## **GM** Issues

## ORGANIC FARMING AND GENE TRANSFER FROM GENETICALLY MODIFIED CROPS

Catherine L. Moyes and Philip J. Dale

John Innes Centre, Colney Lane, Norwich, NR4 7UH

MAFF Research Project OF0157 Copyright © 1999 John Innes Centre. All rights reserved.

Published by the John Innes Centre, UK; May 1999.

Archived at http://orgprints.org/8260

## **Executive Summary.**

- 1. Genetically modified (GM) crops cannot be released into the environment and used as food, feed, medicines or industrial processing before they have passed through a rigorous and internationally recognised regulatory process designed to protect human and animal health, and the environment.
- 2. The UK body that oversees standards in organic farming, the United Kingdom Register of Organic Food Standards (UKROFS), has ruled that genetically modified (GM) crops have no role to play in organic farming systems. They, therefore, have concerns about the possibility and consequences of the mixing of GM crops with organic crops.
- 3. The two main sources of mixing are through pollen and seed. Pollen from GM crops may pollinate an organic crop. Seed from a GM crop, or plants established from them, may become mixed with organic crops or their products.
- 4. Minimising genetic mixing is an important feature of the production of all high quality seed samples of plant varieties supplied to farmers. Extensive experience has been obtained over many decades in the production of high purity seed samples. Crop isolation distances, and crop rotational and management practices are laid down to achieve this. These procedures for the production of seed of high genetic purity could be used for the production of organic crops.
- 5. No system for the field production of seed can guarantee absolute genetic purity of seed samples. Very rarely long distance pollination or seed transfer is possible, so any criteria for organic crop production will need to recognise this. There has always been the possibility of hybridisation and seed mixing between organic crops and non-organic crops. Organic farming systems acknowledge the possibility of spray or fertiliser drift from non-organic farming systems, and procedures are established to minimise this. In practice, detecting the presence of certain types of GM material in organic crops, especially quantification, is likely to be difficult.
- 6. Some seed used by organic farmers are currently obtained from abroad. After January 2001, or a modified deadline thereafter, UK organic farmers will be required to sow seed produced organically. There is little or no organic seed produced in the UK at present, so it has to be obtained from abroad. Seed obtained from outside the UK or the European Union, may have different seed production criteria. This may make it difficult to guarantee that it is absolutely free from any GM material.
- 7. Organic farmers and/or GM crop producers will need to ensure that their crops are isolated from one another by an appropriate distance or

barrier to reduce pollen transfer if the crop flowers. To reduce seed mixing, shared equipment will need to be cleaned and an appropriate period of time allowed before organic crops are grown on land previously used for GM crops. Responsibility for isolation will need to be decided before appropriate measures can be implemented. The report highlights the need for acceptable levels of the presence of GM material in organic crops and measures identified to achieve this.

## ACKNOWLEDGEMENTS

We thank the Ministry of Agriculture, Fisheries and Food for supporting this project (OF 0157) and BBSRC for use of their facilities. We thank Professor Martin Wolfe of the Elm Farm Research Centre, Hamstead Marshall, Newbury, Berkshire, RG20 0HR for acting as a consultant on organic agriculture and John MacLeod (Director) and Tim Cox (Seed Production Officer) of the National Institute of Agricultural Botany Cambridge for their helpful discussions. We also thank colleagues at the John Innes Centre for discussion and Helen Ghirardello for preparing the manuscript.

## ABBREVIATIONS AND DEFINITIONS

- Contamination The term is not used in a pejorative sense. It is defined in this report as "the unintended presence of a plant or plant part". A single non-GM seed in a GM seed sample would be a contaminant, as would a single GM seed in a non-GM seed sample.
- GM Genetic Modification
- IFOAM International Federation of Organic Agricultural Movements. An international body that regulates organic standards.
- UKROFS United Kingdom Register of Organic Food Standards.

## 1. BACKGROUND

Organic farming organisations have ruled that genetically modified (GM) crops have no role to play in their farming systems. Statements to this 1.1 effect have been incorporated in the United Kingdom organic farming guidelines issued by the UK Register of Organic Food Standards (UKROFS). Relevant extracts from the UKROFS Production Standards document are given in **Appendix 1**. Organic farmers have become concerned that contamination of organic crops, by GM crops, may lead to loss of their organic certification (Massood, 1998). In addition, the EU will require the use of organically produced seed in organic farming from 1 January 2001, although it is possible that this deadline will be modified.

1.2 Contamination by pollen or seed from any source is an issue that has always faced seed producers, and regulations concerning the growth of seed production crops exist to counter this problem. A summary of the routes of crop contamination is provided in **Figure 1**. This report will review the likelihood and consequences of contamination of an organic crop by all of these routes. The report will also include a summary of the GM crops that are most likely to be released in the UK during the foreseeable future.

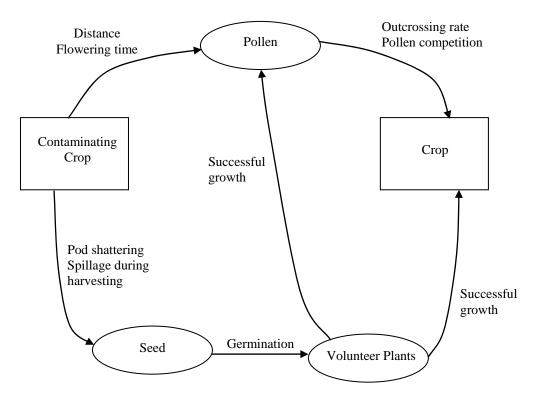


Figure 1. Routes of crop contamination. The main factors influencing the different routes of genetic contamination are shown.

## 2. CONTAMINATION THROUGH POLLEN DISPERSAL

- 2.1 The amount of cross contamination between varieties by pollen is controlled by a number of factors. The most important of these are the physical distance between the pollen donor plants and the crop, the amount of outbreeding in the crop, the overlap in flowering period and the area of the crops grown. All of these factors are important in determining contamination levels in the field. The fourth factor, area of the crop grown, is particularly relevant to many of the small-scale trials set up to investigate pollen contamination. Pollen dispersal over distance forms a leptokurtic curve with a tail containing long distance dispersal events. Small trials almost always truncate the tail. Because the tail contains more dispersal events than an equivalent normal distribution, extrapolation to longer distances may be misleading (Ellstrand 1992). Trials with transgenic plants have been necessarily cautious and limited to small plots. However, other studies have measured dispersal from existing widespread crops (Timmons et al., 1995; Wilkinson et al., 1995) and data are available on contamination of existing seed production crops (see below).
- 2.2 Most field investigations have concentrated on isolation distances and pollen dispersal over distance. These studies have either looked at pollen grains directly or used trap plants to detect pollen. The first approach does not allow for pollen mortality and so may provide overestimates of maximum distances travelled where only viable pollen is of interest. Values of 45, 220 and 80 minutes for pollen longevity have been found for wheat, rye and triticale, respectively (Fritz & Lukaszewski, 1989) and 20min-2hr for maize (Dumas & Mogensen, 1993). The life span of grass pollen may be as short as 30 minutes and even in insect-pollinated species with sticky pollen, longevity rarely exceeds 1 day (Richards, 1986).
- 2.3 Studies using trap plants to detect pollen often do not consider factors such as outcrossing rate (the proportion of seed not resulting from self pollination) so the results obtained cannot be compared across species, or even varieties, where they vary in outcrossing rate. Many of these trials have used small plots where the ratio of pollen donor plants to pollen recipient plants is likely to have an effect on the contamination rates found. If a small number of trap plants are used to detect pollen from a large source this could give high contamination rates particularly in insect-pollinated plants where edge plants are more likely to be visited by pollinators. However, these trials do measure viable pollen and this is important when considering contamination.

- 2.4 Despite the variability in results from these studies, and the different models developed to predict pollen dispersal (Di-Giovanni *et al.*, 1990; Peart, 1985; Bateman, 1947c; Lavigne *et al.*, 1996), the main conclusions agree. Pollen concentration decreases rapidly close to the source but low levels can be detected at much longer distances. This is true for wind and insect pollinated species.
- 2.5 Below is a summary of the studies carried out on pollen dispersal in different crop plants. Because of the difficulties in comparing the actual levels of contamination found across studies (discussed above) we describe the key parts of the dispersal curve instead. That is, the distance over which levels drop rapidly and the distance over which low levels persist. We give the *level contamination declines to as a percentage of the amount found at the pollen source and the maximum distances at which pollen is detected*, although this is often the maximum distance tested. To differentiate between the two methods of measuring pollen dispersal (described above), we refer to *pollen concentration* where pollen concentration was measured directly and to *contamination rates* where pollination was measured following hybridisation with trap plants. We have only quoted an *actual contamination rate* (as opposed to the change in rate/concentration) where whole fields of source plants were used.

