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Genetically modified crops and hybridization with wild relatives: a UK perspective

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Summary

1. It has been suggested that genetic modification could lead to crops with enhanced invasiveness and persistence. These new genotypes could invade natural ecosystems and cause undesirable change, either through spread of the crops themselves or through hybridization with wild relatives.
2. We review the progress made in the genetic modification of the major UK crops, and identify those crops and traits in which genetic modification is most advanced.
3. Data on the potential for the spread of transgenes through pollen movement and the relative performance of modified and unmodified crops are examined. It is concluded that the spread of modified crops and their hybrids with wild relatives can be modelled in the same manner as for unmodified crops.
4. Evidence for hybridization between crop and wild species in the UK is reviewed. We identify three categories of crop according to the likelihood of formation of hybrids with wild relatives.
5. The categorization of crops is used to suggest some simplifications to the procedures for permission to release genetically modified crops into the environment.
6. We suggest research priorities for ecologists based on the traits that are of most interest to plant breeders using genetic modification and the most likely route of escape of transgenes from a particular crop.

Key-words: disease resistance, gene flow, genetic modification, herbicide resistance, invasions.

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Introduction

The science, some would say the art, of plant breeding involves the creation of new plant genotypes and the subsequent selection of those showing desired characteristics. For centuries the only technique for introducing genetic variation into crops was sexual hybridization. Two parental types, each having traits of interest, would be crossed, and the progeny examined and selected for use or further rounds of hybridization. Often this process was carried out unwittingly in early agriculture, but present-day breeding can entail highly complex crossing designs.

Although this approach has been enormously successful in improving crop quality and productivity, there are certain disadvantages associated with the process. First, the speed of the method is dependent on the generation time of the plants concerned — this can be a major constraint on the breeding of

timber and fruit trees, for example. Secondly, if one wants to introduce a single new character into a plant variety, say from a primitive wild relative, then one has repeatedly to backcross the products of hybridization to the crop in order to remove unwanted traits linked to the trait of interest. This can take several years, depending on the generation times of the species. Finally, the gene pool available to the breeder is limited to those species with which the crop is sexually compatible.

In the twentieth century new techniques have become available to the plant breeder that have both speeded up the process and increased the range of available genetic variation. These include the induction of mutations by treatment with chemicals or X-rays; anther and ovule culture allowing the production of completely homozygous plants which can short-cut some of the requirements for backcrossing; and embryo rescue techniques which

permit previously incompatible species to produce viable offspring. These methods have been termed 'classical' genetic modification (National Research Council 1989).

A second type of modification is described by the National Research Council (1989) as 'cellular'. The generation of variants through tissue culture and the production of novel hybrids by cell fusion fall into this category. Again, these increase the gene pool available to breeders and can produce results much more rapidly than hybridization in plants with long generation times.

Finally, are the 'molecular' techniques which form the subject of this review. Essentially, these involve the insertion and integration of a short segment of DNA into the genome of a plant. The DNA usually consists of the coding sequences ('genes') for one or a small number of proteins, and flanking regions allowing the correct expression of the protein in plant cells. The gene and its flanking sequences are generally termed 'constructs'. The DNA can be introduced by essentially natural processes using bacteria or viruses as 'vectors', or by physically inserting the DNA directly into plant cells.

The process of insertion and integration of DNA has been described variously as genetic engineering, genetic manipulation, genetic modification or transformation. The last two are the preferred terms at the moment of writing. Similarly, the plants produced can be described as genetically engineered, genetically manipulated, genetically modified, transformed or transgenic.

Genetic modification has, at least in theory, the advantage of allowing the addition of a single character to a tried and tested variety without the need for backcrossing to remove unwanted genetic linkages. Also, because the transforming DNA can come from any source, biological or artificial, the gene pool available is virtually limitless.

Although the products of genetic modification are often indistinguishable at the whole plant level from those of classical and cellular techniques, there are perceived to be special risks associated with their use in agriculture. The first is that the products coded by the inserted DNA (the 'transgene') may be toxic to humans or animals. These effects are not considered here but they have been addressed in the literature (e.g. Knight 1989; Goldberg & Tjaden 1990; Flavell *et al.* 1992; Kessler *et al.* 1992).

The second concern is that the transgene will 'escape' from the confines of agriculture and lead to undesirable environmental change. A transgene may be regarded as having escaped from the crop if any of the following criteria can be applied.

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

1b. The plant containing it persists in the disturbed habitats associated with agriculture or other human

activities (e.g. headlands, verges, ditches, roadsides, waste tips).

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

or

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

2b. As above, but the plant occupying disturbed habitats.

2c. As above, but the plant invades semi-natural habitats.

or

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

3b. As above, but the plant occupying disturbed habitats.

3c. As above, but the plant occupying semi-natural habitats.

The first route involves either vegetative persistence or transmission of the genetic modification in seed from generation to generation. Plants produced by (1b) and (1c) should be referred to as feral populations. Route 2 is similar in some respects to route 1, apart from the transmission of the transgene via pollination to another crop. This could include the transfer of a construct from a genetically modified to an unmodified plant of the same crop variety, of different varieties, or even to transfers between different crop species.

Although the release of genetically modified organisms is a relatively new issue, the possibility of escape of transgenes has already accrued a large and burgeoning literature in the areas of potential environmental effects and risk assessment. For example, ecologists have attempted predictions on theoretical grounds and by analogy with introduced, exotic or invasive species (Sharples 1983; Regal 1986; Williamson 1988, 1992; Keeler 1989; NRC 1989; Tiedje *et al.* 1989; Crawley 1990; Levin 1990; Mooney & Drake 1990; Hoffman 1990; Williamson, Perrins & Fitter 1990; Rees *et al.* 1991; and various papers in Ginzberg 1991). It is not proposed to rehearse this debate here but to focus instead on the data available to assess the likelihood of hybridization between a transgenic crop and a related species, with special reference to crops of the United Kingdom and their wild relatives (route 3 above).

First, we will outline briefly the progress made in the genetic modification of UK crop species. Secondly, we summarize the conditions necessary for the formation and persistence of hybrids, and determine whether transgenic crops have any properties that may make them behave differently from non-transgenics with respect to hybridization. Thirdly, data on the occurrence of hybrids between UK crops and wild relatives are presented. In the final section we use the information to examine

whether the part of the risk assessment of the release of transgenics concerned with hybridization can be simplified. In the small number of releases to date, the investigators have been required to minimize the spread of pollen from the transgenics, regardless of the presence of close relatives. This lengthens the time from application to permission to release and complicates and increases the work involved in field trials, and in a rapidly developing and potentially highly lucrative business this can prove expensive and frustrating. Any simplification of risk assessment should be valuable both scientifically and commercially.

Genetic modification of UK crops

The progress made in the genetic modification of UK crops is summarized in Table 1. The information comes from an extensive literature search and personal communications with scientists involved in genetic modification work. In a field where new advances are being made regularly, and where results are often not published for reasons of commercial confidentiality, we cannot claim that the information is completely comprehensive. It does, however, illustrate several trends.

METHODS

The first feature to highlight is the methodology used in genetic modification. It can be seen that *Agrobacterium tumefaciens*-mediated transformation is the commonest transformation procedure. *A. tumefaciens* is a soil-borne bacterium that causes crown gall disease. The disease is caused by the bacterium transferring DNA from its Ti (tumour-inducing) plasmid into plants cells, usually at a wound site. The DNA becomes integrated into the plant's genome and is stably inherited. The transferred DNA (T-DNA) codes for enzymes involved in the production of plant hormones, causing plant cells to proliferate and form the tumours charac-

teristic of crown gall disease. The T-DNA also codes for enzymes involved in the synthesis of amino acid derivatives called opines, which the bacteria, but not the plant, can utilize as a carbon source. Binns & Thomashow (1988) and Zambryski (1988) provide excellent reviews of the process of T-DNA transfer and expression.

