Review

Tiered tests to assess the environmental risk of fitness changes in hybrids between transgenic crops and wild relatives: the example of virus resistant *Brassica napus*

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Over the last 20 years, there has been much research aimed at improving environmental risk assessment of transgenic crops. Despite large amounts of data, decisions to allow or prohibit the release of transgenic crops remain confused and controversial. We argue that part of the reason for confusion is the lack of clear definitions of components of the environment that should be protected, and, as a consequence, there is no way to judge the relevance of data collected under the auspices of 'environmental risk assessment'. Although this criticism applies to most aspects of environmental risk assessment of transgenic crops, it is most pertinent to effects that might result from an increase in plant fitness, often referred to as increased weediness. Environmental risk assessment of weediness is regarded as complicated: an increase in the fitness of a transgenic plant compared with non-transgenic counterparts will be the result of an interaction between the altered plant phenotype and an enormous number of environmental variables. This has led to the idea that risk assessment of weediness needs to "understand" these interactions, with the implication that exhaustive data are required. Here we argue that environmental risk assessment of the weediness of transgenic plants need not be complicated. Analysis of the conditions that must be met for increased weediness to occur suggests a series of studies that starts with simple tests in the laboratory under "worst case" assumptions, and becomes increasingly complex and realistic should the simpler studies not indicate negligible risk with sufficient certainty. We illustrate how the approach might work for assessing the risks of increased weediness using the example of possible introgression of a gene for *Turnip mosaic virus* (TuMV) resistance from oilseed rape to certain wild *Brassica* species.

Keywords: Risk assessment / hazard / exposure / fitness / virus tolerance / ecological release

INTRODUCTION

An effective risk assessment seeks to minimize the amount of data required to reach an accurate prediction of the likelihood that harm might result from a proposed action. The collection of superfluous data that do not improve decision-making diverts effort from more worthwhile activities, and may confuse rather than clarify. Indeed, collection of data is not free of risk; overall risk may be increased if the replacement of a product with a less harmful alternative is delayed while data are collected (Cross, 1996).

In this paper we propose that stepwise assessment, using a framework similar to that which is standard for the testing of pesticides, can be used to assess the risks of increased weediness of wild species following introgression of transgenes from a transgenic crop. We argue that a detailed simulation of the natural ecosystems into which the transgenes may spread is not necessary. Our aim is to show that the environmental risks associated with gene flow from transgenic crops can begin with desk or laboratory studies and be followed, if justified, by small,

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contained experiments. If these initial studies fail to indicate negligible risk with sufficient certainty, field observations may be justified. To illustrate how such a scheme might work, we use data on the ecology of *Turnip mosaic virus* (TuMV) in populations of wild *Brassica* species to assess risks from TuMV tolerant *Brassica napus* L. subsp. *oleifera* (DC.) Metzger (oilseed rape).

THE STRUCTURE OF ENVIRONMENTAL RISK ASSESSMENTS

The structure of an environmental risk assessment is simple: decide what needs protection from harm; assess how a proposed action might cause harm; and collect data, from the literature or from new studies, to predict the likelihood and magnitude of harm following that action. Environmental risk assessment of transgenic plants appears complicated because these simple concepts are not followed. There is confusion about what needs protection (*e.g.,* are weeds harmful, or are they valuable because they contribute to biodiversity?), vague ideas about how transgenic plants might cause harm (the effects of "unknown unknowns"; see Gray, 2004, for a critique of this concept in risk assessment), and data are collected without any indication of how they should be used to assess risk. In the jargon of risk assessment, there is poor "problem formulation".

Our purpose is to show how clarification of a problem can lead to simple and effective experiments to assess the risk of something as apparently complicated as the potential increase in weediness of plants that become resistant to disease through introgression of genes from transgenic crops. To do this, we introduce definitions of vital elements of risk assessments with illustrations from the assessment of pesticides: management objectives, assessment endpoints, hazard, exposure, test endpoints and trigger values. Although pesticides and transgenic crops pose different hazards and have different routes of exposure, the concepts applied to the environmental risks of chemicals can be usefully applied to transgenic crops (Raybould, 2005; Raybould and Wilkinson, 2005).

Management objectives

The assessment of risk of an action, such as the cultivation of a transgenic crop, is usually undertaken to meet some general objectives that are set by law. In the United States, transgenic plants that express pesticidal proteins are regulated under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). The purpose of FIFRA is to "protect the public health and environment from the

misuse of pesticides by regulating the labeling and registration of pesticides and by considering the costs and benefits of their use". Also, transgenic plants that contain or are derived from plant pests (in practice all transgenic plants), whether expressing pesticidal proteins or not, are regulated under the Federal Plant Protection Act (FPPA). Transgenic plants are regarded as "regulated articles" under the FPPA unless it can be demonstrated that the plants do not present a plant pest risk. A plant pest is defined as an organism or substance that "can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants" (APHIS, 1987). Transgenic plants must also comply with the Endangered Species Act (ESA), which "prohibits any action that can adversely affect an endangered or threatened species or its habitat".

Similar management objectives are prescribed in the European Union. Directive 2001/18/EC covers the release of transgenic organisms ("GMOs"), and requires that risk assessments "identify and evaluate potential adverse effects of the GMO, either direct [or] indirect, immediate or delayed, on human health and the environment which the deliberate release or placing on the market of GMOs may have". The "Habitats Directive" (92/43/EEC), fulfils a similar function to the USA's ESA, and obliges countries ensure the "preservation, protection and improvement of the quality of the environment, including the conservation of natural habitats and of wild fauna and flora". The Directive lists habitats (in Annex I) and species (in Annex II) that require "the designation of special areas of conservation", and also lists species (in Annex IV) of EU "interest in need of strict protection". Cultivation of transgenic plants should comply with this Directive. Therefore in the USA and the EU, the protection of human health and the environment, including endangered species and their habitats, are the management objectives that must be covered by an environmental risk assessment for a transgenic crop.

Assessment endpoints

Management objectives are general concepts. To be useful, they must be expressed as measurable phenomena as unambiguously as possible; in other words, they require operational definitions. Assessment endpoints are operational definitions of management objectives that describe components in the environment to be protected. Assessment endpoints should comprise an entity (*e.g.,* a population of a particular species in a particular area) and a property of that entity (*e.g.,* the population size) (Newman, 1998). A typical assessment endpoint for pesticide risk

assessment is the population size of non-target arthropods in the area where the pesticide was applied, one year after application.

Hazard

The hazard of a substance or action is its ability to cause harm. The main hazard of pesticides is their toxicity, although properties such as flammability and corrosiveness are other potential hazards.

Exposure

Exposure is the probability of being exposed to a hazard. A pesticide may be highly toxic, but pose no risk because all the assessment endpoints are not exposed to concentrations or doses of the chemical that cause harm.