#### Apples

Wertheim (1991) found that contamination declined to 6-13% within 5-15m and was detectable at 40m. Johnson (1993) found that pollen-borne viruses are carried up to 56m by bees in apple orchards.

#### Beans

Stoddard (1986) quoted a distance of 194m from the hive as the maximum distance at which bees enhanced fertilisation in faba beans. Ibarra-Perez (1996) found that in common beans 1.9-2.7% of seed were cross-pollinated at a distance of 0.76m.

#### Beets

Bateman (1947b) found that contamination declined to 4% at 4.6m and was detectable at 20.8m. van Raamsdonk & Schouten (1997) found that pollen concentration declined to 17% over 250m, resulting in an actual contamination rate of 2%, and pollen could be detected at 1000m.

#### Brassica crops

Bateman (1947a) found that contamination rate declined to 5% at 6.1m and was detectable at 18.5m. Stringham & Downey (1978) found that contamination rate dropped sharply in the first 137m and was still detectable at 366m. The actual contamination rate at 137m was 5.8%. Mesquida & Renard (1982) found that pollen concentration declined to 7-10% at 24-32m. McCartney & Lacey (1991) looked solely at airborne pollen and found that

concentration at the ground was 2-10% at 100m and predicted an actual contamination rate of 0.6-3%. Darmency & Renard (1992) detected low levels of pollen at 800m. Scheffler *et al.* (1993) found that contamination declined to 0.4% at 12m and was detected at 47m. Pauk *et al.* (1995) found that contamination declined by two orders of magnitude in 32m. Scheffler *et al.* (1995) found that low levels of pollen could be detected at 400m. Timmons *et al.* (1995) found that airborne pollen concentration declined to 10% at 360m and low amounts were detected at 1500m. Wilkinson *et al.* (1995) found that 100 rapidly within 32m, then stabilised after 32m giving actual contamination rates of 0.03-0.05%. Contamination could be detected at 100m. Lavigne *et al.* (1996) predicted pollen concentration would decline to 6% at 54m and low levels would be found at 160m.

#### Carrot

Van Raamsdonk & Schouten (1997) quote a mean forage distance of 1663m for bees visiting carrot flowers and a maximum of 6117m. No indication is given of the implications for pollen dispersal.

#### Grasses

Griffiths (1950) found that perennial ryegrass contamination declined to 5.2% over 277m which gave a contamination rate of 5% when donor plants were grown on a field scale and trap plants were limited in number. Rows of plants between the crops reduced this contamination by 24%. Van Raamsdonk & Schouten (1997) quote a concentration decline to 17% at 250m and detectable levels at 1000m for grasses. Rhebergen et al. (1991) detected pollen at 32m and found that pollen dispersal in red fescue is very hard to predict. Giddings et al. (1997) found that perennial ryegrass pollen concentration declined to 17% at 8m and 4% at 80m. Nurminiemi et al. (1998) found that meadow fescue contamination declined sharply within 50m and was detectable at over 300m.

#### Legumes

Goplen et al. (1972) found contamination at 1609m for sweet clover but where insect-attracting crops (oilseed rape) were grown in the vicinity they found low contamination at 46m.

#### Maize

Bateman (1947b) found that contamination declined to 4% at 9.2m and was detectable at 24.6m. Greenpeace commissioned work by two independent laboratories in Germany and found that contamination of organic maize, by GM maize, declined to 16% at 5m and to 2% at 10m. This resulted in an actual contamination rate of 0.05-0.2% at 10m in the main direction of the wind, but no overall field contamination rate was calculated (Greenpeace International, pers. comm.).

#### Oats (wild oat)

Rai & Jain (1982) found that contamination declined rapidly over the first 2.5m and was still detectable at 6m for the slender wild oat. Andrews (1998) found that pollen flow in wild oats was low and that movement of seed within a field was more important.

#### Onion

Van Raamsdonk & Schouten (1997) quote a mean foraging distance of 557m for bees visiting onion (Allium cepa) flowers and a maximum of 4246m. No indication is given of the implications for pollen dispersal.

#### Potato

McPartlan & Dale (1994) found that contamination dropped to 0.14% at 10m. Schittenhelm & Hoekstra (1995) found that contamination declined rapidly within 20m and was detectable at 80m. Many potato varieties are at least partly male sterile which reduces the risk of contamination considerably (Eijlander, 1994).

#### Radish

Bateman (1947a) found that contamination declined to 5% at 46 m and was detectable at 178.5m.

#### Rye

No data were found for rye crops but wild rye species show extensive gene flow between populations (Sun & Corke, 1992).

#### Wheat

de Vries (1974) found that contamination declined to 10% at 3m and was detectable at 20m. In spelt wheat the very tight glumes prevent cross-pollination (Winzeler, 1994).

- 2.6 The above papers span 50 years and include both studies aimed at identifying isolation distances that will give varietal purity of a particular level and studies investigating the transfer of transgenes. Overall, the two sets of studies do not differ in their results despite their different goals. Where transgenic and non-transgenic pollen of the same variety have been compared directly, their dispersal did not differ (Hokanson *et al.*, 1997). None of the transgenic plants used would have been expected to differ from non-transgenic plants in their pollen dispersal. In the future plants may be developed with altered pollen characteristics or breeding systems.
- 2.7 In general, studies that have looked at the dispersal of pollen directly gave lower rates of decline and higher distances travelled than studies that used pollination of trap plants to give contamination rates. Not all pollen will result in contamination because of factors such as the outcrossing rate of the recipient species/variety. Further reductions will result from competition from pollen intended to pollinate the crop (Skogsmyr, 1994). For example, millet is predominately self-pollinating and only 2% of the seed arise from cross-pollinations. When Wang et al. (1997) investigated pollen dispersal from millet they found that the amount of pollen that could potentially fertilise trap plants declined to 13% at 20m. For every 100 pollen grains released outside the source only 1.5 seed were produced and the contamination rate at 20m was less than 0.01%. When pollen competition was removed by using male sterile plants the contamination at 20m was over 0.1% of the potential seed harvest. The maximum distance at which contamination was detected was also increased by the use of male sterile plants. This example illustrates how low outcrossing rates

result in low contamination rates and pollen competition reduces contamination still further.

- 2.8 Some crops are perennial and seed propagated, therefore pollen contamination could accumulate over several years (see section 4.5). Very few studies have considered gene flow from crops over many seasons. Luby & McNicol (1995) investigated the transfer of genes from raspberry crops to wild *Rubus* spp. over 20-30 years. They found that some genes could not be detected at all whereas others were found at low frequencies (0.004) but only at sites within 2000m of the crop sites.
- 2.9 All of these factors have been taken into account to produce the MAFF regulations on minimum isolation distances for seed production crops. Seed production crops are classified into breeders, pre-basic, basic, certified 1<sup>st</sup> generation and certified 2<sup>nd</sup> generation seed. Breeder's seed is used for the production of pre-basic and basic seed. Pre-basic and basic seed are used for further multiplication to produce the certified seed supplied to farmers. Because basic seed is used for multiplication it must be of a higher standard than certified 2<sup>nd</sup> generation seed. Higher voluntary standards for cereals can be applied to all categories except pre-basic. **Table 1** gives details of the standards for the production of basic and certified (1<sup>st</sup> generation) seed, outcrossing rates and mode of pollination for each crop. This provides a summary of the regulations. More detailed information is given in the MAFF Guide to Seed Certification in England and Wales (1998).
- 2.10 The basic and certified seed of hybrid varieties of rape (*B. napus, B. rapa*) should have a genetic purity of 99.9% and 99% respectively (MAFF 1998). Values of 99.7% for basic seed and 99% for certified seed of beans are quoted (Crofton, 1997). Ten years ago Standard seed was introduced as a class for vegetable seed. Standard seed does not require field inspections, which include isolation distances, but the seed must still reach set standards for varietal purity (Neil Stern, Seed Certification, NIAB, *pers. comm.*).
- 2.11 The European Community (EC) Seed Marketing Directives are implemented in the MAFF regulations therefore the isolation distances used in the UK are similar to those in other member states. Some additional requirements are also incorporated in the UK. The UK also participates in the OECD schemes for Herbage and Oilseed, Cereal seed and Beet seed. The isolation distance required in Canada, New Zealand and the USA are given in **Appendix 2**. These are also very similar to those implemented in the UK.
- 2.12 The scientific studies largely found that pollen concentration/contamination rate dropped to low levels within the MAFF isolation distances. Where the isolation distances have been tested with seed production crops and the results published, no contamination above the allowable rate has been found (Apostolides & Goulas, 1998

(beet); Stringham & Downey, 1978 (turnip)). However, conditions could give rise to contamination rates above acceptable levels. The National Institute of Agricultural Botany (NIAB), responsible for policing certified seed production, report high standards in crops entered for certification (Annual Report 1996/7).