The utilization of *A. tumefaciens* in genetic modification of plants is based on the removal from the T-DNA of the genes leading to hormone and opine production (referred to as 'disarming') and their replacement with genes of interest to plant breeders and marker genes, such as antibiotic resistance, that allow transformed tissue to be selected (see Draper & Scott (1991) for an introduction to the design of Ti plasmids for plant genetic modification). The T-DNA is transferred in the usual way and the introduced genes are expressed by the plant. Most transformation protocols are based on the method of Horsch *et al.* (1985) in which small tissue explants, often leaf discs, are incubated with disarmed bacteria carrying genes of interest. After 2–3 days the tissue is transferred to a selective medium and transgenic plants are regenerated. This has proved enormously successful in producing a wide range of transgenic plants, especially in the dicotyledons.

Several groups, notably the grasses (including the world's major grain crops), are not amenable to transformation by *Agrobacterium*. The main problem is believed to be a difference in the wound response of these species. Many plants respond to wounding by undergoing cell division at the wound site. This is the basis of plant regeneration from the tissue explants. In the grasses, however, cells at a wound site accumulate phenolic compounds and die. Thus, although *Agrobacterium* can transfer T-DNA to grasses (Grimsley *et al.* 1987) the transformed cells do not divide and so cannot be used to regenerate transgenic plants (Potrykus 1990a).

In order to circumvent the problems with *Agrobacterium*-mediated transformation in the grasses, and other recalcitrant groups such as the

Table 1. Genetic modification of UK crop plants

Crop	Transformation status	Modified traits
Sugar beet (<i>Beta vulgaris</i> ssp. <i>vulgaris</i>)	Transgenic plants produced by <i>At</i> /shoot culture ¹ and <i>At</i> /embryogenic callus ² .	Herbicide-resistant plants ² . BNYVV resistance in root cultures ³ and protoplasts ⁴ . Interest in nematode resistance and insect resistance ⁵ .
Oilseed rape (<i>Brassica napus</i> ssp. <i>oleifera</i>)	Transgenic plants produced by several means, e.g. <i>At</i> /cotyledon petioles ⁶ , <i>Ar</i> /hypocotyls ⁷ , protoplasts/PEG ⁸ , protoplasts/electroporation ⁹ , microinjection/microspore-derived embryoids ¹⁰ .	Herbicide-resistant plants ^{11,12} . Plants with increased methionine in seed meal ¹³ . Plants showing male sterility ¹⁴ and restorer genes ¹⁵ . Heavy metal tolerant plants ¹⁶ . Interest in resistance to club root and other fungal diseases and insect resistance ¹⁷ .

Table 1. Continued

Crop	Transformation status	Modified traits
Cabbage, cauliflower, etc. (<i>Brassica oleracea</i>)	Transgenic plants produced by several means, e.g. At/hypocotyls ¹⁸ , Ar/seedlings ¹⁹ , protoplasts/PEG ²⁰ .	Interest in fungal, viral and insect resistances ¹⁷ .
Potato (<i>Solanum tuberosum</i>)	Transgenic plants produced by, e.g. At/leaf discs ²¹ , At/tuber discs ²² , At/callus ²³ , Ar/leaf discs ²⁴ .	Virus resistant plants, e.g. to PVS ²⁵ , PVX and PVY ²⁶ , and PLRV ²⁷ . Insect-resistant plants based on Bt ²⁸ , CpTI ²⁹ , or p-lec ²⁹ . Herbicide-resistant plants ³⁰ . Plants producing novel compounds in their tubers ³¹ . Plants with altered carbohydrate metabolism, e.g. to prevent the browning of crisps during manufacture ³² . Interest in nematode resistance based on cloned potato genes ³³ or Bt ³⁴ .
Tomato (<i>Lycopersicon esculentum</i>)	Model plant for transformation studies based on the At/leaf disc method ³⁵ .	Plants with fruits with prolonged shelf life based on expression of antisense RNA to polygalacturonase ^{36,37} or enzymes in ethylene biosynthesis ^{38,39} ; also genes for enzymes that degrade ethylene precursors ⁴⁰ . Virus resistant plants, e.g. to TMV ⁴⁴ . Herbicide resistant plants ⁴² . Insect resistant plants based on Bt ^{43,44} . Herbicide resistant plants ^{47,48,49,20} . Plants with altered seed storage proteins ²⁰ .
Maize (<i>Zea mays</i>)	Transgenic plants produced by At/embryos ⁴⁵ , protoplasts/electroporation (plants sterile) ⁴⁶ , biolistics/cell culture ^{47,48} .	Herbicide resistant plants ⁵⁰ . Interest in manipulation of grain quality, especially with respect to baking characteristics ⁵¹ . There will probably be interest in drought, salt, virus and insect resistances.
Wheat (<i>Triticum aestivum</i>)	Transgenic plants by biolistics/embryogenic callus ⁵⁰ .	Likely characters of interest include increasing lysine content of grain ⁵³ , production of heat stable glucanases in grain to improve brewing qualities ⁵⁴ and disease, salt and drought resistances ⁵⁵ . No information, but probably similar to wheat and barley.
Barley (<i>Hordeum vulgare</i>)	Stably transformed callus produced by protoplasts/PEG ⁵² .	Suggestions include herbicide resistance, reduction of pollen antigens that cause hay fever, male sterility and increase in protein quality of leaves ⁵⁹ .
Rye (<i>Secale cereale</i>)	Report of transgenic plants produced by macroinjection of DNA into floral tillers ⁵⁶ , considered by many to be an artifact ⁵⁷ .	Suggestions include resistance to <i>Sitona</i> weevil based on Bt, improvement of nutritional quality (especially reduction 'bloat'), herbicide resistance and alteration of <i>Rhizobium</i> host range ⁶³ . Herbicide-resistant plants ⁶⁵ . Plants resistant to AIMV ⁶⁶ . Plants with improved nutritional quality following transformation with pea albumins ⁶⁸ or chicken albumins ⁶⁹ .
Ryegrass (<i>Lolium perenne</i> & <i>L. multiflorum</i>)	Transgenic callus by protoplasts/heat shock ⁵⁸ .	Interest in resistance to aphids, <i>Sitona</i> weevils, fungi and viruses ⁷¹ . Interests similar to broad bean ⁷¹ .
Clover (<i>Trifolium repens</i>)	Transgenic callus produced by At/stolon segments ⁶⁰ , hairy root cultures Ar/seedlings ^{61,62} .	Interests similar to broad bean ⁷¹ . No information.
Lucerne (<i>Medicago sativa</i> ssp. <i>sativa</i>)	Transgenic plants from At/stem sections ⁶⁴ , At/petioles ⁶⁵ , At/leaf slices ⁶⁶ , Ar/seedlings ⁶⁷ .	
Broad bean (<i>Vicia faba</i>)	Transgenic root cultures from Ar/seedlings ⁷⁰ .	
Runner bean (<i>Phaseolus vulgaris</i>)	Transgenic plants(?) produced by At/stem sections ⁷² .	
Pea (<i>Pisum sativum</i>)	Transgenic plants produced by At/epicotyls ⁷³ .	
Carrot (<i>Daucus carota</i> ssp. <i>sativus</i>)	Transgenic plants from At/cell suspension cultures ⁷⁴ .	