Test endpoints

The risk to assessment endpoints is not usually assessed directly, at least in the early stages of a risk assessment. Instead, measurements of hazard and exposure are made in the laboratory and used to predict effects on the assessment endpoints. The measures of hazard and exposure are called test endpoints. Test endpoints for hazard include the concentration or dose of a pesticide required to kill 50% of a test population (the LC_{50} and LD_{50} respectively). Test endpoints for exposure include predicted environmental concentrations (PECs) of the pesticide in water or in food.

Risk

Risk is estimated by combining the hazard and exposure measurements. For chemicals, the risk estimator may be as simple as the ratio of the PEC and the LC_{50} (the hazard quotient, HQ).

Trigger values

Once the risk has been estimated, a decision must be made as to whether negligible risk has been estimated with sufficient certainty ("acceptable risk"). When risk is assessed as an HQ, values below a certain threshold are defined as "acceptable risk" and values above as "unacceptable risk". The threshold is called the "trigger" because different actions are triggered by values of the risk estimator either side of the threshold.

TIERED ASSESSMENTS IN ENVIRONMENTAL RISK ASSESSMENT

Risk assessment begins with problem formulation, in essence a conceptual model that links the proposed action (*e.g.,* cultivation of a transgenic crop, application of a pesticide, building a road) to possible harm to the assessment endpoints, and the assessment endpoints to a series of test endpoints to predict to what extent that harm will be realized. Test endpoints will be measures of the hazards of, and exposure to the proposed action.

If estimates of the test endpoints already exist, the risk assessment can be completed without additional studies. If data do not exist, studies to obtain hazard or exposure data, or both, are required. Usually, the first measurements of hazard and exposure are made under "worst case" conditions. For instance in the risk assessment of pesticides, the exposure may be set as the concentration of the active ingredient in the formulated product and the hazard assessed under conditions where contact with the pesticide is unavoidable; these are known as 'tier I' tests and are combined to give a tier I risk assessment.

If the risk judged under tier I conditions is below that which triggers concern, by definition no further testing is necessary to conclude that the risks are acceptable because the risks have been assessed under worst case conditions. The trigger value may be set conservatively (a low value for HQs), so that false negatives (compounds that appear to have negligible risk and require no further testing, when further testing is necessary) are minimized. When the risk is greater than the trigger value, "higher tier tests" that introduce more realism may be justified. These higher tier tests seek to identify false positives that are likely to result from tier I assessments. For each tier, a new trigger value that accounts for the greater realism of the test is set, and revised decisions are made about the acceptable amount of risk in these circumstances.

Tier I tests are not intended to be realistic: their purpose is to aid early decision making and thereby minimize unnecessary costs of testing of substances (including transgenic crops) that present very low hazard. Although direct costs (expense of doing the work, delay in selling the product) are borne by the developer of the product, they may be borne more widely if additional testing delays the introduction of an efficacious product that reduces environmental risks compared with current practice.

For the testing of chemical pesticides, a more-or-less standard procedure is now used within the European Union to predict effects on non-target arthropods (Candolfi et al., 2000; EPPO, 2003). The hazard of the pesticide to a small number of representative indicator

organisms is measured using protocols that have been rigorously evaluated for consistency of results among many laboratories. Exposures are also estimated using a common set of experiments and models and combined with the hazard estimates to give a standard set of risk estimators with agreed trigger values. If tier I assessments all fall below the trigger values, the expectation is that regulators from different countries will reach the same decision: the pesticide poses negligible risk under the assessed pattern of use. Similar methods can be used to assess the safety of insecticidal protein expressed in plants (Dutton et al., 2003; US EPA, 2001) and many studies indicate are these methods are predictive of effects in the field (Naranjo et al., 2005).

A tiered approach to the risk assessment of genetically modified (GM) crops has been proposed (Poppy, 2000; Wilkinson et al., 2003b). However advocacy of tiered tests stops short of the recommendation that risk assessment should seek the minimum necessary information to help make a decision. Often a tiered risk assessment for transgenic crops refers to the order in which tests are done: characterization of the protein, then characterization of the plant, and finally an assessment of the effects of cultivation of the plant; the results of earlier studies being used to interpret the field data. Strictly, this is not tiered testing because under a tiered system the objective is to assess risk at each tier (not just collect data) and only proceed to a high tier (less worse case) if necessary.

In addition to assessing risk, studies of hazard and exposure can be used to design and assess the efficacy of risk management. For example, if plants are transformed with a pesticidal protein, events with the lowest pollen expression may be selected to reduce exposure of non-target organisms. Risk management includes the discontinuation of the development of a product should data indicate that risks are high and that exposure and hazard cannot be reduced without the product becoming ineffective.

PEST RESISTANT CROPS AND "ECOLOGICAL RELEASE"

One of the main concerns about transgenic crops is that they will hybridize with wild plants, and that the traits conferred by the transgenes will increase the abundance or distribution of the wild species (Bergelson et al., 1998; Butler and Reichhardt, 1999; Ellstrand, 2001; Ellstrand et al., 1999; Raybould and Gray, 1994; Scheffler and Dale, 1994). This concern arises from the concept of "ecological release".

Some plant species that are not invasive in their native range become invasive when introduced into new areas. The ecological release concept proposes that in the native range, pests and pathogens control the abundance and spread of these plants. Outside the native range, these pests and pathogens are absent, "releasing" the plants from controls on their abundance and spread (Mitchell and Power, 2003). A similar release could occur if the plants became tolerant or resistant to the pests and pathogens through introgression of transgenes, or indeed acquire non-transgenic resistance through hybridization or mutation.

Transgenic virus resistance in Brassica

Since 1986, many crops have been transformed with DNA sequences derived from viruses (and also non virus-derived sequences) to obtain tolerance or resistance to economically damaging diseases caused by virus infection (Cooper and Walsh, 2003); some virus tolerant transgenic varieties, notably of papaya, squash and tobacco have been grown commercially. TuMV causes serious disease in many crops, including cabbage and other *Brassica* vegetables, oilseed rape, chicory, horseradish, lettuce, peas and rhubarb (Shattuck, 1992), and transgenic approaches have been used to obtain resistance to TuMV (Dinant et al., 1993; 1997; Jan et al., 2000). In anticipation of the possible field release of transgenic TuMV resistant *B. napus* (Lehmann et al., 1996) we assessed whether the introgression of genes for TuMV tolerance could lead to ecological release in wild *Brassica* species.

We studied the ecology of TuMV in three wild brassicas in England: *Brassica oleracea* L. (wild cabbage), a long-lived perennial that grows on sea cliffs; *Brassica nigra* (L.) Koch (black mustard), an annual that grows in ruderal habitats; and *Brassica rapa* L. subsp. *sylvestris* (L.) Janchen (wild turnip), an annual or biennial species that grows on riverbanks (Mitchell and Richards, 1979; Preston et al., 2002; Rich, 1991; Wilkinson et al., 2003a). The weedy forms of *B. rapa* that infest *Brassica* crops are not considered here. These species have the genome designations 'CC', 'BB' and 'AA' respectively. Ancestors of these species are the progenitors of important tetraploid oilseed crops *B. napus* (AACC), *B. juncea* (AABB) and *B. carinata* (BBCC) (U, 1935). *B. rapa* and *B. oleracea* can hybridize with *B. napus* under field conditions (Wilkinson et al., 2000; M.J. Wilkinson, personal communication) and, because *B. napus* is widely commercially cultivated in the UK (Heritage, 2003), there is potential for introgression of genes from transgenic oilseed rape in these species. Indeed, a detailed map of the

predicted incidence of hybridization between oilseed rape and *B. rapa* in the UK has been published (Wilkinson et al., 2003a). There are no records from the field of hybrids between *B. nigra* and *B. napus*, although the species can be crossed in the laboratory (Chèvre et al., 2004; Scheffler and Dale, 1994). These data are discussed in more detail below in the section on exposure assessments.

