

The release of genetically modified crops into the environment

Part II. Overview of ecological risk assessment

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Summary

Despite numerous future promises, there is a multitude of concerns about the impact of GM crops on the environment. Key issues in the environmental assessment of GM crops are putative invasiveness, vertical or horizontal gene flow, other ecological impacts, effects on biodiversity and the impact of presence of GM material in other products. These are all highly interdisciplinary and complex issues. A crucial component for a proper assessment is defining the appropriate baseline for comparison and decision. For GM crops, the best and most appropriately defined reference point is the impact of plants developed by traditional breeding. The latter is an integral and accepted part of agriculture. In many instances, the putative impacts identified for GM crops are very similar to the impacts of new cultivars derived from traditional breeding. When assessing GM crops relative to existing cultivars, the increased knowledge base underpinning the development of GM crops will provide greater confidence in the assurances plant science can give on the risks of releasing such crops.

Keywords: agricultural biotechnology, biodiversity, ecological impact, gene transfer, plant breeding.

'None of the emperor's clothes had ever been such a success. "But he doesn't have anything on" said a little child' (Hans Christian Andersen, 1835).

Introduction

Throughout the history of plant breeding, 'new technologies' have regularly been utilised to develop new gene combinations for improving crop cultivars (Simmonds *et al.*, 1999). These included: the artificial manipulation of chromosome number; the development of addition and substitution lines for specific chromosomes; chemical and radiation treatments to induce mutations and chromosome rearrangements; as well as cell and tissue culture approaches such as embryo rescue, *in vitro* fertilisation and protoplast fusion to allow the recovery of inter-specific and -generic hybrids. The genetic gains from the

integration of these technologies into mainstream plant breeding have substantially improved the performance of the resulting cultivars. They continue to make a major contribution to genetic improvements in yield, environmental adaptation, resistance to specific diseases and pests, and specific quality attributes that are constantly demanded by farmers, the food industry and consumers.

Scientific advances in cell and molecular biology have now culminated in the genetic engineering or modification of crops. The resulting novel germplasm is anticipated to

allow plant breeders to respond more quickly to increasing consumer demands.

Despite the potential benefits of this new technology to improve the reliability and quality of the world food supply, public and scientific concerns have been raised about the environmental and food safety of GM crops. It is feared that the technology will harm people by undesired impacts on environment, health and/or the economic order at the expense of the poor. The public concern is becoming increasingly more vocal and sometimes violent. In Europe (and New Zealand), consumer acceptance of commercial gene technology products seems further away than ever. Consumers in the US are awakening to the controversy (McHughen, 2000; SDCMA, 2000) and regulators follow (CAST, 2001; NAS, 2002). The coming years may, therefore, prove decisive for the commercial and economically viable application of GM crops in agriculture and food production. Without the consent of society at large, GM crops will fail in the marketplace. At the same time, the United Nations (UN) and other international organisations announce that the world is facing such serious problems with global food and nutrition security that it cannot afford to turn away from GM crops (Schrope, 2001). Similar assessments can be found at various other places (Chrispeels, 2000; Leisinger *et al.*, 2002; NABC, 1999; NAS, 2000). In such assessments, GM crops are not presented as the only solution, but as a possible contribution to an array of necessary measurements and incentives to a problem growing in urgency.

Current discussions in western countries mainly focus on GM food and feed as well as on consumer safety. This topic is reviewed in depth elsewhere (Kuiper *et al.*, 2001). To some extent, this has diverted public attention from ecological and environmental concerns about the impact of growing and processing GM crops. The topic, however, is likely to be a dormant volcano, ready to erupt the moment the food issues are agreed upon, if ever, to the sufficient satisfaction of most stakeholders. The temporary calm may be a good moment to reflect on the rational debate on the environmental impact of GM crops and focus on the science used to assess that impact.

The problem is partly that the relevant questions have been repeatedly asked for over 15 years, and keep being asked, despite the fact that all supposedly relevant research has been performed. The answers given are apparently not satisfactory. This may indicate that many of the concerns raised about GM crops reflect more the concerns about the changing nature of agriculture at large (Beringer, 2000), which draws on values and philosophical positions that are not readily changed upon the presentation of technical information. We acknowledge the prime importance of socio-economic and other issues for a proper technology assessment (Bruce and Bruce, 1998; EFB, 1999; NCB, 1999) and realise that prudent and transparent linking of science and politics may be the biggest challenge for the overall

evaluation of GM crops (Levidow and Carr 2000). Despite this, we limit the scope of this review to the issues of plant science we consider to be most relevant.

In addition to the accompanying paper (Nap *et al.*, 2003) on the current status and regulation of environmental release of GM crops around the globe, we will here give a description of GM crop risk assessments and the way these are performed. This is followed by a thorough discussion of the perceived risks associated with the release of GM crops. The baseline taken in this review is the impact of non-GM crops and the effects of agriculture in general.

The science of risk and risk assessment

'Risk' means many different things to many people. It depends on their social, cultural and economic backgrounds and values (Kaplan and Garrick, 1981). Risk also means many different things to one person. It depends on the issue and the particular situation (Slovic, 1987; 1993). For most people, risk is most easily understood in terms of economy or insurance: the chance of (financial) loss or physical injury. A common description of risk is 'probability of harm'. When taking the magnitude of the potential harm into account, risk is expressed in a mathematical form as:

$$\begin{aligned} \text{Risk} &= \text{probability} \times \text{consequence} \\ &= \text{likelihood of event} \times (\text{negative}) \text{ impact of event.} \end{aligned}$$

The negative or undesired impact of an event is commonly referred to as 'hazard' (Wachbroit, 1991). Risk can be expressed as rate or probability. The probabilistic interpretation of risk implies that managing probability or consequence or both can influence risk. This way risk is a two-dimensional definition that should be considered a simplified interpretation of what is, in reality, a multidimensional concept (Kaplan and Garrick, 1981). It is also apparent that whoever controls the definition of risk, also controls the rational solution (Slovic, 1999). This interpretation of risk requires understanding and mastering of the concepts of harm and consequence in terms of statistics and (subjective) probabilities (Wachbroit, 1991). These are difficult topics to master. The difficulty with which the general public and scientists deal with numbers and chance (Purchase and Slovic, 1999) has prompted the term 'innumeracy' as equivalent to 'illiteracy' (Paulos, 1988). Risk assessment tries to find answers on the following three questions for each individual case:

Question 1: what can go wrong? (=the possibility of harm),
Question 2: how likely is that to happen? (=the probability of that harm occurring), and

Question 3: what are the consequences if it happens? (=the consequence of that harm).

The concept of risk is often implicitly taken as the possibility of harm, rather than the probability of harm. This

makes a small but essential difference: the 'possibility of harm' is only part of the relevant questions to be answered. This difference is often neglected, especially in relation to the assessment of the environmental impact of GM crops. It is wrong to consider that any effect automatically results in an undesired, negative impact. If the probability of a given harm is not zero, for worst-case scenarios the probability of occurrence could be taken as 1 and the focus should be on the potential detrimental consequences of the event.

Obviously, the decision whether a certain risk is acceptable and/or tolerable under a particular set of conditions is not part of the risk analysis itself. It is a choice that is based on political, social, cultural and economic considerations. This choice is often based more on the perceived outcome of a risk analysis than on the probabilistic calculation of a risk. Unfortunately, risk calculation and risk perception are not very (cor)related. What experts measure is generally not what most people perceive as risk. It is now generally accepted, for example, that the perception of risk of a given issue differs greatly between experts on that issue and non-experts (Bostrom, 1997). Relevant risk factors are weighed differently and often subconsciously. In the face of uncertainty, the road to decision and choice is generally paved with irrationality, inconsistency and incompetence (Bernstein, 1998). The GM crop discussion also shows that within groups of experts, risk perceptions differ dramatically (Wachbroit, 1991), showing that differences in individual contexts, motives and values are as important as knowledge and expertise. This further affects the public's trust (Slovic, 1999). Moreover, the very nature of the concept 'newsworthiness' in the public press gives a strong bias to risks and hazards over benefits. All this adds to the complexity of risk communication.

The issues of risk of GM crops deal with the ecology and toxicology of GM crops upon release and use. It is a continued discussion whether more broad 'risks' should be part of the basic biosafety assessment (Commandeur *et al.*, 1996; Sagar *et al.*, 2000). Countries and stakeholders still disagree considerably about the extent to which issues such as sustainability, globalisation, ethics and socio-economics should be part of a GM crop risk assessment. It may be worthwhile to reconsider if any issue is truly an intrinsic aspect of the GM crop technology (technology inherent; CAST, 1999; Leisinger, 1996) that is being considered, or only the implied consequences of developments that could also take place without GM crops (technology transcending; CAST, 1999; Leisinger, 1996). Sometimes, the demand for involvement of higher order socio-economic dimensions appears to be part of a strategy to ensure a pre-determined conclusion rather than an attempt to contribute towards informed, responsible and consensus decisions about the risk and safety of GM crops.

Concepts for ecological risk assessment: familiarity and the precautionary principle

We will here concentrate on the ecological and environmental issues of the release and use of GM crops. The ecological probabilities of harm focus on weediness, spread of the transgene by either vertical or horizontal gene flow, and the potential for any unintended, or pleiotropic, effects. These issues will be addressed in more detail below. Two general concepts have been proposed to guide the ecological risk assessment in regulatory and associated procedures: the concept of familiarity and, more recently, the precautionary principle. The latter is part of the Cartagena Protocol on Biosafety (SCBD, 2000) and is now the basis of regulation in the EU. The concept of familiarity considers whether the GM phenotype is novel for the ecosystem under study (NRC, 1989; OECD, 1993a,b). In general, the concept of familiarity seems too loosely defined to be very useful for risk assessments (Levidow *et al.*, 1996; Regal, 1999; Torgersen, 1996). Similar problems with respect to its full meaning and implications are also true for the precautionary principle. This principle was first introduced in the preamble of the Rio Declaration of the Convention of Biological Diversity (CBD) as "*where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimise such a threat*" (CBD, 1992). It has seen many different and much more generalised forms since (Goklany, 2000). In the recently adopted Cartagena Protocol on Biosafety (SCBD, 2000), it reads:

"Lack of scientific certainty due to insufficient scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity... , taking also into account risks to human health, shall not prevent... from taking a decision, as appropriate, with regard to the import of the living modified organism in question... , in order to avoid or minimize such potential adverse effect."

There is considerable controversy on the meaning, scope and application of this principle (Ammann, 2000; Goklany, 2000; Wiener and Rogers, 2002). One of the more extreme interpretations of this difficult concept can be that it implies: 'in case of doubt, do not or do nothing'. This interpretation implicitly reflects a demand for a risk-free world. In such an interpretation, the principle seems not a very suitable or decisive principle to base decisions and regulation on. The main argument against this interpretation is that 'doing nothing' is a decision too, with its own premises and consequences.

We should take care in defining existing circumstances in agricultural practice as 'natural' and 'desirable' and

new circumstances such as GM crops as 'unnatural' and 'undesirable' (Editorial, 1999). An associated problem is to define the science required for such comparisons (Levidow and Carr, 2000). In case of GM crops it may be worthwhile to have the precautionary principle work both ways and require its application to the overall situation of potential costs and potential benefits (De Kathen, 1998; Goklany, 2000). In addition to applying the precautionary principle to a GM crop, it should/could also be applied to each alternative that is proposed as an alternative solution, including currently used technology. The precautionary principle, therefore, becomes an approach to have the risk of the new technology assessed appropriately. In this context, during risk analysis of a given GM crop, the fourth issue to consider in addition to the probability of harm discussed above would then become:

Question 4: what are the consequences if we do NOT allow this GM crop?

The consequence of non-use, tentatively defined as 'the probability of harm of non-use × the impact of harm of non-use', may make cost/benefit analyses more transparent in assessments and discussions.

