

# Safety of Genetically Engineered Plants: an Ecological Risk Assessment of Vertical Gene Flow

Authors: **Klaus Ammann, Yolande Jacot, Pia Rufener Al Mazyad**  
**University of Bern, Botanical Garden**  
**Altenbergrain 21, CH - 3013 BERN**

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## 1. Introductory remarks

Genetically engineered plants are a hot topic in the public debate in Europe. One concern that gives rise to controversy is the potential longterm environmental risk of the large scale application of genetically engineered crops.

The environmental risks of genetically engineered crops have been categorised as follows (Journal of Molecular Ecology, vol.3, 1994):

- Invasiveness of the transgenic crop (in the agricultural system as a weed or in natural habitats).
- Invasiveness of transgene itself (vertical gene flow<sup>1</sup> through hybridisation with wild relatives).
- Environmental side effects of the transgenic products (for instance effects on non-target organisms).

This study will only deal with the first two environmental risk categories of the use of transgenic crops. The third category is dealt with elsewhere in this report in the paper by Julian Kinderlerer. This study will describe what factors determine the successful hybridisation with wild relatives and it will describe some concepts that are thought to be important for determining the invasiveness of the transgenic crop or the transgene itself. These are the concepts of weediness, fitness and selective advantage. Wherever possible it will be tried to make a healthy comparison with facts known from conventional plant breeding, without jumping to preliminary conclusions or generalisations.

The risk assesment process is very important in determining the possible and actual ecological consequences of the use of transgenic crops. This study will try to gather relevant risk assessment concepts and it will give the example of gene flow indexes as a means of categorising crops into risk classes.

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<sup>1</sup> **Vertical gene flow** is the flow of genetic material from parent plants to their descendants, where these descendants are either clones from their parental plants (for instance a potato plant resulting from a tuber), or where these descendants are the result of mating between sexually compatible organisms. This is in contrast to **horizontal gene flow** which is defined as the transfer of genetic material from one organism to another non-sexually compatible organism (for instance from a potato to a bacterium), and where the organism that has taken up the genetic material is not a descendant from the donor of the genetic material. Horizontal gene transfer is dealt with elsewhere in this report in the paper by Philippe Gay.

## 2. Gene flow and hybridisation

### 2.1. Definition

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The vertical flow of transgenes from genetically engineered crops to wild relatives is a major concern in the environmental risk assessment of genetically engineered crops. Vertical gene flow is a well-known phenomenon in population genetics. It is by no means something new and it is not connected solely to transgenic plants. Vertical gene flow from transgenic plants to wild relatives requires successful hybridisation to take place between the transgenic crop and a wild relative. "Hybridisation" can be defined as the cross-breeding of different individual plants. These individuals may be genetically similar, differ in a few or in numerous genes or be very different genetically. They may belong to various populations or races of the same taxonomic species (intraspecific or interpopulational hybridisation) or to different species (interspecific hybridisation). From an agronomic point of view hybridisation is very important as the major mechanism for generating new varieties of crops through cross-breeding. Most of the current varieties are the result of hybridisation between different existing varieties or hybridisation with wild relatives.

### 2.2. Factors of hybridisation

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The first and major requirement for hybridisation to occur between two individual plants is that they have to be sexually compatible. But even if two individual plants are sexually compatible this does not mean that the dispersal of pollen in nature will lead to hybridisation in every case. The probability of successful pollination depends on a great number of interrelated factors, such as:

- Level of pollen production of (transgenic) plants.
- Rate of self- and cross-fertilisation of receptor plants.
- Rate of dispersion of donor pollen.
- Properties of pollinating agents<sup>2</sup>, where plants sometimes use highly specialised insect pollinators and have highly adapted flowers, resulting in a very effective genetic isolation (mechanical isolation).
- The existence of spatial distance between the pollen donor and the wild recipient population (spatial isolation). How large the distance is within which pollination can occur depends on factors like: wind turbulence, speed and direction and/or the flying range of insects and the time during which pollen is viable.
- Local density of recipient population.
- A difference in flowering season between the crop and the wild population (phenological isolation).

In addition to these factors a number of other factors are sometimes responsible for the fact that even though plants are sexually compatible, they do not hybridise in practice, or that the formed hybrid is not viable (Hadley and Openshaw, 1980). These are prevention of fertilization, hybrid weakness or inviability, hybrid sterility, and hybrid breakdown.