RISK ASSESSMENT FRAMEWORK FOR VIRUS RESISTANCE GENES

Assessment endpoints

Risk assessment begins by identifying the potential harmful consequences of a proposed action; in this instance we will consider the cultivation of oilseed rape with transgenic resistance to TuMV and the harm that may result from spread of the resistance gene to wild relatives. Harm from introgression of virus-derived virus resistance transgenes could occur through the evolution of new diseases, via recombination of transgene mRNA with the RNA of viruses that infected the transgenic wild relative, or through ecological release of the wild relative (Cooper and Raybould, 1997). Here, we will consider harm from ecological release only.

The harm caused by the ecological release of a wild relative is analogous to that produced by the spread of an introduced invasive species. Pimentel et al. (2001) identified the main problems caused by invasive plants, other than weeds of crops:
- Displacement of native plant species (and presumably other taxa that use those plants as food, shelter, etc.);
- Physical changes, including reduced water supply, increased frequency of bush fires and changed nutrient cycles;
- Detrimental effects on recreation (this is a particular problem with aquatic plants that affect fishing, boating, swimming, etc.);
- Loss of yield in semi-natural pastures;
- Costs of control.

The problems identified by Pimentel et al. are probably not an exhaustive list of possible harmful effects of ecological release of wild relatives; however they are likely to be the most important. Also, they are very close to being operational assessment endpoints: for example, physical changes could be quantified in terms of litres of water, or area burnt; displacement of native species could be defined in terms of population sizes of taxa with legal protection, and so on (Raybould, 2005; Raybould and Wilkinson, 2005). Therefore it may be possible to devise

assessment endpoints that are the ultimate concerns about ecological release. Nevertheless, we use a simpler assessment endpoint: the population size of wild relatives. The reason for this is that theory linking the size of the population of a wild relative to displacement of other species or physical changes in its habitat is lacking, therefore we take a conservative approach and assume that any increase in the population size of a wild relative is harmful. In other words, we suppose that a trigger for action is an indication that the population size could increase if the resistance gene were to introgress.

Test endpoints and trigger values for exposure

The simplest way to define exposure in relation to ecological release is the likelihood of hybridization between the crop and wild relative in the area covered by the risk assessment. There are several reasons for considering hybridization, rather than gene frequency, as the endpoint for exposure: hybridization is more conservative (introgression does not follow automatically from hybridization); gene frequency will be influenced by the properties of the transgene, which are conceptually easier to consider as hazards rather than components of exposure; hybridization is easy to test in a tiered manner; and if hybridization can be ruled out there is no risk and hence no need for hazard testing.

The possibility of hybrid formation can be assessed using a tiered approach as advocated by Raybould (2004):

Tier I: Test for hybrid production using laboratory methods (hand pollination, embryo rescue, etc.).
No hybrids, stop testing; hybrids, go to tier II;

Tier II: Test for 'spontaneous' hybrid production (lab/field).
No hybrids, stop testing; hybrids, go to tier III;

Tier III: Search for naturally-produced hybrids.
No hybrids, stop testing; hybrids, base risk assessment on hazard in regions where hybrids are found.

In well-studied flora such as that of the UK, there is, in effect, Tier III data for most crop and wild relative combinations (Preston et al., 2002; Raybould and Gray, 1993). However, this scheme could be followed in regions where there is uncertainty over whether a crop and a wild species are sexually compatible. If negligible risk can be established because of the low likelihood of hybridization, hazard testing may be unnecessary. If hybridization cannot be ruled out with high certainty, hazard testing will be required to assess risk.

Test endpoints and trigger values for hazard

We defined an unacceptable effect of introgression of disease resistance genes as any increase in the population size of a wild relative. For this to occur, several conditions need to be satisfied:

1. The wild relative must be infectible and sensitive to the pathogen against which the transgene provides resistance. If the plant species is already immune, additional resistance gene (s) will have no effect (except possibly if the pre-existing resistance mechanism has detrimental pleiotropic effects).

2. The pathogen must increase mortality or reduce reproduction, or both. Plant population size will be unchanged unless enhanced resistance increases the persistence or reproductive rate of infected individuals.

3. The wild relative must be infected by the pathogen naturally in the field. Although the wild crop relative may be experimentally infectible by a specific pathogen, the plant species may not become infected in the field, for example because appropriate vectors are absent.

4. No density dependent mortality should operate before introgression. Density dependent mortality operates when an increase in density leads to an increase in mortality; in simple terms the population size in an area is limited by space. If the size of the population is already controlled by density dependent factors, increased numbers of plants resulting from fewer pathogen induced deaths will not result in a stable increase in population size.

In addition to these 'event independent' factors, other conditions relating to the specific construct used to create the transgenic crop must also apply:

5. The transgene must function in the genetic background of the wild relative.

6. The 'cost' of transgene resistance in the absence of the pathogen must be small. If the transgene confers reduced fitness when the plant is not challenged by the pathogen, the gene may not persist in the population, especially if the pathogen is distributed patchily in space or time.

The first four statements suggest a tiered framework for testing whether hybridization (introgression) of resistance gene would lead to ecological release (*i.e.,* an assessment of hazard). Below we describe the general principles of tiered testing for ecological release following introgression of a gene for virus resistance; similar experiments could be designed for other pathogens. After describing the tests, we illustrate them with examples from our research on TuMV in wild *Brassica* species.

Tier I laboratory study

This study assesses the infectibility/ sensitivity of the wild relative to high doses of the viruses potentially controlled by the transgene. For convenience, and to achieve worst case doses of virus, inoculation should be mechanical (manual), where possible. A range of plant and pathogen genotypes should be used in these tests to minimize the possibility of mistaking gene-for-gene resistance to virus replication for immunity or field resistance (see Cooper and Jones, 1983, for definitions). Similarly, if an animal vector is obligatory for virus transmission, a range of vector genotypes should be tested. For example, the whitefly *Bemisia tabaci* is the only known vector of Begomoviruses (a genus of Geminiviruses). However, biological "races" of *B. tabaci* occur and differences in vector competence may absolutely determine the host range of specific virus isolates (Bedford et al., 1994; Burban et al., 1992). Sensitivity can be judged by visual symptoms; if no symptoms are visible, immunity should be distinguished from tolerance using serological or other methods specifically to detect the target virus. We have set the unacceptable effect to be any increase in the population size of the wild relative; therefore the trigger for higher tier testing is any indication of susceptibility. Other triggers could be set depending on other definitions of unacceptable change in the assessment endpoint.