Botanical files in the ecological risk assessment of GM crops

To assess the potential ecological impact of field or commercial releases of GM crops in a given region, the likelihood and impact of vertical gene flow for that crop in that region should be taken into consideration. To guide this assessment, the concept of gene flow indices or botanical files (Ammann *et al.*, 2001; Frietema de Vries *et al.*, 1992, 1994; Frietema de Vries, 1996) was developed. Gene flow indices or botanical files give an indication of the likelihood of a given species to hybridise with wild relatives and the impact this may have. Obviously, such botanical files should be established for each region considered. Vice versa, such files are also only applicable for the region considered. Botanical files consist of data on a particular plant and provide an index of the likelihood for:

Factor 1: dispersal of pollen, Dp,

Factor 2: dispersal of reproductive plant parts, such as seeds or fruit, collectively called diaspores, Dd, and

Factor 3: the distribution frequency of wild relatives, Df.

Each code is subdivided in seven levels of potential (or unknown) risk that are given a numeric code. These numerical values range from '0' (no chance of any effect), '1–5' (a scale from low to high probability of effect), and 'N' (unknown or uncertain, meaning that further research is needed). The three-digit code combines to form the so-called Dpdf score, which classifies the likelihood and scope of gene flow from a certain crop in a given region. If any one

of the Dp, Dd or Df values is '0', no ecological effects are to be expected from the cultivated plants. Based on the triple Dpdf score, five categories of risk probability have been defined, which can be and are used to set safety standards on field trials and confinement requirements. These five categories are: (i) no gene flow effect; (ii) minimal gene flow effects; (iii) low and local gene flow effects; (iv) substantial but local gene flow effects; and (v) substantial and wide-spread gene flow effects.

Botanical files indicate the likelihood of gene flow from a particular GM crop plant to its wild flora, but ignore the potential impact of the transgene on crop and recipient wild relative. Therefore, botanical files have to be combined with knowledge about the transgenes used for transformation and the particular transformation event (Ammann *et al.*, 2001). This will allow the evaluation of issues such as the contribution of the transgene to the weediness and fitness of the host plant. For each transgene, a 'transgene file' with all relevant information should be put together in an approach previously coined as the 'transgene-centred approach to biosafety' (Metz and Nap, 1997; Metz *et al.*, 1998). It may be worthwhile to consider extension of the code with a fourth parameter, called:

Factor 4: description of gene, Dg,

that is assessing the ecological impact of a gene on a given crop (Ammann *et al.*, 2001). As particular attention goes to the use of GM crops for human consumption, a next addition could be the 'food files', in which all nutritional and food safety aspects of a given plant product/consumer combination are evaluated and classified in a fifth parameter, called:

Factor 5: description of nutrition, Dn.

A five-digit 'D' code would then summarise all safety considerations for the growth and consumption of a particular product from a transgenic crop grown in a given region. Such a code, or derivative of it, linked to books or websites where all codes are defined, may give all desired consumer information in a way of labelling that is equivalent to current labelling of the presence of food additives. A recent OECD designation of an identifier for transgenic plants (OECD, 2002) aims to define a unique key for each product. The main aim of this identifier is for use within the databases of the OECD, rather than for more information towards consumers.

Assessment of perceived environmental and agricultural risks

In this part, we will outline the various concerns regarding environmental release of GM crops. Issues covered are the putative occurrence of, and the consequent impact on, the invasion of ecosystems, out-crossing, horizontal gene transfer, secondary ecological impacts, superpests, biodiversity and other agricultural practices.

Will transgenic crops invade agricultural and natural ecosystems?

There are concerns that the release of GM crops will result in such plants becoming agricultural weeds and, therefore, add to the already large agricultural weed management burden of farmers. It is also feared that such plants may invade natural habitats and, as a consequence, compromise their biodiversity values. Considering whether GM crops can gain the attributes of weedy species should assess these issues. This presents the first problem to be resolved; what is a weed and what are weedy attributes. These terms often have different meaning to different groups, depending on whether the perspective is associated with intensive agricultural production or ecological monitoring of (semi-)natural plant communities. This is further complicated because what is considered a weed may differ between regions. While some plant species may be recognised as serious weeds in one environment (a specific habitat, climatic zone, or geographic area) they may be entirely absent, a casual curiosity, a species of little ecological or economic significance, a delightful 'wild-flower', or an important component of biodiversity in another environment. For example, purple loosestrife (*Lythrum salicaria*) is well loved as a wildflower in the UK, but is now impacting on large areas of wetland habitats in the US (Thompson *et al.*, 1987).

In this context ecological definitions seem most appropriate. Weeds generally exhibit a preference for habitats disturbed by human activities, such as cultivated fields, field margins, gardens, roadsides, soil dumps, and waste sites recently cleared of vegetation (Harlan and de Wet, 1965). One feature that all weeds appear to share is a high phenotypic plasticity that allows continuous adaptation to changing environments. Although weeds are well adapted to disturbed sites, they often succeed due to varying life history strategies. Sometimes, closely related species can be highly successful weeds by possessing different combinations of weedy characteristics (Williamson, 1993). Consequently, lists of typical weedy characteristics (Baker, 1965; 1974; Hill, 1977) do not necessarily apply to all weed species, but instead represent a list of consensus traits. However, it has been emphasised that the presence of such characteristics cannot be considered a measure for predicting weediness (Williamson, 1994). For example, the weediest of 49 annual British weeds possessed only half of these characters (Perrins *et al.*, 1992; Williamson, 1993). Whether a plant species becomes a serious weed in a new environment may relate more to its ability to grow well in the new environment, coupled with the absence of effective enemies such as herbivores and diseases (Williamson, 1994). The relevance of assessing weedy characteristics when considering the invasiveness of GM crops has been the subject of much debate (Fitter *et al.*, 1990; Keeler, 1989;

1990). While it is important to be highly cautious when predicting weediness based on plant attributes for the deliberate introduction of new species into a new environment, an assessment of such characteristics can be made with much greater confidence when assessing whether well-established crops can invade agricultural or natural ecosystems. When modern crop cultivars no longer possess weedy characteristics that represent important life history strategies for wild races of the specific crop, their ability to become weeds has been severely retarded in the absence of gene introgression from the wild races.

Common distinctive attributes of weeds such as seed dormancy, phenotypic plasticity, indeterminate growth, continuous flowering and seed production, and seed dispersal (Baker, 1965; 1974), have been bred out of the most important crop plants over thousands of generations. Such changes appeared early in the domestication of crop plants and arose as a consequence of repeated sowing and harvesting cycles of plants by early civilisations without any conscious selection for change (Harlan, 1992). These characters are not candidates for gene transfer back into crops, whether by genetic modification or traditional breeding, because they would severely reduce the agronomic performance of a crop for modern farming practices. Furthermore, these attributes do not arise from the single or few gene transfers of genetic modification. Therefore, genetically modified crops are no more likely to become weeds outside farming situations than crop cultivars have in the past.

There has been a close association between the evolution of weeds and the domestication of crops (Harlan, 1992). Indeed some weeds have evolved important characteristics associated with their life history strategies through mimicry of crop characteristics such as growth form and maturity dates (Barrett, 1983). These are aspects that have no doubt been facilitated by the unconscious human selection for weed populations along with the repeated sowing and harvest cycles of crops. Weeds continue to evolve in man-made habitats via a range of strategies (de Wet and Harlan, 1975). They may evolve from coloniser species by selection for adaptation to habitats that are continually being disturbed, from the abandonment of domesticated races or escape of crops from cultivation followed by selection away from cultivated attributes, from derivatives of hybrids between wild and cultivated races of crop species, and from gene introgression between the wild and cultivated races. Only the second mechanism seems relevant to the question whether transgenic crops can invade agricultural or natural ecosystems. The latter two are important for assessing the possibility of out-crossing combined with increased weediness. This is discussed in the next section. Wild races of most crop plants are well known to exist, not only at the centre of origins for specific crops, but occasionally as naturalised plants in the flora of regions

throughout the world. The existence of such wild races of crops raises the question of how easily modern crop cultivars can revert to a weedy condition.

The ability of cultivated plants to escape is recognised to decline with a longer history associated with their domestication (Zeven and de Wet, 1982). Most crops have been dramatically altered in general phenotype following their long history of domestication. Modern cultivars are highly unlikely to revert to weedy derivatives, with or without further genetic modification. The rare occurrence of spontaneous 'weedy types' within crop fields is usually associated with chance hybridisation with wild races during seed production (Wijnheijmer *et al.*, 1989). Some crops, such as forage grasses and legumes, have a much shorter history of domestication and are more likely to revert to a weedy condition. Similarly, oilseed rape is recognised as being domesticated relatively recently (McNaughton, 1995) and is often associated with a potential to escape from cultivation and revert to a weedy condition. This is a consequence of high seed production, the frequent appearance of volunteers, especially along roadsides near crop production fields, the occurrence of wild races, and induced seed dormancy associated with seed burial. Oilseed (rape) has been the subject of the majority of investigations on the potential invasiveness of transgenic crops. It represents a useful 'model system'; it is a potential worst-case scenario with an apparent risk associated with numerous large-scale GM crops on which to base experiments.

Virtually all crops have an inherent capacity to appear as volunteers within fields following the loss of seed during harvest or the incomplete destruction of vegetative propagules following subsequent cultivation (e.g. Lutman, 1993). However, the extent to which this occurs is highly dependent upon crop management and local environmental conditions (e.g. Lumkes and Beukema, 1973). Despite the potential for crop volunteers, crop plants are rarely seen as weeds, and when they are it is usually on disturbed soils within or alongside farmers' fields (de Wet and Harlan, 1975). In such environments they usually survive for only one season. The observed persistence of such populations is usually the result of a continuing influx of new volunteers, rather than the ongoing self-maintenance of the population.

In order to assess the potential weediness of a transgenic crop the key issue is invasiveness. An appropriate measure of invasion risk for a plant is its finite rate of increase (λ) (Crawley, 1986), the multiplicative constant at which a population increases over time, assuming a stable age distribution and the absence of density-dependent constraints. In crops with discrete non-overlapping generations, such as annual vegetable and arable crops, λ in its simplest form is defined as:

$$\lambda = S_{t+1}/S_t$$

where S_t is the number of seeds in generation t and S_{t+1} the number of seeds in the subsequent generation. In this way, λ represents the number of seeds produced in one generation for every seed in the previous generation. For $\lambda > 1$, crop volunteers will increase in abundance or invade under the given set of environmental conditions; whereas for $\lambda < 1$, the crop will decline to extinction (Crawley, 1986). This risk assessment tool encapsulates all the demographic processes regulating population growth, such as fecundity, seed survival, seed germination, and seedling survival to maturity (Parker and Kareiva, 1996). Inherent in λ are all demographic processes that may not be individually recognised as being important for the survival and maintenance of plant populations. It is, therefore, far more valuable than estimates of a single demographic process (i.e. performance at any particular life history stage) which provide no information about invasiveness when used in isolation. Furthermore, the transfer of a gene conferring a particular character, whether by genetic modification or traditional breeding, may have a positive influence on one component of the overall demographic processes under some environmental conditions and a negative influence under other conditions. For example, GM seeds of oilseed rape with modified oil content (high-stearate) can have enhanced longevity in soil (Linder, 1998), but the high-stearate gene also conferred reduced vigour on seedlings and presumably also reduced fecundity (Linder and Schmitt, 1995). The latter may well cancel out any advantage resulting from enhanced seed longevity. Changes in the rate of a single demographic process cannot be taken alone as a measure of plant invasion, a methodological concern often overlooked when enhanced invasiveness of GM crops is considered.