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<sup>2</sup> Many plants make use of insects to transport the fertile pollen to another flower. In some cases there is a highly specialised relationship between the plant and the pollinating agent

## 2.3. Hybridisation of crops with their wild relatives

Even though the barriers to hybridisation exist in nature as described above, many economically important crops show low hybridisation barriers. Such low hybridisation barriers have been important for agriculture because it has enabled plant breeders to develop new varieties using cross-breeding. It has been shown that hybridisation was a common technique in the breeding history of 31 out of 39 plant families, and in 70 out of 90 plant genera, i.e. 75-80 percent of the cases (Simmonds, 1976). To make new varieties plant breeders often use wild plant material as a source of genetic variation. The cultivation area of a particular crop in many cases overlaps the area in which these wild, sexually compatible relatives occur, and sometimes even the centre of origin of the crop is within that overlap. Ellstrand et al. (1999) demonstrated that 12 of the 13 worldwide most important crops hybridise with wild relatives somewhere in their cultivation area (Table 1). So if one of these crops is genetically modified and grown worldwide, the newly incorporated transgene will most probably be transferred to a wild relative somewhere in the world.

Table 1: Hybridisation of crops with wild relatives. Sugar beet are added to this list of Ellstrand et al. (1999) due to their European importance.

Crop	Relative(s)	Crop	Relative(s)
1. WHEAT	Wild <i>Triticum turgidum</i> subspecies, some <i>Aegilops</i> species	8. MILLETS	<i>Eleusine coracana</i> ssp. <i>Africana</i> , Wild <i>Pennisetum</i> species
2. RICE	Wild <i>Oryza</i> species	9. BEANS	Wild <i>Phaseolus</i> species
3. MAIZE	Wild <i>Zea mays</i> subspecies	10. OILSEED RAPE	Some wild <i>Brassicaea</i> species
4. SOYBEAN	<i>Glycine gracilis</i> , <i>Glycine Soya</i>	11. PEANUT	No report
5. BARLEY	<i>Hordeum spontaneum</i>	12. SUNFLOWER	Wild <i>Helianthus annuus</i>
6. COTTON	Wild <i>Gossypium</i> species	13. SUGARCANE	Wild <i>Saccharum</i> species
7. SORGHUM	Wild <i>Sorghum</i> species	14. SUGARBEET	Wild <i>Beta</i> species

## 2.4. Hybridisation with wild relatives in Europe

On a smaller scale, the perspectives for cross to hybridise with wild relatives may change dramatically. In Europe for instance, not all crops have wild relatives with which they can hybridise successfully. Table 2 gives an indication of the hybridisation possibilities for a number of crops in Europe. But even within Europe there still are large regional differences, where in one area crops can hybridise with wild relatives and in other regions it cannot, for instance sugarbeet.

Table 2: Possibilities for successful hybridisation of some European crops with wild relatives

Crop	Wild relative(s) within Europe	Crop	Wild relative(s) within Europe
1. POTATO	None	8. SUGARBEET	Wild <i>Beta</i> species
2. SOYBEAN	None	9. SUNFLOWER	None
3. MAIZE	None	10. CARROT	Wild <i>Carota</i> species
4. BRASSICA SPP.	Wild <i>Brassica</i> species	11. GRASSES	Wild grasses
5. TOMATO	None	12. WHEAT	None
6. RICE	None	13. COTTON	None
7. BARLEY	<i>Hordeum spontaneum</i>	14. BEANS ( <i>Phaseolus</i> )	None

Table 2 shows that in Europe in the risk assessment for crops like potato, tomato, soybean, sunflower, rice, wheat, cotton, beans and maize, the invasiveness of the transgene is not a concern, but only the possible invasiveness of the crop itself. This of course starts from the assumption that the genetic modification itself does not alter the possibilities of the crop to hybridise in a positive sense.

## **2.5. Genetic engineering and effects on hybridisation and reproduction**

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The possibilities for crops to hybridise with wild relatives as described in the former paragraphs are not a static situation. Reproductive isolation barriers can break down or be built up. Many examples of changes in reproductive isolation barriers resulting from classical breeding are known, especially in compositae, grasses, orchids and other ornamentals. Genetic engineering, like classical breeding, has the potential to change characteristics of a plant resulting in changes in the possibilities for successful hybridisation.