Tier II laboratory or field study

This study determines whether the pathogen reduces survival or reproduction of the wild relative. Plants should be inoculated with pathogen strains to which they are susceptible, and survival and reproduction compared with mock-inoculated controls. It may be obvious from the results of the Tier I study that survival and reproduction are reduced by the pathogen, however effects of some pathogens are subtle and may only be revealed by long-term observations. The trigger for a higher tier study is any statistically significant reduction in survival or reproduction, though other triggers could be set.

Tier III field study

This study determines whether the wild relative is infected with the pathogen in the field. Susceptibility to the pathogen in the laboratory does not demonstrate that the wild relative will be infected in the field (in the same way as sexual compatibility between two plant species in the laboratory does not mean that the species will form hybrids in the field). Estimates of the proportion of plants

infected in the field may show that the pathogen is absent, perhaps because vectors are absent. Pathogen infection should be measured by genetic or immunological methods because some pathogens can infect asymptomatically. In our scheme, a higher tier study would be triggered by the discovery of infected plants.

Tier IV demographic field studies

The purpose of this study is to determine whether density dependent factors are controlling population size; an increase in survival or seed production due to protection from a virus may not lead to an increase in population growth rate (Bergelson, 1994). There are several ways this could be tested. The pathogen could be excluded from field sites using pesticides and the population dynamics compared with equivalent plots under pathogen pressure; seeds could be sown into field sites to simulate additional seed production from pathogen resistant plants and the population dynamics compared with control plots; seedling densities in the field could be estimated and laboratory experiments could examine whether adult plant density is increased if seeds are sown at higher density than that in the field. In each case, the experiment is attempting to simulate additional survival or reproduction resulting from pathogen resistance and testing whether this results in changes in population dynamics.

In addition to this general scheme, hazards could also be assessed for particular events using experiments to test whether pathogen resistance genes function in the wild genetic background and whether there are costs to possessing the resistance gene in the absence of the pathogen. We do not refer to these tests here, but an example of an experiment to detect a cost of conventional major gene resistance to TuMV is described by Raybould et al. (2003).

RISK ASSESSMENT FOR INTROGRESSION OF TUMV RESISTANCE FROM OILSEED RAPE INTO WILD *BRASSICA* POPULATIONS IN THE UK

Assessment of exposure

Hybridization between oilseed rape and its wild relatives has been the subject of intensive research over the last 15 years (see Chèvre et al., 2004; Scheffler and Dale, 1994, for reviews). *B. nigra* and *B. napus* will hybridize when pollinated manually (Tier I), but no spontaneous hybrids have been recorded in laboratory or field experiments (Bing et al., 1996; Tier II), and no hybrids

have been recorded in the field (Tier III). Therefore, the likelihood of any hybridization between *B. nigra* and a virus resistant *B. napus* under field conditions is very low.

B. oleracea and *B. napus* will form hybrids when pollinated manually (Scheffler and Dale, 1994), and there is one unconfirmed record of spontaneous hybrid formation in a field experiment (Chèvre et al., 1998). Recently, hybrids have been found in a *B. oleracea* population in Dorset, UK (M.J. Wilkinson, personal communication), but at low frequency. Therefore, hybrids between transgenic oilseed rape and *B. oleracea* might occur sporadically if oilseed rape were grown close to populations of wild *B. oleracea*.

B. rapa and *B. napus* hybridise readily in the laboratory (Scheffler and Dale, 1994), and hybrids are found where *B. rapa* occurs as a weed of oilseed rape (Hansen et al., 2001) and in wild populations of *B. rapa* near to oilseed rape fields (Wilkinson et al., 2000). Using a combination of remote sensing, field surveys, molecular genetics and mathematical modeling, Wilkinson et al. (2003a) estimated that in *B. rapa* populations in the United Kingdom about 50,000 *B. napus* × *B. rapa* hybrids form annually.

Assessment of hazard

In this section we present the results of experiments designed to assess whether ecological release could occur if a TuMV resistance gene introgressed into a population of *B. nigra*, *B. oleracea* or *B. rapa*. The data are presented according to the tiered testing scheme described above. Although some data are unpublished, full details of the experimental methods and most results are presented elsewhere (Maskell et al., 1999; Pallett et al., 2002; Raybould et al., 1999a; 2000; 2003; Thurston et al., 2001). Therefore only data relevant to risk assessment are given here.

Brassica nigra

Tier I laboratory study of infectibility. A total of 40 seedlings from 4 *B. nigra* populations from Dorset on the south coast of England were grown in a glasshouse. At 4 weeks old, the seedlings were challenged with TuMV isolated from a plant of wild *B. oleracea* growing at Chapman's Pool, Dorset. Eighteen seedlings were inoculated mechanically by rubbing sap from TuMV-infected *B. juncea* into the leaves; 17 plants became infected. Twenty-two seedlings were exposed to aphids that had recently fed on the TuMV-infected *B. juncea*; 20 became infected. All infected seedlings developed systematic necrosis within

10 days and were dead 2 to 3 weeks after inoculation (Thurston et al., 2001). Eight-week-old plants of *B. nigra* were also challenged mechanically; necrosis took longer to develop, but 8 weeks after inoculation 15 out of 19 plants had died and the remaining four were stunted and had severe necrosis of the apical meristem (Thurston et al., 2001). The data show that at least some populations of *B. nigra* are highly susceptible and sensitive to local isolates of TuMV and hence ecological release is possible and higher tier studies are triggered.

Tier II study for reduction in survival and reproduction. Because of the obvious symptoms in the inoculated plants in the tier I study, further studies to measure whether TuMV reduces the reproduction of *B. nigra* were not necessary.

Tier III study of infection in the field. Adult *B. nigra* plants were collected in 3 consecutive years from 4 Dorset populations and tested for the presence of TuMV by ELISA. Of 597 plants tested, only 5 gave positive ELISA readings. However, these data are a 'snapshot' and it is not possible to be certain whether *B. nigra* is infected rarely, or infected frequently with subsequent rapid death. To distinguish between these possibilities, we tested for the presence of TuMV infection in natural seedling cohorts in two sites in Dorset. At each site, the number of infected plants in a 20 cm × 20 cm quadrat was estimated fortnightly for 16 months. TuMV was detected in 3 of 62 samples, and at a maximum frequency of 2% (54 of 2201 plants infected). These data suggest that TuMV rarely infects *B. nigra* in Dorset (Raybould et al., 2003).

