougemont, 1989).

#### 8.1.2. Genetic modification

The production of cultivars with resistance to different abiotic stresses, particularly to early spring frosts, is important. Firsov & Dolgov (1999) reported the agrobacterial transformation and transfer of the antifreeze

protein gene of winter flounder to the strawberry. *Agrobacterium*-mediated transformation of strawberry has been reported using a modification of the leaf-disc system developed by Horsch *et al* (1985). James *et al* (1990) used petiole explants co-cultivated with *Agrobacterium tumefaciens* to produce plants showing kanamycin resistance.

Plants transformed with the cowpea trypsin inhibitor gene for insect resistance have also been described (Raybould & Gray, 1993). Being a clonally propagated plant, virus resistance genes would also be attractive to growers.

#### 8.1.3. Wild relatives of *Fragaria x ananassa*

At least 46 species of *Fragaria* have been described, but many are not distinct. The species form a polyploid series, from diploid to octoploid, with a basic chromosome number of  $x=7$ .

*F. vesca* (wild strawberry), the commonest wild diploid, and *F. muricata* (Hautbois strawberry) are distributed throughout Europe although *F. muricata* is thought to be rare and over-recorded. *F. moschata*, the only hexaploid species, exists in central Europe extending to N. W. France, Italy, Turkey and C. Russia. This species is also widely naturalised from gardens in N. Europe. *F. viridis* extends to most of Europe except the islands and the extreme north. Another species recorded by Tutin *et al* (1968) is *F. virginiana*, a type that is cultivated and naturalised locally in E. Europe. All of these species as well as many of their hybrids are or have been cultivated.

#### 8.1.4. Hybridisation

Several diploids, including *F. vesca*, are monoecious, self-compatible and mostly inbreeding, but three diploid species are self-incompatible. Most of the polyploid crop varieties are entirely or predominantly dioecious, although hermaphrodite forms occur and have been selected in cultivation; these are self-compatible, but cross-pollination is either essential or advantageous for full fruit set. This suggests that there is some risk of gene flow from GM crops to non-GM crops.

Experimental hybrids have been produced between *F. x ananassa* and *F. vesca* (Stace,

1975) but all died before flowering. Hybridisation of strawberry with this native species may occur but hybrid viability appears to be limited. A study of potential ecological impacts of GM crops in the Netherlands (De Bokx & van der Want, 1987) suggests that transgenic strawberries can be expected to have minimal impacts by gene introgression on the Netherlands flora. However, this risk should be further evaluated for biotic and abiotic stress resistance characters.

## 8.2. Apples (*Malus x domestica*)

### 8.2.1. Crop use and distribution

The *Malus* genera belongs to the subfamily Pomoideae of the family Rosaceae, all with the basic chromosome number of  $x=17$ . Apple is of hybrid origin, probably derived from *M. sylvestris*, *M. dasyphylla*, *M. praecox* and some Asiatic species. Many cultivars of apple that are grown have been produced by hybridisation with wild species, as opposed to intervarietal hybridisation.

Apple is the most widely grown of the fruit crops. The world crop is estimated to be 40 million tonnes per annum (Smartt & Simmonds, 1995). Production in the EU represents 20 % of world production. Apple is a heterozygous crop ( $2n=34$ ) with strong self-incompatibility tendencies. Honey bees and bumblebees are used as pollinators in commercial orchards. Self-pollination of apple tends to result in lower fruit set.

### 8.2.2. Genetic modification

Current production of apples is intensive, requiring continuous use of chemical sprays against insect pests and fungal diseases. The Bt toxin gene has been used to confer resistance to codling moth. Other serious pests for which insect resistance would be welcome by growers are rosy apple aphid, woolly apple aphid and fruit tree red spider mite (DoE, 1994). Fungal diseases, such as mildew and scab are also important (King *et al*, 1991).

The transformation of apple by infecting wounded leaf explants with *Agrobacterium tumefaciens* has been reported (De Bondt *et al*, 1994; Maximova *et al*, 1998). Early attempts resulted in low efficiency of transformation although 'solid' transgenic lines have been achieved. Chimaeric apple trees with transgenic roots transformed by wild type *Agrobacterium rhizogenes* has also been used (Lambert & Tepfer, 1991), possibly allowing the production of new rootstocks for the grafting of recalcitrant varieties.

### 8.2.3. Wild relatives of *Malus x domestica*

Most of the 33 species of *Malus* are diploid and cross-pollinated. Relatives of *Malus x domestica* recorded by Stace (1991) are *M. sylvestris*, *M. x purpurea*, *M. baccata* L. and *M. floribunda*. *M. sylvestris* (crab apple) is distributed throughout most of Europe and is thought to have some parentage of many cultivars. The species is commonly used in commercial orchards as a pollinator for self-incompatible apple varieties. Other wild species noted by Tutin *et al* (1968) are *M. trilobata*, an evergreen shrub of N. E. Greece, and *M. florentina*, a very localised species of Italy and N. Greece. *M. dasyphylla* inhabits damp lowland woods on the Balkan peninsula, and *M. praecox* exists in deciduous woods along rivers in Russia.