- 2.13 The criteria used to assess contamination levels are usually those that readily identify the variety and therefore may not always detect contamination from a variety with similar traits. A transgene transferred from a GM crop may not have a readily identifiable effect. Other non-GM traits may be transferred from the GM crop alongside the GM-trait, but contamination may still not be easily distinguishable. In addition to visual characterisation, starch gel electrophoresis is used to characterise seeds biochemically e.g. maize seed (McDonald, 1998). The development of GM crops has led to research on the more accurate but expensive DNA-based methods (McDonald, 1998) and NIAB plan to introduce DNA methods that identify varieties as part of their laboratory services (Annual Report 1996/7).
- 2.14 Contamination can occur outside the official isolation distances and pollen was detected at long distances (1000m or more) for beet, brassicas, grasses and legumes. The frequency of this contamination is low and it is not possible to quote an actual figure from the data available. Organic farmers often double or treble the stringency of precautionary measures. If this practice were to be applied to isolation distances then contamination may be reduced further as levels do decline with distance. However, at these distances pollen levels have reached the tail of the dispersal curve where low levels are observed and do not decrease rapidly over distance. Doubling or trebling isolation
- 2.15 The above data consider the risks of cross contamination between crops of the same species. However, some closely related crop species may also cross-pollinate. This may be of relevance to organic farmers because although oilseed rape is not produced organically (Martin Wolfe, *pers. comm.*) it is widely grown and may pollinate certain other *Brassica* crops (e.g. Bing *et al.*, 1996). The MAFF regulations on isolation distances take account of this factor. *Brassica* crops, grasses and cereals have been grouped into species which cross-pollinate, and a seed production crop must be isolated from all plants within the same group.
- 2.16 The probability of contamination by GM pollen may be reduced by placing the transgene in the cytoplasmic genome (plastid or mitochondria DNA), as opposed to the nuclear genome. These organelles are generally maternally inherited and so the likelihood of transgene transfer by pollen contamination is greatly reduced. However some species, for example carrot and most conifers, do transmit plastids through their pollen (Reboud & Zeyl, 1994; Cato & Richardson, 1996). Furthermore, in

species where organelles are maternally inherited this is not always completely true. Rare paternal transmission of mitochondria has been found in oilseed rape and in barley x rye crosses (Reboud & Zeyl, 1994; Erikson & Kemble, 1993). Rare paternal transmission of plastids has been found in runner beans, peas, potato and annual meadow grass (Corriveau *et al.*, 1989; Reboud & Zeyl, 1994). Plastids are inherited from both parents in rye (Reboud & Zeyl, 1994). This has not been investigated thoroughly for many species but levels of rare paternal transmission seem to be less than 10% with a mean of about 2% (Cornu, 1988; Sewell *et al.*, 1993).

## 3.CONTAMINATION THROUGH SEED DISPERSAL

- 3.1 In addition to pollen, seed can also be dispersed from their field of origin. Seed are produced and dispersed from crops in much lower numbers than pollen grains. However, if contaminant seed go on to establish volunteer plants that produce pollen within a new crop, this contaminating pollen source will not be isolated by distance from the crop. In this way an individual seed dispersal event may have a greater effect than an individual pollen dispersal event. The information is not available to quantify the relative importance of contamination by seed and pollen but we can say that both routes need to be controlled to minimise contamination.
- 3.2 There are a number of mechanisms by which seed may be dispersed. In many wild plant species, animal and wind dispersal are important in long distance seed dispersal (Thill & Mallory-Smith, 1997). However, many of the crop plants grown in this country have no specialised structures adapted for seed dispersal. Furthermore, many of our crop plants have been bred to reduce pod shatter and hold their seed on the plant (Morgan *et al.*, 1998). Where seed are lost they will usually fall next to the mother plant.
- 3.3 However, the agricultural environment does provide alternative mechanisms for long distance seed dispersal. Cultivation methods differ in their effect on seed dispersal but movement is mostly limited to within the field (Rew & Cussans, 1997). Seed can be transported between fields by sowing, cultivation or harvesting equipment which is not thoroughly cleaned between uses (Strykstra *et al.*, 1997; Thill & Mallory-Smith, 1997). Longer distances can be covered when the harvested seed are transported and spillages occur. This has been found to be an important factor in the dispersal and density of feral rape populations (Crawley & Brown, 1995). Other mechanisms include seed movement with manure, transport on the wheels/bodies of vehicles and movement with topsoil (Thill & Mallory-Smith, 1997; Clifford, 1958; Hodkinson & Thompson, 1997).
- 3.4 Seed can also be dispersed in time through delayed germination or dormancy. This applies to seed lost during harvesting and may involve large numbers (Price *et al.*, 1996). Although crops have largely been bred to germinate as soon as they are sown, low levels of dormancy can be detected (Adler, 1993). Organic crops grown in fields previously sown to GM crops may be contaminated in this way. Volunteers of high erucic acid oilseed rape were recorded in the years following their use as a crop and a mean of 1 plant per 3m<sup>2</sup> was found 3, 4 and 5 years later (Ramans, 1995). Young & Youngberg (1996) found that 2-year rotations between perennial ryegrass seed production crops were insufficient to meet the variety purity required for certified seed.

3.5 Volunteer plants and the time period that must elapse before a field is sown with a seed production crop are included in the regulations for basic and certified seed production. Table 2 gives acceptable numbers of volunteers and minimum numbers of years between crops of the same species. The relevant data for Canadian, New Zealand and USA crops are given in Appendix 2.

Table 2. The minimum previous cropping is given in years between crops of the same species. The maximum permissible number of plants which do not conform to the variety type (off-types) are also given. These could be volunteers or result from a contaminated seed batch.

	Basic Seed		Certified Seed 1st generation	
Сгор	Previous Cropping	Nos Off- types	Previous Cropping	Nos Off- types
Barley	2	0.1%	2	0.3%
Beet + Mangels	5	[a]	5	[a]
Brassica Crops	5	0.3-0.1%	5	0.3-2.0%
Broad Beans	4	0.3%	4	1%
Field Beans + Field Peas	2	0.3%	2	1%
Grasses	6	1 per 20- 50m <sup>2</sup>	4	1-6 per 10m <sup>2</sup>
Legumes	6	1 per 30- 50m <sup>2</sup>	4	1 per 10m <sup>2</sup>
Oats	2	0.1%	2	0.3%
Peas	4	0.005%	4	0.005%
Radish/Brown Mustard	5	1 per 30m <sup>2</sup>	5	1 per 10m <sup>2</sup>
Rye	1	1 per $30m^2$	1	1 per $10m^2$
Triticale	2	0.3%	2	1%
Wheat	2	0.1%	2	0.3%
White Mustard	5	0.3%	5	1%

[a] These need to have "sufficient variety purity and identity".

## 4. CONSEQUENCES OF CROSS CONTAMINATION

## A. Fate of the crop

- 4.1 The consequences of a transgene entering an organic crop are affected by both the fate of the contaminated crop and the nature of the transgene. Crop fate will be dealt with first. There is a range of isolation distances used for seed production crops (described above) to cover the different uses of seed crops. Seed to be used for further multiplication need to be of a higher purity than seed which go to the farmer for standard crop production. If contamination (by seed or pollen) of a seed production crop occurs, the transgene will then be present throughout the life cycle of the standard crop grown from the contaminated seed.
- 4.2 Crops grown for human / animal consumption of their seed may also be contaminated. These include cereals, pulses, fruits and oilseed crops. In this instance a transgene arriving through pollen contamination will not be present in the crop prior to fertilisation so its impact will be limited to a short period towards the end of the plant life cycle.
- 4.3 A third group of plants is those used for animal consumption of vegetative plants (hay, haylage, silage) or for mulching for soil fertility. Seed for production of such crops may be contaminated, but pollen contamination is of no consequence except in the case of whole crop silage.
- 4.4 A fourth group of plants is those which do not flower and are grown for vegetative parts e.g. root crops and leaf vegetables. These crops will not be affected by contamination from pollen. However, it is possible that volunteer plants might be harvested with the crop and passed on to the consumer.
- 4.5 These are issues that face all farmers. However, in the case of organic farming the issue is complicated by the practice of planting home-saved seed. If part of the seed harvest is kept for sowing, then the status of the crop has changed from a standard crop to a seed production crop. If this process is repeated over many years then this is equivalent to seed multiplication. Acceptable standards of genetic purity (i.e. basic seed standards) will need to be maintained to minimise the accumulation of contaminating genes from all plant sources.
- 4.6 Contaminating transgenes in perennial or self-seeding crops such as forage grasses and legumes could potentially accumulate over a number of years. There is very little information on the rate at which this could occur. The proportion of new plants recruited each year will have a large effect on the rate of transgene accumulation as it will determine what proportion of the crop is derived from seed. It is through seed establishment that contamination (pollen or seed derived) will enter the

crop. Once a transgene is established its rate of spread through the crop will depend in part on the success of plants containing the transgene. If the transgene confers herbicide tolerance, plants containing it will not be at any advantage in organic crops where herbicides are not applied. Other traits are less straight forward. However, organic farmers aim to create crops which are locally adapted and so this may make them stronger competitors than other crops. To our knowledge this is not an area that has been studied.