Tier IV demographic field studies. The tier III infection study suggests that the hazard from any introgression of a TuMV resistance gene is low, and when combined with exposure data suggests that the risk of ecological release of TuMV-resistant *B. nigra* is negligible. No requirement for tier IV data is suggested by these results. However, demographic data from 2 sites were collected during the field infection study, and for the sake of completeness are reported briefly. In 1999–2000, there was a flush of germination in early September (Cohort 1), and seeds continued to germinate in late September (Cohort 2). All seeds that germinated after September were included in cohort 3. At one site (Chapman's Pool) Cohort 3 consisted of a few seeds that germinated in October; at the other site (Kimmeridge) a few seeds germinated in October followed by a huge flush of germination in January and February. At both sites, all flowering plants in 2000 were recruited from cohort 1 (Raybould et al., 2003).

In 2000–2001, the Chapman's Pool population followed a similar pattern to the previous year, although the number of seedlings was about half that in 1999. In February 2001 all surviving seedlings were from Cohort 2, and there was little or no germination in January. At Kimmeridge in autumn 2000 there were also fewer seedlings than the pervious year. By February 2001, all plants from Cohorts 1 and 2 were dead, probably because of the very wet autumn of 2000. Again, there was a strong flush of seedlings in January. We could not monitor the populations after 2001 because of movement restrictions imposed following an outbreak of foot and mouth disease. However, there were seed-producing plants at both sites in August 2001, and these were assumed to be from Cohort 2 at Chapman's Pool and from Cohort 3 at Kimmeridge (Raybould et al., 2003).

In summary, at both sites in 1999, Cohort 1 survived and subsequent cohorts were unable to establish. In 2000, Cohort 1 was destroyed by heavy rain, leaving room for recruitment from later cohorts. Taken together with the virus infection data from the field we can suggest that climatic and density dependent factors are far more important than viruses in controlling recruitment.

Brasssica oleracea

Tier I laboratory study of infectibility. Seven hundred and twenty seedlings were grown in a glasshouse and mechanically inoculated with a single isolate of TuMV when four weeks old. The seedlings comprised 60 half-sib families of 12 individuals, taken from three sites on the coast of Dorset, UK. TuMV was isolated was from a plant of *B. oleracea* at one of those sites. Four weeks after inoculation, the concentration of TuMV in each seedling was measured using quantitative ELISA. No seedlings died during the experiment although the highest concentration of virus in an inoculated seedling was 200,000 times that of the lowest concentration (Raybould et al., 2000). The data show that at least some genotypes of wild *B. oleracea* are infectible and sensitive to TuMV and therefore ecological release is possible.

Tier II for reduction in survival and reproduction. One hundred and eighty five seedlings of *B. oleracea* from five sites in Dorset were grown in a glasshouse until they had produced three to five fully expanded leaves and then inoculated with the same isolate of TuMV as used above. Two hundred and three plants were inoculated with water as a control. Four weeks after inoculation, plants were transplanted into a fallow area of a cereal field. Eighteen months after transplantation, 21.7% of control plants had died, whereas 34.1% of the TuMV inoculated plants had died ($P < 0.01$). Among the surviving plants, there were no significant differences in growth, but the

TuMV-inoculated plants produced significantly fewer seeds than the controls (1426 ± 438 TuMV; 3405 ± 604 control [seeds per plant ± SE]; $P < 0.001$) (Maskell et al., 1999). The data indicate that TuMV-resistant *B. oleracea* might have increased survival and reproduction, indicating the possibility of ecological release, and hence triggering higher tier studies.

Tier III study of infection in the field. We tested for the presence of TuMV at several times and in several populations of *B. oleracea* on the Dorset coast: 5 populations in 1995, 3 in 1998, and 4 in 1999. A total of 723 plant was tested, of which 158 (22%) were positive for TuMV by ELISA. There were statistically significant differences in the proportion of plants infected among sites within years, and among years within sites (Raybould et al., 1999a; 2003).

Tier IV demographic field studies. *B. oleracea* plants can live for over 20 years (Mitchell and Richards, 1979); therefore although TuMV was detected in the field, studies similar to those carried out on *B. nigra* were not attempted for *B. oleracea*.

Brassica rapa

Tier I laboratory study of infectibility. Seedlings from two populations of *B. rapa* from the Thames in Oxfordshire (Abingdon and Culham) were grown in a glasshouse and mechanically challenged with TuMV when 4 weeks old. Two TuMV isolates were used: one from *B. oleracea* growing at Chapman's Pool, Dorset, and the other from *B. oleracea* from Llandudno, North Wales. A total of 30 seedlings were challenged with the Llandudno isolate, and 18 were challenged with the Dorset isolate. All plants challenged with the Dorset isolate showed symptoms, and 9 weeks after challenge 10 plants had died. Twenty plants challenged with the Llandudno isolate were infected, as measured by ELISA, and all showed symptoms, including vein clearing and severe mottling. Nine weeks after challenge with the Llandudno isolate, the infected plants were severely stunted, but not dead (Pallett et al., 2002). The data show that at least some populations of *B. rapa* are highly susceptible and sensitive to local isolates of TuMV and hence ecological release is possible. Consequently, higher tier studies are triggered.

Tier II study for reduction in survival and reproduction. Because of the obvious symptoms associated with virus infection in the inoculated plants in the tier I study, further studies to measure whether TuMV reduces the reproduction of *B. rapa* were not necessary.

Tier III study of infection in the field. Between June 2000 and July 2001, 2644 plants of *B. rapa* were sampled

from Abingdon and Culham and tested for TuMV. No TuMV infections were found. As with *B. nigra*, a more detailed study to supplement this 'snapshot' would increase our certainty that TuMV does not infect *B. rapa* in the field. However given that 2644 plants of various ages were tested, infection with TuMV followed by rapid death is very unlikely and further studies, at least in these Oxfordshire populations, seem unwarranted.

Tier IV demographic field studies. Should it be discovered that the absence of TuMV from *B. rapa* in the field is due to infection and rapid death of a large number of plants, studies of the demography of wild *B. rapa* would determine whether protection from TuMV infection is likely to increase its population growth.

SUMMARY RISK ASSESSMENTS FOR WILD *BRASSICA* IN THE UK

B. nigra

Exposure to TuMV resistance genes will be very low to zero because hybridization between *B. nigra* and *B. napus* under field conditions is extremely unlikely. TuMV resistance poses minimal hazard because *B. nigra* is rarely infected with TuMV, and because climate and density dependent mortality appear to control the population dynamics of *B. nigra*. Therefore, the risk of ecological release of *B. nigra* due to the cultivation of TuMV resistant oilseed rape is negligible in southern England.

B. oleracea

Exposure to TuMV resistance genes will be low to zero; hybridization between *B. oleracea* and *B. napus* is possible under field conditions, but is likely to be rare and sporadic. Laboratory and field data indicate a potential hazard of TuMV resistance genes: wild *B. oleracea* is infected with TuMV in the field and experimental inoculation of *B. oleracea* results in lower survival and seed production compared with mock inoculated controls. It is unclear whether increases in survival and seed production will alter the population dynamics of wild *B. oleracea*.