### 8.2.4. Hybridisation

Hybridisation between a high proportion of wild species within *Malus* occurs readily. It is clear from the origin and development of the crop that hybridisation between *M. x domestica* and *M. sylvestris* is possible. No formal records of hybrids exist and therefore it is difficult to predict how widespread gene flow and introgression of GM apple crops into wild species may be. In many parts of the UK the commonest crab apples show evidence of introgression with cultivated apple (Raybould & Gray, 1993) and thus the likelihood of gene flow between cultivated and crab apples is high. There is also a likelihood of gene flow from GM crops to non-GM crops.

The impacts that transfer of disease resistance, for example, from transgenic apple to crab apple might have are difficult to predict. DoE (1994) considers that gene flow effects of this particular trait may be minimal since the consequence of disease in fruit trees often amounts to unsightly fruit rather than disease vigour.

*Malus* and *Pyrus* (pear) belong to the same subfamily though hybridisation between the two genera is difficult and derivatives rarely survive. There is no evidence to suggest that recent hybridisation between the genera has contributed to the evolution of cultivated varieties of either crop.

## 8.3. Grapevines (*Vitis vinifera*)

### 8.3.1. Crop use and distribution

Wild and cultivated grapevines belong to the genus *Vitis* L. in the family *Vitaceae*. Grapes are the world's most widely grown fruit crop

with over 9 million ha grown. The grapevine is a perennial, woody vine climbing by coiled tendrils. The fruit (a berry) is juicy and rich in sugar (15–25 %), in roughly equal proportions of dextrose and levulose. It is the commercial source of tartaric acid and is rich in malic acid. 80 % of the crop is used for wine production and the rest for table grapes and raisins (DoE, 1994). *Vitis vinifera* is the predominant commercial species, and is largely concentrated in regions with a Mediterranean-type climate, with hot dry summers and a cool, rainy winter period. The European grapevine originated around the Mediterranean Basin and the Middle East and has been cultivated for at least 4000 years.

### 8.3.2. Genetic modification

Grapevines are difficult to breed by conventional methods and genetic innovation in viticulture is subject to constraints that are not encountered in other crops. The application of biotechnology to grape breeding should help to overcome these problems.

Much progress has been made in the last few years in the genetic manipulation of grape as reviewed by Gray & Meredith (1992) and Torregrosa (1995). Targeted traits for genetic improvement include resistance to pests (particularly aphid) or diseases, improved ripening and enhanced flavour of the fruit. Tsvetkov & Atanassov (2000) recently presented research on a genetic transformation system by *Agrobacterium tumefaciens* vector based on grapevine repetitive embrogenesis and introduction of genes intended to confer cold and virus resistance.

Early transformation involved the co-cultivation of hypocotyl explants from somatic embryos of the rootstock cultivar, with a disarmed *Agrobacterium tumefaciens* strain encoding  $\beta$ -glucuronidase (GUS) and kanamycin resistance (Mullins *et al*, 1990). Buds and plants were produced with cultivar Rupestris St. George, though with Cabernet Sauvignon and Chardonnay, using petiole explants, only transgene buds were produced. Integration of the foreign DNA was unconfirmed.

Gölles *et al* (2000) induced virus resistance by introducing constructs containing chimeric coat protein (CP) genes of grapevine fanleaf virus (GFLV), including nontranslatable and truncated forms of the CP gene, arabis mosaic virus (ArMV), grapevine virus A (GVA), and grapevine virus B (GVB) into embryogenic cultures of *Vitis*

*vinifera* by *Agrobacterium*-mediated transformation. Integration of the different CP genes was confirmed by PCR, and parallel experiments showed protection against virus inoculation.

### 8.3.3. Wild relatives of *Vitis vinifera*

*Vitis* contains about 60 species which can be divided into three geographical groups: American, Eurasian and Asian. In Europe *Vitis vinifera* contains the subspecies *vinifera* which is cultivated in S. and C. Europe and is widely naturalised, and subspecies *sylvestris*, a coloniser of river banks and damp woods in S. E. and S. C. Europe. *V. coignetiae* is a Japanese grapevine that is grown in gardens and has been found as a relic in parts of S. E. England. Several other species have been naturalised in Europe from America, including *V. aestivalis*, *V. berlandier*, *V. cordifolia*, *V. labrusca*, *V. rotundifolia*, *V. rupestris* and *V. vulpina*.

### 8.3.4. Hybridisation

All known *Vitis* species can be easily crossed experimentally and the F<sub>1</sub> hybrids are vigorous and fertile. Hybridisation between GM and non-GM crops may occur.

Studies on natural populations indicate that hybridisation has occurred and continues (Smartt & Simmonds, 1995). However, what determines the frequency of establishment and rate of spread of this species is unclear and it is difficult, therefore, to predict whether GM grapevine varieties may be more or less likely to hybridise with and introgress into wild species than conventional varieties.

## 8.4. Plums (*Prunus domestica*)

### 8.4.1. Crop use and distribution

All members of the family Rosaceae have a basic chromosome number of  $x=8$ . *Prunus domestica*, part of the subfamily Prunoideae, is a complex species with several subspecies or varieties being recognised. Plum grows up to 10 m in height as a shrub or tree and is cultivated for its fruits as a field crop in most of Europe except the N. E. and extreme north. Plum is one of the most important fruit crops grown in the cooler temperate regions of the world with an annual crop of 6.6 million tonnes. Many derived forms have been cultivated at least since classical times. Many new varieties were bred in the 19<sup>th</sup> century (DoE, 1994).

