# Estimating the Potential for Ecological Harm from Gene Flow to Crop Wild Relatives

*By* Mike J. Wilkinson and Caroline S. Ford,
*Institute of Biological Sciences, University of Wales, Aberystwyth,*
*Ceredigion, SY23 3DA, UK*
*email: jjw@aber.ac.uk*

## Abstract

The exchange of genetic material between crops and their wild relatives is not new but raises fresh environmental concerns with Genetically Modified (GM) crops because of the novel properties of the transgenes they contain. Consequently, GM crops are invariably subject to regulation based on a case-by-case assessment of the risks presented to the environment. These risks vary according to the crop, the transgene(s) and geographic region. As the number of GM crops-transgene-geographic region combinations grows, the challenge facing scientists and regulators is to avoid overload of the regulatory system. This requires the development of generic approaches to risk assessment and the assembly of information that relates to as many submissions for release as possible. In this review, we explore the importance of focussing on the hazard (i.e. the unwanted environmental outcome arising from gene flow) as a means of directing risk assessment research and of prioritising which scenarios should be subject to most scrutiny. In large measure, our ability to compare and rank hazards depends first on our capacity to identify all that are of any relevance. We compare and contrast three approaches that have been used for hazard identification: inspirational, exposure-based and endpoint species-based. We conclude that concerns raised for a particular GM release are profoundly influenced by the hazards we identify. Furthermore, if all hazards are afforded equal weighting by taking the premise that 'all change is bad', grounds can potentially be found to refuse release of all new cultivars (GM and conventional). We must therefore take some difficult decisions about which ecological or agronomic changes would be unacceptable within the context of a changing environment. For this, we may need to centre our attention on the fate of species and communities that we most value.

Collection of Biosafety Reviews Vol. 3 (2007): 42-63.

Padriciano 99, 34012 Trieste, Italy.
http://www.icgeb.org/biosafety/publications.htm

**Riassunto**

Lo scambio di materiale genetico tra colture agricole e specie selvatiche geneticamente correlate non è un problema nuovo, ma con l'introduzione degli OGM vengono sollevate comunque nuove preoccupazioni per l'ambiente a seguito delle caratteristiche conferite dai transgeni che gli OGM contengono. Come conseguenza di ciò le colture geneticamente modificate sono inevitabilmente soggette a regolamenti basati sulla valutazione "caso per caso" dei rischi che l'OGM comporta per l'ambiente. Questi rischi variano a seconda della coltura, del transgene e della regione geografica in questione. Con l'aumentare del numero di combinazioni tra coltura transgenica, transgene e regione geografica, la sfida con cui si devono confrontare scienziati e legislatori è di evitare un sovraccarico del sistema legislativo. Ciò richiede lo sviluppo di approcci generici alla valutazione del rischio e la raccolta di informazioni in relazione a quante più domande di rilascio possible. In questa recensione viene esplorata l'importanza di focalizzare l'attenzione sul rischio (ad esempio l'effetto indesiderato sull'ambiente derivante dal flusso genico) come mezzo per indirizzare la ricerca nella valutazione del rischio e per dare priorità agli scenari che dovrebbero essere oggetto di più accurata analisi. In larga misura la nostra capacità di confrontare e di classificare i rischi dipende dalla possibilità di identificare tutti quelli di una certa rilevanza. Abbiamo così confrontato e contrapposto tre approcci che sono stati usati per l'identificazione dei rischi: quello ispiratore, quello basato sull'esposizione, e quello basato sulle specie recettori finali. Abbiamo concluso che le preoccupazioni sollevate da un rilascio ambientale di OGM sono profondamente influenzate dai rischi che vengono identificati. Inoltre se a tutti i rischi viene riconosciuto un eguale peso assumendo che "tutto ciò che cambia è male", si può facilmente stabilire che nessuna nuova varietà può essere rilasciata (sia GM che convenzionale). Bisogna quindi prendere alcune decisioni difficili su quali cambiamenti ecologici o agronomici possono essere accettati nel contesto di un cambiamento ambientale. Per questo potremmo aver bisogno di concentrare la nostra attenzione sul destino di specie e comunità che maggiormente teniamo in considerazione.

## 1. INTRODUCTION

The exchange of genetic material between crops and their wild relatives has occurred in both directions since the dawn of agriculture. From the context of crop improvement, the introduction of genes into crops from crop wild relatives (CWRs) has been a formal feature of plant breeding for around a century, with modern plant breeders using a range of increasingly sophisticated techniques in order to transfer desirable features from CWRs. These efforts have naturally focussed on the targeted introduction of genes providing traits of agronomic interest, particularly those relating to disease resistance and tolerance of abiotic stress. Examples are manifold and include (amongst others) the introduction of Potato Virus X, Potato Virus A and Potato Virus Y resistance from *Solanum brevidens* into potato by somatic hybridization (e.g. Valkonen and Rokka, 1998) and the recruitment of drought tolerance into wheat from its relatives by bridging crosses (e.g. Valkoun, 2001). However, it was not until the advent of genetically modified (GM) crops that serious concerns were raised over the possible environmental consequences of gene flow from crops to their relatives. This technology allows for individual genes and small gene complexes to be assembled from any living organism and inserted into the crop of interest. This capacity frees the breeder of the strict constraints imposed by breeding barriers that had hitherto prevented the passage of germplasm between species belonging to different families. The breeder is also able to combine elements relating to the control of gene expression and can even create entirely synthetic genes previously unknown in the natural world. These features confer huge potential benefits to mankind at a time when the combined threats of continuing global population growth, reduced scope for agricultural extensification, and global climate change all threaten global food security. Nevertheless, commercialisation of GM crops is a topic that evokes strong and often polarised views amongst scientists and public alike. Controversy stems from the balance between the clear benefits that may accrue from exploitation of the technology and the many possible risks that could arise from the widespread cultivation of such material. We can broadly divide risks relating to GM crops into those associated with effects on human or animal health, and those impacting on the wider environment. Health risks largely centre on the possible exposure of animals or humans to the protein products of the transgene (e.g. Lack, 2002) and are not examined here. The risks posed by GM crops into the wider environment are more complex to identify and far more difficult to quantify. There are three areas that warrant attention: implications of changed farm practice; economic consequences of crop-to-crop gene flow; and the possible ecological consequences following transgene movement into wild relatives of GM crop plants. In acknowledgment to these concerns, various legislative frameworks have been constructed in stakeholder countries where GM crops are either grown or used for food processing. In all instances, decisions are currently made on a case-by-case basis, in which data provided by the organisation seeking approval for commercial release is considered by regulators, together with that generated by non-affiliated scientists, and used as a basis for the decision to approve, delay, restrict or deny release. Whilst such data encompasses a wide range of scientific disciplines, it is important to emphasise that the majority of works purporting to relate to risk assessment do not actually help in the regulation of submissions, even though they may provide better insights into community ecology, population ecology, pollen dispersal, agroecosystem function and plant/disease interactions. Viewed in this context, it is the primary responsibility of scientists in the field of GM risk assessment to provide generic data that genuinely assists in decision-making process.