Table 1. Continued

Crop	Transformation status	Modified traits
Lettuce (<i>Lactuca sativa</i>)	Transgenic plants from <i>At</i> / cotyledons ⁷⁵ , protoplasts/ electroporation ⁷⁶ .	Insect resistance based on snowdrop lectin gene ⁷⁷ .
Cucumber (<i>Cucumis sativus</i>)	Transgenic plants obtained by <i>At</i> /cotyledons ⁷⁸ , <i>Ar</i> /hypocotyls ⁷⁹ .	No information.
Sunflower (<i>Helianthus annuus</i>)	Transgenic plants produced by <i>At</i> /hypocotyls ⁸⁰ .	Interest in manipulation of oil quality ⁸¹ .
Flax (<i>Linum usitatissimum</i>)	Transgenic plants produced by <i>At</i> /hypocotyls ⁸² .	Herbicide resistant plants ⁸² .
Conifers (<i>Pinus</i> spp.; <i>Picea</i> spp.; <i>Pseudotsuga</i> spp.)	Unconfirmed report of transgenic plants of <i>Picea</i> produced by biolistics ⁸³ , several reports of transgenic calli, e.g. in Douglas-fir by <i>At</i> ⁸⁴ .	Insect resistant plants based on <i>Bt</i> ⁸³ . Suggestions include herbicide resistance, reduction in time to flowering and manipulation of lignin structure ⁸⁵ .
Poplar (<i>Populus</i> spp.)	Transgenic <i>P. alba</i> × <i>P. grandidentata</i> by <i>At</i> /leaf discs ⁸⁶ and biolistics ⁸⁷ . Transgenic <i>P. alba</i> × <i>P. tremula</i> and <i>P. trichocarpa</i> × <i>P. deltoides</i> by <i>At</i> /internode segments ⁸⁸ .	Herbicide resistant plants ^{86,88} . Insect resistant plants based on <i>Bt</i> ⁸⁷ .
Grapevines (<i>Vitis vinifera</i>)	Transgenic plants produced by <i>At</i> /hypocotyls ⁸⁹ .	Suggestions include disease resistances and enhanced flavour of the fruit ⁸⁹ .
Plums (<i>Prunus domestica</i>)	Transgenic plants produced by <i>At</i> /hypocotyls ⁹⁰ .	No information but apricot plants (in the same genus as plums) resistant to PPV have been produced ⁹¹ .
Apple (<i>Malus pumila</i>)	Transgenic plants produced by <i>At</i> /leaf discs ⁹² . Transgenic rootstocks by <i>Ar</i> inoculation ⁹³ .	Plants resistant to codling moth based on <i>Bt</i> ⁹⁴ .
Strawberry (<i>Fragaria</i> × <i>ananassa</i>)	Transgenic plants produced by <i>At</i> /petioles ⁹⁵ , <i>At</i> /leaf discs ⁹⁶ .	Plants transformed with CpTI gene for insect resistance ⁹⁴ .
Blackcurrant (<i>Ribes nigrum</i>)	Transgenic plants produced by <i>At</i> /peeled internode segments ⁹⁷ .	Plants transformed with CpTI and AbMV coat protein genes. Interest in other viral resistances and transfer of anthocyanin genes to improve fruit colour ⁹⁸ .
Raspberries (<i>Rubus idaeus</i>)	Transgenic plants produced by <i>At</i> /leaf discs ⁹⁹ .	Plants transformed with CpTI and AbMV coat protein genes ⁹⁸ .

References: ¹Lindsey & Gallois (1990). ²D'Halluin *et al.* (1992). ³Ehlers *et al.* (1991). ⁴Kallerhoff *et al.* (1990). ⁵I.D.G. Bartle, I.J. Mackay & B. Ford-Lloyd (personal communications). ⁶Moloney, Walker & Sharma (1989). ⁷Damgaard & Rasmussen (1991). ⁸Golz, Kohler & Schieder (1990). ⁹Guerche *et al.* (1987). ¹⁰Neuhaus *et al.* (1987). ¹¹Miki *et al.* (1990). ¹²Mariani *et al.* (1991). ¹³Altenbach *et al.* (1992). ¹⁴Mariani *et al.* (1990). ¹⁵Mariani *et al.* (1992). ¹⁶Misra & Gedamu (1989). ¹⁷P.J. Dale (personal communication). ¹⁸de Block, de Brouwer & Tenning (1989). ¹⁹David & Tempe (1988). ²⁰Mukhopadhyay *et al.* (1991). ²¹An, Watson & Chiang (1986). ²²Stiekema *et al.* (1988). ²³Ooms *et al.* (1987). ²⁴Fladung (1990). ²⁵MacKenzie, Tremaine & McPherson (1991). ²⁶Hemenway *et al.* (1988). ²⁷Kawchuk, Martin & McPherson (1990). ²⁸Cheng *et al.* (1992). ²⁹Edwards *et al.* (1991). ³⁰de Greef *et al.* (1989). ³¹Oakes, Shewmaker & Stalker (1991). ³²A. Kumar (personal communication). ³³P. Whitty (personal communication). ³⁴Feitelson, Payne & Kim (1992). ³⁵Horsch *et al.* (1985). ³⁶Sheehy *et al.* (1988). ³⁷Smith *et al.* (1990). ³⁸Hamilton, Lycett & Grierson (1990). ³⁹Oeller *et al.* (1991). ⁴⁰Klee *et al.* (1991). ⁴¹Nelson *et al.* (1988). ⁴²Filliatti *et al.* (1987a). ⁴³Fischhoff *et al.* (1987). ⁴⁴Delannay *et al.* (1989). ⁴⁵Gould *et al.* (1991). ⁴⁶Rhodes *et al.* (1988). ⁴⁷Gordon-Kamm *et al.* (1990). ⁴⁸Fromm *et al.* (1990). ⁴⁹Spenser *et al.* (1992). ⁵⁰Vasil *et al.* (1992). ⁵¹P.I. Payne (personal communication). ⁵²Lazzeri & Lorz (1990). ⁵³Austin *et al.* (1986). ⁵⁴von Wettstein (1989). ⁵⁵B.P. Forster (personal communication). ⁵⁶de la Pena, Lorz & Schell (1987). ⁵⁷Potrykus (1991). ⁵⁸Potrykus *et al.* (1985). ⁵⁹M.D. Hayward & M.O. Humphreys (personal communications). ⁶⁰White & Greenwood (1987). ⁶¹Diaz *et al.* (1989). ⁶²Webb *et al.* (1990). ⁶³P. Morris & K.J. Webb (personal communications). ⁶⁴Shahin *et al.* (1986). ⁶⁵D'Halluin, Botterman & de Greef (1990). ⁶⁶Hill *et al.* (1991). ⁶⁷Golds *et al.* (1991). ⁶⁸Ford (1988). ⁶⁹Schroeder *et al.* (1991). ⁷⁰Ramsay & Kumar (1990). ⁷¹G. Ramsay (personal communication). ⁷²Mariotti, Fontana & Santini (1989). ⁷³Puonti-Kaerlas, Eriksson & Engstrom (1990). ⁷⁴Scott & Draper (1987). ⁷⁵Michelmoy *et al.* (1987). ⁷⁶Chupeau *et al.* (1989). ⁷⁷Anonymous (1991a). ⁷⁸Chee (1990). ⁷⁹Trulson, Simpson & Shahin (1986). ⁸⁰Everett, Robinson & Mascarenhas (1987). ⁸¹Altenbach & Simpson (1990). ⁸²McHughen (1989). ⁸³Anonymous (1991b). ⁸⁴Dandekar *et al.* (1987). ⁸⁵Cheliak & Rogers (1990). ⁸⁶Filliatti *et al.* (1987b). ⁸⁷McCown *et al.* (1991). ⁸⁸de Block (1990). ⁸⁹Mullins, Tang & Facciotti (1990). ⁹⁰Mante *et al.* (1991). ⁹¹Laimer da Camara Machado *et al.* (1992). ⁹²James *et al.* (1989). ⁹³Lambert & Tepfer (1991). ⁹⁴D.J. James (personal communication). ⁹⁵James, Passey & Barbara (1990). ⁹⁶Nehra *et al.* (1990). ⁹⁷Graham & McNicol (1991). ⁹⁸R.J. McNicol (personal communication). ⁹⁹Graham, McNicol & Kumar (1990).

Abbreviations: AbMV, Arabis mosaic virus; AIMV, Alfalfa mosaic virus; *Ar*, *Agrobacterium rhizogenes*; *At*, *Agrobacterium tumefaciens*; BNYVV, Beet necrotic yellow vein virus; *Bt*, *Bacillus thuringiensis* toxin gene; CpTI, Cowpea trypsin inhibitor gene; PEG, Polyethylene glycol; p-lec, Pea lectin gene; PLRV, Potato leafroll virus; PPV, Plum pox virus; PVS, Potato virus S; PVX, Potato virus X; PVY, Potato virus Y; TMV, Tobacco mosaic virus.

grain legumes (Kumar & Davey 1991) several other methods for DNA transfer to regenerable plant tissues have been devised. These have been extensively reviewed by Potrykus (1990b, 1991) and only the most successful will be mentioned here.