There are several other species of *Prunus* in

cultivation, including apricots, peaches, nectarines, almonds and cherries.

#### **8.4.2. Genetic modification**

Plum has been genetically modified using *Agrobacterium*-mediated transformation of hypocotyl segments derived from the embryonic axes of ungerminated seeds (Mante *et al.*, 1991). Resulting transgenic plants showed kanamycin resistance and GUS expression, and Southern analyses demonstrated integration of DNA into the plum genome.

*Agrobacterium*-mediated gene transfer has been used to produce plants expressing viral coat protein genes for resistance against Plum Pox Virus (PPV) (Korte *et al.*, 1994; Scorza *et al.*, 1994). This virus is one of the most important pathogens in plum, apricot and peach cultivars, having recently spread through central and southern Europe causing heavy yield losses.

#### **8.4.3. Wild relatives of *Prunus domestica***

There are 21 species of *Prunus* recorded in Europe that are mainly found in hedges, copses, scrub and waste ground. Native species include *P. avium* (wild cherry), *P. spinosa* (buckthorn) and *P. padus* (bird cherry). The two main subspecies of *Prunus* are i) plums and ii) damsons and green-gages. Triploid and hexaploid hybrids of this parentage have been found in the Caucasus (Tutin *et al.*, 1968).

#### **8.4.4. Hybridisation**

Hybridisation occurs between the subspecies of *P. domestica*. There are records of intermediates between plums and damsons in areas where the plants are cultivated. It is likely that these intermediates arose in cultivation and subsequently escaped (Stace, 1975). The intermediate forms identified are fully fertile and there are incompatibility barriers preventing the crossing of certain combinations of the subspecies (DoE, 1994). Evidence suggests that gene flow between GM and non-GM crops may occur.

*P. domestica* has hybridised with buckthorn, producing the hybrid *P. x fruticans*. *P. domestica* is also recorded as having hybridised with the cherry plum (*P. cerasifera*), a common ornamental tree. There are records of interspecific crosses between *P. domestica* *sensu lato* and other *Prunus* species.

*P. domestica* is a frequent escape which can form fully fertile hybrids with buckthorn which can then introgress with either of the parental species. The possibility of gene flow

from GM varieties of *P. domestica* to wild types therefore seems high.

### **8.5. Blackberries (*Rubus fruticosus*) and raspberries (*Rubus idaeus*)**

#### **8.5.1. Crop use and distribution**

The European species of *Rubus*, native and naturalised, are placed in 5 sub-genera containing some 2000 species. Many variants of blackberries and raspberries, some unarmed or with simple leaves, are widely cultivated for their edible fruits in most of Europe. Both species have been favourite garden fruits in Europe and North America for several centuries and they have now become important commercial crops, supplying over 100 000 tonnes of fruit annually for jam making, canning, freezing, yoghurt and flavourings. Both species are also commonly found in natural habitats throughout Europe in both wild and feral forms.

#### **8.5.2. Genetic modification**

Transgenic blackberries and raspberries have been produced using leaf disc transformation with *Agrobacterium tumefaciens*. Modified traits include cowpea trypsin inhibitor gene and arabis mosaic virus coat protein genes (Raybould & Gray, 1993).

Another gene of interest is a dominant gene for spinelessness. Attempts are being made to isolate such a gene by transposon tagging (DoE, 1994). Herbicide tolerance has not been contemplated as yet. In general soft fruits are not weeds in the agricultural environment and appear to have minor pest status elsewhere although volunteer raspberry from seed can be a locally serious nuisance in some crop situations (Harding & Harris, 1994).

#### **8.5.3. Wild relatives of *Rubus fruticosus* and *Rubus idaeus***

Taxonomy of the genus *Rubus* is complex and difficult to define. Almost all species of *Rubus* are agamospecies, segregated from *R. fruticosus* L. Many of these species arose during the Pleistocene era as a result of hybridisation and apomixis (Tutin *et al.*, 1968). This ability of *Rubus* to spread over large areas vegetatively means that the slightest variation tends to persist and become recognised as a species. Due to this, *R. fruticosus* is extremely complicated taxonomy, with over 400 microspecies being recognised in the British Isles alone (DoE, 1994). Other species are sexual diploids and

extensive hybridisation within the group further complicates the genus.

There is some uncertainty over the origins of *R. idaeus*. It is considered by de Rougemont (1989) that the raspberry originated in Asia and became naturalised in Europe as an escape of cultivation. However, Stace (1991) states that the species is probably native. *R. idaeus* is a sexual diploid and its taxonomy is straightforward.

#### **8.5.4. Hybridisation**

Hybridisation events in the *Rubus* genus are too numerous to discuss though some examples can be given. *R. idaeus* is known to hybridise with *R. caesius* (dewberry). Hybrids between the two species have been recorded scattered throughout S. England, where *R. caesius* is the more common species. The hybrids, usually sterile, range from triploid to hexaploid, depending on the ploidy of *R. caesius* (DoE, 1994). *R. idaeus* also hybridises with sexual diploid species in the *R. fruticosus* aggregate. Most other hybrids of blackberries are between species in the *R. fruticosus* aggregate (DoE, 1994).