## 2. SIZE OF THE CHALLENGE

The first generation of GM crops are notable for the lack of diversity in the transgenes that they carry and in the number of crops carrying them. Indeed, four crops (maize, soybean, cotton and rapeseed) and just two transgene types (insect resistance and herbicide tolerance) (James, 2006) currently dominate the commercial GM varieties grown worldwide. This simple situation has allowed for a considerable literature to amass on the environmental risks posed by a relatively small number of scenarios. This situation is set to change in the near future; both recent trends toward the generation of GM lines with multiple inserts (Wilkinson *et al.*, 2003) and substantial diversification of the transgenes being introduced (Dunwell, 2002) means that regulation of GM crops will need to become increasingly sophisticated to accommodate the new set of potential hazards that diversification brings (Raybould and Wilkinson, 2005). From the viewpoint of non-affiliated scientists, this means that there is an increasing need to provide data that applies to multiple submissions, ideally those that relate to all GM events produced for a particular crop.

### 2.1. Preparing for risk assessment

The first task for any risk assessment process is to define the nature of the concern. This is known as 'hazard specification'. The aim here is to state what exactly it is that we are concerned about and so wish to evaluate. In the case of GM crops, as described above, concern relates to possible effects on human or animal health, indirect influences on the broader environment mediated by changed farm practice and the possible consequences arising from gene flow from the crop. In this review, we will concentrate on the environmental consequences of gene flow. This can be divided into transgene movement to other (usually non-GM) crops and to CWRs. The former generally causes economic rather than ecological problems but the latter could invoke a wide range of possible unwanted outcomes that can be broadly categorised as follows: gene flow will change the abundance of the CWR within its community; allow the CWR to invade new habitats/geographic ranges; cause change to the genetic diversity of the CWR (by introducing genetic sweeps); dilute the genetic integrity of the CWR (genetic swamping); change fitness aspects of the CWR such that it impacts on the abundance of associated flora, fauna or disease-causing agents.

### 2.2. Risk assessment terminology

At this point, it is useful to define some terms that are central to risk assessment. **Risk** is a balanced evaluation based on the severity of a particular unwanted outcome and the likelihood that it will occur, and can be usefully defined by the formula:

$$\text{Risk} = f\,(\text{hazard, exposure})$$

The '**hazard**' term represents the severity of the unwanted environmental change and often relates to a particular species. This element inevitably involves some subjectivity and is usually semi-qualitatively represented (e.g. severe, moderate or low). In essence, the hazard element defines how unwanted the environmental change is. The '**exposure**' term on the other hand, represents the probability that the hazard will occur and so should be quantifiable, provided the hazard is adequately defined. We quantify the risk by combining a well-defined hazard with the probability of occurrence (exposure). Viewed in this manner, the process of '**risk assessment**' involves the cumulative assessment of all 'risks' that relate to a specified crop in a particular area, with each risk being an assessment of 'how bad' a particular hazard is and how likely is it to occur. The first task when considering the risks posed by a particular GM cultivar in a specified geographic location (generally country) is to assemble a comprehensive list of hazards.

### 2.3. Specifying the hazards

Fish species which have been subjected to GM technology fall into two It is clearly possible to assemble a long list of hazards for all crop-wild relatives given sufficient time and imagination, although many of these may ultimately prove to be trivial or else extremely unlikely. In the past, the process of assembling this list has been rather haphazard but future diversification of the global GM crop portfolio means that system will need to be introduced into this task. There are essentially three methods that can be deployed to identify hazards relating to a specified GM plant:

1. Inspiration
2. Exposure-based
3. Endpoint species-based

We will next examine each of these approaches in turn.

#### 2.3.1. Inspirational hazard identification

The experience and imagination of stakeholders involved in the regulation, provision and risk assessment of GM crops represents by far the most widely applied means of defining hazards associated with the release of a particular event. In many instances the relationship between the transgene and the perceived hazard is both simple and direct, and requires little in the way of inspiration from the workers involved. For example, hazards associated with the spread of GM herbicide tolerant (HT) weeds represent a relatively simple and direct link between hazard and transgene function. In many cases, however, there is also potential for more cryptic influences of a transgene following its movement from the bounds of the GM crops. It is relatively easy to envisage a series of rather fanciful scenarios that can give rise to such indirect hazards, with the potential transfer of transgenes conferring antibiotic resistance to soil micro-organisms via horizontal gene transfer (de Vries *et al*., 2004) and thence to human gut flora by some means representing a particularly germane example (Macovei and Zurek, 2006). Nevertheless, there is genuine scope for indirect hazards of genuine concern being overlooked during the risk assessment process. In this context, the work of Losey and colleagues (1999) undoubtedly provides a stark illustration of the dangers of complacency when assembling a provisional list of hazards on the basis of experience and expert opinion. This highly controversial study argued that pollen shed by GM *Zea mays* containing the *cry3A* gene that confers resistance to lepidopteran pests may inadvertently cause the death of the high profile Monarch butterfly and lead to significant population decline if sufficient quantities of pollen accumulate on milkweed leaves, the favoured foodplant of the Monarch. This study was much criticised because it provided no measure of exposure of the Monarch to the toxin (Gatehouse *et al*., 2002) and an elegant series of subsequent studies clearly demonstrated only a negligible likelihood of this scenario giving rise to significant changes in Monarch numbers (e.g. Sears *et al*., 2001). However, whilst these works clearly demonstrated that *cry3A* posed negligible risk, it could be reasonably argued that the earlier work of Losey *et al*. (1999) was valuable in that it did identify a plausible hazard that warranted investigation. Viewed in this context, then, the study of Losey *et al*. (1999) seems entirely justified as a viable investigation aimed directly at indirect 'hazard-identification'. On the other hand, this case clearly uncovers some serious problems with regards to the risk assessment process. The fundamental flaw with adopting 'inspiration and experience' as the sole basis upon which to define potential hazards lies in the very real prospect that some nuance of trangene function may be overlooked and with it, the potential for highly damaging ecological or agronomic damage. This has been euphemistically termed the danger of failing to consider 'unknown unknowns'. Ultimately there are several inherent problems with the inspirational approach. In particular, this strategy favours:

- The first hazards to be identified
- The dramatic 'eye catchers'
- Direct effects
- Biased by interests of researchers
- Wastes resources on low priority hazards

There is consequently a real and tacit need for a more reliable system for identifying potential hazards associated with the commercial release of specified transgenic events.

### 2.3.2. Exposure-based hazard identification

Collective consideration of all or most hazards relating to a particular recipient species represents a useful alternative to the inspirational method of hazard identification. When taking this approach it is important to remember that the mere presence of a transgene in a hybrid rarely if ever constitutes an ecological hazard in its own right but can do so by triggering a number of sequential events that culminate with the realisation of a potential hazard. An illustrative hypothetical example is given in Figure 1.

There are typically numerous such scenarios for the vast majority of crop-transgene-locality combinations and so a complex matrix of inter-related events provides a more realistic representation of the indirect hazards likely to arise from gene flow (Figure 2).

Despite this complexity, if one is able to discount any of the generic elements of the pathways (initial hybridisation, introgression and transgene spread) then all hazards arising from gene flow can be effectively discounted. This means that any potential recipients that are unable to form hybrids, produce stable introgressants and/or disperse to other populations can be effectively discounted from consideration. It follows that all hazards remaining pertain to recipients with a realistic chance of

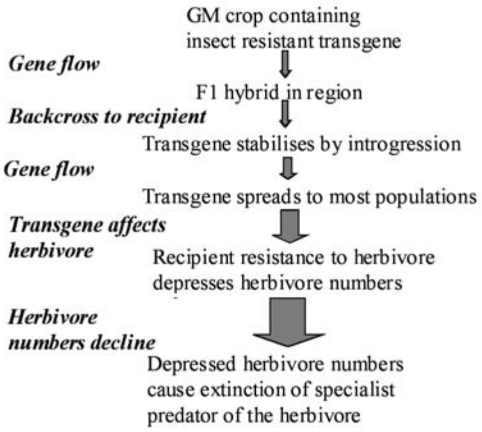

**Figure 1.** *Hypothetical scenario describing how a transgene conferring insect resistance could lead to the local extinction of a specialist predator following gene flow from the crop to a wild recipient*

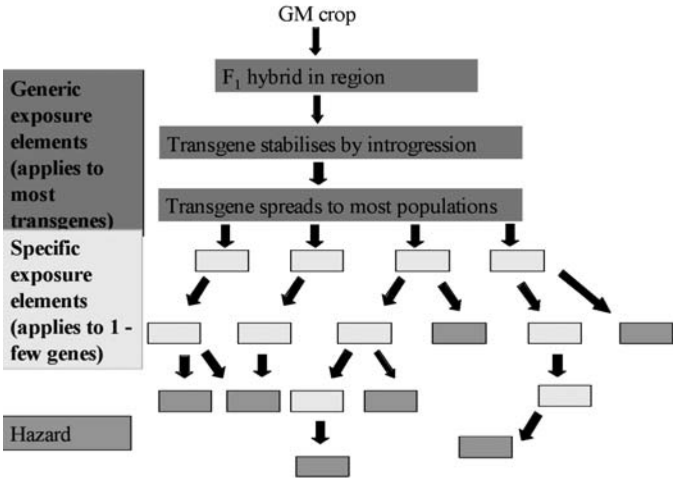

**Figure 2.** *Diagramatic representation of inter-related pathways by which gene flow from a GM crop could lead to various hazards in the recipient and associated flora and fauna. Hypothetical hazards (grey boxes) terminate all pathways, with interim events (exposure elements) either being generic and so applying to all pathways (darkest boxes) or else specific to one or more hazards (palest boxes)*

transgene recruitment. The natural extension and widely adopted ethos of this thinking is to assign the highest priority to those relatives that receive the highest rate of gene flow from the crop. The relatives of rapeseed (*Brassica napus*) in the United Kingdom provide an appropriate example of this kind of informal hazard prioritisation that has been widely adopted by the risk assessment community.

Various workers (Raybould and Gray, 1993; Warwick *et al.*, 2003; Scheffler and Dale, 1994; Ford *et al.*, 2006) have collectively established that there are sixteen wild relatives of rapeseed in the UK into which transgene recruitment via gene flow is a plausible possibility. These can be crudely ranked by the relative ease with which these species hybridise with rapeseed and so with the frequency with which transgenes are likely to be recruited by each recipient (Table 1).

*Brassica rapa* is widely acknowledged to head this ranking and, as a consequence, has been subjected to more intensive GM risk assessment.

| **Recipients Species** | **Hybridisation Ranking*** |
|---|---|
| _Brassica rapa_** | 1 |
| _Brassica juncea_* | 2 |
| _Brassica oleracea_** | 3 |
| _Brassica carinata_* | 4 |
| _Brassica nigra_** | 5 |
| _Raphanus raphanistrum_* | 6 |
| _Diplotaxis muralis_* | 7= |
| _Diplotaxis erucoides_* | 7= |
| _Sinapis arvensis_* | 9= |
| _Sinapis alba_** | 9= |
| _Brassica tournefortii_* | 11= |
| _Diplotaxis tenuifolia_* | 11= |
| _Eruca vesicaria_* | 11= |
| _Raphanus sativus_* | 11= |
| _Erucastrum gallicum_$^{+}$ | 15= |
| _Hirschfeldia incana_** | 15= |

**Table 1.** Wild relatives of rapeseed in the United Kingdom ranked according to their ease of hybridisation with the crop.