Many plant species can be regenerated from protoplasts (Roest & Gilissen 1989) and protoplasts can be induced to take up and integrate foreign DNA, either by treatment with chemicals such as polyethylene glycol (Larkin *et al.* 1990) or electric shocks ('electroporation') (Joersbo & Brunstedt 1991). Protoplast transformation has allowed the production of several genetically modified grasses including rice (e.g. Datta *et al.* 1990), maize (Rhodes *et al.* 1988), *Dactylis glomerata* (Horn *et al.* 1988) and *Festuca arundinacea* (Wang *et al.* 1992).

The other important method of plant transformation is called biolistics. This is the firing of DNA-coated particles of tungsten or gold into plant tissues. Again, this DNA can be integrated into the plant cell's genome and by careful selection of the target tissue, transformed plants can be regenerated (Sanford 1990; Klein *et al.* 1992). Biolistics has allowed the production of transgenic soybean (McCabe *et al.* 1988), cotton (Finer & McMullen 1990), rice (Christou, Ford & Kofron 1991) and wheat (Vasil *et al.* 1992), for example.

From Table 1 it can be seen that *Agrobacterium*-, protoplast- and biolistics-based methods have allowed the genetic modification of nearly all of the major UK crops. There are still problems, however, as many of the protocols are very variety- or even genotype-specific. For example, the published methods for the transformation of oilseed rape tend to work well with spring, but not winter varieties. Nevertheless, it appears that the foundations for the routine transformation of UK crops are in place.

TRAITS

Turning to the modified traits, it will be noticed that herbicide resistance is the most commonly modified character. Although herbicide resistance is a useful trait, the reasons for its being used widely in transformation studies are that the physiological basis is well characterized, that resistance is often a dominant single-gene trait and that it can be used as a selectable marker (Mazur & Falco 1989).

Two other areas where great progress has been made are insect and virus resistance. Again both are frequently dominant single-gene traits. Insect resistance is based on genes from the soil bacterium *Bacillus thuringiensis* that produces a crystalline protein on sporulation. The protein is non-toxic until it is hydrolysed in the gut of certain insects to produce polypeptides that affect membrane functions (Gill, Cowles & Pietrantonio 1992) and there is a variety of genes coding for toxins specific to Diptera, Lepidoptera and Coleoptera (Feitelson,

Payne & Kim 1992). Whole bacteria have been used in insecticidal sprays for over 20 years but they have limited use due to high production costs and instability of the protein under field conditions (Boulter *et al.* 1990); thus, transgenic plants expressing the toxin in their tissues offer an attractive alternative. The other important gene that has been used in the genetic modification of insect resistance is the proteinase inhibitor from cowpea seeds (Hilder *et al.* 1987). Plants have an enormous variety of proteinase inhibitors (Ryan 1990) and transgenic plants resistant to herbivores other than insects could also be produced.

Virus resistance has been produced in the main by transforming plants with the coat protein gene from the virus against which protection is required (Beachy, Loesch-Fries & Tumer 1990). Although the mechanism of protection is not clear, evidence points to the disruption of uncoating of the viral nucleic acid (Nelson, Powell & Beachy 1990) and it may be that the coat protein mRNA transcript blocks uncoating, rather than the coat protein itself, since protection has been obtained with little or no protein present (e.g. Hemenway *et al.* 1988; Kawchuk, Martin & McPherson 1990; van der Wilk *et al.* 1991). There have been suggestions that expression of viral coat protein genes in plants could lead to the production of new viruses following viral infection (de Zoeten 1991). Postulated mechanisms include the infecting viral genome being packaged in the coat protein produced by the plant (trans-capsidation) and the infecting virus's replicase switching from the viral genome to the mRNA of the transgenic coat protein gene (template switching). These processes are outside the scope of this review but they might have important implications for determining virus-vector relationships.

Perhaps the most important conclusion to draw from the data in Table 1 is that the traits introduced into crops are not new, although the mechanisms used to produce the trait might be (virus resistance through the expression of coat protein genes, for example). Disease and pest resistances are widespread in natural populations (Burdon 1987), and over 100 species have evolved herbicide resistance (Warwick 1991). This suggests that ecologists can best contribute to the debate about the ecological risks of genetically modified crops by attempting to understand the dynamics of these traits in natural populations, rather than becoming bogged down in the minutiae of small-scale field trials of a few transformed plants. As Burdon (1987) has illustrated, our knowledge of diseases in natural populations is very limited so this should be an area of future research.

Formation and persistence of hybrids between transgenic crops and wild relatives

HYBRIDIZATION AND GENE FLOW

Despite the widespread existence of breeding barriers, interspecific hybridization is a ubiquitous process. In the British flora, comprising roughly 1500 native and 1000 alien species, about 770 hybrids have been described, and the number is increasing (C.A. Stace, personal communication). However, hybrids are generally rare, often sterile and relatively few populations persist, except where the parents remain in contact or where they are able to spread vegetatively.

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. Studies of pollen flow almost universally describe highly leptokurtic distributions of pollen from the source plants (reviewed by Levin & Kerster 1974; Levin 1984) with most pollen grains moving less than 2 m in herbaceous plants. The small amount of evidence suggests that transgenic pollen behaves in exactly the same way. For example, Tynan, Williams & Connor (1990) found that when transgenic potatoes were grown surrounded by wild-type potatoes, no transgenic seeds were recovered from the wild-types further than 4.5 m away from the transgenic pollen parents. Data from other crops are lacking, but there is no *a priori* reason to believe that transgenic pollen will move in a different manner from non-transgenic pollen. Definitions of 'leptokurtic' and 'platykurtic' (see below) are given by Sokal & Rohlf (1981, p. 114).

Nevertheless, such estimates of pollen movement may give a misleading picture, both of gene flow (of which pollen movement is but one component), and of the rate of spread of a gene within and among populations. This is for a number of well understood reasons, including pollen 'carryover' in insect-pollinated species (Schaal 1980), the differential contribution of pollen grain clusters to pollen-mediated gene flow in wind-pollinated species (producing platykurtic distributions, for instance in the plantain *Plantago lanceolata*; Tonsor 1985), the disproportionate importance of low-frequency long-distance seed and pollen dispersal (which may be measured in miles in some tree species; Lanner 1966), and yearly or seasonal changes in pollinator behaviour associated with flower density (Campbell & Waser 1989). Other extrinsic factors affecting gene exchange between populations include the shape, size and density of both the donor and recipient populations, the height of plants and the characteristics of intervening vegetation, as well as spatial and temporal variation in pollen and seed production. Moreover, the breeding system

and life-history characteristics of the species will fundamentally affect amounts of gene flow. For example, Govindaraju (1988) demonstrated a significant positive correlation between outcrossing rates (largely determined by pollination mode) and gene flow variables among 32 plant species. The relationship between gene flow and the breeding system is reflected in the different isolation requirements for various crops (see below).

A number of recent studies, focusing on gene flow between populations or 'patches' of plants using allozyme markers and various forms of paternity exclusion analyses, provide quite a different picture of the rates of gene exchange between populations. They have demonstrated: (i) considerable variation in gene flow between different patches not necessarily directly related to interpatch distance; (ii) occasionally high amounts of gene flow between patches; and (iii) gene flow occasionally occurring over very long distances. In one series of investigations the number of individuals (seeds) produced within natural and experimental populations of wild radish *Raphanus sativus* that must have been sired by pollen from outside the patch averaged around 10% and varied from 3.2% to 18% (Devlin & Ellstrand 1990; Ellstrand & Marshall 1985; Ellstrand Devlin & Marshall 1989). The equivalent figures for small patches of cultivated *Cucurbita pepo* and the wild species *C. texana* were an average of 5% and a range of 0–15% (Kirkpatrick & Wilson 1988), and for natural populations of *Cucurbita foetidissima*, an average of 8.5% and a range of 0–48.3% (Kohn & Casper 1992). These studies detected gene flow at distances up to 700 m in *C. foetidissima*, up to 1000 m in *Raphanus sativus*, and up to 1300 m between *C. pepo* and *C. texana*. The species concerned are all insect pollinated. Whilst making estimates of gene flow of a similar order of magnitude, the authors of these studies stress that gene flow may be so highly idiosyncratic from species to species and place to place that it is far too early for any generalizations (Kohn & Casper 1992).