The demographic parameters of GM oilseed rape with resistance to the herbicide glufosinate and conventional oilseed rape were estimated over a 3-year period in twelve natural habitats involving a range of climatic conditions (Crawley *et al.*, 1993). No evidence was obtained to indicate that oilseed rape is invasive of undisturbed natural habitats. Furthermore, there was no evidence that the GM lines were more invasive of, or more persistent in, disturbed habitats. When there were significant differences between the genetic lines, the GM lines tended to be less invasive and less persistent than their non-GM counterparts. This study clearly established the relative invasiveness of non-GM and a given glufosinate-resistant GM oilseed rape in the absence of selection pressure from glufosinate in the environment. The scientific merits of this study were widely debated at the time, with emphasis on the nature of the experimental design and the validity and generality of the conclusions (see Metz and Nap, 1997, for discussion). The issues raised can be equally applied to other ecological data relating to the performance of transgenic crops, as well as to ecological experimentation in general. In many respects

the points of discussion largely reflected the maturing of ecological experimentation with an ongoing refinement of methodology required to address and resolve more definitive questions associated with ecological parameters relevant to risk assessment of crops. A more recent comprehensive study compared the results from monitoring conventional and GM lines of four different crops in field experiments established in twelve habitats and over Ten years (Crawley *et al.*, 2001). The GM lines included oilseed rape and maize exhibiting resistance to the herbicide glufosinate, sugar beet exhibiting resistance to the herbicide glyphosate, and potato containing insecticidal Cry proteins or pea lectin. In none of these cases, the GM plants were found to be more invasive or more persistent than their conventional counterparts.

When assessing the invasiveness potential of GM crops the key issue to address is whether their weedy characteristics are likely to be different when the expression of the transgene is taken into account. In this context, the transgene-centred approach to biosafety is important (Metz and Nap, 1997), and the concept of Dg parameters suggested as part of the 'botanical files' concept mentioned in an earlier section becomes relevant. Experimental studies investigating invasiveness need to be established in appropriate and well-defined environments, measure parameters that encompass all demographic processes, and carefully address the use of appropriate controls.

Will transgenes outcross to other species and increase weediness?

Concerns have been expressed that GM crops will hybridise with related species and result in the introgression of transgenes to weedy relatives. For transgenes conferring resistance to pests, diseases, and herbicides it is often suggested that this may result in enhanced fitness, survival and spread of weeds (Ellstrand, 2001). This too has the potential to add to the agricultural weed management burden by farmers, and/or may result in further invasion of natural habitats and compromise the biodiversity values of these habitats.

The potential for a crop to hybridise with a weed is highly dependent on sexual compatibility and relatedness between the parent species. While plant breeders have repeatedly crossed crops with related species to introgress a wide range of beneficial traits (Harlan, 1976), many of the hybrid combinations developed in this manner would not occur in nature because of the barriers within the plants to prevent normal embryo or endosperm development. The occurrence of interspecific and intergeneric hybrids as a result of manual hybridisation plus the use of biotechnology approaches such as embryo and ovule culture, only give a possible indication of sexual compatibility and the potential for hybridisation in nature. The opportunity for

Table 1 Factors determining the likelihood of gene introgression from crop plants to related species

Pre-zygotic barriers to hybridization
1. Spatial isolation of parent populations
2. Synchrony in flowering
3. Direction of the cross (the parent from which the pollen and ovules originate)
4. Specific parental genotypes
5. Method of pollen dissemination and presence of pollen vectors
6. Pollen competition from maternal population
7. Environmental conditions
Post-zygotic barriers to hybridisation
8. Mitotic compatibility of the two parental genomes
9. Ability of endosperm to support hybrid embryo development
10. Direction of cross (maternal effects on seed/fruit development)
11. Number and viability of hybrid seeds
Establishment of hybrid plants
12. Seed dormancy
13. Direction of cross (maternal effects influencing seedling vigour)
14. Growth vigour of hybrid plant
15. Habitat conditions (natural, ruderal, cultivated)
16. Competition from other plants
17. Influence of pests, diseases, predators
Propagation of hybrid plants
18. Ability to propagate vegetatively
19. Persistence, dissemination and invasiveness of vegetative propagules
20. Pollen and ovule fertility (meiotic stability and chromosome pairing)
21. Ability to produce sexual progeny (selfed and backcrossed)
22. Ability to survive over subsequent generations
23. Seed number, viability and dormancy
24. Habitat conditions, plant competition, pests, diseases and predators

natural hybridisation between two species in nature depends on many pre- and post-zygotic factors (Table 1).

If an interspecific or intergeneric hybrid does develop in nature, the success of this hybrid and its progeny is also highly dependent upon another series of factors (Table 1). Gene introgression from one species to another, or from a crop to a weed of the same species also requires repeated backcrossing to effect the incorporation of alleles from the gene pool of one population to another recipient population. The key issue of whether gene introgression can occur from a crop to a weed is the fitness of any possible hybrid populations and their persistence through several generations. Such fitness is based on the cumulative effects of all the above factors, with a poor performance at any step severely limiting gene introgression to weeds.

Many combinations of crops and species of the same and related genera are unlikely to naturally hybridise and affect the introgression and establishment of transgenes in natural populations. However, the situation is different when the combination involves sympatric populations of crops

and their progenitor species. The natural hybridisation of crops with related plants was documented very early in plant science (Darwin, 1876; de Vries, 1912). Darwin (1876, p. 378) refers to cabbage seed stocks as being 'seriously affected with purple bastards, by some plants of purple kale which flowered at a distance of half a mile'. The possibility of repeated hybridisation cycles leading to allele introgression from cultivated crops to weedy relatives has also been recognised for many years (Anderson, 1949). It is considered to have played an important role in both the domestication of crops and the evolution of weeds (de Wet and Harlan, 1975). The extent to which natural allele introgression occurs between many crop–weed combinations has been highlighted with the recent use of allozyme and DNA-based genetic markers (Ellstrand *et al.*, 1999; Ellstrand, 2001). These events occur where the distribution of a wild species overlaps with the cultivation of the related crop and are, especially common in the centres of diversity for specific crops, where hybrid swarms are often found (de Wet and Harlan, 1975).

When considering the potential impact of gene transfer between GM crops and other species it is important to assess whether GM crops have any different capacity for gene transfer than their non-GM counterparts. The characters influencing natural gene transfer between species are complex and will not, in general, change as a consequence of transgene expression. However, it is conceivable that transgenic changes in flower colour may have either a positive or negative influence on insect vectors of pollination. Depending on crop management, male sterility may remove pollen competition and provide a greater opportunity for foreign pollen to effect hybridisation. For the greater majority of transgenic traits, GM crops are no more likely to transfer either their transgenes or any other gene to other species than crop cultivars have done in the past. If gene introgression from a crop to natural populations does occur, the key issue to consider is whether the impact is any different for cultivars derived from genetic modification or traditional breeding. When considering the ecological concerns about transfer of transgenes to weedy species, it is the phenotype conferred by a gene that is important, not whether it was derived by GM processes or traditional plant breeding. Are the characteristics of weeds likely to be different when an introgressed transgene is expressed This can be considered in the same context as evaluating the potential weediness of a GM crop, where the concept of a transgene-centred approach to biosafety is important (Metz and Nap, 1997). In particular, the extension of the 'botanical files' approach to include a 'description of gene' (Dg) parameter to assess the ecological impact of a gene becomes relevant.

If transgenes conferring resistance to pests, diseases and environmental stress are introgressed into weedy relatives

of crops there is a concern that they may enhance particular fitness components of the weed in particular environments (Ellstrand *et al.*, 1999). However, plant breeders have released many cultivars with new genes for resistance to pests, diseases and environmental stress over many years. Any impacts resulting from the introgression of such traits into weedy species are equally likely for the products of plant breeding and genetic modification. The risks are no different and the uses of resistance genes in cultivars from traditional breeding have not been noted to enhance the survival and spread of weeds during the past history of crop breeding. When serious weeds have arisen following hybridisation of crops and wild species, their aggressive nature has arisen from a coupling of morphological traits conferring weedy attributes and the synchrony of development with the crop (de Wet and Harlan, 1975), rather than a gain in fitness from resistance genes.

The release of GM crops with notably herbicide resistance has often raised concerns about possible increase in fitness of weeds if the transgenes are introgressed into wild species. This may result if the selective herbicide continues to be used on the derived weedy populations. While this is a potential concern, it must be remembered that the development of weedy populations with herbicide resistance is not a new situation for agriculture since herbicide-resistant plants have also been developed by traditional plant breeding and arise by entirely natural means (Conner and Field, 1995). The agricultural industry is generally well aware of the problems that this can impose on weed management practices. Clearly, growing herbicide-resistant crops that are capable of hybridising with weedy relatives can enhance the risk of this situation. It is well-recognised that in geographical regions where crops are sympatric with their progenitor species, the risks may outweigh the benefits. Since many different herbicide resistances can be developed in crops via both GM and non-GM approaches (Conner and Field, 1995), this is an important issue that needs to be resolved from the perspective of agricultural strategy rather than a concern associated with GM crops (Dale, 1994).

Another problem highlighted by natural hybridisation between crops and their wild relatives is the increased potential of extinction of wild taxa. Some 'genetically aggressive' species, referred to as compilospecies (Harlan and de Wet, 1963), may completely assimilate another locally rare species through repeated cycles of hybridisation and introgression, causing it to become extinct. The highly successful crop wheat is considered to have assimilated germplasm from more than one species of *Aegilops* (Harlan and de Wet, 1963). Extinction by hybridisation does not depend on relative fitness, but only on patterns of mating (Ellstrand *et al.*, 1999). The impact of the release of GM crops will, in this scenario, be no different than the impact of existing non-GM crops.

Will GM crops contribute to horizontal gene transfer?

Horizontal gene transfer (HGT) is defined as the transfer of genetic material from one organism (the donor) to another organism (the recipient) which is not sexually compatible with the donor (Gay, 2001). HGT between bacterial species is particularly common when it involves plasmids and transposons (Courvalin, 1994; Landis *et al.*, 2000; Lorenz and Wackernagel, 1994). Now that full genomic sequences are becoming available, more and more potential candidates for HGT between species, genera and even kingdoms are being identified, including putative HGT events in *Arabidopsis thaliana* (Bevan *et al.*, 2001). HGT is considered a significant source of genome variation in bacteria (Ochman *et al.*, 2000) and may be a common route for evolution of bacterial populations and possibly also eukaryotes (De la Cruz and Davies, 2000). This may call for a revision of the concept of classification of species (Doolittle, 1999). Although this suggests that HGT is much more common than previously assumed, detailed phylogenetic analyses based on the presence of specific DNA sequences does not necessarily support the involvement of HGT (Stanhope *et al.*, 2001). Independent gene loss (Roelofs and van Haastert, 2001; Salzberg *et al.*, 2001) or other mechanisms (Eisen, 2000) have still to be considered.

The general concern with respect to GM crops is that the novel genes in such crops will result in a transfer of that material to other species and cause harm. Of particular concern are putative recipient micro-organisms in soil or in the digestive track of humans and livestock (Dröge *et al.*, 1998, 1999). The initial debate on HGT from GM crops focused on the presence of antibiotic marker genes in the plants. Due to the strong selection pressure of unwise prescription regimes in human and animal therapy, as well as use in farming (as feed additives), spontaneous resistance through mutation, coupled with some HGT between bacteria, has resulted in the spread of antibiotic resistance to such an extent that the medical/veterinary use of antibiotics as therapeutic agents is being seriously compromised (Austin *et al.*, 1999). Bacterial antibiotic resistance is currently one of the serious threats to public health (Kumin, 1993). Could the presence of antibiotic resistance genes in GM crops enhance existing problems with drug-resistant bacteria in human therapy

Once antibiotic selectable markers could be removed from GM plants and/or alternative selectable markers were developed for plant transformation, the HGT debate shifted to involve all transgenes in GM plants. Could HGT affect the intestinal microflora upon consumption of GM crops Or could HGT transform intestinal cells and change their phenotype In the environment, HGT could affect the soil microflora and create novel pathogens, or have other influences detrimental to either agricultural productivity or biodiversity.