Rankings follow those given by Scheffler and Dale (1994) as adapted by Ford *et al.* (2006). Source references are indicated as follows: **Raybould and Gray, 1993; $^{+}$Warwick *et al.*, 2003; *Scheffler and Dale, 1994

However, there are tangible problems with using such lists even as a start point from which to select a recipient for study. One cause for concern relates to instances where hybridisation is unreported in the wild but is known from laboratory experiments to be possible (albeit difficult). *Brassica oleracea* provides a suitable example of this kind for rapeseed. Although this species is widely believed to be a progenitor of the *B. napus* crop, hybridisation between the two species was known to be technically demanding and at the time that Scheffler and Dale compiled their species ranking (Table 1) there were no reports of any spontaneous hybrids in the wild. Partly as a consequence of such works, hazards relating to *B. oleracea* have been largely overlooked by the risk assessment community. In no small measure, the absence of hybrids and introgressants could be attributed to the considerable effort required to find such rare individuals on a landscape scale. However, Ford *et al.* (2006) recently discovered F1 hybrids and introgressed *B. oleracea* plants growing in close proximity to rapeseed fields, thereby demonstrating the genuine scope for transgene recruitment by this species in the wild should GM rapeseed be grown in this area. More significantly, this finding means that groups of hazards previously overlooked because of the low perceived probability of stable transgene recruitment (hybridisation and introgression) now warrant serious examination. Ford *et al.* (2006) identify the acquisition of the Lepidoptera-specific *cry1A* transgene from *Bacillus thuringiensis* (Bt) into *B. oleracea* as being one possible cause of concern because of the presence of a national red book 3 (RDB3)-listed micro-moth species *Selenia leplastriana* (Kent County Council, 2006) that uses *B. oleracea* as a larval food source within the introgressed populations.

Problems of this kind associated with the informal and passive prioritisation of potential recipients becomes even more pronounced for widespread weeds, where even extremely low hybridisation frequencies could be expected but contact with the crop is so vast that even miniscule rates of introgression could still yield many hybrids on a landscape scale. For instance, whilst 1 in 100 pollinations produce hybrids between rapeseed and the very common weed, *Sinapis arvensis* using ovule culture (Lefol *et al.*, 1996), until recently extensive screening of millions of naturally set seeds by several groups had yet to uncover a single confirmed hybrid (Bing *et al.*, 1996; Lefol *et al.*, 1996; Chèvre *et al.*, 2003; Moyes *et al.*, 2002). However, the unconfirmed report of one putative transgenic hybrid containing resistance to glufosinate ammonium by Daniels *et al.* (2005) raises significant cause for concern. Faced with such cases, regulators are either to adopt a precautionary stance and assume that gene flow does occur and assess risk on the basis of consequence(s) or else discount transgene movement unless proved otherwise. Should the regulators elect to take the former approach, then there is a need to decide how much research effort is directed to hazards relating to a possible recipient with only theoretical exposure to transgene recruitment. In any event, the production of even one fertile spontaneous hybrid would radically change how such recipients are viewed.

When considered in this context, formal or passive ranking of hazards based on the likelihood of gene flow into a particular recipient seems fraught with difficulty. Indeed, many authors have long argued that the amount of gene flow is largely irrelevant since the appearance of a single fertile hybrid has the theoretical capacity to spread and cause ecological or agronomic harm (e.g. Ellstrand *et al.*, 1999; Chapman and Burke, 2006). This line of thinking is extremely persuasive in an ecological or evolutionary context where there are effectively no limits on timeframe over which rare events may occur. However, the commercial agricultural environment differs profoundly from most natural communities in the time frame and context in which most events operate. The commercial life expectancy of cultivated varieties of most economically important crops, including those for which GM varieties are currently available, is generally less than 1-2 decades. This provides a finite limit to the temporal exposure of a recipient to any transgenes. Likewise, the physical space occupied by many crops is defined (by field boundaries), often partially isolated from natural or semi-natural communities and frequently subject to regular change (e.g. by crop rotation or changed farm practice). These factors also limit exposure of some recipients (e.g. non-weedy relatives) to transgene recruitment via gene flow.

There is now increasing interest in whether natural and agronomic factors constraining gene flow could be augmented by genetic or physical interventions (known as biocontainment measures) to effectively discount the possibility of trangene recruitment. Before such technologies can be adopted with confidence, however, it is vital that we gain some measure of their likely efficacy. The pertinent question being posed is "does this biocontainment technology effectively remove the possibility of transgene recruitment and spread to a specified recipient in a specified region within a given time frame?" Evaluation of biocontainment therefore requires an estimate of the extent to which hybridisation is reduced for a given crop-recipient combination and, crucially, a realistic estimate of the annual number of hybrids expected between these species within the geographic region under consideration. The latter has relevance to all biocontainment techniques and so should be the first element to be estimated.

In order to have maximum utility to the regulatory process, any estimate of hybrid number must match the scale at which legislative decisions are being practiced; in most cases this will be at the national or regional scale. This is a demanding task and will almost inevitably require extensive modelling given the impracticability of identifying all hybrids across a landscape for most crop-recipient combinations. The aim of the model should be to both establish a realistic broad estimate of hybrid number, together with an estimate of the error terms involved.  As with all predictive models, the key to success lies in the assembly of appropriate empirical measures for parameterisation. In order to assemble estimates of hybrid number it is necessary to obtain; realistic descriptions of the size and spatial distribution of crop and recipient populations, measures of the gene dispersal characteristics of the crop, a description of the various contexts of contact (i.e. as a weed or as allopatric or sympatric wild populations; see Box 1.) and a measure of the survival of hybrids as living plants.