The levels of gene flow expected to occur between GM and non-GM crops are difficult to predict. It would seem reasonable to assume that hybridisation will occur between GM *Rubus* and its wild relatives. There are large feral and naturalised populations, there is limited self-incompatibility, the plants are wind and insect pollinated and the seeds can also be dispersed by birds. In an experiment designed to assess the risk associated with the testing and large-scale deployment of transgenic raspberries, Luby & McNicol (1995) surveyed wild and feral populations for evidence of the escape genes introduced into raspberry cultivars by traditional breeding. Escape of the semi-dominant L1 gene, affecting fruit size and morphology, could not be detected after 30 years in test plots. The recessive gene *s*, conferring spinelessness, was detected at very low frequencies in wild populations within the commercial production locales where cultivars carrying this gene had been introduced on a large scale 21 years previously. This gene was not, however, found in areas remote from commercial production. The authors concluded that escape does occur following large-scale deployment but that gene flow events are probably infrequent and spread is localized for genes having probable neutral selective value. A similar extent of gene flow would be expected from transgenic raspberries.

## **8.6. Blackcurrants (*Ribes nigrum*)**

### **8.6.1. Crop use and distribution**

The genus *Ribes* comprises some 150 species, all diploid ( $2n=2x=16$ ), distributed mainly in the temperate regions of Europe, Asia and North and South America. *R. nigrum* is native to central and eastern Europe except the Mediterranean. The crop is vegetatively propagated and grown on a limited scale in most cool temperate countries. The bulk of the blackcurrant crop is processed, particularly for juice, but also for canning, jamming, liqueurs, pie fillings and pastilles. The blackcurrant is especially valued for its high vitamin C content.

### **8.6.2. Genetic modification**

Transgenic blackcurrants have been developed by inoculating peeled internodal stem segments with *Agrobacterium tumefaciens* (Graham & McNicol, 1991). In this particular experiment integration of DNA into the blackcurrant genome was not proved although dot blots did show the presence of the transforming DNA.

Similarly to blackberry and raspberry, blackcurrant has also been transformed with the cowpea trypsin inhibitor gene and arabis mosaic virus coat protein genes.

There is some interest in using raspberry bushy dwarf virus coat protein-mediated virus protection, and anthocyanin genes from snapdragon for improving fruit juice colour (DoE, 1994).

### **8.6.3. Wild relatives of *Ribes nigrum***

There are numerous wild relatives of *R. nigrum* scattered across parts of Europe. *R. aureum* is often cultivated and is partly naturalised in C. Europe. *R. multiflorum* is restricted to Italy and the Balkan peninsula. *R. spicatum* exists in N. and E. Europe and is sometimes cultivated in the east, but rarely naturalised outside its native territory. *R. petraeum* inhabits the mountains of C. Europe and extends southwards to Italy and Bulgaria. *R. uva-crispa* (gooseberries) is native to C. and W. Europe and is frequently naturalised by bird dispersal. *R. alpinum* (mountain currant) extends from N. and C. Europe southwards to Spain and C. Italy. *R. orientale* is restricted to C. and S. Greece. Other European species are *R. sardoum* and *R. rubrum* (red currants) (Tutin *et al.*, 1964).

### **8.6.4. Hybridisation**

Classification of *Ribes* based on morphology into subgenera and sections or series has

been remarkably successful in delineating the possibilities of interspecific hybridisation. Most intrasectional (and some intrasubgeneric) combinations produce vigorous, fertile F1s, while intersubgeneric and many intersectional combinations either fail or produce sterile hybrids which invariably show meiotic irregularities (Smartt & Simmonds, 1995). The levels of gene flow expected to occur between GM and non-GM crops are difficult to predict.

No records of hybridisations between *R. nigrum* and its related species are evident in Europe. Although gene flow from GM *R. nigrum* to its wild relatives is improbable, escape of the crop from cultivation is common and therefore the risk of gene flow cannot be ruled out where GM varieties are concerned.

## 9. Evaluation and conclusions

### 9.1. Oilseed rape

The majority of pollen is deposited at very short distances from the pollen source. Pollen can travel considerable distances by means of both wind and insects. Low frequencies of cross-pollination have been recorded at distances of up to 4 km from the source.

While pollen is important in the spatial dispersal of transgenes from oilseed rape, it has a short life-span and provides little temporal dispersal. Seed is also very important in the spatial dispersal of transgenes through dispersal along transport corridors within and between countries. It also allows GM plants to persist at sites for several years.

On a farm-scale the current recommended isolation distance of 100 m will maintain cross-pollination levels at below 0.5 % in the majority of fully fertile crops.

Varieties and lines containing male sterile components will outcross with neighbouring fully fertile GM oilseed rape at higher frequencies and at greater distances than was previously thought. Varietal associations will require considerably greater isolation distances from GM crops than conventional varieties.

Gene flow will occur to and from volunteer and feral populations which can act as gene pools carrying over the contamination into subsequent rape crops.

Gene stacking in volunteers has been observed in GM crops. It is predicted that plants carrying multiple resistances will become common once GM herbicide tolerant rape is widely commercialised. Volunteers may become more difficult to control with herbicide treatments in certain situations, though the current range of selective herbicides used in cereal crops is effective in controlling single and multiple tolerant volunteers.