The changing of flower shape could for instance have consequences in the case that mechanical isolation is the actual reproductive isolation barrier, resulting in hybridisation between plants that did not occur before. The changing of the flowering season could result in new types of hybridisation where phenological isolation forms an effective isolation barrier. Flowering period is a multigenic trait and up to now, transformation has not resulted in alterations in flowering period. There are other examples. Research on for instance flower morphology is being done and genes involved in the development of flowers are being isolated. There are no transgenic plants in field trials in which a reproductive isolation barrier has been broken down.

Even more research is done to introduce new reproductive isolation barriers into crop plants. The improvement of the biosafety of transgenic crops is one of the driving forces. Examples are male sterility, apomixis, or gene switch technologies that require the addition of an external factor to allow germination. The latter is often also referred to as 'terminator technology' and is being abandoned by many companies because of societal pressure as a consequence of negative socio-economic and ethical considerations.

## **2.6. Gene flow from transgenic plants**

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In relation to gene flow from transgenic plants there is a true flood of smaller and more extensive review papers produced (Abbott 1994a, 1994b, Ammann 1995, Bazin et al. 1995, Bevan et al. 1995, Ellstrand 1992, Gregorius et al. 1993, Raybould et al. 1993, 1994, Snow et al. 1995, Tiedje et al. 1989, van der Meer 1993). Lots of ingenious pollen release experiments have been performed, over 1600 field trials have been counted until 1994 according to extracts from GIBiP Database on field trials 1986 - 1994 (Green Industry Biotechnology Platform 1995).

### **Transgene spread from oilseed rape**

Oilseed rape has been the first major transgenic crop for which gene flow to wild plants is a genuine concern. Much attention has gone in determining the actual gene flow from transgenic oilseed rape. The minimum average safety distance between transgenic and non-transgenic oilseed rape in order to avoid unwelcome gene flow is determined by different groups and differs from 70 to 400m (Dale et al. 1993, Scheffler et al. 1993, Sinemus 1994). Such isolation distances are already known from traditional breeding, in particular for the growing of certified seed that must have a high degree of purity. In the case of oilseed rape, isolation distances of several hundred meters are known to give a seed purity of 99% or more. Still, there is a possibility, that the partially entomophilous oilseed rape can spread its pollen over distances of several kilometers, but successful pollination and fertilization over such big distances must be considered as very rare events (Eckert 1933).

Recent publications by Mikkelsen et al. (1996) and Timmons et al. (1996) show the evidence of transgene spread in the case of oilseed rape. The results suggest a rapid spread of transgenes through interspecific backcrossing under field conditions. The occurrence of fertile, transgenic weed-like plants after just two generations of hybridization should be taken into account when considering the consequences of transferring new traits to oilseed rape. The wild species mentioned in this article is *Brassica rapa ssp. campestris* (often

referred to as *B.campestris*). The authors themselves give a rather pragmatic interpretation of their own results: it depends strictly on the nature of the transgene whether there will be a rapid spread of the new transgenic weed or not.

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## 2.7. Conclusion

Sexual reproduction among crops, weeds and wild plants is possible on the conditions that (1) the crop and the weed or the wild relative are within a distance that pollination can occur, and (2) the plants are sexually compatible. Sexual compatibility is hampered by the existence of external and internal reproductive isolation barriers which determine whether or not viable hybrids are formed. In practice this means for a number of crops like maize, potato and tomato that there are no wild relatives in Europe with which successful hybridisation could occur. But it should be realised that genetic engineering, like classical breeding, has the potential to change reproductive isolation barriers. The experience with transgenic plants like transgenic oilseed rape shows that gene flow from these plants does take place as predicted from the knowledge on the sexual reproduction of these plants. Even though isolation barriers can limit the chances of hybridisation, in many cases chances of hybridisation are not zero and for risk assessment purposes they then have to be considered to be one.

## 3. Codes to help the evaluation of risk regarding field release of transgenic crops

### 3.1. Gene flow indices as a help in risk assessment

To be able to evaluate the risk of the field release of a genetically engineered crop it is important to have knowledge on the possibilities of gene flow for a certain crop in a certain region. Frietema De Vries ([Frietema] De Vries et al. 1992 and 1996) has introduced **gene flow indices** that give an indication of the possibilities of a certain plant to successfully hybridise with wild relatives and the impact this may have. These gene flow indices were not specifically developed for transgenic crops, but apply to them just as well. These indices can help in the risk assessment, in particular the risk assessment for field trials. The higher the gene flow indices, the more containment measures one will have to take if one wants to prevent outcrossing to wild relatives. For the risk assessment in case of the marketing of a genetically engineered crop these gene flow indices are less relevant, because for this particular risk assessment it is more a black-and-white situation: either plants are able to hybridise with wild relatives (even if chances are low), or they are not. This is because after the marketing of a particular genetically engineered crop, when it is grown on a very large scale, it will be very difficult or even impossible to prevent outcrossing with wild relatives.