### BOX 1: Definition of ecological terms

| | |
|---|---|
| **Allopatric populations** | Pairs or groups of populations occurring in separate, non-overlapping areas. Often used of populations of related organisms unable to crossbreed because of large physical separation in distance |
| **Sympatric populations** | Pairs or groups of populations occupying the same, overlapping or physically adjacent sites such that the distance between them presents no or minimal impediment to crossbreeding |

To date, Wilkinson *et al*. (2003) have provided the only example of a national scale estimate of hybrids between any crop and wild relative when they surveyed the capacity for hybridisation between rapeseed and B. rapa in the UK. In doing so, the authors distinguished between hybrids formed locally during sympatry and those formed over longer distances. This action was justified on the basis of the sample sizes required to produce hybrid frequency estimates. Moreover, whilst it is tractable to collect sufficient local hybrids to generate reasonably robust estimates of short-range hybrid formation, this is not possible over longer distances because the extremely low frequencies of hybrid formation mean that even large screens of individuals are likely to yield insufficient statistical power to allow meaningful estimates of hybrid abundance.  For this reason, local hybrids were parameterised empirically whereas long-range hybrids were predicted on the basis of pollen dispersal curves. The broad strategy adopted by the authors to estimate local and long-range hybrids between rapeseed and *B. rapa* is briefly outlined below:

### *2.3.2.1. Estimating hybrid frequency*

When examining global numbers of hybrids formed with *B. rapa*, a distinction was made between the two settings in which *B. rapa* commonly occurs in the UK; as an agricultural weed, and as wild populations growing along the margins of water courses. These settings differ profoundly in the nature of contact with the crop and so were considered separately.

In the case of weedy *B. rapa*, the recipient populations are typically dispersed plants surrounded by an excess of crop plants. In this situation, it should be remembered that *B. rapa* is self-incompatible and receives large quantities of rapeseed pollen via insect vectors, mechanical contact and wind compared to relatively small amounts from occasional conspecific individuals within the field. Long-range hybrids could therefore be effectively discounted from the model. However, it should also be noted that hybrid seed abundance within fields does not necessarily translate into hybrid plant frequency since hybrids may exhibit differential survival characteristics and seed dormancy profiles. Indeed, a survey of five dispersed fields revealed just 1.9% (± 0.5% 2SEM) F1 hybrid plants within the weedy population. This seems at wide variance to the earlier seed-based estimate of 60% hybrid seed amongst isolated *B. rapa* individuals surrounded by rapeseed, although cognisance should be taken of the clusters of *B. rapa* plants and critically, of the dual effects of seed dormancy and crop rotation. Linder (1998) reported that whilst weedy *B. rapa* exhibits 60% seed dormancy, only 10% of $F_1$ hybrids are usually dormant. This difference introduces unexpected bias into hybrid seed estimate since these seeds were not treated to break dormancy and so many of the *B. rapa* seeds remain ungerminated. Correcting for this factor provides a revised estimate of 17% hybrid seed. This is remarkably close to an observation of 11-14% taken from *B. rapa* plants growing in the above five populations, but is still some distance from the 1.9% of living plants observed in these sites. This requires further thought on the consequences of differential seed dormancy between *B. rapa* and $F_1$ hybrids. Such divergence means that weedy B. rapa and the $F_1$ hybrids will show different seedling emergence characteristics, with >90% of hybrids appearing in the year following hybridisation compared to 0-40% of weedy *B. rapa*. Given that rapeseed is typically followed by wheat in the 3-4 year crop rotation prevalent in the UK, this means that hybrids will predominantly appear as plants in wheat where they are extremely effectively controlled by the use of various broadleaf herbicides. Furthermore, the remaining years of rotation also usually feature wheat or another cereal such as barley. Thus, the time that rapeseed next appears as a crop (and so the *B.rapa*/$F_1$ hybrids can survive) will be 3-4 years following hybridisation, by which time the proportion of $F_1$ hybrids should have declined to 0.1% (0.01/8.9)-1.1% (0.012/11.1) of the total population size (Table 2). This range shows a remarkably close concordance with the empirical observation of 1.9 (± 0.5) %.

Having established the expected proportion of hybrid plants within an average population, the estimate of hybrid numbers was established by combining estimates of the frequency of infested fields (from sample surveying) with the observed mean and variance in weed population size. In this case, it was estimated that 128 (0.35%) of the 37,000 rapeseed fields contain *B. rapa*, and that a mean national population size lies at 195,000 plants (128 populations x 1523 plants/ population). Combining these figures allowed a global estimate of 3,800 hybrids *per annum* (195,000 plants x 0.019 hybridisation rate).

Wild, riverside *B. rapa* posed a different array of problems. The first requirement was to establish how many fields of rapeseed lay adjacent to rivers containing *B. rapa*. Riverside rapeseed fields were identified by overlaying information from remote sensing onto digital river location information. Rivers containing *B. rapa* and their distribution along those rivers

| Relative Seed Band Constitution (%) | | | | |
|---|---|---|---|---|
| | *B. rapa* | | $F_1$ hybrids | |
| Year | % residual not germinating | % germinating | % residual not germinating | % germinating |
| 0 | 87 | 0 | 13 | 0 |
| 1 | 69.6 | 17.4 | 1.3 | 11.7 |
| 2 | 55.7 | 13.9 | 0.13 | 1.17 |
| 3 | 44.6 | 11.1 | 0.013 | 0.012 |
| 4 | | 8.9 | 0.001 | 0.001 |

**Table 2.** Decline in relative abundance of $F_1$ hybrids with years in rotation assuming 20% germination *per annum* for *B. rapa* and 90% for year 1 for hybrids. 100% mortality assumed during emergence in wheat/barley

was determined through a combination of foot, literature and herbarium surveys. This information allowed for a global estimate of 1.8 million riverside *B. rapa* plants growing within 30m of a field of rapeseed. Hybridisation rates at such sites of sympatry were determined empirically, with 47 hybrids found among 3230 plants from eight sympatric populations. Given 1.8 million sympatric *B. rapa* plants and this hybridisation rate of 1.46% (± 0.43%, 2SEM Poisson error estimate), it was thereby estimated that 26 (± 22, 2SEM) thousand hybrids occur within sympatric waterside *B. rapa* populations.