The risk of hybridisation between oilseed rape and some wild relatives, particularly *B. rapa*, *B. juncea*, *B. adpressa*, *B. oleracea*, *Raphanus raphanistrum* and *Hirschfeldia incana* is high. Long term introgression of

transgenes into some of these Brassica species is likely to occur though the rate and level of introgression will be determined by the enhanced fitness conferred by the transgene. The creation of a herbicide tolerant, competent weed is possible. Gene introgression into other hybridising related species is unlikely since backcross plants fail to persist due to cytoplasmic incompatibility.

Oilseed rape can be described as a high risk crop for pollen mediated gene flow from crop to crop and from crop to wild relatives.

### 9.2. Sugar beet

Pollen from sugar beet seed crops is primarily wind dispersed and has been recorded at distances of more than 1 km at relatively high frequencies. Appropriate atmospheric conditions combined with peak pollen release times can account for longer distance dispersal. Sugar beet flowers are visited by a range of pollinating insect species.

The current recommended isolation distance for GM beet seed production of 1000 m may not guarantee the prevention of seed contamination in the long term.

While pollen is important in the spatial dispersal of transgenes from sugar beet, it has a short life-span and provides little temporal dispersal. Seed is very important in the spatial dispersal of transgenes through dispersal along transport corridors within and between countries. Also, seed can survive in soil from one beet crop to another causing contamination of subsequent crops.

Cross-pollination in root crops is not usually considered an issue since the crop is harvested before flowering. However a small proportion of plants in a crop will bolt and transgene movement between crops may occur in this way. GM bolters occurring in a following crop of conventional beet may pollinate bolters in the current crop and be taken up with the crop at harvest, causing contamination.

Hybridisation between bolting GM beet and weed beet could lead to the transfer of GM traits, in which case GM weed beet may



become more difficult to control with chemical treatments.

Hybridisation and introgression between cultivated beet and wild sea beet has been shown to occur. GM traits could therefore introgress into wild beets.

In one population of wild sea beet gene flow from cultivated beet did not lead to the erosion of genetic diversity of that particular population. In some cases crop-to-wild gene flow will have limited evolutionary effect on wild populations. However, certain transgenes may be more likely to alter the fitness of hybrid or introgressed individuals and change niche relationships between populations.

Sugar beet can be described as a medium to high risk crop for pollen mediated gene flow from crop to crop (especially seed crops) and from crop to wild relatives.

### 9.3. Potato

Wind is considered a more important vector than insects in effecting cross-pollination, though pollen dispersal is generally very restricted due to high self-fertility. Isolation distances of 20 m were recommended for experimental releases.

High frequencies of outcrossing at distances up to 1 km may have been shown to occur in one instance where pollination was by pollen beetle. However this research was criticised for having a significant proportion of false positives.

Cross-pollination between GM and non-GM production crops would not result in the harvested potato tubers becoming transgenic. Furthermore, the crop is usually sown with seed tubers rather than true seed. In this situation the introgression of transgenes into non-GM crops or true seed crops nearby is unlikely.

If GM volunteer tubers, plants and true seed are allowed to persist after a crop the risk of introduction of GM volunteers into following conventional crops exists.

In true seed production crops the likelihood of cross-pollination leading to contamination of neighbouring and subsequent crops is higher unless effective isolation and crop hygiene is carried out.

Feral plants present little or no risk of acting as either a GM pollen source or recipient, though research should continue to determine whether increased feralisation is likely in future GM varieties.

Naturally occurring hybridisation and introgression between potato and its related wild species in Europe is unlikely. Evidence suggests that even if cross-pollination occurred, post-zygotic barriers would prevent the formation of a viable hybrid.

Potato can be described as a low risk crop for pollen-mediated gene flow from crop to crop and from crop to wild relatives.

### 9.4. Maize

Maize is primarily wind pollinated although there is evidence to suggest that bees and other insects collect pollen from maize. The majority of airborne pollen is shown to fall within a short distance of the pollen source, though outcrossing has been recorded at up to 800 m. It is predicted that under suitable atmospheric conditions maize pollen has the potential to travel over much longer distances.

Incoming maize pollen is rapidly diluted by local pollen so that cross-pollination occurs mostly in the first few rows. The recommended isolation distance of 200 m will maintain crop purity at 99 % in most cases. There is no evidence that any current variety is not interfertile with another variety, for example cross-pollination data between maize and sweetcorn exists.

There is no indication that hybridisation between maize and other European crop species can occur.

Maize has poor survival characteristics as a feral plant in much of Europe. The crop is incapable of sustained reproduction outside cultivated areas and is non-invasive of natural habitats.

There are no known wild species in Europe with which maize can hybridise.

Maize can be described as a medium to high risk crop for pollen mediated gene flow from crop to crop, but low risk for gene flow to wild species.

### 9.5. Wheat

Wheat is typically self-pollinated and produces small amounts of pollen with a short viability period. Cross-pollination under field conditions normally involves less than 2 % of all florets. The combination of these factors means that any outcrossing normally occurs with adjacent plants.

In some cases strong winds can disperse pollen widely. Cross-pollination has been recorded at distances of 20 m from the source.

There are no records of naturally occurring hybrids between wheat and any crop relatives. Hybrids formed between wheat and several wild barley and grass species generally appear to be restricted to the F1 generation with little evidence for subsequent introgression due to sterility.