The gene flow indices are built up from data for a particular plant on its possibilities for dispersal of pollen, dispersal of diaspora<sup>3</sup>, and the presence and density of wild relatives in a given region. These data are classified into three different codes: the Dp-code (dispersal of pollen), the Dd-code (dispersal of diaspora) and the Df code (frequency of distribution of wild relatives). Together they are a measure for the possibilities of gene flow of a certain crop in a certain region and a measure of how widespread this gene flow may be. The Dpdf gene flow indices are always applicable for a given region, and this region therefore has to be mentioned in all cases where gene flow indices are used.

Table 3: The Dpdf gene flow indices after Frietema De Vries (1992, 1996), adapted to European needs as a whole

#### ***Classification of the codes of dispersal of pollen (Dp)***

Dispersal of pollen hybridisation potential, including a differentiation of possible negative ecological effects of the inserted gene itself. Categories 0 (lowest risk) to 5 (highest risk) and U (unknown).

**Dp 0:** No wild relatives in the area (country) under consideration

**Dp 1:** No compatible wild relatives in the area (country) under consideration

**Dp 2:** No records of spontaneous hybrids in the area (country) under consideration

**Dp 3:** Occasional natural hybridisation, no backcrosses observed in the area (country) under consideration

**Dp 4:** Natural hybridisation occur and hybrids are fertile and do backcross

**Dp 5:** Natural hybridisation occurs fairly often, hybrids are fertile and do backcross frequently

**Dp U:** Data too scanty or lacking at all, no evaluation possible.

#### ***Classification of the codes for the dispersal of diaspores (Dd)***

**Dd 0:** No chance for diaspora dispersal (seeds are sterile or deficient)

**Dd 1:** Diaspora dispersal possible occasionally under very favourable and exceptional conditions

**Dd 2:** Diaspora dispersal possible under favourable conditions

**Dd 3:** Diaspora dispersal occurs, fruiting is usually undesirable and is normally suppressed by various methods

<sup>3</sup> Diaspora are reproductive plant parts, such as seeds, fruit, or spores, that are modified for dispersal.

**Dd 4:** Diaspore dispersal is important, fruiting occurs normally during cultivation  
**Dd 5:** Diaspore dispersal is the rule, fruiting occurs very frequently and is very abundant  
**Dd U:** Data too scanty or lacking at all, no evaluation possible.

### ***Classification of the codes for Df (frequency of distribution)***

**Df 0:** Wild relatives not known in the wild or as feral populations in the area (country) under consideration  
**Df 1:** Wild relatives extremely rare in the wild and do not occur as feral populations in the area (country) under consideration  
**Df 2:** Wild relatives very rare in the wild and/or they occur sporadically as feral populations in the area (country) under consideration  
**Df 3:** Wild relatives and/or their feral populations not very common in the wild in the area (country) under consideration  
**Df 4:** Wild relatives and/or their feral populations not frequent in the wild but well distributed over the whole plateau in the area under consideration  
**Df 5:** Wild relatives and/or their feral populations common in the wild and well distributed over the whole area (country) under consideration  
**Df U:** Data too scanty or lacking at all, no evaluation possible.

## **3.2. Classification by combination of the three codes**

The codes have been translated into a risk classification of gene dispersal probability from transgenic crop to the wild flora. After an evaluation of the three single factors (see above, dispersal codes), the combination of these codes enables us to estimate the impact of gene flow on the wild flora. Five categories of risk probability have been developed, which give guidance to the safe execution of field trials:

### ***1. No gene flow effect***

- No related species or no compatible related species of the crop are known in a given region. Field releases of species belonging to this category are possible without any containment or short term monitoring.
- Certain transgenes have to be tested in medium term field experiments regarding their secondary effects on ecosystems: Sustainable resistance must be achieved. To reach this goal a long term monitoring is required.