Projecting the long-range hybrid numbers presents more of a challenge because of the extremely low numbers of hybrids encountered over even modest distances (none were found beyond 20m of the crop margin). One route around this problem is to use airborne pollen dispersal patterns as a proxy for gene flow, assuming that the relative success of the inter-specific pollination does not decline disproportionately with distance. For rapeseed, the added assumption is that insect-borne dispersal patterns will be broadly similar to those expected via wind. Reference to the remote sensing imagery (providing the location of rapeseed fields) and to the spatially explicit survey data of riverside *B. rapa* allowed for the classification of plant number across various isolation distances from rapeseed fields. Pollen dispersal equations were then used to estimate the amount of airborne pollen delivered at these distances but does not provide a direct basis upon which to estimate hybrid numbers. Calibration of airborne pollen density to hybrid seed frequency was therefore performed from three sites where crop and *B. rapa* were separated by 1m. This allowed a relationship to be inferred between pollen density and a robust measurement of hybrid frequency. When this strategy was applied across all distances, the cumulative total estimate of long-range hybrids was just 5,600 (± 4300, 2SEM) across the entire UK. This dominance of short-range hybrid formation over long-range pollination can be explained by the relatively high instance of sympatry between rapeseed and riverside *B. rapa.* Taken collectively, therefore, this study predicted 32,000 (± 26,000) hybrids form annually on riverside *B. rapa* and 17,000 (± 16,000) form on weedy *B. rapa* across the UK, with most hybrids forming in Central and Eastern England. This value sets a demanding target for any biocontainment measure aiming to prevent hybrid formation in the UK and requires a hybrid repression of at least $10^{-6}$ before one can confidently predict no hybrids will arise within the 5-10 life expectancy of a GM cultivar. Given that this species is the recipient that is most exposed to gene flow from the crop, lower hybrid repression efficiencies may still have value for some of the potential recipients that are less easily crossed with the crop. The example also serves to highlight the importance of considering context and geographic area when considering the feasibility and value of biocontainment strategies; one would expect much greater repression efficiencies would be required for GM crops in large countries with vast areas of crop cultivation such as the USA, Canada or China.

#### 2.3.3. Hazard prioritisation based on endpoint species

Whilst the informal or formal ranking of recipients based on ease of hybridisation has value for biocontainment and other risk management strategies, it has limited value in terms of prioritising which hazard warrants detailed or tiered examination. This is because exposure of the recipient to gene flow does not indicate the number or severity of ecological, evolutionary or agronomic hazards that may result from transgene assimilation. That is, the likelihood of transgene presence provides little or no measure of the scope for environmental harm (see Raybould and Wilkinson, 2005). Instead, this capacity resides in the nature of the recipient itself and in the scale and nature of interactions between the recipient and associated flora and fauna. Collectively, these species can be considered as potential endpoint species, since any could conceivably be the species negatively impacted upon by the transgene. Indeed, Ford *et al*. (2006) proposed that the conservational, cultural, or ecological scarcity of possible endpoint species as an alternative basis upon which to rank the possible recipients of gene flow from GM crops. In this study, they compared the diversity and conservation status of species associated with potential recipients of transgenes from GM rapeseed. The work focused particularly on the two closest relatives of the crop; *B. rapa* and *B. oleracea*. The most direct impacts of any transgene would be to cause a decline in the recipient itself, although one needs to question whether this outcome is as important as less obvious, indirect hazards pertaining to more valued species or to the community as a whole.

In this instance, it was noted that none of the potential recipients of gene flow from rapeseed that grow in the UK are rare and the majority are not native and/or problematic weeds. Furthermore, all are relatively common in a global context. Only *B. oleracea* was sufficiently abundant in some of its native habitats to be considered as a defining species of its communities (Rodwell, 2000). Thus, whilst dramatic decline in *B. oleracea* may be cause for concern in affecting community structure and function, decline would need to be extensive and widespread to be of highest concern. Here also, the severity of the harm caused depends on the importance placed on the community affected. Ford *et al*. (2006) examined this element by comparing the number and legal conservation status of plant and insect species associating with *B. rapa* and *B. oleracea* by reference with the plant community structure literature and by direct surveys. They reported that *B. oleracea* dominates sea cliff communities in the White Cliffs area of Kent that contain 321 plant species, 53 species of bird and 27 butterflies. This population occupies a location designated as a Site of Special Scientific Interest (SSSI) and contains several species of national conservational importance protected under the UK Wildlife and Countryside Act, 1981. The area was also found to support 12 red-listed bird species (Gregory *et al*., 2002) and four plants categorised as 'endangered', three as 'vulnerable' and six as 'near threatened' according to IUCN threat categories (Cheffings *et al*., 2005). Extreme care must be exercised in interpreting such findings because the simple co-location of a recipient with a 'red-listed' species does not constitute a problem in itself and only provides a starting point for hazard identification. There was one relationship identified as needing further evaluation; the RDB3-listed micro-moth species *S. leplastriana* (Kent County Council, 2006) uses *B. oleracea* as a larval food source. Acquisition of *cry1A* into *B. oleracea* plants used by these moths was judged to have the potential to cause local decline in moth numbers. However, the true value of such figures lay in allowing direct comparisons between recipients. For instance, when the community associates of *B. oleracea* were compared with those of the much studied *B. rapa*, there was some cause to re-examine existing research priorities. Moreover, the 19 *B. rapa* riverside communities studied included just 110 plant species, none of which is protected by law or possessed recognised conservational status (Cheffings *et al*., 2005). Whilst the diversity and scarcity of associates does not relate directly to ecosystem function and does not consider the social importance of some species, it is nevertheless a valuable perspective from which to compare recipients of gene flow. Viewed in this context, the authors argued that GM *B. oleracea* introgressants apparently possess greater scope to harm species of conservational significance than do those of *B. rapa*. For this reason it was maintained that *B. oleracea* probably deserves greater attention than it has received to date.