The most popular technology of gene transfer to plants using *Agrobacterium tumefaciens* is based on HGT. Whereas the mechanisms of HGT from *A. tumefaciens* to plant cells is known in considerable detail, there is no known mechanism for HGT from plants to other organisms. In theory, HGT of plant DNA requires:

Availability. During growth, decay, herbivory or consumption of GM crops the introduced transgene (s) should be available for transfer. This requires the existence of free DNA that should be of sufficient length and persist long enough for uptake.

Uptake. A (bacterial) recipient should be competent for DNA uptake and a mechanism for uptake should be in place. Bacterial strains may be naturally competent for DNA uptake during some stage of development, such as *Ralstonia solanacearum* (Gay, 2001). Transformation mechanisms developed in the laboratory for *Escherichia coli*, such as those operating during PEG-mediated DNA uptake or electrophoration (Sambrook *et al.*, 1989), may have counterparts in nature. As GM-crop DNA will not be released as plasmids, conjugal transfer can be excluded and other mechanisms will have to occur.

Establishment. The recipient cell should incorporate, maintain and use the incoming DNA. This genetic alteration should pose no selection against the recipient organism.

Each of these steps has a likelihood that depends on several assumptions and/or data. Although DNA in decaying plant cells is rapidly degraded, DNA of the appropriate length can survive in some soils (Lorenz and Wackernagel, 1992), aquatic environments (Paul *et al.*, 1989) or the digestive tract of mice (Schubbert *et al.*, 1997) long enough to be available for uptake. The intestinal tract of cows and other ruminants is likely to be more hostile towards free DNA (Duggan *et al.*, 2000; Gay, 2001). Competence of bacteria in natural surroundings is difficult to assess, but is unlikely to approach the efficiencies reached in optimised laboratory conditions. The maintenance and integration of incoming DNA is mediated by (and may require) sequence homology with DNA of the recipient bacterium (de Vries and Wackernagel, 1998; de Vries *et al.*, 2001). The given transfer of a plant gene to a bacterium does not imply functionality in the bacterium. Regulatory sequences (promoters, enhancers) may not work and introns, if any, may not be recognised in the recipient.

Several experimental studies have been published that all failed in demonstrating HGT from transgenic plants to bacteria (Bertolla and Simonet, 1999; Gebhard and Smalla, 1999; Nielsen *et al.*, 1998; Schlüter *et al.*, 1995). This by itself is quite remarkable because in plant science negative results are not often considered publishable or published. In more elaborate marker-rescue approaches with large stretches of homology, the kanamycin resistance gene from GM maize could be retrieved in an *Acinetobacter* strain (de Vries and Wackernagel, 1998). Without the

artificially introduced homology in the recipient strain, no HGT was detected, indicating that the transformation frequency is very low. Such systems confirm that HGT can occur, be it at exceptionally low frequencies. Few data suggest HGT to plant-associated fungi (Hoffmann *et al.*, 1994), but evidence for stable integration and inheritance is lacking.

Another route for HGT could be a plant virus in a process equivalent to transduction in bacteria. It is known that plant viruses can acquire host sequences, but in the case of RNA viruses it would seem highly unlikely that such DNA will become integrated in the genome of a related plant (Tepfer, 1993), although RNA recombination itself may be an HGT issue (Malno *et al.*, 1999). In the case of DNA viruses, there is some evidence for transfer of genetic material from virus to plants (Bejarano *et al.*, 1996; Harper *et al.*, 1999; Jakowitsch *et al.*, 1999). Feeding mice with M13 phage did not exhibit any transformation of potential microbial hosts, but 400-bp M13 DNA fragments could be recovered from faeces, blood and even from cells in offspring developing in female mice (Schubbert *et al.*, 1997; 1998). In this manner, M13 DNA, and probably DNA in general, may have a mutagenic (carcinogenic) role in mammals, but the relevance for HGT of transgenic DNA is farfetched. In all such estimations and considerations, it should not be forgotten that the transgene of the GM crop constitutes only a fraction of the total plant DNA, whereas all plant-derived DNA will be subject to the same likelihood of decay and HGT. If the average length of a transgene is 3 kb, three transgenes in *Arabidopsis thaliana* would constitute $7 \times 10^{-6}\%$ of the total genome. The statistical likelihood that 'a' piece of DNA undergoes HGT is obviously considerably higher than the likelihood that a given piece of transgene DNA undergoes HGT.

Given that HGT to microbes or cells is not impossible, as shown by the marker rescue systems mentioned above, the next relevant question becomes 'so what' To what extent is the transgene added to the genetic material of GM crops different from the plant DNA in which it is integrated Are there specific consequences from, or issues for, a given transgene that need to be addressed How should this be taken into account Clearly, these issues cannot be generalised and should be assessed on a case-by-case and gene-by-gene basis. As indicated above, many concerns have focused on antibiotic resistance genes and their potential to compromise human or veterinary therapy. Previously, detailed risk assessments have been given for the kanamycin resistance gene *nptII*, still the most popular selectable marker gene for plant transformation (Fuchs *et al.*, 1993; Gay, 2001; Nap *et al.*, 1992). For the detailed considerations, we refer to these papers. In summary, the widespread occurrence of kanamycin resistance in the microbial soil and intestinal flora (e.g. Smalla *et al.*, 1993), combined with the very low occurrence of HGT, suggests that the likelihood of a bacterium in conditions selecting for kanamycin

resistance receiving the gene from another (bacterial) source is much greater than the likelihood that a bacterium receives the gene from a transgenic plant. In combination with the limited therapeutic value of kanamycin as an antibiotic, concerns about kanamycin resistance in plants compromising human therapy would seem to be not sufficiently supported by scientific evidence. Similar arguments hold for hygromycin resistance as a selectable marker in GM crops: hygromycin is too toxic an antibiotic for any therapeutic use (Gay, 2001). The EU decision to require the phasing out of the use of these two markers is, therefore, not in agreement with the policy intention of science-based decision making. For any other antibiotic-resistant selectable marker gene, it will remain an issue until concluded otherwise. For example, the *nptIII* gene gives some resistance against amikacin, an antibiotic in use to combat nosocomial infections. A potato fortuitously containing this gene was withdrawn from commercial application because of perceived problems with HGT (Gay, 2001).

Overall, the likelihood and impact of HGT with parental plant DNA compared to transgenic plant DNA would seem to indicate that HGT deserves less attention in the regulatory process compared to other concerns. Unless there is *a priori* strong evidence for impacts from HGT of a plant gene, such as in the case of a useful antibiotic resistance, HGT from GM plants to other organisms should be considered a calculable risk.

Will GM crops have secondary ecological impacts?

The view that GM crops are 'unnatural' has contributed to a perception that widespread use of such plants will lead to secondary or indirect ecological effects with undesirable consequences. The concept 'secondary ecological effects' is a broad, umbrella-like concept covering any impact on ecological relationships as diverse as effects on non-target or beneficial insects to food webs and the integrity of populations of soil biota. This is a relatively new area of research that promises to yield interesting and useful new insights in ecological relationships. As it is a research field in development, what to measure and how to measure are still debated. In particular, defining the relevance of what is measured for the environmental assessment of GM crops needs to be improved. At the moment, there is some tendency to consider any secondary ecological effect by definition a negative effect. The increasing demand for extensive evaluation of secondary ecological effects seems sometimes more a strategy to question and delay the applications of the GM technology than a reflection of genuine and relevant concerns. Any ecological impact of GM crops should involve a comparison of the perceived ecological benefits and potential threats of the crops they intend to replace. The important issue is, again, whether any potential secondary ecological impact of GM crops is

qualitatively and/or quantitatively different from possible impacts from crops created by traditional breeding. In this context, we will here give an overview of the various research lines into secondary ecological effects of GM crops.

Considerable ongoing research attention has focused on the secondary effects of insect-resistant, *Bacillus thuringiensis* toxin (Bt)-containing GM crops. Potential impacts are two-fold:

1. a direct effect on non-target insects (or other organisms) due to toxicity through exposure to GM plant material; and
2. an indirect effect on non-target insects (or other organisms) via so-called multi-trophic food chains.

Direct effects on non-target insects imply toxicity of Bt to non-target insect species. Although Bt has a high specificity, it is specificity towards insect groups, such as the lepidopteran insects, rather than towards particular insect species. Therefore, any non-target species from the same group may also be affected. The obvious relevant issue is whether that non-target species will ever encounter the Bt. If it is a species that is also feeding on the plant, it may be affected. Such direct toxicity to a non-target, non-pest species is rare. If it is a species that feeds on parts of the plant, such as pollen, it may also be affected. The latter issue is highlighted by the case of Bt-maize pollen and the Monarch butterfly (*Danaus plexippus*). When pollen from a commercial variety of Bt-maize (N4640) expressing a lepidopteran-specific Bt gene in the whole plant including pollen, was spread onto milkweed leaves (*Asclepias syriaca*, the feed plant of Monarch butterfly larvae) and fed to Monarch butterfly caterpillars in the laboratory, the caterpillars died (Losey *et al.*, 1999). This study led to considerable debate over the environmental impact and relevance for the potential risks from Bt maize. Follow-up studies to investigate the impact of widespread plantings Bt-maize on the Monarch butterfly essentially concluded that the impact of Bt-maize pollen from current commercial hybrids on Monarch butterfly populations is negligible (Hellmich *et al.*, 2001; Oberhauser *et al.*, 2001; Pleasants *et al.*, 2001; Sears *et al.*, 2001; Stanley-Horn *et al.*, 2001; Zangerl *et al.*, 2001). This is based on the low expression of Bt toxin genes in pollen for most maize hybrids, lack of acute toxicity at expected field rates, limited overlap of pollen shed and larval activity, and the limited overlap in distribution of Bt-maize and milkweed. In view of these follow-up studies, it must be concluded that the Losey *et al.*, (1999) paper describes a phenomenon that is in no way representative for the field situation. It shows that extra careful consideration applies when translating laboratory experimental results in the laboratory to the real-life situation in the field.

Another species that may be affected directly by GM crops is the honey bee (*Apis mellifera*), a beneficial insect which collects pollen and is therefore heavily exposed. A

number of studies have investigated the possible impacts of GM plants and purified recombinant proteins on bees. Direct toxicity is extremely rare and evidence from the most widely grown commercial crops has found no effect on colony performance (e.g. Malone and Pham-Delègue, 2001). At high doses, serine protease inhibitors, however, have been shown to inhibit bee gut proteases, which may result in reduced adult longevity (Malone *et al.*, 2000). However, the expression level in pollen from GM plants is not likely to reach the high dose required. In one study, pollen expression of cowpea trypsin inhibitor (CpTI) reduced the ability of bees to learn a conditioned response to floral odour (Picard-Nizou *et al.*, 1997), although other studies involving the expression of two other serine proteases (Girard *et al.*, 1998) or the cysteine protease inhibitor, oryzacystatin (Girard *et al.*, 1998; Jouanin *et al.*, 1998; Sandoz, 1996), found no effects on learning or foraging behaviour of bees. Overall, the ecological relevance of such effects, if any, in agricultural fields or beyond is unlikely to have any further undesirable or important consequences.