### ***2. Minimal gene flow effects***

- No records of spontaneous hybridization between the crop and the wild relatives are known in a given region. Field releases are possible after a thorough clarification of the biogeographical situation. Short term monitoring in confinements should be done prior to large scale field releases.
- Certain transgenes have to be tested in medium term field experiments regarding their secondary effects on ecosystems (pest and insect resistance genes).

### ***3. Low but local gene flow effects***

- Gene flow occurs towards wild or feral species existing also outside agricultural environment and control. Release experiments should first be done in confinements and afterwards in small scale releases closely monitored.
- This statement is restricted to transgenes not causing enhanced competitiveness outside agricultural environment, such as herbicide tolerance. Any other transgenes should be carefully tested in confinements.

### ***4. Substantial but local gene flow effects***

- Gene flow is high and substantial, but still locally controllable.
- Field releases could be done within strict confinements. A case by case analysis including the potential effects of the transgene is required before any field releases are done.
- Long term monitoring of field releases under strict biological or geographical confinement conditions is necessary in order to study competitiveness of the transgenic crop. Risky transgenes have to be avoided.

### ***5. Substantial and wide-spread gene flow effects***

- Gene flow is high, substantial, and widespread and will not be controllable by any means.



- No field releases of species belonging to this fifth category are possible.
- Medium term monitoring under strict confinement conditions is necessary in order to find out about competitiveness of the transgenic varieties.
- Experiments with less risky crop varieties (e.g. with male sterility) having the same favourable effect desired.

In Europe, crops like tomato, maize or potato would fall into the 'no gene flow effect' category. Crops like carrot would probably fall into the fourth or fifth category (substantial effects), which means that field trials with carrot should be performed with extreme care, especially when the newly introduced trait is considered to be risky.

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### 3.3. Probability of gene dispersal versus gene effects

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The gene flow indices give a good idea of the probability of gene dispersal from a particular transgenic crop to the wild flora, but they do not tell what will happen if a gene is dispersed to the wild flora. Will the gene be maintained in the wild flora or not? Will it have detrimental effects on the wild plant? Will it give the plant a selective advantage? This will all depend on the particular gene and the properties it confers to the plants containing it. The system of gene flow indices could perhaps be extended with a factor that could be called the *Dg-code* (the 'gene-factor', named in line with the other three codes). Distinctions could perhaps be made between 'risky' genes and less risky genes. Whether such a factor could work in practice remains to be seen, because genes that can be risky in one particular crop do not have to pose a risk in another crop at all. With regard to the risk of transgenes themselves the concepts of weediness, selective advantage, and fitness are thought to be relevant for the estimation of the level of risk. These concepts will be dealt with in the following two chapters.

## 4. Weediness of transgenic crops

### 4.1. A definition of weeds

There are various concepts defining weeds and there is no classical approach generally accepted. Lambelet-Haueter (1990) divides weed definitions into popular, economical and ecological concepts whereas Holzner (1982) groups them similarly into subjective and ecological ones. *Popular* as well as *subjective concepts* define weeds as plants of *any* kind growing in the wrong place, causing damage, being of no benefit and suppressing cultivated plant species. *Economical concepts* reflect the view of agronomists who concentrate on the reduction of yield, thereby stressing the damage aspect. A weed problem is solved as soon as the plant no longer creates considerable damage in the fields, a state which is reached by means of adjusted weed control (crop rotation, tillage, herbicide application).

In contrast to the previous concept, *ecological definitions* include habitats outside agrosystems colonised by weeds. The usual preference of weeds for habitats disturbed by man is stressed, like cultivated fields, gardens, disturbed areas on road sides, recently built artificial slopes, etc.. An aggressive weed can cause damage not only in agrosystems but also in (semi-)natural plant communities by outcompeting weak species.

Following Holzner (1982), it is sometimes difficult to call a plant a weed because one and the same species may be considered in some parts of its area as a harmless component of natural vegetation, in others as a weed and again in others, even as a useful plant species. Williamson (1988) pays attention to the fact that 17 out of 18 most feared „World’s Worst Weeds“ (Holm et al. 1977) are also cultivated.