It is important to emphasize that pollen-mediated gene flow, as investigated in the work outlined above, comprises only one element in the assessment of the rates of spread of genes within and among populations. Apart from gene flow as seed (typically over shorter distances than pollen, although occasionally over very long distances and with the added condition that establishment of a plant containing the gene is more likely) the effects of both time and selection are crucial (see below). Thus, in order to model rates of spread predictively we need to estimate gene flow distances on a per generation basis and as a function of the relative fitness of individuals containing the gene. Manasse & Kareiva (1991) point out that quantification of rate of spread is an essential part of risk assessment for genetically modified organisms and urge that

experimental designs to measure gene movement from blocks of crop plants are sufficiently sensitive to characterize dispersal.

For similar reasons more insights may be gained into gene dispersal by employing so-called 'indirect' or 'inferential' estimates based on the methods of Wright (1951) or Slatkin (1985). In such methods the spatial structure of genotypes in natural populations (measured by variation at several isoenzyme loci) is used to infer underlying patterns of gene flow. Such patterns will be the net result of pollen- and seed-mediated gene flow, and establishment and selection over several generations. Coupled with parental analyses (e.g. Meagher & Thompson 1987) such methods offer the prospect of quantifying microevolutionary processes in populations of plant species with a wide range of breeding systems, life histories and ecological characteristics.

Some of the experiments which have led to specific minimal isolation distances for the maintenance of varietal purity in seed crops are described by Levin & Kerster (1974) who also list these distances for several species (based on data from Kernick 1961) (see Table 2). These have essentially used markers of the type described above to estimate the amounts of inter-varietal hybridization at various distances from a pollen source, usually a central block of marked plants. In many crops total isolation can apparently be achieved by distances of a few hundred

metres. The average isolation distance for self-fertilizing species is around 300 (± 150) m and that in primarily or exclusively out-crossing species is 800 (± 240) m.

Whilst these distances are likely to be useful guidelines to the actual distances of gene flow, the investigations of natural populations described above suggest that gene flow is likely not only to be crop-specific but, to some extent, variety-specific, site-specific and season-specific too. For example, the work on natural populations of *Raphanus sativus* by Ellstrand *et al.* (1989) suggests that the recommended isolation distances could allow anything up to 10% of 'foreign' pollinations. The distances indicated for *Cucurbita* species appear similarly inadequate.

YIELD PENALTIES

As was stated above, selection is an important factor when considering the maintenance or spread of a gene in a population. In assessing the impact of transgenic plants on agriculture and the natural environment, it is important to know whether plants carrying a transgene are at a disadvantage under conditions where there is no selection for the product of the introduced gene. If plants expressing a gene under such conditions show inferior performance in comparison with plants not expressing the gene,

Table 2. Isolation requirements of seed crops (based on Levin & Kerster 1974): S, predominantly self-fertilizing; SC, selfing and cross-fertilization of equal importance; C, mainly or exclusively cross-fertilizing; I, insect pollination; W, wind pollination

Species	Breeding system	Pollination mode	Isolation requirements (m)
<i>Linum usitatissimum</i>	S	I	100–300
<i>Lupinus</i> spp.	S	I	500
<i>Lactuca sativa</i>	S	I	30–60
<i>Avena sativa</i>	S	W	180
<i>Hordeum vulgare</i>	S	W	180
<i>Triticum aestivum</i>	S	W	1.5–3.0
<i>Capsicum</i> spp.	SC	I	360
<i>Apium graveolens</i>	SC	I	1100
<i>Phaseolus</i> spp.	SC	I	45
<i>Vicia faba</i>	SC	I	90–180
<i>Pastinaca sativa</i>	SC	I	500
<i>Nicotiana tabacum</i>	SC	I	400
<i>Poa</i> , <i>Bromus</i> , <i>Festuca</i> , <i>Phleum</i> spp.	SC	W	540–1000
<i>Helianthus annuus</i>	C	I	800
<i>Brassica campestris</i>	C	I	900
<i>Daucus carota</i>	C	I	900
<i>Lycopersicon esculentum</i>	C	I	30–60
<i>Medicago</i> , <i>Trifolium</i> , <i>Lotus</i> spp.	C	I	720–1600
<i>Brassica oleracea</i>	C	I	600–970
<i>Allium cepa</i>	C	I	900
<i>Raphanus sativus</i>	C	I	270–300
<i>Cucurbita</i> spp.	C	I	400
<i>Zea mays</i>	C	W	180
<i>Secale cereale</i>	C	W	180
<i>Beta vulgaris</i>	C	IW	3200

then there is said to be a yield penalty to the expression of that gene. There has been much debate over whether transgenics will show yield penalties due to pleiotropic effects of the transgene. Two lines of evidence will be examined to estimate the likelihood of yield penalties. First, we will consider direct evidence from experiments on transgenic plants and, secondly, we will examine the pleiotropic effects of naturally occurring disease and herbicide resistance genes in plants.

Transgenic plants

Most plant transformation strategies involve tissue culture and this can produce large phenotypic variation in the regenerated plants (Larkin & Scowcroft 1981). Many transgenic plants will perform poorly and be discarded because of this, rather than because they are transgenic. In experiments to assess yield penalties, therefore, one must use transgenic plants which perform well under conditions which select for the transgene. These plants can then be compared with the untransformed parental lines under conditions which do not give an advantage to the transgene.

Very few experiments have been designed specifically to measure yield penalties in transgenics, but some conclusions can be drawn from trials to test the performance of transgenics under field conditions. In general there is little evidence for yield penalties in the absence of an advantage for the introduced gene.

Potatoes expressing potato virus X and Y coat protein sequences (Kaniewski *et al.* 1990; Jongedijk *et al.* 1992) and tomatoes expressing tobacco mosaic virus coat protein genes (Nelson *et al.* 1988) have been grown in field trials. Yield and other characteristics such as growth rate and flowering time were not, in general, significantly different between uninfected controls and uninfected transgenics. Transgenic herbicide-resistant oilseed rape (de Greef *et al.* 1989), herbicide-resistant flax (McHughen & Rowland 1991; McHughen & Holm 1991) and antibiotic-resistant oilseed rape (Arnoldo *et al.* 1992) have all shown similar performance to non-transgenic controls in field trials.

Hilder & Gatehouse (1991) performed an experiment to test specifically the phenotypic cost of the expression of an extra gene. They grew tobacco plants transformed with the CpTI gene (both homo- and hemizygotes), plants transformed with an anti-sense construct of the CpTI gene (these plants do not express the CpTI protein), and untransformed controls. Plants were placed in growth chambers without insect infestation. Small, but significant, differences were found between transformed and control plants, but there were no differences between transformants expressing the transgene at amounts up to 1% of total leaf protein and the non-expressing

transformants. In other experiments no significant differences were found between plants homo- and hemizygous for the CpTI gene. A similar pattern of results was found when the experiments were repeated under conditions of nutrient and water deficiency designed to simulate environmental stress.

It was concluded from these experiments that the process of transformation and regeneration can affect plant phenotype but that the expression of the transgene imposed no additional cost. The range of variation in the transgenics usually lay within the range of the control plants. This suggests that if any of the changes caused by the regeneration process were to prove undesirable then they could be restored by selective breeding.

Non-transgenic plants

As was described above, two of the commonest traits produced by genetic modification are disease and herbicide resistances. In natural plant populations there is much polymorphism for disease resistance genes (e.g. Burdon 1987). One mechanism suggested for the maintenance of polymorphisms is that the resistance genes have harmful pleiotropic effects in disease-free conditions. Also, over 100 species have evolved herbicide resistance (Warwick 1991) yet these plants spread very slowly, if at all, in natural populations. Thus, both disease and herbicide resistance in natural populations may impose a yield penalty and may be useful in predicting the behaviour of transgenes conferring these traits in natural populations. However, one must be careful about making generalizations since the mechanisms for producing resistance can differ widely, and some modes of transgenic resistance (coat protein-mediated virus resistance, for example) have no wild equivalent.