Wheat can be described as a low risk crop for pollen mediated gene flow from GM crops to other crops and to wild relatives. However, GM wheat grown in rotation with conventional wheat could cause some contamination of the latter if volunteers are allowed to persist.

### 9.6. Barley

Barley reproduces almost entirely by self-fertilisation. Small amounts of pollen are produced and the bulk of outcrosses occur between closely adjacent plants.

Rare cross-pollination events are known to occur at distances up to 60 m from the source. However, a crop isolation distance of 1m is deemed sufficient in maintaining seed contamination within acceptable levels for most materials.

Strong hybridisation barriers exist between *Hordeum* species. There are no records of naturally occurring hybrids between barley and any wild relatives in Europe.

Barley can be described as a low risk crop for pollen mediated gene flow from crop to crop and from crop to wild relatives. However, GM barley grown in rotation with conventional barley could cause some contamination of the latter if volunteers are allowed to persist.

### 9.7. Fruit crops

Strawberry has around five wild relatives distributed throughout Europe. Hybridisation may occur but hybrid viability appears to be limited. On the basis of present research transgenic strawberries are expected to have minimal impacts by gene introgression on wild flora.

Apple is a heterozygous crop with strong self-incompatibility tendencies. Hybridisation between apple crops and between apple and some wild species is possible, though it is difficult to predict how widespread gene flow and introgression of GM apple crops into wild species may be.

Grapevine has very few related wild species with which it could hybridise, although hybridisation does occur. It is possible that some gene flow from GM grapevine varieties to conventional varieties and to wild species will occur.

Plum is a complex species with several subspecies or varieties being recognised. There are 21 species of *Prunus* recorded in Europe. Cultivated plum is a frequent escape and therefore there is a high likelihood of gene flow from GM varieties to wild types occurring.

The European species of *Rubus* are placed in 5 sub-genera containing some 2000 species. Hybridisation events in wild and feral populations are numerous, but gene flow from cultivated blackberries and raspberries to wild populations does not seem to occur to any significant degree. GM *Rubus* varieties should be monitored prior to release to determine whether outcrossing to wild species would be more likely to occur.

Cultivated blackcurrant has numerous wild relatives scattered across parts of Europe, though there are no records of hybridisations. Gene flow from GM blackcurrants to wild species is unlikely but cannot be ruled out.

There is limited information on crop to crop gene flow for the fruit crops and therefore definite conclusions cannot be made at present. However, there is some likelihood of gene flow from GM crops of strawberry, apple, grapevine and plum to other crops occurring. Crop to crop gene flow in blackberry, raspberry and blackcurrant is more difficult to predict although the reproductive characteristics of these species make it a possibility.

# 10. Future considerations and recommendations

## 10.1. Gene flow: Crop to crop

At farm and regional scale gene flow can occur over long distances and therefore complete genetic purity will be difficult to maintain within the official isolation distances, for crops such as oilseed rape, maize and sugar beet. Here we present some recommendations for farm practice to minimise crop contamination.

- i) The current isolation distances should be reviewed for some crop types and further stringency applied in order for levels of gene flow, albeit low, to be further reduced. This especially applies to seed production crops because any genetic impurity will then be present throughout the life cycle of the standard crop grown from the contaminated seed, and may be multiplied to higher levels.
- ii) It is now apparent that varieties and lines containing male sterile components will outcross with neighbouring fully fertile GM varieties at higher frequencies and at greater distances than previously thought. Therefore varietal associations such as Synergy will require considerably greater isolation distances from GM crops than conventional varieties.
- iii) As well as isolation distances, barrier crops could be used as standard where they are thought to be effective in reducing cross-pollination levels (see section 10.4.2) and where genetic purity is most essential (e.g. seed production crops).
- iv) Neighbouring farms should inform each other of their planting intentions in order for appropriate isolation measures to be considered.
- v) Gene flow can occur to and from volunteer and feral populations which act as gene pools carrying over the contamination into subsequent crops. Management systems should be used to minimise GM seed spread on a farm and to minimise seed bank and volunteer populations. Allowing GM volunteer populations to discharge viable seed will cause a large increase in the burden for following crops (Harding & Harris, 1994) through gene exchange from volunteers to crops, and the possibility that GM volunteer plants could be harvested with the crop and passed on to the consumer.

- vi) As well as removing any volunteers from a field that has previously been cropped with a GM type, when sowing conventional types volunteer contamination can be prevented by taking into account whether GM crops were grown previously in a field and whether farm practices were likely to have moved seed to that field from other fields.
- vii) The development of GM plants which incorporate biological methods to restrict the spread of transgenes between crops should be encouraged (see section 10.3).