### 4.2. Weed characteristics

Weeds are well adapted to life conditions in areas disturbed by man, which means that surviving strategies are so variable that any list of weed characteristics remains incomplete, even the famous one from Baker (1967, 1974):

1. Germination requirements fulfilled in a broad range of habitats
2. Discontinuous germination (internally controlled) and great longevity of seeds
3. Rapid growth through vegetative phase to flowering
4. Continuous seed production for as long as growing conditions permit
5. Self-compatible but not completely autogamous<sup>4</sup> or apomictic<sup>5</sup>
6. When cross-pollinated, unspecialized visitors or wind utilised
7. Very high seed output in favourable environmental circumstances
8. Produces some seed in wide range of environmental conditions; tolerant and plastic
9. Adaptations for short- and long-distance dispersal
10. If a perennial<sup>6</sup>, vigorous vegetative reproduction or regeneration from fragments
11. If a perennial, brittleness<sup>7</sup>, so not easily drawn from ground

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<sup>4</sup> Autogamous plants are plants where all seeds are always the result of selfing.

<sup>5</sup> Apomicts are plants that require pollination to trigger embryo formation, but there is no actual fusion of gametes. The embryo is a clone from the mother plant.

<sup>6</sup> Perennial plants are overwintering plants.

12. Has ability to compete interspecifically by special means (rosette, choking growth, allelochemicals).

A weed never possesses all these characteristics, therefore we have to speak of a weed-*syndrome* with even additional characteristics not mentioned here. The only attribute which all weeds might have in common is a marked plasticity enabling adaptation to continuous environmental changes.

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### 4.3. Weediness in relation to other concepts in risk assessment

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From the above it is clear that the concept of weediness can be used either very broadly or within more limited terms. In the public debate the term 'superweed' is sometimes used for transgenic plants that have become very noxious and have a strong ability to overgrow other plants. In this same public debate, distinctions between effects within the field or outside the field in natural habitats are not very often made. In this paper we want to use the term weediness next to the terms 'selective advantage' and 'fitness', and use the term weediness starting from a basically economical perspective. Without unwanted effects in the field, a particular transgenic crop is not considered to be weedy. If a transgenic crop only has unwanted effects outside the field we will use the concepts of selective advantage and fitness in the risk assessment of this transgenic crop.

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### 4.4. Genetic engineering of crops and weediness

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It is known that crops can give rise to weeds in three different ways, and this applies to transgenic crops as well (Zoldan, 1993 and Rauber, 1977):

1. Hybrids between the crop and wild relatives evolve into weeds.
2. Crops evolve into weeds.
3. Crops appear as unwanted volunteers in the subsequent culture where they are considered to be weeds.

#### Weeds resulting from hybridisation between crops and wild species

Gene flow from conventional crops to weeds has had important practical and economic consequences since it promotes the evolution of more aggressive weeds (e.g. Anderson 1949, Barrett 1983). There are many recorded examples of crops becoming weeds after hybridisation with wild relatives, for instance:

- Annual weedy beets resulting from hybridisation between sugarbeet and wild beet in Western Europe. These beets can be very difficult to control in fields of sugarbeet (Boudry et al. 1993, Desplanques et al. 1999, Mùcher et al., in press). The weedy variant differs only in one single allele from the cultivar *Beta vulgaris ssp. vulgaris* (Hoffmann et al 1970);
- *Secale cereale* in California, where a weedy rye probably derived from a cross between *S. cereale* and *S. montanum* is leading to the abandonment of rye cultivation;
- Squash (*Cucurbita pepo*) - in the Southern United States,
- "Hybrid Grain Sorghum" and others (NRC Report on Field Testing, 1989 and NAS Report 2000 p. 81 ff).

In crops that are known to hybridise with wild species to form a weedy variant, the addition of a transgenic trait will in many cases result in the transfer of this trait to the weedy variant. If this trait provides a selective advantage to the weedy variant, or lead to an improved fitness of the weedy variant, the weedy variant may become more difficult to control.

For Western Europe herbicide tolerant sugarbeet is a relevant case to look at in more detail. Herbicide tolerant beets will most probably be the first transgenic beets to come on the market. For these sugarbeets the

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<sup>7</sup> Brittle plants are vulnerable plants, that, when drawn from the ground, break in such a way that the roots remain in the soil.

following is a likely scenario. Introduction of glyphosate tolerance (only used as an example here) into sugarbeet will lead to the outcrossing of the glyphosate tolerance to the weedy variant, but only if transgenic bolters<sup>8</sup> are not controlled. This glyphosate tolerant weedy variant would then no longer be controllable in fields with glyphosate tolerant sugarbeets by means of application of the herbicide. This would mean a return to the original situation where weedy beets can only be controlled by mechanical means and a specific combination of herbicides. Therefore without the strict control of transgenic bolters the introduction of herbicide tolerance in sugarbeet in regions where there are many weedy beets will only lead to a temporary improvement of the situation until the moment that this herbicide tolerance has successfully outcrossed to the weedy beets. On the other hand if transgenic bolters can be controlled – and this would require quite a commitment from the farmer –, transgenic herbicide tolerant sugarbeet can be helpful in controlling the weedy variant.