Experiments to test the theory that disease resistance imposes a yield penalty on wild plants are rare, but those that have been performed suggest no cost of resistance. Simms & Rausher (1987, 1989) found no evidence for a correlation between genetic variation for resistance to herbivorous insects and variation for yield in the absence of predation in the annual morning glory *Ipomoea purpurea*. Also, Parker (1990) found that hogpeanuts (*Amphicarpaea bracteata*) resistant to the fungal pathogen *Synchytrium decipiens* were no less fit than susceptible types in a disease-free environment. Brown (1988) tested the cost of producing inducible proteinase inhibitors in tomatoes under a range of nitrogen amounts. The inhibitors were induced by chitin injections and plants were grown in the absence of herbivores. Inhibitor production had no detectable effect on a range of fitness-related characters.

In contrast to the above experiments, data from crop plants grown in the absence of pathogens have

suggested a yield penalty to some forms of disease resistance. Chaplin (1970) found significant yield reductions associated with resistance to tobacco mosaic virus and fusarium wilt in 466 F₅ breeding lines of tobacco. Simons (1979) showed a similar yield penalty to resistance to *Puccinia coronata* in 84 lines of *Avena sterilis*. In isogenic lines of *Avena sativa*, however, Frey & Browning (1971) discovered that some genes for resistance to *Puccinia* were associated with a yield increase whilst others were associated with a yield reduction.

Numerous studies have shown that herbicide-resistant biotypes are at a competitive disadvantage in comparison with their susceptible counterparts (see Holt 1990; Warwick 1991 for reviews). This would suggest that transgenic herbicide-resistant plants would have a yield penalty. This may not be the case, however, since the herbicide-resistance experiments present several problems. First, very few experiments used near-isogenic lines, so that the poor performance of the herbicide-resistant biotypes may be due to genetic factors unrelated to herbicide resistance. Nevertheless, the studies of Gressel & Ben-Sinai (1985) on oilseed rape and Reboud & Till-Bottraud (1991) on foxtail millet used near-isogenic lines and both showed a cost to herbicide resistance. A second and more important proviso is that the genetic basis of herbicide resistance is often a mutation causing a loss of sensitivity to, rather than the detoxification of the herbicide. This is especially so in the case of triazines which are the best studied herbicides with respect to the performance of resistant biotypes (Warwick 1991). Resistance is based on mutant chloroplast membrane proteins that are less sensitive to triazines, but are also less efficient than the wild-type protein in the absence of herbicide. Thus, the competitive disadvantage of resistant types in the absence of herbicide is not due to the cost of synthesizing superfluous protein, as would be the case with most transgenics, but to the loss of efficiency of an essential protein. There are some cases of naturally occurring herbicide resistances in plants based on detoxification enzymes (Warwick 1991) but there are no data on the relative performance of resistant and susceptible biotypes.

In summary, the limited amount of data considered above suggests little cost to transgenics of synthesizing extra proteins in the absence of a selective advantage, and that effects due to transformation *per se* are minimal. Thus, the spread of transgenes can be predicted using similar methodology to any other single gene trait. In order to improve predictions the importance of disease in controlling the dynamics of plant populations should be an prominent target for further research.

Hybridization between UK crops and wild relatives

Data on the incidence of hybridization between UK crops and their wild relatives are summarized in Table 3. We have excluded three categories of plant: (i) those UK crops about which we have no information on transformation experiments (e.g. oats); (ii) species that have been transformed but for which genetic modification attracts little or no commercial interest in the UK (e.g. *Dactylis glomerata*); and (iii) genetically manipulated ornamental species (e.g. *Petunia* and chrysanthemum [*Dendranthema* spp.]). From this survey three groups of crops can be identified on the basis of cross-compatibility with wild relatives, suggesting that there is scope for differential regulatory treatments of genetically modified crops to limit the escape of transgenes.

In group 1 ('minimal' probability of gene flow to wild relatives, Table 3), which includes potato, maize, wheat, rye, tomato, several grain legumes, cucumber, sunflower, and grapevines, the escape of transgenes from agricultural situations via hybridization is not an issue. Here concern should be concentrated on escape of the crop itself and on whether the proposed genetic modifications in some way alter the persistence, weediness or invasiveness of the plant. Research should, therefore, focus on this area, rather than on the maintenance of the genetic purity of natural populations. If the plant is utilized by herbivores, toxicity of the products of the transgene to the local fauna may be a concern although, if the crop is a food plant, this will probably have been addressed previously with respect to human safety and is easily tested.

Group 2 species ('low' probability of gene flow) have, in general, no wild conspecifics but do have close, usually congeneric, wild relatives with which there is limited sexual compatibility. This group includes oilseed rape, flax, raspberry, blackcurrant, lettuce and barley. It is a difficult group to assess with respect to transgene escape through hybridization, because the probability of such an escape varies from species to species and, indeed, from site to site, season to season and genotype to genotype, and in many instances may be vanishingly low. Nevertheless, the lesson from plant evolution is that they should be treated differently from those species where hybridization with a wild relative is impossible. For example, the production of allopolyploids from sterile hybrids is a method of speciation which is both well established, and has generated successful and invasive species in recent times (Gray, Marshall & Raybould 1991). The major research questions in this group are the nature and strength of the breeding barriers within the species complex, the persistence of hybrid and/or feral populations which may contain the transgene (a problem of particular interest in

Table 3. UK crop plants and their wild relatives (nomenclature follows Stace 1991)

Crop	UK wild relatives	Evidence of hybridization	Probability of gene flow from crop to a wild species
Sugar beet (<i>Beta vulgaris</i> ssp. <i>vulgaris</i>)	Sea beet (<i>Beta vulgaris</i> ssp. <i>maritima</i> [= <i>Beta maritima</i>])	Contamination of sugar beet seed lots with 'bolting genes' ^{1,2} . Contamination of beet varieties with red stem genes ³ . Repeated back-crosses of sugar beet and sea beet in breeding programmes ⁴ . Hybrids 'occur readily wherever the parents occur close together, with no apparent loss of fertility' ⁵ .	High
Oilseed rape (<i>Brassica napus</i> ssp. <i>oleifera</i>)	Wild turnip (<i>Brassica rapa</i> [= <i>Brassica campestris</i>])	Cross achieved experimentally, especially with <i>Brassica napus</i> as the female parent. Cross produces seed and shows sterility or reduced fertility in the F ₁ , F ₂ and back-cross generations produced by manual pollination ^{6,7} . In the wild, the hybrid (<i>Brassica</i> × <i>harmstana</i>) occurs 'sporadically in crops of <i>B. napus</i> when exposed to pollination by <i>Brassica rapa</i> ' ⁸ .	Low
	Wild cabbage (<i>Brassica oleracea</i>)	Cross achieved with extreme difficulty by manual pollination ^{6,9,10} . Hybrid obtained by embryo rescue ¹¹ . No evidence of hybrid in the wild ⁵ .	Low
	Hoary mustard (<i>Hirschfeldia incana</i>)	Cross at diploid level very difficult; some success using <i>Brassica nigra</i> with chromosome number doubled by colchicine ¹² . No evidence of hybrid in the wild ⁵ .	Low
	Black mustard (<i>Brassica nigra</i>)	Hybrid achieved by embryo rescue only ¹³ .	Low
Cabbage, cauliflower, etc. (<i>Brassica oleracea</i>)	White mustard (<i>Sinapis alba</i>)	Hybrid achieved experimentally only ¹⁴ .	Low
	Charlock (<i>Sinapis arvensis</i>)	Hybrid achieved by embryo rescue only ¹⁵ .	Low
	Wild cabbage (<i>Brassica oleracea</i>)	Same species.	High
	Wild turnip (<i>Brassica rapa</i>)	Very occasional hybrids achieved by manual pollination ⁷ .	Low
	Black mustard (<i>Brassica nigra</i>)	Hybrids only achieved by manual pollination with <i>Brassica oleracea</i> as pollen parent. Hybrids sterile ¹³ .	Low
	Cruciferous species in several genera e.g. <i>Eruca</i> , <i>Erucastrum</i> , <i>Diplotaxis</i> .	Hybrids by embryo rescue only ¹⁶ .	Minimal