Although TuMV inoculation at the seedling stage produced a clear reduction in survival and fecundity, we found no correlation between TuMV and fitness components in the field. Therefore, infection at seedling stage may have over-estimated the effect of TuMV (Raybould et al., 1999b). Also, Raybould et al. (2000) found high heritability for immunity to TuMV in Dorset populations of *B. oleracea*. These data suggest that selection for

resistance to TuMV is not strong, and that susceptible plants persist alongside immune plants through tolerance of TuMV as adults. Therefore, these data suggest that while ecological release of TuMV resistant *B. oleracea* is possible, the risks are low in southern England.

B. rapa

Exposure to TuMV resistance genes will be relatively high due to the sexual compatibility of *B. rapa* and *B. napus* and the co-occurrence of these species in many parts of the UK (Wilkinson et al., 2003a). Preliminary experiments suggest that the TuMV resistance poses low hazard in *B. rapa* populations. Although TuMV can infect *B. rapa* in the laboratory, no infected plants have been found in the field. Of course this could indicate that infected plants die extremely rapidly and so are not detected. However, this seems unlikely, as some experimentally inoculated *B. rapa* survive for at least 9 weeks. Detailed observations similar to those carried out on *B. nigra* would increase our certainty that the absence of TuMV from *B. rapa* populations is due to lack of infection rather than infection and rapid death. Observations by Wilkinson (pers. comm.) suggest that flooding controls the establishment and persistence of *B. rapa* populations on river banks. Therefore even if TuMV were shown to cause mortality in *B. rapa*, the effect of TuMV resistance genes of the population dynamics of wild *B. rapa* is likely to be minimal (weedy *B. rapa* might be different depending on its population dynamics). Thus, the risk of ecological release of *B. rapa* due to the cultivation of TuMV resistant oilseed rape, at least in southern England, is low, but not negligible.

DISCUSSION

Assessment of the environmental risks of agriculture is not new; for example, the potential impacts of pesticides on non-target arthropods have been the subject of standard, relatively uncontroversial, and apparently successful risk assessments for many years (Barrett et al., 1994; Candolfi et al., 2000; EPPO, 2003). However, risk assessments for the release of transgenic plants, which have similar objectives to those for pesticides, remain controversial. We discuss possible reasons for this in the light of our methods for assessing the risks of the introgression of TuMV resistance.

An environmental risk assessment is a way of organizing information to help decide whether a course of action will lead to unacceptable environmental harm. The risk assessment does not constitute the decision; rather it analyses the probability that harm will occur, the likely magnitude of the harm and the uncertainty associated with those predictions. Decisions are made by weighing the environmental predictions with other relevant information, such as the economic, legal, social and political implications of the proposed course of action.

The risk assessment is not necessarily a method for setting a research agenda (Hill and Sendashonga, 2003), because sufficient information may be available already to assess risk satisfactorily. The risk assessment does not seek to develop theory or generate new data unless the expense and delay is warranted by a high probability of improving the decision (selecting the right decision, or having greater certainty that the decision is correct). In fact, an effective risk assessment seeks to minimize the amount of data required to reach a sound judgment because collection of superfluous data often confuses decision-making and diverts effort from more worthwhile activities (Raybould, 2005). Indeed, if the collection of additional data delays the introduction of a beneficial product, overall environmental risk may be increased rather than reduced (Cross, 1996).

In this paper, we show that it is possible to design a set of simple experiments to assess the potential risks of introgression of TuMV resistance from oilseed rape into related wild species. We do not claim that our data provide a comprehensive risk assessment for TuMV resistant oilseed rape worldwide; for example tolerance or resistance to TuMV may vary locally because of genetic variation in the pathogen and its hosts. However, our work does show that tiered testing for the risk assessment of increased weediness (fitness) following the introgression of disease resistance genes is feasible. Assessments can be made using results from simple laboratory experiments and field observations and without comprehensive knowledge of ecosystem dynamics.

This view is not common. The more widespread opinion among ecologists is that research addressing possible harm from transgenic crops should do more than generate a minimum set of data for decision making. Instead, it should aim to increase "understanding" of ecological processes (Elvin et al., 2003; Hails and Morley, 2005). We disagree with this view because we believe it confuses rather than helps risk assessment.

Peters (1991) argues that ecology has failed to become a predictive science because practitioners tend not to study whole phenomena, but break them into components and analyse them mechanistically. Detailed data on the components are collected, but no theory is built to predict the phenomenon of interest; or if a theory is built, its complexity leads to predictions that are little better than random. A detailed critique of mechanistic analysis in

ecology and its philosophical basis can be found in Peters (1991). It is sufficient here to note that Peters associates mechanistic analysis with realism, the philosophy that science captures the reality of natural phenomena; this leads ecologists to seek exhaustive depictions of complexity to "understand" these phenomena. Peters argues that understanding is subjective, and that the only objective way to evaluate science is by the success of its predictions. Science should, therefore, develop methods to predict natural phenomena, rather than build detailed descriptions of them; this view is called instrumentalism. Like Peters' view of pure science, we believe that risk assessment should seek prediction, not understanding.

The consequences of searching for understanding, when combined with misinterpretation of risk, hazard and exposure, have been detrimental for risk assessment of transgenic crops: many data are produced without a clear idea of how they will be used, and risk assessment becomes stuck in a morass of data of unknown relevance. A good example is the many studies that were made of the breeding system and life cycle of oilseed rape in the 1990s, ostensibly to predict rates of gene flow. Many components of gene flow were measured: the distance pollen is dispersed; the mechanism of dispersal (insects or wind); pollen longevity in relation to temperature and humidity; intraspecific hybridization between male sterile oilseed rape and other crucifers; the relative growth rates of oilseed rape pollen and pollen of other species; seed longevity and dormancy; and population persistence of feral populations of oilseed rape. These studies were carried out to "assess the risks" of releasing GM oilseed rape in Europe. Presumably, the authors of these studies saw the studies' relevance to risk assessment as providing data to predict gene flow from GM oilseed rape to non-GM rape and to other species. However, no theory exists to link the observations to an estimate of gene flow (Raybould, 2004), and therefore the data are of unknown relevance to any risk assessment. This contrasts with the work of Wilkinson et al. (2003a), which had a clear theory to link observations to the endpoint of interest, the number of *B. napus* × *B. rapa* hybrids formed annually in the UK.

A related problem is that even if estimates of gene flow were made, they would only comprise an estimate of exposure. However, the careless presentation of an exposure estimate in the absence of other components necessary for risk assessment can create the impression that a serious risk has been demonstrated. Similarly, demonstration of a hazard in the absence of data on exposure should not be taken as a demonstration of risk. Furthermore, if relevance to risk assessment is claimed, it should be made clear what risk is being evaluated;

a definition of unwanted change to assessment endpoints should be a minimum requirement to give the results context. If this cannot be supplied, claims of relevance to risk assessment are spurious, however good the science, and serve only to increase confusion and unease about the risks of transgenic crops.