- 4.7 A final factor is whether the crop will be used as animal feed and if so whether the transgene or products thereof will be passed on. This is an issue that is being considered by the Advisory Committee for Novel Foods and Processes (ACNFP) and the Advisory Committee on Releases to the Environment (ACRE) and will become the responsibility of the new Advisory Committee on Animal Feedingstuffs that is currently being established. Current studies aim to quantify the risk of a transgene being passed from feed to gut microflora. At the present time it can be concluded that if such transfer does occur, it is a very rare event.
- 4.8 In conclusion, the fate of the crop will determine where in the plant life cycle the transgene is present. This will help to determine whether the genetic modification is expressed in the growing crop, feed for livestock and food for the consumer.

## B. Nature of the Transgene

- 4.9 We will now consider how the consequences of contamination might be affected by the nature of the transgene. The presence of a gene can lead to the production of a specific product only if the gene is expressed (switched on) but this is not always the case. Each transgene construct will include a piece of DNA called a promoter. This promoter plays a role in controlling expression of the gene. Promoters may be constitutive (the gene is expressed all of the time), inducible (the gene is only expressed on under certain conditions) or tissue-specific (the gene is expressed in certain plant parts). The time and place where the gene is expressed is an important factor in determining the consequences of contamination. We give examples of different parts of the life cycle where genes may be expressed, below, and then go on to discuss the possible consequences of that expression.
- 4.10 Some transgenes may be expressed in pollen (Twell *et al.*, 1990; Albani *et al.*, 1992; Weterings *et al.*, 1992; Mascarenhas, 1992; Davies *et al.*, 1992; Richter & Powles, 1993; van der Geeste*t al.*, 1995; Hong *et al.*, 1997) and the compounds produced will usually be detected in low quantities (Wilkinson *et al.*, 1997).

- 4.11 The first stage of plant growth after fertilisation is seed development. Some of the compounds found in the seed are produced by the mother plant and imported into the seed. Therefore, genes obtained through the pollen will not affect these compounds in this generation of seed. Production of other seed compounds may be affected by expression of genes from both parents (from both pollen and egg). An example of both of these mechanisms is provided by double-low oilseed rape. This type of rape produces seed-oil low in glucosinolates and erucic acid. Aliphatic glucosinolates are imported from the mother plant (Magrath & Mithen, 1993) so pollen contamination does not alter aliphatic glucosinolate levels in the seed. If this seed is grown to produce a plant, the contaminating genes are present in the maternal parent and so have an effect on glucosinolate levels. In contrast, erucic acid production is controlled by genes from both parents and so pollen contamination from high erucic acid varieties results in increased levels of erucic acid in the seed (Ramans, 1995, Bilsborrow, 1998). In both cases the relevant gene(s) from the pollen are present in the seed. Another example involves the Dap mutation in maize that results in seed dappled with a purple pigment. When the Dap mutation is derived from the female parent, it is expressed but when it is derived from the male parent, it is not. Some Dap mutations are never passed on via the pollen so even the offspring resulting from the pollination will not produce dappled seed (Gavazzi et al., 1997). Other gene products are not found in the seed at all, whether the gene is maternally or paternally derived (see below).
- 4.12 The above examples are taken from non-GM plants but there is a range of seed traits that have been genetically modified. These include nutritive value (Molvig *et al.*, 1997; Pickardt *et al.*, 1995; Saalbach *et al.*, 1995; Hood *et al.*, 1997; Raina & Datta, 1992), oil quality (Murphy, 1995), seedlessness (Tomes, 1997) and pharmaceuticals (Parmenter *et al.*, 1995) in a range of crops. However, details of paternal expression of these individual transgenes are not always given in the scientific literature.
- 4.13 If the contaminated seed is grown on, the transgene is likely to be expressed at some point in the crop s development. Genes can be specifically expressed in seed (examples above), leaves (Yamamoto *et al.*, 1994; Stockhaus *et al.*, 1987), roots (Neuhaus *et al.*, 1994), nodules (Mett *et al.*, 1996), vascular tissue (van der Mijnsbrugge *et al.*, 1996; Raho *et al.*, 1996) and various combinations of plant parts. Transgenes can be induced by anaerobic stress (Kyozura *et al.*, 1991), light (Quandt *et al.* 1992), nitrates (Rastogi *et al.*, 1993), heat stress (Severin, 1995), drought (Wei & O Connell, 1995), heavy metals such as copper (Mett *et al.*, 1996), natural and artificial chemicals (Gatz, 1996), wounding (Hansen *et al.*, 1996) and plant hormones (Bommineni *et al.*, 1998). In addition to the production of novel products or increasing levels of certain plant products, transgenes can also give reductions in certain plant products (Hofgen & Willmitzer, 1992; Gorschen *et al.*, 1997).

- 4.14 All of these factors can work together in different combinations. For example, induction and tissue-specificity are not mutually exclusive. Thus, transgene effects are variable and should be considered on a case by case basis, as no generalisations can be made.
- 4.15 When the two factors, crop fate and nature of the transgene, are combined it can be seen that the consequences of contamination depend on both factors. If a seed production crop is contaminated, the effect of contamination is determined by the nature of the transgene. If a crop that produces seed as an edible end product is contaminated by pollen, then it is only expression of the transgene in the seed that will have an effect. Transgene expression in the seed is not important where volunteer plants contaminate vegetative crops which do not produce seed. Here it is only transgene expression in vegetative plant parts which have an effect.
- 4.16 In this section we have considered the different ways in which a transgene may affect a crop. The types of crop traits that are being genetically modified are summarised in Section 5. However, it may be argued that it is the very presence of a transgene, rather than the effect that it has, which is of concern. If the presence of the transgene alone is of concern, then the fate of the crop and the nature of the transgene are less relevant. In this instance the consequence of contamination is always the same: the transgene is present in the harvest.

# 5. GENETICALLY MODIFIED CROPS LIKELY TO BE RELEASED IN THE UK

5.1 **Table 3** gives the crops that have been genetically modified and grown in experimental field releases in the UK. It also includes details of the types of traits that have been modified.

Table 3. The categories of GM plants which were given permission to be grown under field conditions in the UK from 01/02/93 - 13/05/98. The numbers refer to the numbers of applications approved and may include repeat applications for the same GM crop.

Сгор	GM Trait	Number
Apple	Fungal Resistance	1
	Insect Resistance	1
Barley	Malting Quality	1
Beet (Fodder)	Herbicide Tolerance	3
Beet (Sugar)	Herbicide Tolerance	22
	Male Sterility and Fertility Restorers	1
	Sugar Content	1
	Virus Resistance	2
Chicory	Herbicide Tolerance	1
	Male Sterility and Fertility Restorers	2
Eucalyptus	Herbicide Tolerance	1
Maize	Herbicide Tolerance	4
Oilseed Rape	Fungal Resistance	4
	Herbicide Tolerance	37
	Male Sterility and Fertility	21

	Restorers		
	Oil Content	17	
	Pod Shatter Resistance	3	
Poplar	Pulping Quality	2	
Potato	Fungal Resistance	1	
	Insect Resistance	3	
	Nematode Resistance	1	
	Sugar Content	9	
	Virus Resistance	5	
Strawberry	Insect Resistance	1	
Tobacco	Nematode Resistance	1	
	Shading Response	3	
Tomato	Fruit Ripening	1	
Wheat	Baking Quality	1	
	Fungal Resistance	1	
	Herbicide Tolerance	4	
	Pollen Disruption	1	
	Starch	1	

## 6. ACCEPTABLE LEVELS OF CONTAMINATION

- 6.1 Acceptable levels of varietal purity are given in The Cereal Seed Regulations, The Fodder Plant Seeds Regulations, The Oil and Fibre Plant Seeds Regulations, The Beet Seeds Regulations and The Vegetable Seeds Regulations. The isolation distances, provided by MAFF, aim to ensure that these levels are met, although the chance that a crop may be contaminated above an acceptable level can never be entirely excluded. Levels of contamination acceptable to organic farmers are not clear. The UKROFS do not quote a figure for contamination by GM or non-GM crops. However, if the level of contamination of seed production crops acceptable to organic farmers is less than the levels indicated by the MAFF standards, then a new set of isolation distances and other procedures will need to be considered. If no level of contamination is acceptable then this clearly cannot be guaranteed.
- 6.2 For crops in which the seed is the end product, and the seed are not grown on, there are a few examples where contamination has been considered to be a sufficient problem to be studied. These arise in oilseed crops where varieties produce different oils suited to specific uses. It is important that edible oils do not contain the anti-nutritional compounds that may be found in other oils. Here contamination has been considered. In the case of high erucic acid oilseed rape (a non-GM crop) the isolation distance of 50 m is used (see also Ramans, 1995; Bilsborrow *et al.*, 1998).
- 6.3 In the case of crops that do not set seed, contamination by pollen will not be a problem. However, the presence of GM volunteers may lead to contamination of the harvest and acceptable levels need to be decided upon. It is important to define acceptable levels because complete isolation cannot be guaranteed.