Even more difficult to study are any indirect effects on non-target insects (or other organisms) via so-called multi-trophic food chains. Investigations of multi-trophic effects were initiated with studies of the impact of Bt GM plants on predators and parasites of the pest insect targeted by Bt. These groups of organisms may be good indicators of potential secondary ecological impacts of Bt crops. The main issue in this concern is whether a whole food chain can be negatively affected, for example by slowly accumulating Bt as previously shown for DDT (Carson, 1962). Given the relative instability of the Bt protein, it may seem a rather theoretical scenario. Conventional agriculture aims to spray the target species to death with broad-spectrum chemicals. In this case, the availability of the target species as prey for other predator insects or food for other parasites seems less an issue. Generally, field data from insect-resistant GM crops expressing *cry* genes have failed to find impact on predator numbers between GM-Bt cotton and non-GM cotton and, in some cases, numbers have increased in GM plots (e.g. Schuler *et al.*, 1999a). However, in particular cases, such as the European corn borer (*Ostrinia nubilalis*) in maize, no truly appropriate chemicals exist and the appropriate comparison is not sprayed versus GM, but damaged versus GM. From the point of view of predators and parasites of target insects, Bt crops may affect the quantity of food (i.e. the number of prey insect individuals available) and/or the quality of food (the condition and composition of prey insect individuals available).

Commonly, predators and parasites reared on GM plant-fed prey insects do not grow to the same weight as prey-fed non-GM plants (e.g. Jørgensen and Lövei, 1999; Lövei *et al.*, 2000). When direct toxicity has not found in the laboratory to either purified toxin or GM plant material, it would be unexpected to find any ecological effect from field use,

unless there was a change in the quantity and quality of food. This view is now supported by field studies on crops expressing Bt toxins. Lepidopteran-active Cry proteins from *Bacillus thuringiensis* (Bt) lack direct toxicity to predators and parasites (Glare and O'Callaghan, 2000). Consequently, any impact in the field would be through indirect effects, such as reduction in food quantity or quality. The fecundity of the two-spot ladybird (*Adalia bipunctata*) was reduced when fed with aphids that were reared on potatoes expressing the snowdrop lectin GNA (Birch *et al.*, 1999). The ladybirds showed reductions in fecundity, egg viability and longevity. However, in another study, no effect was found on *A. bipunctata* from consuming aphids reared on GNA-containing diet, if the aphid weight used was the same as the controls (Down *et al.*, 2000). Retarded development and extended time to pupation of the ladybird is therefore directly attributable to the reduced weight of aphids reared on GNA-expressing potato. Some parasitoids use herbivore-induced plant volatiles to find their host. Wind tunnel experiments investigating parasitoid host-finding behaviour found that less parasitoids were attracted to the leaves of Bt-plants being fed to Bt-susceptible caterpillars. This was attributed to reduced feeding by Bt-susceptible caterpillars, since there was no difference for Bt-resistant caterpillars on wild-type or Bt-plants (Schuler *et al.*, 1999b).

In contrast, the mortality of lacewing (*Chrysoperla carnea*) larvae occurred when fed with (high) concentrations of solubilised and trypsinised Bt toxin (Hilbeck *et al.*, 1998a) or with prey raised on Bt maize (Hilbeck *et al.*, 1998b). Although Hilbeck *et al.*, (1999) suggest that the transgenic expression of Bt toxin gene in maize may have altered the specificity of the toxin, there are no convincing data to support such a suggestion. The prey used was *Spodoptera littoralis*, whereas in field situations lacewing larvae tend to prefer aphids as prey. In another study, lacewing larvae development or mortality was not effected when fed on aphids reared on the same Bt maize line (Lozzia *et al.*, 1998). It seems much more likely that *S. littoralis* is a less optimal food source, similar to the GNA-ladybird case. Not all interactions will result in negative impacts. Delayed development of the prey may increase the time frame in which parasitism or predation occurs, giving a positive synergy between the GM host plant resistance and parasitism/predation (Johnson, 1997). Beneficial secondary effects have been also observed. The increases in the numbers of natural beneficial invertebrates in GM crops where less pesticides have been applied may not be considered truly unexpected (Lacey *et al.*, 1999; Luttrell *et al.*, 1995; Parker and Huffman, 1997; Xia *et al.*, 1999). An unexpected beneficial effect was the reduced infestation of mycotoxin-producing fungi and associated reduced levels of fumonisin in Bt maize compared to unmodified maize (Artim *et al.*, 2002). This stipulates that not each secondary ecological effect is necessarily a negative or undesired effect.

In addition to insects, concerns about the secondary ecological impacts of GM crops are starting to focus on soil ecosystems. Analogous to insect-resistant GM crops, GM plants expressing antimicrobial proteins could affect soil microbial communities with undesired consequences. Glandorf *et al.*, (1997) reviewed the effects of GM plants expressing a range of antimicrobial proteins for plant pathogen control, such as chitinases and glucanases, on saprophytic soil microflora. Studies completed to date have not really answered key questions and investigating relevant effects on soil communities is a major challenge for the future. Soil organisms are generally heavily exposed to GM plant material, either through leaf shedding, root exudates or decomposition. Soil is a highly complex and variable ecosystem of primary importance in the redistribution of nutrients. The potential impact of any GM plant on soil organisms includes potential toxicity to a range of organisms (most of which are not tested under standard conditions, as many can not be cultured), the persistence in soil of any transgene product with undesirable effects, and the likelihood of such products ending up in soil.

The most studied system for persistence of transgene products is with Bt-toxins expressed in cotton and maize. The persistence of extractable Bt toxins from soil around GM plants is estimated at half-lives of around 10–30 days. Degradation is largely due to biotic factors, being highly dependent on soil type (Crecchio and Stotzky, 1998; Glandorf *et al.*, 1997). Clay particles were shown to bind and inactivate Bt irreversibly. Bound Bt is not taken up and accumulated by other plants (Saxena and Stotzky, 2001). Rhizosphere microbes are particularly exposed to decomposing plants and exudates from GM plant roots. Many studies have reported no changes in microbe populations in the rhizosphere over a range of many different GM plant modifications (e.g. Griffiths *et al.*, 2000, for lectin-producing potatoes).

In a few cases, changes in the populations of bacteria, fungi and soil invertebrates have been detected, even though no direct toxicity to the organisms has ever been demonstrated. For example, Bt cotton produced transient increases in soil bacteria and fungi compared to non-transgenic cotton (Donegan *et al.*, 1995), although studies on Bt as biopesticides and purified toxins do not show such effects (Glare and O'Callaghan, 2000). GM peroxidase-producing alfalfa had an altered shoot weight and higher nitrogen and phosphorus content of lignin compared to the parent non-GM line. The GM line appeared to change soil properties around the GM plants: soil pH was increased and activity of soil enzymes dehydrogenase (indicating bacterial activity) and alkaline phosphatase (organic phosphorus mineralisation and plant nutrition) were reduced (Donegan *et al.*, 1999). GM tobacco plants modified for decreased lignin showed an increased decomposition rate and soil bacterial communities associated with

lignin-peroxidase-producing plants differed from parental plants. This was attributed to a reduction in the degree of protection from microbial attack provided by the lignin to the polysaccharides and other labile plant components (Hopkins *et al.*, 2001). Other GM plants, such as opine producing legumes, can be specifically designed to alter rhizosphere bacterial populations (Oger *et al.*, 1997). The development of GM plants that produce specific exudates designed to give selective advantage for beneficial soil bacteria that can utilise the new exudate, is considered to be a useful way to enhance plant-bacterial associations by providing a trophic link (e.g. Savka and Farrand, 1997).

It can be very difficult to detect changes in bacterial soil numbers as result of the cultivation of GM crops, due to the high natural variability between samples. Biological indicators such as soil-dwelling nematodes are thought to be better indicator organisms for changes in soil communities (Bardgett and Griffiths, 1997). Such nematodes are a diverse group of bacterial-, plant- and invertebrate-feeders and can respond quickly to any perturbations. In a litter bag study comparing the decomposition of GM tobacco leaves expressing a protease inhibitor gene with that of non-GM tobacco leaves (Donegan *et al.*, 1997), nematode populations were increased, whereas populations of springtails, insects important in mineralisation of plant material, were reduced in soil surrounding transgenic litter compared to the numbers in soil surrounding non-GM litter. These differences were assumed to result from changes in the microbiota utilising the differences in the carbon substrates between the GM and non-GM plant litter (Donegan *et al.*, 1997). In other studies populations of springtails were unaffected by Bt crops (Sims and Martin, 1997; Yu *et al.*, 1997).

All studies in which significant differences in microbial ecology have been reported, raise questions over the cause of the differences. Inadvertent changes (e.g. carbon and nitrogen content) in GM plants during the process of producing GM crops are common (e.g. Donegan *et al.*, 1995; 1997; Escher *et al.*, 2000), and may result from (minor) metabolic changes due to mutational events from transgene insertion or somaclonal changes induced during the tissue culture phase used generate GM plants (Conner and Christey, 1994). Obviously, this only applies to minor changes that have not been discarded in the stringent selection process towards commercialisation. The observed impacts may not be relevant for ecological impacts upon release of GM crops. Differences in the decomposition of any plant material may occur and such changes are well known to affect the decomposition rates and soil processes/organisms. Similar changes are also likely to occur as a result of release new cultivars developed by traditional plant breeding approaches. The use of specific crops, such as certain Brassica species, for the purpose of biofumigation is seen an important basis for sustainable

agriculture, especially as an alternative to the use of methyl bromide (Rosa and Rodrigues, 1999). For this purpose, traditional breeding efforts are now being directed at the specific development of new cultivars for the primary purpose of changing the balance of microbial and invertebrate species within soil ecosystems.

Given current knowledge, it is too early to conclude whether GM crops can impact on agricultural and natural ecosystems by means of secondary ecological effects to an extent that will present undesirable harm. GM crops are as likely to give such effects to the same extent as any other human activity related to agriculture and traditional plant breeding. Each modification to agricultural practice has the potential to cause ecological impact. The data collected to date on the use of GM crops has found few examples of secondary effects, which would be deemed negative enough to result in actual problems at the ecosystem level. Large-scale field trials and commercially grown GM crops, with appropriate comparisons with non-GM crops and 'normal agricultural practice', provide the most appropriate systems to investigate whether there is any ecological impact or effect on non-target organisms. Many such studies are currently underway (e.g. Firbank *et al.*, 1999; Woilwood *et al.*, 2000). Any effect noted for ecological impact from GM crops must be viewed against a background that any alteration in agronomic practice would alter the microbial and invertebrate community structure. In this perspective, GM crops are not less 'unnatural' than agriculture itself.

Will GM crops lead to superpests and superdiseases?

The release and widespread cultivation of GM crops with pest or disease resistance has raised concerns that this will impose intense selection pressure on pest and pathogen populations to adapt to the resistance mechanism. This might result in the development of superpests and superdiseases that would be difficult or impossible to control. Obviously, this is not really a new issue. The development of pest- and disease-resistant cultivars has been one of the primary objectives of plant breeding for many years (Simmonds *et al.*, 1999). The history of plant breeding has clearly established that pest and pathogen populations can quickly adapt to crop cultivars with new resistance genes (e.g. Bonman *et al.*, 1992; McIntosh and Brown, 1997). In this respect, crop improvement is an 'ongoing battle'. If plant breeders stopped breeding for pest/disease resistance simply because the target pest or disease might overcome the resistance developed, the world's food supply would be under significantly more pressure. Pest and disease adaptation is therefore a problem well recognised by plant breeders and the agricultural industry. The development of cultivar management strategies to minimise the establishment of pest and disease populations that

overcome the resistance genes in new cultivars has been ongoing for many years. Nowadays, this is usually a component of an overall integrated pest/disease management strategy involving the use of resistance genes in cultivars, some chemical applications, biocontrol agents, and crop management practices. In order to establish an appropriate management plan it is important to understand the nature of the interactions between the host crop, the pest/pathogen population(s), and the control mechanisms involved. The key to maintaining an effective management plan is regular monitoring of the response of the pest/pathogen populations to the control measures.