Table 3. Continued

Crop	UK wild relatives	Evidence of hybridization	Probability of gene flow from crop to a wild species
Potato (<i>Solanum tuberosum</i>)	Woody nightshade (<i>Solanum dulcamara</i>)	No records of hybrids in the UK and controlled pollinations have failed to produce hybrids ¹⁵ .	Minimal
	Black nightshade (<i>Solanum nigrum</i>)	As for <i>Solanum dulcamara</i> .	Minimal
Tomato (<i>Lycopersicon esculentum</i>)	As for <i>Solanum tuberosum</i>	As for <i>Solanum tuberosum</i> .	Minimal
Maize (<i>Zea mays</i>)	None	N/A	Minimal
Wheat (<i>Triticum aestivum</i>)	Barley species (<i>Hordeum</i> spp.)	Wheat successfully hybridized with several <i>Hordeum</i> species ¹⁷ , but none of these are native species in the UK.	Minimal
	Couch grasses (<i>Elytrigia</i> spp.)	Wheat– <i>Elytrigia</i> hybrids formed by embryo rescue ¹⁸ . None involve UK species of <i>Elytrigia</i> .	Minimal
	Lyme grass (<i>Leymus arenarius</i>)	Wheat– <i>Leymus</i> hybrids formed by embryo rescue ¹⁹ . None involve <i>L. arenarius</i> .	Minimal
Barley (<i>Hordeum vulgare</i>)	Barley species (<i>Hordeum</i> spp.)	<i>Hordeum vulgare</i> can be hybridized with several other <i>Hordeum</i> species under controlled conditions ²⁰ . Several of these species grow wild in the UK, but no <i>Hordeum</i> hybrids recorded from UK ⁵ .	Low
	Couch grasses (<i>Elytrigia</i> spp.)	Several <i>Hordeum</i> – <i>Elytrigia</i> hybrids recorded from North America but none involve UK species ⁵ .	Minimal
Rye (<i>Secale cereale</i>)	Barley species (<i>Hordeum</i> spp.)	Rye can be hybridized with <i>Hordeum vulgare</i> ²¹ . No hybrids with UK species.	Minimal
Ryegrass (<i>Lolium perenne</i> & <i>Lolium multiflorum</i>)	Feral/wild ryegrass (<i>Lolium perenne</i> & <i>Lolium multiflorum</i>)	Same species.	High
	Fescue species (<i>Festuca</i> spp.)	<i>Lolium</i> × <i>Festuca</i> hybrids readily formed artificially. Hybrids show fertility and can backcross to parents. Tend to be uncommon in the wild. Most frequent is <i>Lolium perenne</i> × <i>Festuca pratensis</i> (= × <i>Festulolium loliaceum</i>) ^{5,22} .	High
White Clover (<i>Trifolium repens</i>)	Feral/wild white clover (<i>Trifolium repens</i>)	Same species.	High
	Clover species (<i>Trifolium</i> spp.)	Breeding barriers between <i>Trifolium</i> spp. very strong ²³ . No <i>Trifolium</i> hybrids recorded from UK.	Minimal
Lucerne (<i>Medicago sativa</i> ssp. <i>sativa</i>)	Sickle medic (<i>Medicago sativa</i> ssp. <i>falcata</i>)	Ssp. <i>sativa</i> and <i>falcata</i> do hybridize and the hybrid (= ssp. <i>veria</i>) is fertile ^{5,24} . Ssp. <i>veria</i> relatively common in East Midlands and East Anglia ⁵ .	High
Broad bean (<i>Vicia faba</i>)	Vetch species (<i>Vicia</i> spp.)	All attempts at producing interspecific hybrids with <i>Vicia faba</i> have failed ²⁵ .	Minimal

Table 3. *Continued*

Crop	UK wild relatives	Evidence of hybridization	Probability of gene flow from crop to a wild species
Runner bean (<i>Phaseolus vulgaris</i>) & French bean (<i>Phaseolus coccineus</i>)	None	N/A	Minimal
Pea (<i>Pisum sativum</i>)	None	N/A	Minimal
Carrot (<i>Daucus carota</i> ssp. <i>sativus</i>)	Wild carrot (<i>Daucus carota</i> ssp. <i>carota</i>)	Essentially the same species. Evidence for hybridization from morphology, phenology ²⁶ and isozymes ²⁷ .	High
Lettuce (<i>Lactuca sativa</i>)	Prickly lettuce (<i>Lactuca serriola</i>)	Fully interfertile with controlled pollinations ^{28,29,30} . No <i>Lactuca</i> hybrids recorded from UK ⁵ .	Low
	Great lettuce (<i>Lactuca virosa</i>)	Hybrids produced by controlled pollinations but died prematurely ³¹ or sterile ³² . Hybrids also produced by protoplast fusion ³³ .	Low
	Least lettuce (<i>Lactuca saligna</i>)	Hybrids produced by controlled pollinations, limited fertility allows production of F ₂ generations ³⁰ .	Low
Cucumber (<i>Cucumis sativus</i>)	None	N/A	Minimal
Sunflower (<i>Helianthus annuus</i>)	None	N/A	Minimal
Flax (<i>Linum usitatissimum</i>)	Pale flax (<i>Linum bienne</i>)	There are no records of flax hybrids in the British Flora ⁵ .	Low
	Perennial flax (<i>Linum perenne</i>)	See above.	Low
	Fairy flax (<i>Linum catharticum</i>)	See above.	Low
Scots pine (<i>Pinus sylvestris</i>)	Scots pine (<i>Pinus sylvestris</i>)	Cultivated and wild Scots pine are the same species, although outside Scotland 'wild' Scot's pines are often introductions from Russia ³⁴ .	High
Spruce (<i>Picea</i> spp.)	Scots pine (<i>Pinus sylvestris</i>)	None: interspecific hybridization in conifers is extremely rare ³⁵ .	Minimal
Douglas fir (<i>Pseudotsuga menziesii</i>)	Scots pine (<i>Pinus sylvestris</i>)	As for <i>Picea</i> .	Minimal
Poplar (<i>Populus</i> spp.)	Black poplar (<i>Populus nigra</i>)	The breeding of cultivated poplar has used extensive hybridization programmes between <i>P. nigra</i> , <i>P. deltoides</i> , <i>P. trichocarpa</i> and <i>P. maximowiczii</i> . <i>P. alba</i> is also planted widely in the UK. These species hybridize freely with each other and other <i>Populus</i> species. Cultivated and feral <i>P. nigra</i> are the same species and wild <i>P. nigra</i> will hybridize with the other species mentioned ³⁶ .	High

Table 3. *Continued*

Crop	UK wild relatives	Evidence of hybridization	Probability of gene flow from crop to a wild species
Poplar (<i>Populus</i> spp.)	Black poplar (<i>Populus nigra</i>)	The hybrid black poplar, <i>P.</i> × <i>canadensis</i> (<i>P. nigra</i> × <i>P. deltoides</i>), occurs throughout the UK, although most specimens are planted ⁶ .	High
	Aspen (<i>Populus tremula</i>)	As above ³⁶ . The grey poplar, <i>Populus</i> × <i>canescens</i> (<i>P. alba</i> × <i>P. tremula</i>) has arisen throughout the UK ⁵ .	High
Grapevines (<i>Vitis vinifera</i>)	None	N/A	Minimal
Plums (<i>Prunus domestica</i>)	Blackthorn (<i>Prunus spinosa</i>)	Extensive hybridization (hybrid = <i>Prunus</i> × <i>fruticans</i>) and introgression with full range of intermediates between the species ^{5,8} .	High
	Wild cherry (<i>Prunus avium</i>)	No hybrids recorded.	Low
Apple (<i>Malus pumila</i>)	Bird cherry (<i>Prunus padus</i>)	As above.	Low
	Crab apple (<i>Malus sylvestris</i>)	No formal records of hybrids, but crab apple is used as a pollinator in commercial orchards and in many parts of the UK the commonest crab apples show evidence of introgression with cultivated apple ³⁷ .	High
	Wild strawberry (<i>Fragaria × ananassa</i>)	Experimental hybrids have been produced but all died before flowering ⁵ .	Minimal
Black currant (<i>Ribes nigrum</i>)	Downy currant (<i>Ribes spicatum</i>)	No hybrids recorded.	Low
	Mountain currant (<i>Ribes alpinum</i>)	As above.	Low
Raspberries (<i>Rubus idaeus</i>)	Blackberry (<i>Rubus fruticosus</i> agg.)	<i>Rubus fruticosus</i> taxonomy is highly complex, it being an aggregate of sterile polyploid apomicts and fertile diploid microspecies. No evidence of introgression of characters of commercial raspberries into wild populations of diploid <i>Rubus fruticosus</i> agg., despite widespread sampling of wild populations ³⁸ .	Low
	Dewberry (<i>Rubus caesius</i>)	Sterile hybrids scattered throughout southern England ⁵ .	Low