## 7. RESPONSIBILITY FOR ISOLATION

- 7.1 GM crops will not be released into the environment without first going through a rigorous and internationally recognised regulatory process designed to protect human health and the environment. The Advisory Committee on Novel Foods and Processes (ACNFP) must be satisfied that these crops will not harm human health, while the Advisory Committee on Releases to the Environment (ACRE) must be satisfied that they will not harm human and animal health and the environment. Nonorganic agriculture makes up about 98.5% of UK agriculture. This means that GM crops could eventually become widespread. The regulatory process acknowledges this and so regulation is strict. Therefore, any GM crops that are released have to be accepted as a legitimate part of agriculture in the UK.
- 7.2 The organic farming movement have set themselves strict standards. These include the exclusion of synthetic chemicals. To achieve this, organic farmers must provide an effective windbreak to minimise the risk of spray-drift contamination and ensure equipment is free of non-permitted substances (UKROFS 1997). To exclude transgenes, comparable measures will need to be taken. Organic farmers and/or GM producers will need to ensure that their crops are isolated from other crops by an appropriate distance or barrier to reduce pollen contamination if the crop flowers. To reduce seed contamination, shared equipment will need to be cleaned and an appropriate period of time allowed before organic crops are grown on land previously used for GM crops.
- 7.3 Responsibility for isolation needs to be decided upon before appropriate measures can be implemented. In a draft code of practice the Supply Chain Initiative on Modified Agricultural Crops (SCIMAC) propose GM farmers adopt the isolation distances observed for the production of certified seed crops (maize 200m, oilseed rape 200m, sugar and fodder beet 600m). These proposals are being considered by the UKROFS Board. SCIMAC also suggest that neighbouring farms should inform each other of their planting intentions in order to consider appropriate isolation measures.

## 8. CONCLUSION

- 8.1 Genetic contamination of various kinds is inevitable in field grown crops. This is an issue faced by farmers growing seed production crops and regulations already exist to ensure contamination is below an agreed level. The current status of organic crops relies on the exclusion of GM contamination. This means that contamination is an issue for all classes of organic crops, not only seed production crops.
- 8.2 Contamination may arise through pollen or seed dispersal and both mechanisms are important. The degree of pollen contamination will depend upon physical distance, sexual compatibility between the crop and the contaminating source, relative flowering times, crop areas and outcrossing rate of the crop. The degree of seed contamination will be influenced by seed handling procedures, seed dormancy and rotation practices.
- 8.3 Pollen cannot be contained but the best means of reducing levels is to provide isolation distances between crops and other sexually compatible plants. The effects of seed dispersal can be reduced by allowing a time period between similar crops and by removing volunteers. All of these factors are covered by the regulations for seed production crops. Seed handling and transport is not regulated and spillages leading to feral populations (particularly of oilseed rape) may cause a problem. Unlike the case of pollen contamination where close neighbours are most at risk, seed spillages could lead to contamination at long distances from the original source. The seed production regulations do include the removal of any potential sources of foreign pollen in the area while the crop is flowering, which includes weeds and feral plant populations.
- 8.4 Neither source of contamination, pollen or seed, can be entirely eliminated. If levels of contamination similar to those set for certified seed are acceptable, then the same growing practices can be adopted. It would then be possible to draw on the experience of many years of seed production in the UK. A number of factors may mean organic farmers require higher standards of genetic purity. Firstly, they may require higher standards in order to provide the consumer with an acceptable level of GM-free food. Secondly, the practise of using home-saved seed may lead to the accumulation and multiplication of contamination so initial levels of contamination will need to be minimised. Lower levels of contamination can be achieved by increasing the precautions required by the regulations for seed production crops. However, the information is not available to quantify the effect of increasing these measures.
- 8.5 In order to implement measures to minimise mixing of organic and GM crops, it is necessary to decide who will carry these measures out. For

instance, should isolation distance be the responsibility of the organic or GM farmer, or both? Other measures, including physical barriers, years between land use and cleaning of equipment, need not necessarily be carried out by the same party.

- 8.6 In order to ensure that the measures taken achieve acceptable levels of contamination, it may be necessary to test for and quantify the presence of GM material. Estimates of contamination are already required for seed production crops and are carried out by the National Institute of Agricultural Botany (NIAB). However, the methods available may not always detect contamination, and quantification of the level of GM contamination in organic crops is likely in some instances to be very difficult to achieve. Firstly, because the GM trait may not be readily apparent and secondly, because the genetic diversity already within some organic crops may make additional traits from a contaminating crop harder to identify.
- 8.7 If the plant trait a transgene confers is used to test for transgene presence, this could lead to underestimates as not all transgenes will be active at all times. To test for the transgene(s) directly would be costly, time-consuming and require detailed knowledge of the transgenes in question. However, if it is the transgene itself that is of concern, as opposed to any products thereof, then direct detection of the transgene is the only reliable method available.
- 8.8 It is impossible to generalise about the effects of individual transgenes once contamination occurs. Factors that are likely to be important are the time of contamination, fate of the crop and the nature of the transgene construct, particularly the promoter which controls when the gene is switched on in the plant.
- 8.9 Initially, releases of GM crops will involve a limited range of modifications, which will make testing for contamination and consideration of the consequences easier. However, if the technology takes off in the UK, as it is already doing in North America, Argentina and China, then the range of modifications could increase dramatically.
- 8.10 Some of the seed used to sow organic crops in the UK are obtained from abroad. The possibility of pollination or mixing of these seed means that GM crops grown outside of the UK are relevant to organic farming here. Regulations on GM and organic crops in other EU countries are in line with those in the UK. This may not be true for seed obtained from further afield. For example, seed companies obtain seed of some species of forage grasses from around the world. Genetic modification of this group of plants is the subject of much research internationally (although field trials with GM forage grasses have not been carried out in the UK). Another factor is that after 1<sup>st</sup> January 2001, or a modified deadline thereafter, organic farmers in the UK will be required to use organically produced seed. Little or no organic seed is produced in the UK at present but it can be obtained from other countries. The situation

regarding GM crops across the globe is constantly changing. Although the IFOAM regulations are used widely, organic farmers need to be aware that other countries, particularly those outside Europe, are likely to have different regulations regarding the separation of GM and organically produced seed.

- In conclusion, once GM crops are released they, like all crops, cannot be completely contained. The same principle is true for spray or fertiliser drift from one farming system to another. There has always been the possibility of hybridisation and seed mixing between organic crops and conventional crops. There is a rigorous regulatory process in the UK governing the production and commercial approval of GM crops for release into the environment and for their use as food and feed. The areas highlighted in this report are:
- •
- The need for acceptable levels of contamination of organic crops to be decided, and measures identified to achieve them. It is important to define acceptable levels because complete isolation cannot be guaranteed.
- •
- The need for easy and reliable methods of identifying and quantifying GM contamination, which in practice may be very difficult to achieve.
- •
- The importance of checking sources of seed for organic crop production, particularly those obtained from outside Europe.
- •

## REFERENCES

Adler, L.S., Wikler, K., Wyndham, F.S., Linder, C.R. & Schmitt, J. (1993) Potential for persistence of genes escaped from canola: germination cues in crop, wild and crop-wild hybrid *Brassica rapa. Functional Ecology*, **7**, 736-745.

Albani, D., Sardana, R., Robert, L.S., Illimar, A., Arnison, P.G. & Fabijanski, S.F. (1992) A *Brassica napus* gene family which shows sequence similarity to ascorbate oxidase is expressed in developing pollen. Molecular characterization and analysis of promoter activity in transgenic tobacco plants. *Plant Journal*, **2**, 331-342.

Andrews, T.S., Morrison, I.N. & Penner, G.A. (1998) Monitoring the spread of ACCase inhibitor resistance among wild oat (*Avena fatua*) patches using AFLP analysis. *Weed Science*, **46**, 196-199.

Apostolides, G. & Goulas, C. (1998) Seed crop environment and processing effects on sugar beet (*Beta vulgaris* L.) certified hybrid variety seed quality. *Seed Science and Technology*, **26**, 223-235.

Bateman, A.J. (1947a) Contamination of seed crops I. Insect pollination. *Heredity*, **1**, 258-275.

Bateman, A.J. (1947b) Contamination of seed crops II. Wind pollination. *Heredity*, **1**, 235-246.

Bateman, A.J. (1947c) Contamination in seed crops III. Relation with isolation distance. *Heredity*, **1**, 303-336.