If "understanding" the ecology of TuMV in natural populations of *Brassica* species were necessary for risk assessment, there are endless studies we could carry out, because there is always something we could study in more detail, and we could claim all the studies were relevant. However, as the discussion above shows, this will end in confusion. Risk assessment cannot rely on an undefined feeling that we understand something; and will not be helped by data of unspecified relevance to predicting changes to assessment endpoints, or confusing hazard and exposure with risk. Rather risk assessment depends on a clear formulation of the problem: the management objectives, the assessment endpoints (operational definitions of the management objectives), a conceptual model to link changes in the assessment endpoints to estimates of exposure and hazard (test endpoints), a method for evaluating risk from the values of the test endpoints, and a value of risk on which a decision is made.

In this paper, we show that it is possible to define these terms for the risk assessment of introgression of a TuMV resistance gene from oilseed rape into its wild relatives:

- management objectives are set by law and include the protection of human health and the environment, including endangered species and their habitats;
- the assessment endpoint is the population size of a wild relative;
- the conceptual model is that the assessment endpoint will be affected if:
  o hybridization is possible between oilseed rape and the wild relative;
  o and the wild relative is not immune to TuMV;
  o and TuMV reduces survival or reproduction of the wild relative;
  o and TuMV infects the wild relative in the field;
  o and the population size wild relative is presently limited by density;
  o and the transgene confers resistance to TuMV to the wild relative;
  o and there is no cost of resistance in the wild relative.
- Test endpoints for exposure are:
  1. production of hybrids in the laboratory by any means;
  2. production of spontaneous hybrids in laboratory or field experiments;

3. presence of hybrids in the wild.
- Test endpoints for hazard are:
  1. infection with TuMV of the wild relative in the laboratory;
  2. lower survival and reproduction of the wild relative infected with TuMV;
  3. infection of the wild relative with TuMV in the wild;
  4. high population growth rates of the wild relative at high plant densities.

These definitions lead to a set of simple experiments, the results of which allow a decision to stop testing or carry out further studies. When considering hazard or exposure, a positive result in "experiment 1" triggers "experiment 2", and so on. A risk is identified if hybridization is detected and ecological release cannot be ruled out with confidence.

We offer this scheme as an illustration of how an apparently complicated problem, predicting the likelihood and effects of an increase in fitness of a wild plant, can be simplified by clear problem formulation. As discussed above, the conclusions may not hold worldwide. However we believe that they are reasonable for southern England at least, particularly as an increase in population size is a very conservative endpoint (we might only be concerned about an increase if it harms something of value) (Raybould and Wilkinson, 2005). Finally, we should point out that the risks the introgression of virus resistance genes apply equally to non-transgenic major gene resistance. If the risks of ecological release are judged acceptable for conventional resistance, there is no logical reason to require disproportionately onerous testing of transgenic resistance (Miller and Conko, 2005).

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REFERENCES

APHIS (Animal and Plant Health Inspection Service) (1987) 7 CFR Parts 330 and 340, Plant pests; introduction of genetically engineered organisms or products; final rule. *Fed. Register* **52**: 22892–22915

Barrett KL, Grandy N, Harrison EG, Hassan S, Oomen P (1994) Guidance Document on Regulatory Testing Procedures for Pesticides with Non-Target Arthropods. Society for Environmental Toxicology and Chemistry, Brussels

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG (1994) Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol.* **125**: 311–325

Bergelson J (1994) Changes in fecundity do not predict invasiveness – a model study of transgenic plants. *Ecology* **75**: 249–252

Bergelson J, Purrington CB, Wichmann G (1998) Promiscuity in transgenic plants. *Nature* **395**: 25

Bing DJ, Downey RK, Rakow G (1996) Hybridizations among *Brassica napus*, *B.rapa*, and *B. juncea* and their weedy relatives *B. nigra* and *Sinapis arvensis* under open pollination conditions in the field. *Plant Breed.* **115**: 470–473

Burban C, Fishpool LDC, Fauquet C, Fargette D, Thouvenel J-C (1992) Host associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae). *J. Appl. Ent.* **113**: 416–423

Butler D, Reichhardt A (1999) Long-term effect of GM crops serves up food for thought. *Nature* **398**: 654–656

Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection products with Non-Target Arthropods. Society for Environmental Toxicology and Chemistry, Pensacola, FL

Chèvre AM, Eber F, Baranger A, Boucherie A, Broucqsault LM, Bouchet Y, Renard M (1998) Risk assessment on crucifer species. *Acta Hort*. **459**: 219–224

Chèvre AM, Ammitzboll H, Breckling B, Dietz-Pfeilstetter A, Eber F, Fargue A, Gomez-Campo C, Jenczewski E, Jørgensen R, Lavigne C, Meier MS, den Nijs HCM, Pascher K, Séguin-Swartz G, Sweet J, Stewart Jr CN, Warwick S (2004) A review on interspecific gene flow from oilseed rape to wild relatives. In den Nijs HCM, Bartsch D, Sweet J, eds, Introgression from Genetically Modified plants into Wild Relatives. CAB International, Wallingford, UK, pp 235–251

Cooper JI, Jones AT (1983) Responses of plants to viruses: proposals for the use of terms. *Phytopathology* **73**: 127–128

Cooper JI, Raybould AF (1997) Transgenes for stress tolerance: consequences for weed evolution. *Proceedings of the Brighton Crop Protection Conference – Weeds*, pp 265–272

Cooper JI, Walsh JA (2003) Genetic modification of disease resistance, viral pathogens. In Thomas B, Murphy D, Murray B, eds, Encyclopedia of Applied Plant Sciences. Academic Press, London, UK, pp 257–262

Cross FB (1996) Paradoxical perils of the precautionary principle. *Wash. & L. Law Rev.* **53**: 851–925

Dinant S, Blaise F, Kusiak C, Astier-Manifacier S, Albouy J (1993) Heterologous resistance to potato virus Y in transgenic

tobacco plants expressing the coat protein of lettuce mosaic potyvirus. *Phytopathology* **83**: 818–824

Dinant S, Maisonneuve B, Albouy J, Chupeau Y, Chupeau M-C, Bellec Y, Gaudefroy F, Kusiak C, Souche, S, Robaglia C, Lot H (1997) Coat protein gene-mediated protection in *Lactuca sativa* against lettuce mosaic potyvirus strains. *Mol. Breeding* **3**: 75–86

Dutton A, Romeis J, Bigler F (2003) Assessing the risks of insect resistant plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *BioControl* **48**: 611–636

Ellstrand NC (2001) When transgenes wander, should we worry? *Plant Physiol.* **125**: 1543–1545

Ellstrand NC, Prentice HC, Hancock JF (1999) Gene flow and introgression from domesticated plants into their wild relatives. *Annu. Rev. Ecol. Syst.* **30**: 539–563

Elvin DE, Welsh R, Batie SS, Capentier CL (2003) Towards an ecological systems approach in public research for environmental regulation of transgenic crops. *Agric. Ecosyst. Environ.* **99**: 1–14

EPPO (2003) Environmental risk assessment scheme for plant protection products. *EPPO Bull.* **33**: 103–111

Gray AJ (2004) Ecology and government policies: the GM crop debate. *J. Appl. Ecol.* **41**: 1–10