The use of host plant resistance genes has been extensively used for pest and disease control in breeding programmes of many crop species, especially in cereal crops (e.g. McIntosh, 1998). Although many resistance genes have been identified in crop germplasm, there has been no easy way to predict the quality or durability of these resistance genes (Leach *et al.*, 2001). The 'breaking down' of disease resistance genes is usually associated with qualitative resistance conferred by single major genes (*R* genes), where resistance versus susceptibility results from a gene-for-gene interaction between the *R* genes in the host and avirulence genes in the pathogen (Flor, 1971). The resistance conferred by many *R* genes has not been durable as a consequence of rapid changes in pathogen populations (Leach *et al.*, 2001). The most widely cited examples of durable resistance against bacterial or fungal pathogens have involved supposedly complex, multigenic quantitative traits (Johnson, 1984; Parlevliet, 2002). However, there are examples where single *R* genes have conferred highly durable resistance, e.g. the *Lr34* gene conferring resistance to leaf rust in wheat (Kolmer, 1996) and the *Xa4* gene conferring bacterial blight resistance in rice (Bonman *et al.*, 1992).

One plant breeding approach to manage the 'breakdown' of disease resistance genes in crops has involved the concept of multi-lines or multi-blends. Multi-lines consist of a mixture of inbred isogenic lines that share an identical genetic background but differ only with respect to a gene(s) for a specific trait. This concept was originally proposed as an approach to promote the durability of a cultivar by providing greater stability of production, broader adaptation to the environment, and greater protection against pathogen populations (Jenson, 1952). In contrast, multi-blends involve a simple mixture of one or more cultivars that differ in a diverse range of traits. Such blends of cultivars have had a long history of use in agriculture and have often been confused with the use of multi-lines (Jenson, 1988). In modern agriculture, the use of multi-blends is more feasible in forage, forestry and perennial horticultural crops, but less appropriate in arable and vegetable crops where uniformity is essential for the mechanisation of crop agronomy and harvest. It is difficult to ensure

the uniform development of plants growing from each component in a seed mix, especially under a range of environmental conditions.

The development of isogenic lines, each possessing a different gene for resistance to a specific race of a pathogen, became a popular approach to buffer against rapid disease development and extend the 'life' of resistance genes in cereals (Borlaug, 1959). However, the application of the multi-line concept never reached its full potential due to difficulties associated with the development of isogenic lines via traditional breeding. This was especially evident for non-inbred crops, where new cultivars are clones, hybrids or open pollinated populations. The development of GM lines, isogenic for the presence/absence of various genes targeting specific pests or pathogens, offers a new opportunity for more efficiently implementing multi-line strategies and durable approaches to minimise the 'breakdown' of pest and disease resistance genes in crops. The efficacy with which isogenic lines can be developed in crops by GM approaches may also allow the concept of multi-lines to be effectively extended from inbred crops to clones, hybrids and open pollinated populations (Conner and Christey, 1994). This can provide attractive management options, especially when populations of pests and pathogens are anticipated to overcome the resistance introduced to GM cultivars. Since multi-lines can be reconstructed each year prior to seed sowing, it is possible to change the composition of isogenic lines within each multi-line as changes occur in pest and pathogen populations. Alternatively, in annual crops, isogenic lines for different resistance mechanisms could be alternated each year. In addition to the spatial or temporal mixing of GM crop lines with different genes, the pyramid of multiple genes, each conferring a different mechanism of resistance, into the same GM cultivar may also help the prevention of disease resistance breakdown. A prudent management mix of these approaches may well be most effective.

Resistance management strategies associated with the release of GM crops have been well established for the commercial release of insect resistance conferred by *cry* genes originating from *Bacillus thuringiensis*. The predominant approach has involved the combination of a consistent and high expression level of the *cry* gene with the deployment of a refuge (Roush, 1996; Gould, 1998). A refuge is an area of non-GM crop that is placed either within a crop as a seed mixture (a multi-line approach) or as a separate block within close proximity. The purpose of the refuge is to maintain a population of the target insects with susceptible alleles to the Cry proteins. The high dose/refuge strategy assumes that development of resistance to Cry proteins by insects is conferred by recessive mutations (Tabashnik *et al.*, 1997) and that the presence of these alleles is rare in insect populations (Andow and Alstad, 1998). On this basis, insects homozygous for resistance

alleles will be very rare. Random mating of these rare survivors with susceptible insects maintained in the refugia will ensure that all progeny of any resistant survivors will be heterozygotes, which are also susceptible when feeding on GM plants with high *cry* gene expression. It is also assumed that there is relatively little interplant movement of larvae between the GM and non-GM plants, a consideration more important for refugia involving seed mixtures. There is greater potential for heterozygotes to recover from sub-lethal doses of Cry protein when such movement occurs. Although no management strategy can guarantee that insect populations will not evolve resistance to Cry proteins, simulation models and laboratory/greenhouse studies suggest that the use of appropriate refugia coupled with high *cry* gene expression will delay the development of resistance for many decades (Gould, 1998; Roush, 1996). This can be further enhanced by incorporating the management strategy into a wider framework of integrated pest management. This exposes the pest to a range of mortality mechanisms, which reduces the selection pressure on the most important mortality factor (McGaughey and Whalon, 1992).

The experience gained from plant breeding over many years will help define the appropriate management approaches for pest- and disease-resistant GM crops to prevent or minimise the establishment of pest and disease populations that overcome the resistance mechanism underpinning the resistance genes. It is not the GM versus non-GM status of the crop plants that may result in projected problems arising, but the way the crops are grown and managed. The widespread cultivation of GM crops with pest or disease resistance is no more likely to result in the development of difficult-to-control superpests and super-diseases than that experienced by traditional breeding in the past. Moreover, the efficacy with which isogenic GM lines can be developed with different (trans)genes targeting specific pests or pathogens, may provide new opportunities for more efficiently implementing durable approaches to minimise the 'breakdown' of pest and disease resistance genes in crops.

Will GM crops impact on biodiversity?

Another major concern about the introduction of GM crops into the environment is that such crops will affect and/or destroy biodiversity. Fear for the loss of biodiversity is the focal point of opposition of several influential environmentalist groups against genetic modification and GM crops. The impact of GM crops on biodiversity is a complex and complicated issue that has so far mainly contributed to more forests being destroyed in order to produce the paper on which the arguments have been presented. While the issues of whether GM crops affect biodiversity can be debated, a better and more valuable discussion would be

whether GM crops pose threats to biodiversity that are qualitatively and/or quantitatively different from conventional crops. Assessing the influence of GM crops on biodiversity should then involve a comparison of the perceived benefits and potential threats of such crops to the conventional crops they are intended to replace.

The Convention on Biological Diversity defined biodiversity as 'the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems' (CBD, 1992; Johnson, 1993). The very broad, umbrella-like character of the definition is sometimes taken as an indication that biodiversity is still an ill-defined concept. This has resulted in various other organisations developing their own more specific views on subareas of biodiversity. Alternative definitions differ in the particular aspects they emphasise; diversity of ecosystems and biotopes (OTA, 1988), diversity at the species level (OECD, 1996), or diversity at the level of genetic material (Jenkins, 1992). In addition to defining biodiversity, it is equally difficult to quantify biodiversity or any perceived loss in biodiversity. For example, it is essentially impossible to indicate where one ecosystem stops and the next begins. Species diversity is, in practice, considered central to the evaluation of biodiversity (OECD, 1996). In the case of higher plants and animals, the number of given species present at a given site (species richness) is currently the most straightforward and most used measure of biodiversity. However, it underexposes the (large) categories of invertebrates and micro-organisms and is also an insufficient basis for properly comparing sites across different environments. Biodiversity cannot be reduced to a single number, despite the explicit wishes of policy makers (Purvis and Hector, 2000). The disappearance of a given species from an ecosystem may either affect that ecosystem seriously, or another species may take over its function and place (Grime, 1997; Tilman *et al.*, 1997). In total not more than 2 million species have been described (Wilson and Peter, 1988; ten Kate and Laird, 1999) of the 3–100 million species estimated to be present on Earth (May, 1988; 1992; WCMC, 1995), with 5–15 million being considered a 'best estimate' (Stork 1997). From a gene point of view, there may be about 10^9 different genes present in all living organisms on Earth (Leemans, 1996; WCMC, 1992). When all the alternative alleles for all genes are considered across all species, then from a genetic diversity point of view the numbers become mind-boggling. An extreme conclusion from attempts to quantify biodiversity at the gene level is that each living organism is essentially a unique individual.

The importance of conserving biodiversity is motivated in a variety of ways, depending on the particular connotation of biodiversity. Various governments are in the process

of defining biodiversity action plans (BAPs). Often a resource-centred point of view is taken, in which loss of biodiversity means loss of capital and potentially useful resources (OECD, 1996). Biodiversity is the source of many of the world's products, including foods, fibres, pharmaceuticals, and chemicals (ten Kate and Laird, 1999). It forms the basis for existing cultivars of crops and breeds of livestock, their genetic improvement and the development of new cultivars and breeds. Biodiversity is important for the persistence, regulation and maintenance of planetary conditions. It offers aesthetic, scientific, cultural and other values (Kunin and Lawton, 1996), which can seem intangible and non-monetary, but which are almost universally recognised. The overall value of the world's biodiversity has been estimated to amount up to US\$ 33 trillion per year (Costanza *et al.*, 1997), but such estimates should be used cautiously (ten Kate and Laird, 1999). Admittedly, not every species is worth protecting from a human perspective (e.g. malaria-carrying mosquito, HIV), which is why priorities must be set for biodiversity protection.

Several scenarios predict 'irreversible' and 'catastrophic' harm to biodiversity as a result of the use of GM crops. Equally, several scenarios predict the opposite. Obviously, each scenario depends on the particular characteristics of a given GM crop, as well as on the socio-economic and legal context of the agricultural system into which the crop is introduced. Only a case-by-case approach will allow appropriate assessment of the potential or putative influence of a given GM crop on biodiversity. Here, we will describe some more general considerations and conclusions. Fears with respect to the influence of GM crops on biodiversity at large question the ecological impact of the GM plants. GM crops could threaten the centres of crop diversity (Rissler and Mellon, 1993) or outgrow a local flora to the detriment of native species. The putative presence of transgenes in Mexican maize landraces and the interpretation that this presence may reduce the value of maize genetic resources (Quist and Chapela, 2001) has fuelled the debate about genetic resources in centres of crop diversity. The investigation may not only suffer from experimental artefacts, but also the interpretation given is doubtful (Christou, 2002; Editorial, 2002). Any gene from commercial maize varieties, whether transgenic or not, may introgress into landraces. Of concern here are the potential occurrence and consequences of increased weediness and gene flow due to the introduction of the additional genetic material. These issues are discussed in earlier sections. In general, it seems reasonable to conclude that spread of modern agriculture based on genetically narrow populations of uniform hybrids is a much bigger threat to the genetic diversity in maize than introgression of transgenes now present in commercial maize. Furthermore, the putative presence of transgenes in gene bank accessions is neither uncontrollable nor irreversible (Louwaars *et al.*, 2002).

Where GM crops are adapted to new environmental conditions, notably environmentally marginal conditions such as salt or drought, indigenous plant communities may be at stake (Kareiva and Parker, 1994), but to no greater extent than for similar crops developed through traditional breeding. The possibility of adapting GM crops to conditions currently marginal for farming could also be highly advantageous for biodiversity conservation. A major threat to biodiversity is habitat loss due to the conversion of natural ecosystems to agricultural land in response to food demands (Tilman *et al.*, 2001; World Bank, 1995). GM crops that could give higher yields per surface area and/or produce on suboptimal soils may alleviate the threat of habitat loss, thereby contributing considerably to sustained biodiversity.