Bilsborrow, P.E., Evans, E.J., Bowman, J. & Bland, B.F. (1998) Contamination of edible double-low oilseed rape crops via pollen transfer from high erucic cultivars. *Journal of the Science of Food and Agriculture*, **76**, 17-22.

Bing, D.J., Downey, R.K. & Rakow, G.F.W. (1996) Hybridizations among *Brassica napus*, *B. rapa* and *B. juncea* and their two weedy relative *B. nigra* and *Sinapis arvensis* under open pollination conditions in the field. *Plant Breeding*, **115**, 470-473.

Bommineni, V.R., Bethune, T.D., Hull, K.L. & Dunstan, D.I. (1998) Expression of uidA under regulaton of the ABA-inducible wheat Em promoter in transgenic white spruce somatic embryos. *Journal of Plant Physiology*, **152**, 455-462. Brown, A.H.D., Zohary, D. & Nevo, E. (1978) Outcrossing rates and heterozygosity in natural populations of *Hordeum spontaneum* koch in Israel. *Heredity*, **41**, 49-62.

Brown, C.R. (1993) Outcrossing rate in cultivated autotetraploid potato. *American Potato Journal*, **70**, 725-734.

Carre, S., Tasei, J.N., Badenhauser, I., Le Guen, J., Morin, G. & Pierre, J. (1998) Gene dispersal by bumblebees between two lines of Faba bean. *Crop Science*, **38**, 322-325.

Cato, S.A. & Richardson, T.E. (1996) Inter- and intraspecific polymorphism at chloroplast SSR loci and the inheritance of plastids in *Pinus radiata* D.Don. *Theoretical and Applied Genetics*, **93**, 587-592.

Clifford, H. T. (1956) Seed dispersal by motor vehicles. *Journal of Ecology*, **47**, 311-315.

Cornu, A. & Dulieu, H. (1988) Pollen transmission of plastid-DNA under genotypic control in *Petunia hybridia* Hort. *Journal of Heredity*, **79**, 40-44.

Corriveau, J. L., Plans, N. O., and Coleman, A. W. (1989) Cultivar variability for the presence of plastid DNA in pollen of *Pisum sativum* L.: Implications for plastid transmission. *Current Genetics*, **16**, 47-51.

Crawley, M.J. & Brown, T.M. (1995) Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. *Proceedings of the Royal Society of London B*, **259**, 49-54.

Crofton, G.R.A. (1997) The principal seed characters of field beans (*Vicia faba* L. (Partim)) in relation to variety classification. *Plant Varieties and Seeds*, **10**, 81-94.

Darmency, H. & Renard, M. (1992) Efficiency of safety procedures in experiments with transgenic oilseed rape. *Proceedings 2nd International Symposium on the Biosafety Results of Field Tests of GM Plants and Microorganisms* (ed. by R. Casper and J. Landsmann), p.54.

Davies, S.P., Singh, M.B. & Knox, R.B. (1992) Identification and *in situ* localization of pollen-specific genes. *International Review of Cytology*, **140**, 19-34.

de Vries, A. (1974) Some aspects of cross-pollination in wheat (*Triticum aestivum* L.). *Euphytica*, **23**, 601-622.

di-Giovanni, F. & Beckett, P.M. (1990) On the mathematical modelling of pollen dispersal and deposition. *Journal of Applied Meteorology*, **29**, 1352-1357.

Dumas, C. & Mogensen, H.L. (1993) Gametes and fertilization: Maize as a model system for experimental embryogenesis in flowering plants. *The Plant Cell*, **5**, 1337-1348.

Eijlander, R. & Stiekema, W.J. (1994) Biological containment of potato (*Solanum tuberosum*): outcrossing to its related wild species black nightshade (*Solanum nigrum*) and bittersweet (*Solanum dulcamara*). *Sexual Plant Reproduction*, **7**, 29-40.

Ellstrand, N.C. (1992) Gene flow by pollen: Implications for plant conservation genetics. *Oikos*, **63**, 77-86.

Eriksson, G. and Kemble, R. (1993) The effect of genotype on pollen transmission of mitochondria in rapeseed (*Brassica napus*). *Sexual Plant Reproduction*, **6**, 33-39.

Fritz, S.E. & Lukaszewski, A.J. (1989) Pollen longevity in wheat, rye and triticale. *Plant Breeding*, **102**, 31-34.

Gatz, C. (1996) Chemically inducible promoters in transgenic plants. *Current Opinions in Biotechnology*, **7**, 168-172.

Gavazzi, G., Dolfini, S., Allegra, D. & Castiglioni, P. (1997) Dap (defective aleurone pigmentation) mutations affect maize aleurone development. *Molecular and General Genetics*, **256**, 223-230.

Giddings, G.D., Sackville Hamilton, N.R. & Hayward, M.D. (1997) The release of genetically modified grasses. Part 2: the influence of wind direction on pollen dispersal. *Theoretical and Applied Genetics*, **94**, 1007-1014.

Goplen, B.P., Cooke, D.A. & Pankiw, P. (1972) Effects of isolation distance on contamination in sweetclover. *Canadian Journal of Plant Science*, **52**, 517-524.

Gorshen, E., Duneava, M., Reeh, I. & Wasternack, C. (1997) Overexpression of the barley jasmonate-inducible 23kDa protein (JIP23) from barley in transgenic tobacco leads to the repression of leaf proteins. *FEBS Letters*, **419**, 58-62.

Griffiths, D.J. (1950) The liability of seed crops of perennial ryegrass (*Lolium perenne*) to contamination by wind-borne pollen. *Journal of Agricultural Science Cambridge*, **40**, 19-38.

Hansen, E., Harper, G., McPherson, M.J. & Atkinson, H.J. (1996) Differential expression patterns of the wound-inducible transgene wunl-uidA in potato roots following infection with either cyst or root knot nematodes. *Physiological and Molecular Plant Pathology*, **48**, 161-170.

Hodkinson, D. J. and Thompson, K. (1997) Plant dispersal: the role of man. *Journal of Applied Ecology*, **34**, 1484-1496.

Hofgen, R. & Willmizter, L. (1992) Transgenic potato plants depleted for the major tuber protein paptin via expression of antisense RNA. *RNA Plant Science*, **87**, 45-54.

Hokanson, S.C., Hancock, J.F. & Grumet, R. (1997) Direct comparison of pollen-mediated movement of native and engineered genes. *Euphytica*, **96**, 397-403.

Hong, H.P., Gerster, J.L., Datla, R.S.S., Albani, D., Scoles, G., Keller, W. & Robert, L.S. (1997) The promoter of a *Brassica napus* polygalacturonase gene directs pollen expression of B-glucuronidase in transgenic *Brassica* plants. *Plant Cell Reports*, **16**, 373-378.

Hood, E.E., Witcher, D.R., Maddock, S., Meyer, T., Baszczynski, C., Bailey, M., Flynn, P., Register, J., Marshall, L., Bond, D., Kulisek, E., Kusnadi, A., Evangelsta, R., Nikolov, Z., Wooge, C., Mehigh, R.J., Hernan, R., Kappel, W.K., Ritland, D., Li, C.P. & Howard, J.A. (1997) Commercial production of avidin from transgenic maize: characterization of transformant production, processing, extraction and purification. *Molecular Breeding*, **3**, 291-306.

Hucl, P. (1996) Out-crossing rates for 10 Canadian spring wheat cultivars. *Canadian Journal of Plant Science*, **76**, 423-427.

Ibarra-Perez, F.J., Ellstrand, N.C. & Waines, J.G. (1996) Multiple paternity in common bean (*Phaseolus vulgaris* L., Fabaceae). *Amercian Journal of Botany*, **83**, 749-758.

Johnson, K.B., Stockwell, V.O., Burgett, D.M., Sugar, D. & Loper, J.E. (1993) Dispersal of *Erwinia amylovora* and *Pseudomonas fluorescens* by honey bees from hives to apple and pear blossoms. *Phytopathology*, **83**, 478-484.

Knapp, E.E. & Teuber, L.R. (1993) Outcrossing rates of alfalfa populations differing in ease of floret tripping. *Crop Science*, **33**, 1181-1185.

Kyozuka, J., Fujimoto, H., Izawa, T. & Shimamoto, K. (1991) Anaerobic induction and tissue-specific expression of maize Adh1 promoter in transgenic rice plants and their progeny. *Molecular and General Genetics*, **228**, 40-48.

Lavigne, C., Godelle, B., Reboud, X. & Gouyon, P.H. (1996) A method to determine the mean pollen dispersal of individual plants growing within a large pollen source. *Theoretical and Applied Genetics*, **93**, 1319-1326.

Luby, J.J. & McNicol, R.J. (1995) Gene flow from cultivated to wild raspberries in Scotland: developing a basis for risk assessment for testing and deployment of transgenic cultivars. *Theoretical and Applied Genetics*, **90**, 1133-1137.