## 3. CONCLUSION

Clearly the potential for a particular GM release are profoundly influenced by the hazards we compile as part of the risk assessment process. There are two dangers inherent with the hazard identification part of the risk assessment process: firstly, that some important hazards will be overlooked and secondly, that so many potential hazards are identified that grounds can potentially be found to refuse release of all new cultivars (GM and conventional). As we become more proficient at hazard identification, the dangers of the 'ill-founded' rejection of a submission also increase if all hazards are afforded equal weighting. This problem is exacerbated if we adopt the premise that 'all change is bad change'. We must therefore take some difficult decisions about which ecological or agronomic changes would be unacceptable within the context of a changing environment. For this, we may need to centre our attention on the fate of species and communities that we most value. In doing so, however, we may uncover some surprising changes to our current risk assessment activity. This is perhaps best illustrated by extending the approach adopted for *B. rapa* and *B. oleracea* above to consider the associates of all cross-compatible UK relatives of rapeseed. Interestingly, when this is done the species ranking second (*Dipotaxis muralis*) has to our knowledge, yet to feature in any risk assessment study for the crop. If this tenet was to be adopted more generally, we are first faced with a series of difficult choices to make, not least of which being "what changes to our current environment are most and least acceptable?". It is therefore our view that the assembling of associate lists for crop relatives should represent an essential part of generic risk assessment and should feature early in the process as the technology spreads to new areas and as the number of GM crop species grown broadens.

## 4. REFERENCES

**Bing** DJ, **Downey** RK & **Rakow** GFW 1996. Hybridizations among *Brassica napus, B. rapa* and *B. juncea* and their two weedy relatives B. nigra and Sinapis arvensis under open pollination conditions in the field. *Plant Breeding* 115: 470-473.

**Chapman** MA & **Burke** JM 2006. Letting the gene out of the bottle: the population genetics of genetically modified crops. *New Phytologist* 170: 429-443. http://www.blackwell-synergy.com/doi/pdf/10.1111/j.1469-8137.2006.01710.x

**Cheffings** CM & **Farrell** L (eds), **Dines** TD, **Jones** RA, **Leach** SJ, **McKean** DR, **Pearman** DA, **Preston** CD, **Rumsey** FJ, **Taylor** I 2005. *The Vascular Plant Red Data List for Great Britain*. Species Status 7: 1-116. Joint Nature Conservation Committee, Peterborough, UK. ISSN 1473-0154. http://www.jncc.gov.uk/pdf/pub05_speciesstatusvpredlist3_web.pdf

**Chèvre** AM, **Eber** F, **Jenczewski** E, **Darmency** H & **Renard** M 2003. Gene flow from oilseed rape to weedy species. *Acta Agriculturae Scandinavica*, *Section B - Plant Soil Science* 53: 22-25 Supp 1. DOI: 10.1080/16519140310015102

**Crouch** JH, **Lewis** BG & **Mithen** RF 1994. The effect of A-genome substitution on the resistance of *Brassica napus* to infection by *Leptosphaeria maculans. Plant Breeding* 112: 265-278.

**Daniels** R, **Boffey** C, **Mogg** R, **Bond** J & **Clarke** R 2005. The potential for dispersal of herbicide tolerance genes from genetically-modified, herbicide tolerant oilseed rape crops to wild relatives. *Final report to DEFRA, contract reference EPG 1/5/151*. DEFRA, HM Government, London, UK. http://www.defra.gov.uk/science/project_data/DocumentLibrary/CB02006/CB02006_2730_FRP.pdf

**de Vries** J, **Herzfeld** T & **Wackernagel** W 2004. Transfer of plastid DNA from tobacco to the soil bacterium *Acinetobacter* sp. by natural transformation. *Molecular Microbiology* 53(1): 323-334. DOI: 10.1111/j.1365-2958.2004.04132.x

**Dunwell** JM 2002. Future prospects for transgenic crops. *Phytochemistry Reviews* 1: 1-12. DOI: 10.1023/A:1015812332763

**Ellstrand** NC, **Prentice** HC & **Hancock** JF 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30: 539-563.

**Ford** CS, **Allainguillaume** J, **Grilli-Chantler** P, **Cuccato** G, **Allender** CJ, **Wilkinson** MJ 2006. Spontaneous gene flow from rapeseed (*Brassica napus*) to wild *Brassica oleracea. Proceedings of the Royal Society B: Biological Sciences* 273(1605): 3111-3115. DOI: 10.1098/rspb.2006.3686

**Gatehouse** AMR, **Ferry** N & **Raemaekers** RJM 2002. The case of the Monarch butterfly: a verdict is returned. *Trends in Genetics* 18(5): 249-251. http://www2.biologie.unihalle.de/genet/plant/staff/koebnik/teaching/biotech2004/Talks/9_Bt/9_Literatur/Gatehouse_249.pdf

**Gregory** PJ, **Ingram** JSI, **Andersson** R, **Betts** RA, **Brovkin** V, **Chase** TN, **Grace** PR, **Gray** AJ, **Hamilton** N, **Hardy** TB, **Howden** SM, **Jenkins** A, **Meybeck** M, **Olsson** M, **Ortiz-Monasterio** I, **Palm** CA, **Payn** TW, **Rummukainen** M, **Schulze** RE, **Thiem** M, **Valentin** C, **Wilkinson** MJ 2002. Environmental consequences of alternative practices for intensifying crop production. *Agriculture Ecosystems & Environment* 88, 279-290. DOI: 10.1016/S0167-8809(01)00263-8

**Herrmann** D, **Boller** B, **Studer** B, **Widmer** F & **Kolliker** R 2006. QTL analysis of seed yield components in red clover (*Trifolium pratense L.*). *Theoretical and Applied Genetics* 112: 536-545. DOI: 10.1007/s00122-005-0158-1

**Hammer** K & **Laghetti** G 2005. Genetic erosion - examples from Italy. *Genetic Resources and Crop Evolution* 52: 629-634. DOI: 10.1007/s10722-005-7902-x

**James** C 2006. Preview: Global Status of Commercialized Biotech/GM Crops: 2006. *ISAAA Brief No. 35.* ISAAA, Ithaca, NY, USA. http://www.isaaa.org/resources/publications/briefs/35/executivesummary/pdf/Brief%2035%20-%20Executive%20Summary%20-%20English.pdf