References: ¹Longden (1976). ²Evans & Weir (1981). ³Hornsey & Arnold (1979). ⁴Bosemark (1979). ⁵Stace (1975). ⁶U (1935). ⁷Nishiyama, Sarashima & Matsuzawa (1991). ⁸Stace (1991). ⁹Honma & Summers (1976). ¹⁰Chiang, Chiang & Grant (1977). ¹¹Ayotte, Harney & Souza Machado (1987). ¹²Turesson & Nordenskiöld (1943). ¹³Ripley & Arnison (1990). ¹⁴Inomata (1988). ¹⁵P.J. Dale (personal communication). ¹⁶Harberd (1976). ¹⁷Sharma & Gill (1983). ¹⁸Lu, Salomon & von Bothmer (1990). ¹⁹Tabaeizadeh, Plourde & Comeau (1990). ²⁰von Bothmer *et al.* (1983). ²¹Finch, Smith & Bennett (1981). ²²Hubbard (1968). ²³Evans (1962). ²⁴Ledingham (1940). ²⁵G. Ramsay (personal communication). ²⁶Wijnheijmer, Brandenburg & Ter Borg (1989). ²⁷St. Pierre & Bayer (1991). ²⁸Lindqvist (1960). ²⁹Kesseli, Ochoa & Michelmore (1991). ³⁰Zohary (1991). ³¹Eenink, Groenwold & Dieleman (1982). ³²Whitaker (1969). ³³Matsumoto (1991). ³⁴Hyde (1961). ³⁵Mitchell (1974). ³⁶A. Beaton (personal communication). ³⁷Clapham, Tutin & Moore (1987). ³⁸R. McNicol (personal communication).

crops such as oilseed rape where feral populations already exist) and, as before, the effects of particular transgenes on the lifetime fitness of individual plants.

Group 3 ('high' probability of gene flow), including sugar beet, carrots, some cabbage cultivars, the trees poplar, pine, apple and plum, and the forage species ryegrass, clover and lucerne, present a quite different problem. Overlapping geographical distributions and reproductive compatibility with wild relatives indicate that, on a commercial scale, the escape of transgenes via hybridization is both possible and, in some species, highly probable. Thus, in addition to any consideration of a weed problem, it is important that research should examine the extent of gene flow and introgression (for example, how much gene flow has occurred in the past in species such as beet, ryegrass and clover? Under what conditions can introgression to the wild conspecific occur? What traits prevent the persistence of hybrids?) and on the effect of specific genetic modifications on the fitness of the plant in natural populations. As stated previously, relatively little is known about the factors which maintain genetic polymorphisms for traits such as disease and insect resistance in natural populations, or whether diseases and insect pests have significant effects on reproductive output or the life span of perennials. Even less is known about the effects of herbicide resistance.

Concluding remarks

This review has shown that considerable progress is being made in the genetic modification of UK crop species and has highlighted traits, such as disease resistance, which are most amenable to modification. These traits are often found in natural populations. Studies on the field performance of transgenic crops suggest that there is no intrinsic yield penalty to the possession of a transgene. An improved understanding of the maintenance of polymorphisms for traits such as disease resistance, herbivore resistance and herbicide resistance in natural populations should allow predictions of the effects of transgenes conferring these properties on natural populations. One can envisage situations where a transgene could be at a selective advantage in natural populations. A gene conferring insect resistance, for example, could spread rapidly in a species in which density and distribution are regulated by pest pressure. However, there is little evidence that insect predation has a significant effect on the limitation of natural plant populations (although the case of feral populations of crops with a substantial pest fauna, oilseed rape for instance, may prove to be an exception).

A survey of the cross-compatibility of UK crops and their wild relatives shows that for some crops hybridization is not an issue, and any concern for

the detrimental effects of transgenes on the environment should concentrate on the establishment of weed and feral populations. Again, experience of these traits in natural populations should provide information on the possible persistence and spread of such populations.

Although we are proposing that groups of crops may be regulated in a similar fashion with regard to the likelihood of hybridization with wild relatives, it is difficult, and potentially misleading, to generalize about the effects of transgenes on the performance of wild species. This may be irrelevant to proposals involving crops which do not hybridize with any wild species, but in species that do hybridize such effects must be considered on a crop-by-crop and construct-by-construct basis. As information on the effects on fitness of traits such as virus resistance becomes available, further generalizations can be made. Until then we feel that the best way to begin simplifying release procedures is on the basis of the crops' relationships to their wild relatives.

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Postscript

After the completion of this review a survey of hybridization between crops and wild species in the Netherlands was published (F.T. de Vries, R. van der Meijden & W.A. Brandenburg (1992) Botanical files: a study of the real chances for spontaneous gene flow from cultivated plants to the wild flora of the Netherlands. *Gorteria Supplement*, **1**, 1–100). Genetic modification of crops was not examined, but the purpose of the study was to provide information on the safety of the release of genetically modified plants. For 42 cultivated species a 'Botanical file' was prepared giving information on the crop (use, origin, etc.), its wild relatives in the Netherlands, reports of hybrids and records of escapes from cultivation. These data were used to give a numerical code to each crop species indicating its 'possible ecological effect'.

There are, perhaps, only two crops which de

Vries *et al.* would reclassify under our system. They consider gene flow from cultivated to wild *Beta* will have a 'small ecological effect' (roughly equivalent to our 'low') due to the wild species' limited, distribution. We regard the impact as potentially greater because of evidence for a hybrid complex between cultivated, weed and wild beets. *Lactuca sativa* is described as having a possible 'substantial ecological effect' (equivalent to our 'high') through gene flow between *L. sativa* and *L. serriola*, though there is no evidence of natural hybridization and few records of *L. sativa* having escaped from cultivation. We feel our classification of 'low' is more realistic on the basis of these data. The conclusions drawn for almost all the other crops were the same as our own, with any discrepancies being due, in the main, to the different amount of overlap in the distributions of crops and relatives in the UK and in the Netherlands.

Addition to postscript

The speed with which transformation technology is developing is shown by the fact that since this review went to press reports of the transformation of two further UK crops have been published. Herbicide-resistant *Avena sativa* (oat) has been produced by particle bombardment of embryogenic callus (D.A. Somers, H.W. Rines, W. Gu, H.F. Kaeppler & W.R. Bushell (1992) Fertile, transgenic oat plants. *Biotechnology*, **10**, 1589–1594); also *Brassica rapa* (turnip, mustard rape, etc.) plants expressing two marker genes have been obtained by *Agrobacterium tumefaciens*-mediated transformation of hypocotyls (S.E. Radke, J.C. Turner & D. Facciotti (1992) Transformation of *Brassica rapa* using *Agrobacterium tumefaciens*. *Plant Cell Reports*, **11**, 499–505). A preliminary assessment suggests a low probability of *A. sativa* hybridizing with the wild oat, *A. fatua*. Stace (1975) states that hybrids occur 'sporadically' in the UK and do not persist. *Brassica rapa* may have a high probability of hybridization with wild turnip (same species) and a low probability of hybridization with other *Brassica* species.