Magrath R. & Mithen R. (1993) Maternal effects on the expression of individual aliphatic glucosinolates in seeds and seedlings of *Brassica napus*. *Plant Breeding*, **111**, 249-252.

Mascarenhas, J.P. (1992) Pollen gene expression: molecular evidence. *International Review of Cytology*, **140**, 3-18.

Massood, E. (1998) Organic farmer takes gene battle to court. *Nature*, **394**, 8.

McCartney, H. & Lacey, M. (1991) Wind dispersal of pollen from crops of oilseed rape (*Brassica napus* L.). *Journal of Aerosol Science*, **22**, 467-477.

McDonald, M.B. (1998) Seed quality assessment. *Seed Science Research*, **8**, 265-275.

McPartlan, H.C. & Dale, P.J. (1994) The transfer of introduced genes from field grown transgenic potatoes to non-transgenic potatoes and related solanaceous species. *Transgenic Research*, **3**, 216-225.

Mesquida, J. & Renard, M. (1982) Etude de la dispersion du pollen par le vent et l'importance de la pollinisation anemophile chez le colza (*Brassica napus* L. var *oleifera* Metzger). *Apidologie*, **13**, 353-366.

Mett, V.L., Podivinsky, E., Tennant, A.M., Lochhead, L.P., Jones, W.T. & Reynolds, P.H.S. (1996) A system for tissuespecific copper-controllable gene expression in transgenic plants: nodule-specific antisense of asparate aminotransferase-P2. *Transgenic Research*, **5**, 105-113.

Metz, P.L.J., Buiel, A.A.M., van Norel, A. & Helsper, J.P.F.G. (1993) Rate and inheritance of cross-fertilization in faba bean (*Vicia faba* L). *Euphytica*, **66**, 127-133.

Metz, P.L.J., Buiel, A.A.M., van Norel, A. & Helsper, J.P.F.G. (1994) Genetic factors controlling outcrossing in faba bean (*Vicia faba*): effects of pollen donor and receptor genotypes and estimates of inter-receptor cross-fertilization. *Journal of Agricultural Science, Cambridge*, **122**, 249-254.

Molvig, L., Tabie, L.M., Eggum, B.O., Moores, A.E., Craig, S., Spencer, D. & Higgins, T.J.V. (1997) Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. *Proceedings of the National Academy of Science*, **94**, 8393-8398.

Morgan, C.L., Bruce, D.M., Child, R., Ladbrooke, Z.I. & Arthur, A.E. (1998) Genetic variation for pod shatter resistance among lines of oilseed rape developed from synthetic *B. napus. Field Crops Research*, **58**, 153-164.

Murphy, D. (1995) The use of conventionally and molecular genetics to produce new diversity of seed oil composition for the use of plant breeders - progress, problems and future prospects. *Euphytica*, **85**, 433-440.

Neuhaus, G., Neuhaus-Uri, G., Katagiri, F., Seipel, K. & Chua, N.-H. (1994) Tissue-specific expression of as-1 in transgenic tobacco. *The Plant Cell*, **6**, 827-834.

Nurminiemi, M., Tufto, J., Nilsson, N.-O. & Rognli, O.A. (1998) Spatial models of pollen dispersal in the forage grass meadow fescue. *Evolutionary Ecology*, **12**, 487-502.

Parmenter, L., Boothe, J.G., van Rooijen, G.J.H., Yeung, E.C. & Moloney, M.M. (1995) Production of biologically active hirudin in plant seeds using oleosin partitioning. *Plant Molecular Biology*, **29**, 1167-1180.

Pauk, J., Stefanov, I., Fekete, S., Bogre, L., Karsai, I., Feher, A. & Dudits, D. (1995) A study of different (CaMV 35S and mas) promoter activities and risk assessment of field use in transgenic rapeseed plants. *Euphytica*, **85**, 411-416.

Peart, D.R. (1985) The quantitative representation of seed and pollen dispersal. *Ecology*, **66**, 1081-1083.

Pickardt, T., Saalbach, I., Waddell, D., Meixner, M., Muntz, K. & Schieder, O. (1995) Seed specific expression of the 2S albumin gene from Brazil nut (*Bertholletia excelsa*) in transgenic *Vicia narbonensis*. *Molecular Breeding*, **1**, 295-301.

Price, J.S., Hobson, R.N., Neale, M.A. & Bruce, D.M. (1996) Seed losses in commercial harvesting of oilseed rape. *Journal of Agricultural Engineering Research*, **65**, 183-191.

Quandt, H.-J., Broer, I. & Puhler, A. (1992) Tissue-specific activity and light-dependent regulation of a soybean rbcS promoter in transgenic tobacco plants monitored with the firefly luciferase gene. *Plant Science*, **82**, 59-70.

Raamsdonk, L.W.D.v. & Schouten, H.J. (1997) Gene flow and establishment of transgenes in natural plant populations. *Acta Botanica Neerlandica*, **46**, 69-84.

Raho, G., Lupotto, E., Hartings, H., Dellatorre, A.P., Perrotta, C. & Marmiroli, N. (1996) Tissue-specific expression and environmental regulation of the barley Hvhsp17 gene promoter in transgenic tobacco plants. *Journal of Experimental Botany*, **47**, 1587-1594.

Rai, K.N. & Jain, S.K. (1982) Population biology of *Avena* IX. Gene flow and neighborhood size in relation to microgeographic variation in *Avena barbata*. *Oecologia*, **53**, 399-405.

Raina, A. & Datta, A. (1992) Molecular cloning of a gene encoding a seed-specific protein with nutritionally balanced amino acid composition from *Amaranthus*. *Proceedings of the National Academy of Science*, **89**, 1774-1778.

Ramans, M. (1995) Winter HEAR volunteer study in East Anglia. *Proceedings of the 9<sup>th</sup> International Rapeseed Congress*, 1369-1371.

Rastogi, R., Back, E., Schneiderbauer, A., Bowsher, C.G., Moffatt, B. & Rothstein, S.J. (1993) A 330bp region of the spinach nitrate reductase gene promoter directs nitrate-inducible tissue-specific expression in transgenic tobacco. *Plant Journal*, **4**, 317-326.

Reboud, X. and Zhan, X. (1994) Organelle inheritance in plants. *Heredity*, **72**, 132-140.

Rew, L.J. & Cussans, G.W. (1997) Horizontal movement of seeds following tine and plough cultivation: implications for spatial dynamics of weed infestations. *Weed Research*, **37**, 247-256.

Rhebergen, L.J., Van Der Schoor, G.J. & Verkleij, J.A.C. (1991) The potential for gene flow in *Festuca rubra* L. occurring in a heterogenous environment. *Flora*, **185**, 133-142. Richards, A.J. (1986) *Plant Breeding Systems*, George Allen & Unwin Ltd, London.

Richter, J. & Powles, S.B. (1993) Pollen expression of herbicide target site resistance genes in annual ryegrass (*Lolium rigidum*). *Plant Physiology*, **102**, 1037-1041.

Saalbach, I., Waddell, D., Pickardt, T., Schieder, O. & Muntz, K. (1995) Stable expression of the sulphur-rich 2S albumin gene in transgenic *Vicia narbonensis* increases the methionine content of seeds. *Journal of Plant Physiology*, **145**, 674-681.

Scheffler, J.A., Parkinson, R. & Dale, P.J. (1993) Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*). *Transgenic Research*, **2**, 356-364.

Scheffler, J.A., Parkinson, R. & Dale, P.J. (1995) Evaluating the effectiveness of isolation distances for field plots of oilseed rape (*Brassica napus*) using a herbicide-resistance transgene as a selectable marker. *Plant Breeding*, **114**, 317-321.

Schittenhelm, S. & Hoekstra, R. (1995) Recommended isolation distances for the field multiplication of diploid tuber-bearing *Solanum* species. *Plant Breeding*, **114**, 369-371.

Severin, K., Wagner, A. & Schoffel, F. (1995) A heat-inducible Adh gene as a reporter gene for a negative selection in transgenic *Arabidopsis*. *Transgenic Research*, **4**, 163-172.

Sewell, M.M., Qiu, Y.-L., Parks, C.R. & Chase, M.W. (1993) Genetic evidence for trace paternal transmission of plastids in *Liriodendron* and *Magnolia* (Magnoliaceae). *American Journal of Botany*, **80**, 854-858.

Skogsmyr, I. (1994) The effects of sexually selected traits on gene dispersal. *Field Crops Research*, **45**, 163-170.

Stockhaus, J., Eckes, P., Rochasosa, M., Scell, J. & Willmizter, L. (1987) Analysis of cis-active sequences involved in the leafspecific expression of a potato gene in transgenic plants. *Proceedings of the National Academy of Science of the USA*, **84**, 7943-7947.