**Kent County Council** 2006. *Kent Red Data Book: A provisional guide to the threatened flora and fauna of Kent.* KCC Environmental Management, Maidstone, UK. pp495. http://www.kent.gov.uk/NR/rdonlyres/45546CD6-4BEC-40AC-9D39-0A6C64D4787E/191/kentreddatabook.pdf

**Lack** G 2002. Clinical risk assessment of GM foods. *Toxicology Letters* 127(1-3): 337-340. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6TCR-45YCMMV-J-1&_cdi=5177&_user=2503378&_orig=search&_coverDate=02%2F28%2F2002&_sk=998729998&view=c&wchp=dGLb-VzWzSkWb&md5=01d6fd83c38d84acc4570367160962ee&ie=/sdarticle.pdf

**Lefol** E, **Danielou** V & **Darmency** H 1996. Predicting hybridization between transgenic oilseed rape and wild mustard. *Field Crop Research* 45: 153-161. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6T6M-3VYTHF3-M-2&_cdi=5034&_user=2503378&_orig=browse&_coverDate=05%2F31%2F199&_sk=999549998&view=c&wchp=dGLbVlb-zSkWz&md5=efec25bdd2a10026521182fc032669af&ie=/sdarticle.pdf

**Linder** CR 1998. Potential persistence of transgenes: Seed performance of transgenic canola and wild x canola hybrids. *Ecological Applications* 8(4): 1180-1195. DOI: 10.2307/2640971

**Losey** JE, **Rayor** LS & **Carter** ME 1999. Transgenic pollen harms monarch larvae. *Nature* 399: 214. DOI: 10.1038/20338

**Macovei** L & **Zurek** L 2006. Ecology of antibiotic resistance genes: Characterization of enterococci from houseflies collected in food settings. *Applied and Environmental Microbiology* 72(6): 4028-4035. DOI: 10.1128/AEM.00034-06

**Moyes** CL, **Lilley** JM, **Casais** CA, **Cole** SG, **Haeger** PD & **Dale** PJ 2002. Barriers to gene flow from oilseed rape (*Brassica napus*) into populations of *Sinapis arvensis*. *Molecular Ecology* 11(1): 103-112. DOI: 10.1046/j.0962-1083.2001.01416.x

**Ortiz** R & **Ehlenfeldt** M K 1992. The importance of Endosperm Balance Number in potato breeding and the evolution of tuber-bearing *Solanum*. *Euphytica* 60: 105-113. DOI: 10.1007/BF00029665

**Preston** CD, **Paerman** DA & **Dines** TD 2003. *New Atlas of the British and Irish Flora*. Oxford University Press, Oxford, UK. ISBN: 0198510675. pp910.

**Raybould** AF & **Gray** AJ 1993. Genetically modified crops and hybridization with wild relatives - a UK perspective. *Journal of Applied Ecology* 30(2): 199-219. DOI: 10.2307/2404623

**Raybould** AF & **Wilkinson** MJ 2005. Assessing the environmental risks of gene flow from GM crops to wild relatives. In: *Gene Flow from GM Plants*. Poppy GM & Wilkinson MJ (eds), Blackwell Publishing, Oxford, UK. ISBN: 9781405122375. pp 169-185.

**Rodwell** JS. 1991. *British Plant Communities, volumes 1-5*. Cambridge University Press for the Joint Nature Conservancy Council, Cambridge, UK. ISBN 0-521-62721-4/ 62720-6/ 62719-2/ 62718-4/ 64476-3

**Sears** MK, **Hellmich** RL, **Stanley-Horn** DE, **Oberhauser** KS, **Pleasants** JM, **Mattila** HR, **Siegfried** BD & **Dively** GP 2001. Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proceedings of the National Academy of Science USA* 98: 11937-11942. DOI: 10.1073/pnas.211329998

**Scheffler** JA & **Dale** PJ 1994. Opportunities for gene-transfer from transgenic oilseed rape (*Brassica-napus*) to related species. *Transgenic Research* 3(5): 263-278. DOI: 10.1007/BF01973586

**Stace** CA. 2001. *New Flora of the British Isles* (Second Edition). Cambridge University Press, Cambridge, UK. pp 1130. ISBN-10: 0521589355

**Steele** KA, **Price** AH, **Shashidhar** HE, & **Witcombe** JR 2006. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theoretical and Applied Genetics* 112: 208-221. DOI: 10.1007/s00122-005-0110-4

**Valkonen** JPT & **Rokka** VM 1998. Combination and expression of two virus resistance mechanisms in interspecific somatic hybrids of potato. *Plant Science* 131: 85-94. DOI: 10.1016/S0168-9452(97)00238-0

**Valkoun** JJ 2001. Wheat pre-breeding using wild progenitors. *Euphytica* 119: 17-23. DOI: 10.1023/A:1017562909881

**Wang** YP, **Sonntag** K, **Rudloff** E, **Wehling** P & **Snowdon** RJ 2006. GISH analysis of disomic Brassica napus-Crambe abyssinica chromosome addition lines produced by microspore culture from monosomic addition lines. *Plant Cell Reports* 25: 35-40. DOI: 10.1007/s00299-005-0031-3

**Warwick** SI, **Simard** MJ, **Legere** A, **Beckie** HJ, **Braun** L, **Zhu** B, **Mason** P, **Seguin-Swartz** G & **Stewart** CN 2003. Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) OE Schulz. *Theoretical and Applied Genetics* 107: 528-539. DOI: 10.1098/rspb.2006.3686

**Wilkinson** MJ, **Elliott** LJ, **Allainguillaume** J, **Shaw** MW, **Norris** C, **Welters** R, **Alexander** M, **Sweet** J & **Mason** DC 2003. Hybridization between *Brassica napus* and *B-rapa* on a national scale in the United Kingdom. *Science* 302: 457-459. DOI: 10.1126/science.1088200

**Zhou** AF & **Xia** GM 2005. Introgression of the *Haynaldia villosa* genome into gamma-ray-induced asymmetric somatic hybrids of wheat. *Plant Cell Reports* 24: 289-296. DOI: 10.1007/s00299-005-0922-3