## 10.2. Gene flow: Crop to wild relatives

It has been recognised that over time even small amounts of gene flow can have important effects on evolutionary change (Wright, 1931). Gene flow between crops and their related wild species may have two potentially harmful consequences: the evolution of increased invasiveness and persistence and the increased likelihood of extinction of wild relatives. It is difficult to predict, however, the precise limits of sexual barriers between individual crop types and their related species, or the likelihood of hybrids forming and persisting in agricultural or natural habitats. There are several areas in which we need to become better informed:

- i) The current levels of hybridisation and introgression occurring between conventional crops and wild species, and the behaviour of these hybrids. This will determine the factors influencing the extent of gene flow and the likelihood of transgenes becoming established in wild populations (Dale, 1992). It will also provide baseline data against which to assess the possible impacts of transgenes.
- ii) The geographical distributions of GM crop types and any wild plant species with which the crop is capable of hybridising.
- iii) The fate and consequences of transferred genes in different species in order to improve understanding of the genetic and ecological principles involved.
- iv) The stability of transgene expression over generations and in different genetic backgrounds, to determine the extent to

which transgene action and stability can be modified by genetic background, particularly in taxonomically wide hybrids (Dale, 1994).

- v) Test protocols to determine the likely effect of a transgene in a hybrid, so that on release of a GM crop the site can be surveyed for wild relatives and a risk assessment undertaken on a case-by-case basis (until we gain a better understanding of the above points).

### 10.3. Gene flow barriers

As well as identifying the agronomic and environmental risks associated with the release of GM crops, of a primary concern is the development of methods to restrict the spread of introduced genes to other crops and to wild plant populations. Developers of transgenic crops also want to limit gene escape so that competing companies cannot acquire unique genetic constructs through pollen dispersal. Here we give an overview of the various biological and physical barriers to gene flow that are being researched and developed for possible future use.

#### **10.3.1. Biological gene flow barriers**

A consideration for minimising crop to crop gene flow and environmental exposure to transgenes is to design and construct GM plants with improved biosafety characters. This could be achieved, for example, by preventing or minimising cross-pollination, avoiding antibiotic resistance marker genes, or switching on inserted genes only when and where they are needed in the plant. There are three ways in which reduced exposure of transgenes to the environment might be accomplished:

- i) Avoid or minimise inclusion of superfluous transgenes or sequences
- ii) Avoid or minimise superfluous expression of the transgene
- iii) Avoid or minimise the dispersal of transgenes in the environment

The emerging technologies for each approach are discussed in more detail in the discussion paper 'Guidance on Best Practice in the Design of GM Crops' (DETR/ACRE, 2000). Here we look at (iii) the methods to avoid or minimise the dispersal of transgenes in the environment. They include:

##### *10.3.1.1. Apomixis*

Apomixis is the production of seeds without fertilisation, a process that occurs naturally

in many plant species. Transfer of the primary transgene to neighbouring crops via pollen would be minimal because plants can be male sterile without compromising seed or fruit production.

##### *10.3.1.2. Cleistogamy*

Cleistogamy occurs naturally in some plant species, a process whereby self-pollination and fertilisation occurs with the flower remaining unopened so pollen is unlikely to escape from the flower. The adoption of this process to minimise transgene dispersal would require modifications to flower design.

##### *10.3.1.3. Hybridisation barriers*

Interspecific hybridisation only occurs between closely related plant species. Hybridisation between more widely diverged species is prevented by two main barriers; interspecific incompatibility at the stigma surface or within the style which prevents fertilisation, and post-fertilisation barriers that cause seed abortion. Strengthening either barrier would potentially prevent hybridisation.

##### *10.3.1.4. Inhibition of flowering to block floral development*

In recent years the molecular basis of the processes that control flowering has been determined. Such studies open up the possibility of manipulating flowering time control genes and blocking or promoting flowering in a range of species.

##### *10.3.1.5. Genetically engineered male sterility so that a plant produces infertile anthers*

Pollen development can be prevented by destroying the tapetum of a developing anther using non-specific nucleases driven by cell-specific promoters. Nuclease inhibitors can be crossed in to restore pollen fertility. Recently, several promoters have been developed that are induced by the application of exogenous chemicals. Such promoters could be used to control flowering or fertility 'restorer genes' when required. Male sterile flowers can still be pollinated by exogenous pollen and produce viable seeds.

##### *10.3.1.6. Seed sterility*

This technology enables crops to be genetically modified so that they produce seed that is incapable of germination, offering a promising technique for genetic isolation. This means, however, that the seed cannot be saved and replanted the next season. At present seed sterility has not been adopted

because several aspects of the technology are unreliable and require further development.

#### *10.3.1.7. Plastid transformation technology*

Daniell *et al* (1998) have obtained high levels of transgene expression by inserting herbicide tolerance genes into the tobacco chloroplast genome. An advantage of this technology is that integration of foreign DNA into chloroplast DNA can be more precise. Also, chloroplast transformation technology may limit transgene dispersal through pollen in crops because chloroplasts are predominantly maternally inherited in most higher plant species, though there may be some paternal transfer, and this would have to be examined in each risk assessment.

### **10.3.2. Physical gene flow barriers**

#### *10.3.2.1. Isolation zones*

An isolation zone is an area between a GM crop and a nearby non-GM crop that is either de-vegetated (a 'barren zone') or planted with a non-insect pollinated crop that would discourage insect pollinators from leaving the GM crop. Recent research on the effects of isolation zones, and to what extent increasing the width of the isolation zone reduces gene flow shows varying results. In experiments with oilseed rape field trials Morris *et al* (1994) found that barren zones less than 8m in width actually increased gene exchange above the amount observed at comparable distances in continuous crops of oilseed rape. The barren zone appeared to 'reset' the zero point of the gene dispersal profile to the end of the barren zone. The same type of effect has also been encountered in maize trials. Because most outcrossing will occur in the first few rows of the crop nearest to the pollen source, this in effect means that the border rows act as 'buffers' to the dispersal of contaminating pollen in the rest of the crop.