When considering the impact of GM crops on biodiversity, it is worthwhile distinguishing between the biodiversity at large, and the biodiversity associated with agricultural practice. There are many species for which farmland or the surrounding natural environment is the main or sole habitat range. The complex of genetic resources present in agro-ecosystems, as well as elements of natural habitats that are relevant to agricultural production systems, is generally referred to as 'agrobiodiversity' (Hardon *et al.*, 2000). Current-day agriculture is the result of a long process of plant domestication, the establishment of landraces and the deliberate breeding of new cultivars. It is only relatively recently that fields with single species or cultivars have become standard agricultural practice (Pretty, 1995). This historical development reflects the continuous activities designed to create new and better agricultural produce for society. The aim of most current plant breeding is market-driven with the aim of improved and more economical production, together with a modern, efficient, and generally large-scale system of agricultural management. The yield-directed breeding developments have resulted in a considerable narrowing of the number and the genetic basis of current crops. Although no less than 7000 plant species have been used for human food (Ehrlich and Wilson, 1991), at present, as few as 20 plant species account for over 90% of human use (Solbrig, 1992). Of these, four crops (wheat, maize, rice and potato) provide one-half of the total world food production and 15 crops contribute two-thirds (Gotsch and Rieder, 1995). Aggressive planting of high yielding GM cultivars may further contribute to the development of high-input agriculture that has been accompanied by a steady decline in agrobiodiversity. In this manner, GM crops may further reduce the number of available crops/cultivars, and contribute to a further narrowing of the genetic basis of world food production.

On a more local scale, GM crops may affect agrobiodiversity by a variety of means. They may eliminate pests and weeds too effectively, leading to a further deterioration and

simplification of agricultural ecosystems. For example, the application of herbicides could result in a reduction of weeds and associated invertebrate populations, contributing to reductions in native wildlife populations at higher trophic levels. To define and set criteria, both the standards and the limits, for decision making in such evaluations will be very difficult, if not impossible (Dale, 1999). In a computer simulation model of herbicide-resistant sugarbeet, it was calculated that skylark (*Alauda arvensis*) populations could drop as much as 90% due to reduced lambsquarter (*Chenopodium album*) populations (Watkinson *et al.*, 2000). This would not be the result of the GM crop itself, but from changing farming practices resulting from growing such a crop. The impact of insect-resistant GM crops on non-target species may have similar consequences (Kinderlerer, 2001). However, the Watkinson *et al.*, (2000) model is based on a single weed and a single bird. Moreover, it takes only harmful effects into account (Firbank and Forcella, 2000) and has not yet been validated with field data. The final results and conclusions, if any, of the large scale evaluations of the effects of GM herbicide-resistant crops in the UK (Firbank *et al.*, 1999) are therefore eagerly awaited. GM crops could also influence the number and type of microorganisms in the rhizosphere or soil. The most important issue is whether any effect on species that rely on agricultural practices for survival should be considered a disruption of a 'natural' equilibrium. Variation by itself will be huge and not necessarily give rise to negative outcomes. It is likely that it will be impossible to consider all possible interactions and effects when assessing applications for approval to field test GM crops. Therefore, post-approval monitoring should be encouraged. The comparison with the impacts of conventional crops grown under modern farming practices is the best baseline for comparisons.

Conversely, GM crops could contribute to increased agrobiodiversity. The use of GM soybean, canola, cotton and maize was estimated to reduce pesticide use by 22.3 million kg of formulated product (Phipps and Park, 2002). Such reduction in the overall amount of pesticides may impact positively on (agro)biodiversity. Genetic modification, as an extension to the tools of plant breeding, could be an asset to the conservation, use and (re)creation of agrobiodiversity. During the breeding of new crop cultivars the vast majority of genotypes are eliminated by selection against minor defects. GM as a breeding tool allows a reassessment of material previously discarded. It could also facilitate the transfer of genes from related species in a more controlled and efficient manner. The perceived speed and accuracy by which the GM plants can be obtained (Conner, 1997) will be an incentive for breeders to prefer this technology over hybridisation when the trait is sufficiently advantageous. Planting a diversity of cultivars or genotypes in a field could help to harness the intrinsic variability in pest and pathogen resistance (Altieri, 1994; Pretty, 1995).

Genetic modification offers an approach to establish multi-lines that are similar in most of their characteristics, but have different genes and/or strategies for resistance (Conner and Christey, 1994). Both in rediscovering and improving old cultivars/genotypes and in the development of new crops, GM has the potential to contribute to enhanced agrobiodiversity. Genetic modification also allows addition of novel genes to a crop's gene pool. When the source of the novel gene is not a sexually compatible species, genetic modification implies the production of a novel genotype and phenotype. In the context of genetic diversity, the use and creation of genetic variation in this manner could be interpreted as an increase in agrobiodiversity beyond the capabilities of traditional breeding.

The potential long-term effects of GM crops on (agro)biodiversity are often considered a major concern. In this context, a central problem is to define the term 'long-term' (van der Meer, 1993). Unfortunately, 'long-term' may vary from months to decades or centuries, depending on the organisms, environments and genes involved. To assess any potential unintended negative impact, information from over 10 to 100 generations of a species may be required (Kasanmoentalib, 1996). In policy making, a period of 10 years is sometimes suggested. This time span does not reflect the scientific issues at hand. Based on available data to date, we estimate it will take decades for current GM crops to have appreciable ecological consequences, if any, on a single agricultural site. Furthermore, it will take centuries for any appreciable ecological consequences, if indeed they exist, to occur on a more global scale. By definition, such consequences do not need to be negative or undesired. It will be, and remain, essentially impossible to assess or predict such long-term effects. What then is the key question Is it truly necessary to know and understand all the complexities of ecological relationships Or it is reasonable and acceptable to conclude that because of the very complexity of all interactions, populations are likely to be sufficiently buffered against any supposedly relatively minor impact imposed by GM crops, over and above the impact and effect of agriculture itself Given a 10-year time scale, the latter would imply the possibility for a considerable simplification or even elimination of the need for long-term assessments with respect to biodiversity. Such a policy decision would be consistent with agricultural policies aimed at increased productivity, would lead to savings in administration that would be welcomed by economy, technology and industry. However, it may negatively affect the public's trust in the evaluation procedures (Torgersen *et al.*, 1998).

Against the background of native land conversion and habitat fragmentation, the putative threats of GM crops to biodiversity would seem to be largely hypothetical and marginal (World Bank, 1995). There is little controversy that the development of human civilisation and human

activities such as agriculture and industry is a major cause of the loss of biodiversity at large (Tilman *et al.*, 2001; Vitousek *et al.*, 1997; WCMC, 2000). Over consumption and waste in wealthy countries and population pressure in poor countries put immense pressure on the ecosystems we depend on (Serageldin, 2002). In this context, GM crops are no more or less likely to affect biodiversity than any other change in agriculture. Given the multidimensional complexity of the biodiversity concept, assessing the impact of a given technological development such as GM crops is, therefore, far from straightforward. It will largely be the social-economic and political context of the application of genetic modification that will determine whether the perceived threats or potential benefits of GM crops on biodiversity become a reality.

Will GM crops affect the purity of other crops?

A further concern about the agricultural use of GM crops involves the possibility that conventional non-GM crops will receive transgenes from GM crops, resulting in situations that are either undesired or unlawful. A well publicised example of the latter has been the presence of the GM Starlink maize containing the *cry9C* gene in non-GM maize grains (Dorey, 2000). The potential inadvertent mixing of GM and non-GM crop through pollen dispersal and seed is a particular concern for the organic farming industry, for both economic and emotional reasons (Moyes and Dale, 1999). In such cases, liability can become a major issue (Moeller, 2001).

Genetic modification *per se* does not change the frequency with which this admixture of genetic material occurs. It is the substantially increased power of detection of modern molecular biological techniques that permits very low levels of genetic mixing to be discovered. It represents the level of mixing that has existed and still exists in current non-GM seed and food production chains. This level was considered to be well within the accepted and acceptable limits.

Maintenance of seed quality is an important basis of modern agriculture, which is evident from the increasing international trade in the seed of modern crop cultivars. The international trade of seed is already considered one of the most regulated agricultural commodities and subjected to strict phytosanitary and noxious weed regulations, as well as certification standards and regulations that cover physical and genetic purity. Seed quality is controlled by the Association of Official Seed Certification Agencies (AOSCA, 1971) or the OECD Seed Certification system (OECD, 2001). These represent comprehensive international quality assurance systems. The accidental presence of impure seed within the seed supply of a cultivar is known as adventitious seed. For commercial cultivars of both non-GM and GM crops the genetic purity of seed represents the homo-

geneity of a single recognised cultivar or its trueness-to-type (Briggs and Knowles, 1967). Strict management guidelines are imposed by all seed quality assurance schemes to allow the multiplication of sufficient seed to sow the large areas associated with commercial crop production (Briggs and Knowles, 1967; Condon, 2001). Without imposing seed production guidelines to maintain the genetic purity of certified seed, a cultivar may quickly deteriorate and become unrecognisable due to factors such as mechanical admixtures, gene flow through natural crossing, mutations, random genetic drift or selection pressures (Condon, 2001).

The monitoring of genetic purity associated with seed certification is based on the phenotype of the plants. This is implicit in the 1991 Act of the UPOV Convention where a cultivar is defined as the expression of the characteristics resulting from a given genotype or combination of genotypes (UPOV, 1991). Quality assurance in the seed industry is based on observing the characteristics of plants and ensuring that they match the standards in the cultivar descriptions. The international seed purity standards require the incidence of admixture and genetic instability of cultivars to be maintained above a minimal threshold value that depends on the reproductive characteristics of each crop. To achieve the standard for each crop, specific seed production guidelines are prescribed that take into account isolation distances, a rotation cycle involving a minimum number of years between crops of the same species, a maximum number of off-types that may arise from volunteers or contaminated seed. These standards have delivered crops with sufficient uniformity and stability to meet the requirements of Plant Variety Rights legislation in UPOV countries (Simmonds *et al.*, 1999).

The seed industry acknowledges that obtaining 100% genetic purity is unfeasible and uneconomical. Uniformity and consistency requirements of seed processors and marketers have been met using existing parameters of quality assurance schemes. Tolerance of a low level of gene transfer by pollen is considered an inherent component of modern day agriculture, especially when growing commercial crops for food production. International seed certification standards require genetic purity levels of 98–99%, or a standard of 1–2% for adventitious genetic impurity (Leask, 2000). These purity levels represent the compromise between the stringency imposed on seed production and the market need for affordable seed, especially for crops grown over large areas.

New molecular and biochemical techniques such as PCR and ELISA allow for more precise testing. Such diagnostic tests allow the presence of a particular gene (or allele) or the products of a particular gene (or allele) to be measured. Consequently, cultivar purity can now be estimated on the basis of genotype, rather than on phenotype. An additional

advantage is that the environment can substantially influence the latter. The more sophisticated diagnostic tests based on genotype are anticipated to reveal higher frequencies of occurrence of adventitious genetic material in commercial seed than previously recognised or anticipated. Existing cultivars that are widely used and traded, and thought to be homogenous, pure and stable at the phenotypic level, may actually contain considerable variability at the genotypic level. This will present new challenges for commercial seed production. Eventually, UPOV legislation is expected to accept purity assessment on the basis of genotype. For both non-GM and GM cultivars this will require a thorough re-assessment of existing quality assurance paradigms, which must be re-assessed given the new opportunities to measure genetic purity.

If adventitious genetic content in commercial seed of non-GM crops is a common event given current seed certification schemes, the appearance of transgenic material within otherwise non-GM cultivars is unavoidable, except by fully prohibiting the cultivation of GM crops. A standard of zero adventitious content in a commercial seed line is unachievable, irrespective of whether the influx is from non-GM or GM cultivars. Moreover, any improvement in seed production technology to further minimise the incidence of adventitious seed content is likely to be at least matched by enhanced sensitivity and precision of the modern diagnostic tests. The European Commission Scientific Committee on Plants (ECSCP) has therefore stated: "*From experience of research on unintended seed mixing, on gene-flow, and from long experience of commercial seed production, it is clear that a zero level of unauthorised GM seed is unobtainable in practice. Field grown crops are always subject to unintended pollen and seeds from various sources*" (ECSCP, 2001). While the use of stringent management approaches can help minimise the opportunity for inadvertent admixtures of GM with non-GM seed, the issue of transgene flow via pollen dispersal presents a more difficult problem. Restricting the movement of pollen between crops is not a new concern and forms an important basis of all seed quality assurance systems.