Stoddard, F.L. (1986) Effects of irrigation, plant density and genotype on pollination, fertilization and seed development in spring field beans (*Vicia faba* L.). *Journal of Agricultural Science Cambridge*, **107**, 347-355.

Stringham, G.R. & Downey, R.K. (1978) Effectiveness of isolation distance in turnip rape. *Canadian Journal Plant Science*, **58**, 427-434.

Strykstra, R.J., Verweij, G.L. & Bakker, J.P. (1997) Seed dispersal by mowing machinery in a Dutch brook valley system. *Acta Botanica Neerlandica*, **46**, 387-401.

Sun, M. & Corke, H. (1992) Population genetics of colonizing success of weedy rye in Northern California. *Theoretical and Applied Genetics*, **83**, 321-329.

Thill, D.C. & Mallory-Smith, C.A. (1997) The nature and consequence of weed spread in cropping systems. *Weed Science*, **45**, 337-342.

Timmons, A.M., O'Brien, E.T., Charters, Y.M., Dubbels, S.J. & Wilkinson, M.J. (1995) Assessing the risks of wind pollination from fields of genetically modified *Brassicanapus* ssp. *oleifera*. *Euphytica*, **85**, 417-423.

Tomes, D.T. (1997) Seedless hopes bode well for winter vegetables. *Nature Biotechnology*, **15**, 1344-1345.

Twell, D., Yamaguchi, J. & McCormick, S. (1990) Pollen-specific gene expression in transgenic plants: coordinate regulation of two different tomato gene promoters during microsporogenesis. *Development*, **109**, 705-713.

van der Geest, A.H.M., Frisch, D.A., Kemp, J.D. & Hall, T.C. (1995) Cell ablation reveals that expression from the phaseolin promoter is confined to embryogenesis and microsporogenesis. *Plant Physiology*, **109**, 1151-1158.

van der Mijnsbrugge, K., van Montagu, M., Inze, D. & Boerjan, W. (1996) Tissue-specific expression conferred by the Sadenosyl-L-methionine synthetase promoter of *Arabidopsis thaliana* in transgenic poplar. *Plant and Cell Physiology*, **37**, 1109-1115.

Visser, T. & Macucci, C.M. (1986) The performance of double or mixed pollinations with compatible and self-incompatible or incongruous pollen of pear and apple. *Euphytica*, **35**, 1011-1015.

Wang, T.Y., Chen, H.B., Reboud, X. & Darmency, H. (1997) Pollen-mediated gene flow in an autogamous crop: foxtail millet (*Setaria italica*). *Plant Breeding*, **116**, 579-583. Wei, T. & O Connell, M. (1995) A drought-inducible and abscisic-acid (ABA)-inducible H1 histone gene from a droughttolerant tomato, *Lycopersicon pennellii* effects of overexpression in transgenic tobacco. *Journal of Cellular Biochemistry*, **S19A**, 159.

Wertheim, S.J. (1991) Malus cv. Baskatong as an indicator of pollen spread in intensive apple orchards. *Journal of Horticultural Science*, **66**, 635-642.

Weterings, K., Reijnen, W., van Aarssen, R., Kortstee, A., Spijkers, J., van Herpen, M., Schrauwen, J. & Wullems, G. (1992) Characterization of a pollen-specific cDNA clone from *Nicotiana tabacum* expressed during microgametogenesis and germination. *Plant Molecular Biology*, **18**, 1101-1111.

Wilkinson, J.E., Twell, D. & Lindsey, K. (1997) Activities of CaMV 35S and nos promoters in pollen: implications for field release of transgenic lines. *Journal of Experimental Botany*, **48**, 265-275.

Wilkinson, M.J., Timmons, A.M., Charters, Y., Dubbels, S., Robertson, A., Wilson, N., Scott, S., O'Brien, E. & Lawson, H.M. (1995) Problems of risk assessment with genetically modified oilseed rape. *Brighton Crop Protection Conference - Weeds*, 1035-1044.

Winzeler, H., Schmid, J.E. & Winzeler, M. (1994) Analysis of the yield potential and yield components of F1 and F2 hybrids of crosses between wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.). *Euphytica*, **74**, 211-218.

Yamamoto, N., Tada, Y. & Fujimura, T. (1994) The promoter of a pine photosynthetic gene allows expression of a Bglucuronidase reporter gene in transgenic rice plants in a lightindependent but tissue-specific manner. *Plant Cell Physiology*, **35**, 773-778.

Young, W.C. & Youngberg, M. (1996) Cropping systems for perennial ryegrass seed production 2. Minimum tillage systems for changing cultivars in certified seed production. *Agronomy Journal*, **88**, 78-82.

## **APPENDIX 1**

Extracts from the UK Register for Organic Food Standards (UKROFS) production standards document that refer to genetically modified organisms.

## Chapter II - UKROFS PRODUCTION STANDARDS

Section 1 - Organic Production and Care of the Environment

1.4 The UKROFS Board have determined that Genetically Modified Organisms (GMOs) have no place in organic production systems. For a definition of GMOs in this context see Chapter III, Section 10, Annex 1A of these standards.

Section 4 - Crop Production

4.15 In accordance with the principles of organic production set out in Section 1 of this Chapter, plants which have been genetically modified must not be used in organic production. For a definition of genetic modification see Chapter III, Section 10, Annex 1A of these standards.

Section 7 - Livestock Production

7.56 Genetically Modified Organisms (GMOs)

In accordance with the principles of organic production set out in Section 1 of this Chapter, livestock which have been genetically modified must not be used in organic production. Ingredients which are genetically modified organisms, or derive from such organisms, must not be used as feed. For a definition of genetic modification see Chapter III, Section 10, Annex 1A of these standards.

## Chapter III - UKROFS PROCESSING STANDARDS

<u>Section 2</u> - Operational and Compositional Processing Requirements

2.16 Genetically Modified Organisms

In accordance with the principles of organic production set out in Section 1 of Chapter II, ingredients, processing aids or additives which are genetically modified organisms, or derive from such organisms, must not be used in organic production. For a definition of genetic modification see Chapter III, Section 10, Annex 1A of these standards.

<u>Section 7</u> - List of Permitted Ingredients of Non-Agricultural Origin

7.4 Micro-organism Preparations

Any preparations of micro-organisms normally used in food processing, with the exception of micro-organisms genetically modified in the meaning of Article 2(2) of Directive 90/220/EEC. The text of Article 2(2) of Directive 90/220/EEC is given in Section 10.

<u>Section 10</u> - References to Other EC Directives mentioned in Section 3.0 and Section 3.1

Annex 1A Techniques referred to in article 2(2)

PART 1

Techniques referred to in Article 2(2)(I) are inter alia:

- 1. Recombinant DNA techniques using vector systems as previously covered by Council Recommendation 82/472/EEC;
- 2. techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation.
- 3. cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

PART 2

Techniques referred to in Article 2(2)(ii) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant DNA molecules or GMOs, are:

- 4. in vitro fertilisation,
- 5. conjugation, transduction, transformation or any natural process,
- 6. polyploidy induction.

#### **APPENDIX 2**

Seed production standards for Canada, New Zealand and the USA.

Individual farmers may adopt higher voluntary standards. Isolation distances have been converted from feet to metres to facilitate comparisons with UK distances. Years between land use are the number of years which the field(s) must be used for different crops before the same crop can be replanted for seed production. Volunteer: Crop plants is the acceptable ratio of volunteer plants (or heads) compared with crop plants. This is the most relevant information but more detail is given for certain cases.

Crop	Isolation Distance	Years between land use	Volunteers: Crop plants
Barley	0m [a]	1	1:1000 - 1:3000
Clover	50.8 - 184.6m	2 - 5	1:100 - 1:1000
Field & flat peas	0m [a]	1	1:500 - 1:2000
Field & garden beans	0m [a]	1	1:500 - 1:2000
Grasses (outcrossing)	50.7 - 276.9m [c]	1 - 5	1:50 - 1:1000
Grasses (selfing)	4.6 - 18.5m [c]	1 - 5	1:50 - 1:1000
Maize	203.1m [b]	0	1:200 - 1:1000
Mustard	203.1 - 406.2m	1 - 4	1:500 - 1:2000
Oats	0m [a]	1	1:1000 - 1:3000
Onion	406.2 - 1624.6m	1	1:200
Rape (outcrossing)	101.5 - 406.2m	2 - 4	1:500 - 1:2000
Rape (selfing)	101.5 - 203.1m	2 - 4	1:500 - 1:2000
Rye	203.1m	1	1:1000 - 1:3000
Strawberry	92.3 - 457.2 m	2	0
Triticale	0m [a]	1	1:1000 - 1:3000
Wheat	0m [a]	1	1:1000 - 1:3000

[a] = isolation distance required to prevent mechanical mixing

[b] = natural barriers can be used to reduce the isolation distance

[c] = border removal is required for areas over 5 acres and may be used to reduce isolation distance

21/5/99