The impact of isolation zones on rates of gene flow in insect-pollinated plants is

ultimately dependent on their influence on the behaviour of insect pollinators, and this will vary between crop types and sites, and with different weather conditions. Further research may establish how widely these parameters are likely to vary between sites and whether or not standard isolation zones could be applied to GM crops. Research must also consider how wide an isolation zone must be before it deters insects from moving from one crop to another, and whether a valid option would be to discard the first few outer rows of the recipient crop as 'buffer' rows.

#### *10.3.2.2. Barrier crops*

A barrier crop is a border of non-GM plants of the same crop surrounding the GM variety that can act as an 'absorber' of the GM pollen. The barrier rows are then destroyed after flowering. Barrier crops appear to function in a number of ways, primarily in producing masses of pollen to dilute pollen being introduced from adjacent fields. The barrier also increases the distance the pollen must travel from a source to a receptor crop, and foraging insects are likely to visit the barrier crop at the edge of the field before moving on to a potential receptor crop. Similarly, a barrier crop around a receptor crop would mean that insects are likely to make their first visit in the field to a plant in the barrier.

In their experiments Morris *et al* (1994) found that barrier crops had a significant influence on gene escape. The authors suggest that if a small area of 4 to 8m is only available for containment methods, the most effective strategy would be to plant the entire area with a trap crop that could be destroyed before seed set. Many experimental releases of GM crops in the UK and Europe have included barrier crops to restrict GM pollen flow from the release site. Barrier crops are also discussed in section 3.3.4 (sugar beet) and section 5.5.4 (maize).

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## Appendix: Assessment of the Impacts of Genetically Modified Plants (AIGM)

The European Science Foundation (ESF) is the European association of research funding agencies, national research organisations and national academies of sciences and letters from 24 European countries. Its role is to stimulate, develop and support research at a European level, principally in the basic part of the research spectrum. It does this through networking researchers from across Europe and, through its Scientific Programme Scheme, receives additional financial contributions from its Member Organisations on an *à la carte* basis. The AIGM programme was started in 1999 and the level of interest in the topic of the impact of genetically modified plants may be seen from the large number of agencies supporting the programme. ESF warmly welcomed the opportunity to work with the European Environment Agency on this important topic, building on the expertise available through the AIGM programme and thus producing an additional European added value.

Genetically modified (GM) plants are approaching commercialisation and widespread deployment in Europe. Risk assessments supporting release applications have largely been based on assumptions that genetic modifications of plants will not alter their behaviour, or that of other organisms, in the natural environment. These assumptions are made from limited information on actual levels of gene flow occurring between crops and wild species and small scale experiments with GM plants and untransformed plants. Large scale releases of GM plants occurring in North America and other countries provide some additional information on risks but are not always relevant to European environments. There is thus concern that risk assessments are based on limited experimental data which do not fully take account of the novelty of the transgenes or the scale and scope of their ultimate commercial deployment. There is also concern at the large number of different releases that are being proposed in Europe. Information on the transgene interactions within and between GM plants is extremely limited, as is information on the environmental impacts of multiple transformations in single plants, many of which could arise unintentionally. An additional

concern is that GM plants may require different agronomic management or may have agricultural consequences that impact on the environment, e.g. changes in agrochemical usage, effects on predators etc. Agricultural impacts are not always considered in environmental risk assessments, and yet agriculture is a significant component of the total European environment.

A workshop in Cambridge, UK, in October 1997, sponsored by the European Science Foundation, brought together European scientists involved in environmental impact research, plant breeders and representatives of organisations involved in the regulation of GM plant releases. They discussed the range of transformations and plant species most likely to have environmental impacts. They agreed on a number of research priorities and also agreed that research in Europe required coordinating and enhancing so that scientific information could be collated and conclusions made more widely available to support risk assessments in European countries and elsewhere.

The AIGM Programme has been established to coordinate the activities of the principal research programmes in Europe, to enhance them by recruiting younger research personnel to study in them, and to encourage these research programmes to respond to the new research priorities identified by the Programme. It publicises the results of the research through conferences and workshops to a wide range of audiences in Europe particularly to countries with little experience with GM plants and risk assessments. Members of this Programme are available to give expert views on risk assessments and to assist with the development of regulations based on sound scientific principles.

The Programme lasts for 5 years, from 1999 to 2003. It is supported by the following ESF Member Organisations:

*Belgium:* Fonds National de la Recherche Scientifique (FNRS) and the Fonds voor Wetenschappelijk Onderzoek - Vlaanderen (FWO)

*Czech Republic:* Akademie ved České republiky and Grantová agentura České republiky

*Denmark:* Statens Naturvidenskabelige Forskningsråd

*France:* Ministère de l'Éducation Nationale, de l'Enseignement Supérieur et de la Recherche

*Germany:* Deutsche Forschungsgemeinschaft (DFG)

*Italy:* Consiglio Nazionale delle Ricerche (CNR)

*Netherlands:* Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)

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*Sweden:* Skogs- och Jordbrukets Forskningsråd (now FORMAS)

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