The production of certified seed of specific cultivars requires the maintenance of minimum isolation distances. There are internationally recognised isolation distances that vary depending on the crop in question and its reproductive characteristics (Briggs and Knowles, 1967; Simmonds *et al.*, 1999). Some seed companies demand greater distances to ensure higher purity of their seed. If zero adventitious seed content cannot be realistically achieved, then a threshold level must be established to enable the seed industry to provide quality seed at an affordable price. The establishment of any threshold level must be based on the mean adventitious seed content, plus a tolerance based on the variance of the population sampled (a measure of the variance is needed, otherwise

50% of the samples would fail to meet the threshold). The threshold must also be within the sensitivity and error rate of routine analytical procedures, which is currently set at 0.1% in case of PCR-based assays. A reasonable threshold for adventitious GM content should take into account the levels of non-GM adventitious content, measured using the same analytical tools, and achieved using the best practice methods of seed production. Such thresholds should therefore be based on the characteristics of a crop, similar to the establishment of the international seed purity standards.

In addition to seed production, isolation distances are also used as a management strategy to maintain quality attributes during crop production. For example, this is important in a crop such as oilseed rape where isolation enhances uniformity in oil quality by minimising mixed oil components arising from cross-pollination with neighbouring crops or weedy species (Bilborrow *et al.*, 1998). Likewise, isolation between production fields of corn are important to prevent 'xenia' effects in which pollen of one cultivar can influence the endosperm characteristics and kernel quality attributes of another cultivar intended as sweet corn, corn flour, feed, or industrial use (Briggs and Knowles, 1967; Rubatzky and Yamaguchi, 1997).

There is, however, one particular application of GM crops where additional care may be required. These are the applications collectively known as 'molecular farming', in which GM crops produce pharmaceuticals, vaccines, biodegradable plastics or speciality (bio)chemicals, for example special oils for the paint industry. Such products should not be mixed with the normal food crops. The environmental release of such GM crops will require more stringent levels of containment for keeping these products out of the food chain. To prevent the inadvertent admixture of such cultivars with those intended for food use by either unintentional seed mixing or inter-cultivar gene flow presents special challenges. The economic feasibility and success of molecular farming approaches will depend to a large extent on the ability to meet these challenges. Either specific areas of the world should be dedicated for specific productions, or additional molecular mechanisms to prevent gene flow should be put in place. Recent advances in the genetic engineering of chloroplasts offers an approach to further limit gene escape through pollen in species of plants in which chloroplasts are maternally inherited (Daniell *et al.*, 1998). Possibly, the applications of molecular farming may require a revival of the interest in, and acceptance of, other approaches known as genetic use restriction technologies (GURTs), in the popular press better known as 'terminator technology' (Visser *et al.*, 2001). Such technologies are currently generally considered 'not done' (CBD, 2002; FAO, 2001; Visser *et al.*, 2001), but their usefulness in preventing truly undesired gene flow might be underestimated.

What is the proper baseline for appropriate ecological experimentation?

When conducting experiments to assess the ecological risk of GM crops, it is critical to use appropriate controls. Where possible transgenic lines should be compared to the same generation null-segregant as a control (Linder and Schmitt, 1994). Null-segregant controls represent a line that has lost the transgene through normal segregation of alleles from plants hemizygous for the transgene. When this is possible, it allows unambiguous assessment of the effect of the transgene on plant fitness or non-target species, since the GM crop and the null-segregant differ only by the presence/absence of the transgene. This approach is, especially valid for crop cultivars based on genetically uniform inbred lines. Care must be taken with GM lines containing multiple insertion events at different loci, to ensure that appropriate comparisons are made. Some insertion events may be non-functional and not contribute to the GM phenotype.

The null-segregant approach is inappropriate for some crops, where the genetic background of the transgenic cultivar will not permit the recovery of an appropriate control through allele segregation (Conner and Christey, 1994). In crops with clonally propagated cultivars (e.g. potato), the recovery of sexual progeny with the desired agronomic attributes is virtually impossible, which is exactly why the cultivar is propagated asexually. In crops with highly heterozygous open pollinated cultivars, in which inbreeding depression is common, the transgene may be linked in coupling with loci that contribute to overall fitness. These loci may involve alleles that either enhance fitness or have a detrimental influence on plant vigour. In such instances the most appropriate control is the non-transgenic parental cultivar. In the case of F_1 hybrid cultivars, the most appropriate approach would be to produce the hybrid F_1 seed from non-transgenic and transgenic versions of the same parental material. In this context, the use of isogenic plant lines with and without a specific gene provides a valuable experimental approach to further develop ecological science and provide new insights into the dynamics and plasticity of ecosystems.

Investigation of secondary ecological effects, such as multi-trophic interactions, is a relatively new area of experimental biology. Isogenic lines are especially valuable for investigating the effects of plant metabolites on multi-trophic interactions as a basis for understanding ecological interactions between species. GM technology allows isogenic plant lines with and without a specific gene to be conveniently developed (Conner and Christey, 1994). When the expression products of such genes are known to have anti-microbial or insecticidal activity, the isogenic lines provide valuable experimental material for the design of definitive experiments to investigate multi-trophic interactions. This allows state-of-the-art ecological impacts to be

investigated at the level of gene ecology. Since an appropriate null-control for the gene is available, and the products of the specific gene are known and can be quantified in the GM plants, the metabolites responsible for the multi-trophic interactions can be measured through the trophic levels to allow more authoritative interpretation of responses of individual species along a multi-trophic conduit. Furthermore, there is the ability to perform a series of tiered experiments on specific species interactions which involve: laboratory studies in which the culture media (diet formulations) of microbes (invertebrates) are supplemented with the target plant metabolite; whole plant investigations on responses of individuals within a species; and field studies at the level of population ecology. Appropriate isogenic plant lines for specific genes in which the expression products are known, available and measurable are not generally available in non-GM plant material. It is therefore critical that when performing such experiments on GM plants, biologists appreciate and acknowledge that the GM plants are really being used as a convenient model system to investigate species interactions at the level of gene ecology. In most instances, any ecological impacts in response to gene expression that are uncovered will be indicative of what already occurs in agricultural and natural ecosystems, rather than any new impact specific to GM crops.

Experiments assessing the fitness or invasiveness of GM crops, or their impacts on non-target species, should also be performed at an appropriate time in the development of a GM cultivar in order that the most appropriate GM line is identified for assessment. Clearly it should be a GM line that has definite potential for commercial release. The early phases of GM cultivar development involve greenhouse evaluations and small scale field tests in order to identify GM lines with stable expression and phenotypic performance of the transgene, as well as the absence of phenotypic changes resulting from either insertional mutagenesis by transgene insertion events or somaclonal variation associated with the tissue culture phase of plant transformation (Conner and Christey, 1994). Depending on the circumstances, such factors may under- or overestimate the invasiveness or impact of GM crops. The use of a transgenic line not identified as a potential candidate for environmental release into commercial agriculture may give a wrong impression of environmental effects resulting from release of the crop.

There has been a much greater emphasis on gaining knowledge on the impacts of GM plants in recent years. This was not always the case. Seven years after the first field tests on GM plants, Kareiva (1993) noted: *"it is a pity that opportunities to obtain appropriate data have been missed in the hundreds of completed field trials, which have emphasised agronomic performance and have been managed in a way that discouraged multigeneration observations of transgenic population"*. It is ironic that the way

field tests on GM plants are managed is often in response to the containment controls imposed as risk management practices by regulatory authorities, the very bodies with most to gain from the information.

Concluding remarks

There is an increasing body of evidence from industrial and developing countries that current GM crops, in conjunction with conventional agricultural practices, can offer a sufficiently safe and effective technology that may contribute to a better, cost effective, sustainable and productive agriculture (James, 2002). Experience of the last 5 years has demonstrated that the promises of current GM crops have met the expectations of large and small farmers in both industrialised and developing countries, and established an appreciable market share. The discussion whether we or others can afford to ignore such benefits deserves more attention and support. The risk of not using GM crops, particularly in relation to developing countries where the technology may have most to offer, should also be considered more explicitly. In such discussions, the uncompromising, almost dogmatic, position against GM crops of a representative from a highly influential environmentalist group (see Trewavas and Leaver, 2001) may be as regrettable as it may turn out to be irresponsible. A ban on GM crops could limit the options of farmers and be imprudent rather than precautionary. Governments, supported by the global scientific and development community, must ensure continued safe and effective testing and implement harmonised regulatory programmes that inspire public confidence.

Nowadays, it is almost impossible to enter any GM crop discussion without preconceptions. Polarisation works well in the media. Media coverage, and a diminished public trust in regulatory authorities may explain why GM crops have met rancorous public resistance in Europe (Gaskell *et al.*, 1999; PABE, 2001). There seems a current tendency in Western societies to take the bearers of bad news more seriously than the bearers of good news. Social change and technical innovation is looked upon with a sense of disquiet, and the expected benefits are given less credence than the feared risks. It is very difficult to change such attitudes, as it depends largely on subjective perceptions, enforced by fairly extreme cognitive dissonance (Leisinger *et al.*, 2002). Focussing on the prime goal of the GM crop and making a clear distinction between goals that could also have been accomplished with plant breeding (at supposedly a time loss) and goals that could not, may depolarise discussions.

Many of the crop traits being modified by transgenes are the same as those being targeted for many years by plant breeding (Dale, 1993; Conner *et al.*, 1997). The impacts identified for GM crops are therefore very similar to the

impact from traditional breeding and have been the integral part of agriculture for many years. Consequently, the risks of growing most GM crops on the environment or ecosystems will be similar to the effects of growing, processing and consuming similar new cultivars from traditional breeding (Conner, 1997). In view of the problems of current day agriculture, it will be largely counterproductive to re-evaluate the potential environmental effects of traditionally bred crops (NAS, 2002; Gewin, 2002). Overall, the potential environmental and ecological impact of GM crops, when framed in the context of current-day crops, technologies and practices, if not neutral or innocuous, should in many cases be judged preferable to the impact of the practices the GM crops are designed to replace. The challenge is then to identify as efficiently and as early as possible, the few examples for which this is not the case. Whenever and wherever unresolved questions arise concerning undesired impacts of GM crops, science-based evaluations should be used on a case-by-case approach to answer them to the best of our ability. The risk assessments conducted to date have used the best available information and should continue to do so.

It is often stated that regulation should be based on the soundest science possible, while acknowledging the limits to certainty. Science may be in itself an ideology, but in our judgement it is the best approach for addressing complex issues in a debate. Science can help to define the kind of evidence that would be sufficient and/or would satisfy sceptics in socio-economic perspective. The increased knowledge underpinning GM crops provides a greater confidence in the assurances that science can give when evaluating and monitoring the impacts of GM crops relative to traditional breeding. The resulting regulation is not a static activity but needs continuous re-visiting based on that increased knowledge and experience.

A major problem arises when the general public demands that 'no risk' can be demonstrated, since more than a training in plant or life sciences is necessary to resolve the issues. In this, the plant scientists have a special responsibility. In the continuous struggle for funding we have become very good in convincing public sources that 'more research is required'. Now that biosafety is 'hot', research issues are translated in this context. We are still learning to realise the impact of such endeavours, and still need to make better distinctions between 'nice to know' and 'need to know' in biosafety research. Perhaps, it is also time for plant scientists to rediscover that "*one of the noblest tasks of a scientist is to make out of fact, public opinion*" (Arendt, in Ammann, 2000).

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