Hails RS, Morley K (2005) Genes invading new populations: a risk assessment perspective. *Trends Ecol. Evol.* **20**: 245–252

Hansen LB, Siegismund HR, Jørgensen RB (2001) Introgression between oilseed rape *Brassica napus* L. and its weedy relative *B. rapa* in a natural population. *Genet. Resour. Crop Evol.* **48**: 621–627

Heritage J (2003) Will GM rapeseed cut the mustard? *Science* **302**: 401–403

Hill RA, Sendashonga C (2003) General principles for risk assessment of living modified organisms: lessons from chemical assessment. *Environ. Biosafety Res.* **2**: 81–88

Jan FJ, Fagoaga C, Pang SZ, Gonsalves D (2000) A single transgene derived from two distinct viruses confers multi-virus resistance in transgenic plants through homology-dependent gene silencing. *J. Gen. Virol.* **81**: 2103–2109

Lehmann P, Walsh JA, Jenner CE, Kozubek E, Greenland A (1996) Genetically engineered protection against turnip mosaic virus infection in transgenic oilseed rape (*Brassica napus* var. *oleifera*). *J. Appl. Gen.* **37A**: 118–121

Maskell LC, Raybould AF, Cooper JI, Edwards M-L, Gray AJ (1999) Effects of turnip mosaic virus and turnip yellow mosaic virus on the survival, growth and reproduction of wild cabbage (*Brassica oleracea*). *Ann. Appl. Biol.* **135**: 401–407

Miller HI, Conko G (2005) NGO war on biotechnology. *J. Commer. Biotech.* **11**: 209–222

Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral pathogens. *Nature* **421**: 625–627

Mitchell ND, Richards AJ (1979) Biological Flora of the British Isles No 145, *Brassica oleracea* ssp. *oleracea*. *J. Ecol.* **67**: 1087–1096

Naranjo SE, Head G, Dively GP (2005) Field studies assessing arthropod nontarget effects in *Bt* transgenic crops: introduction. *Environ. Ent.* **34**: 1178–1180

Newman NC (1998) Fundamentals of Ecotoxicology. Ann Arbor Press, Chelsea, MI

Pallett DW, Thurston MI, Cortina-Borja M, Edwards M-L, Alexander M, Mitchell E, Raybould AF, Cooper JI (2002) The incidence of viruses in wild *Brassica rapa* ssp. *sylvestris* in Southern England. *Ann. Appl. Biol.* **141**: 163–170

Peters RH (1991) A Critique for Ecology. Cambridge University Press

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agric. Ecosyst. Environ.* **84**: 1–20

Poppy G (2000) GM crops: environmental risks and non-target effects. *Trends Plant Sci.* **5**: 4–6

Preston CD, Pearman DA, Dines TD (2002) New Atlas of the British & Irish Flora. Oxford University Press

Raybould AF (2004) A decade of gene flow research: improved risk assessments or missed opportunities? *Aspects Appl. Biol.* **74**: 27–33

Raybould AF (2005) Assessing the environmental risks of transgenic volunteer weeds. In Gressel J, ed., Crop Ferality and Volunteerism. CRC Press, Boca Raton, FL, pp 389–401

Raybould AF, Gray AJ (1993) Genetically modified crops and hybridization with wild relatives: a UK perspective. *J. Appl. Ecol.* **30**: 199–219

Raybould AF, Gray AJ (1994) Will hybrids of genetically modified crops invade natural communities? *Trends Ecol. Evol.* **9**: 85–89

Raybould AF, Wilkinson MJ (2005) Assessing the environmental risks of gene flow from genetically modified crops to wild relatives. In Poppy GM, Wilkinson MJ, eds, Gene Flow from GM Plants. Oxford: Blackwell Publishing, pp 169–185

Raybould AF, Maskell LC, Edwards M-L, Cooper JI, Gray AJ (1999a) The prevalence and spatial distribution of viruses in natural populations of *Brassica oleracea*. *New Phytol.* **141**: 265–275

Raybould AF, Moyes CL, Maskell LC, Mogg RJ, Warman EA, Wardlaw JC, Elmes GW, Edwards M-L, Cooper JI, Clarke RT, Gray AJ (1999b) Predicting the ecological impacts of transgenes for insect and virus resistance in natural and feral populations of *Brassica* species. In Ammann K, Jacot Y, Simonsen V, Kjellsson G, eds, Methods of Risk Assessment of Transgenic Plants III. Ecological risks and prospects of transgenic plants. Birkhäuser Verlag, Basel, pp 3–15

Raybould AF, Edwards M-L, Clarke RT, Pallett D, Cooper JI (2000) Heritable variation for the control of turnip mosaic virus and cauliflower mosaic virus replication in wild cabbage. Beiträge zur Züchtungsforschung – Bundesanstalt

für Züchtungsforschung an Kulturpflanzen. Proceedings of the 7th Aschersleben Symposium – New Aspects of Resistance Research on Cultivated Plants, pp 4–8

Raybould AF, Alexander MJ, Mitchell E, **Thurston MI, Pallett DW, Hunter P, Walsh JA, Edwards M-L, Jones AME, Moyes CL, Gray AJ, Cooper JI** (2003) The ecology of turnip mosaic virus in populations of wild *Brassica* species. In Beringer J, Godfray CHJ, Hails RA, eds, Ecological Dynamics & Genes, final symposium volume. Blackwell Scientific Press, Oxford, UK, pp 226–244

Rich TCG (1991) Crucifers of Great Britain and Ireland. London: Botanical Society of the British Isles

Scheffler JA, Dale PJ (1994) Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. *Transgenic Res.* **3**: 263–278

Shattuck VI (1992) The biology, epidemiology and control of turnip mosaic virus. *Offprints from Plant Breeding Reviews* **14**: 199–238

Thurston MI, Pallett DW, Cortina-Borja M, Edwards M-L, **Raybould AF, Cooper JI** (2001) The incidence of viruses in wild *Brassica nigra* in Dorset (UK). *Ann. Appl. Biol.* **139**: 277–284

U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and the peculiar mode of fertilization. *Shokub utsu Kekyu Zasshi.* **7**: 389–452

US EPA (2001) Biopesticides Registration Action Document – *Bacillus thuringiensis* Plant Incorporated Protectants. http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brald.htm

Wilkinson MJ, Davenport IJ, Charters YM, Jones AE, Allainguillaume J, Butler HT, Mason DC, Raybould AF (2000) A direct regional scale estimate of transgene movement from GM oilseed rape to its wild progenitors. *Mol. Ecol.* **9**: 983–991

Wilkinson MJ, Elliot LJ, Allainguillaume J, Shaw MW, Norris C, Welters R, Alexander M, Sweet J, Mason DC (2003a) Hybridization between *Brassica napus* and *B. rapa* on a national scale in the United Kingdom. *Science* **302**: 457–459

Wilkinson MJ, Sweet J, Poppy G (2003b) Risk assessment of GM plants: avoiding gridlock? *Trends Plant Sci.* **8**: 208–212

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