In contrast to sugarbeet, which is not meant to flower, the outcrossing of transgenic traits from crops like rye or squash to their weedy variants cannot be prevented. These crops are meant to flower to produce grain or fruit. Other means like male sterility and apomixes would have to be introduced to prevent outcrossing of a transgenic trait to the weedy variant.

### **(Transgenic) crops evolving into weeds**

Domesticated crops, such as wheat, maize and soybean, have been modified in traditional breeding to such an extent that they can no longer compete effectively with wild species in natural ecosystems. These crops are unlikely to revert to a weedy condition upon further genetic modification. Weediness is mostly considered to be a multicharacter attribute (Lupi, 1995, and Baker, 1974), which means that it is unlikely that the addition of a few genes will turn these crops into a weed problem. Some crops that have a low degree of domestication such as forage grasses and canola<sup>9</sup> are more likely to revert to a weedy condition (NRC report on Field Testing, 1989). One mutation may cause the weedy form of the crop that then successfully spreads (Sukopp and Sukopp, 1994 and Bartsch et al., 1993). The loss of spikelet spindle toughness of cereals, for example, is sufficient for regaining the ability to spread diaspores.

### **Volunteers**

The third way in which crops can become a weed is when the crop appears as a volunteer in the subsequent culture after remaining in the field by harvest loss. Almost all crops are able to appear as volunteers in the subsequent culture, but it will depend very much on local conditions whether they will do so (Schlink, 1994). In Northwestern Europe for instance maize is very unlikely to appear as a volunteer, while on the other hand in Mexico it is very likely to do so. In Europe especially *Brassica napus* and *Brassica rapa* are problematic in terms of volunteers. These crops make large amounts of seeds of which quite some are lost during harvest and these seeds are able to stay viable in the soil for many, many years. Changes to crops, whether the result of classical breeding or genetic engineering, can lead to volunteers that are more persistent or more difficult to control. For instance if a potato tuber becomes more resistant to cold, it is likely that more potato volunteers will appear in the subsequent culture in regions where the winters are not very cold.

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## **4.5. A particular case: transgenic herbicide tolerant crops and weediness**

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Herbicide tolerance is currently the most widely used transgenic trait with 78% of all transgenic crops worldwide in 1999 carrying a herbicide tolerance gene (ISAAA, 2000). In the public debate on transgenic crops there is often a link made between herbicide tolerance and weediness. To determine whether herbicide tolerance is problematic from a weediness point of view, the following aspects are relevant:

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<sup>8</sup> Bolters are the flowering plants of perennial plants that normally do not flower in the first year of growth.

Vernalisation – exposure to a cold temperature for a certain period – is normally necessary to trigger flower formation in these plants.

<sup>9</sup> Canola is the originally Canadian brandname of oilseed rape lacking eruric acid and glucosinolates, which is comparable to the European 0/0 oilseed rape.

1. Does the outcrossing of herbicide tolerance turn wild plants into weeds?
2. Does the herbicide tolerant crop itself turn into a weed?
3. Do herbicide tolerant crops lead to problems in weed control in any other way?

### **Outcrossing of herbicide tolerance to wild plants**

Herbicide tolerance can occur in wild species by cross-pollination with herbicide tolerant crops. Of course not all crops are able to cross-pollinate successfully with wild species (see chapter 2). This outcrossing will only turn the wild plant into a weed if it is present in places where one wants to control it with the herbicide to which the plant is tolerant. With the use of another herbicide this situation can be overcome in a simple manner. Examples of this principle are known from conventional practices. One example is the emergence in Israel of triazine tolerant *Brachypodium distachyon* in triazine herbicide treated roadsides. The use of other herbicides easily decimated the *Brachypodium* and has made the original situation return where the plant lives in the sandstone hills. In this particular case the herbicide tolerant *Brachypodium* was not the result of outcrossing of the herbicide tolerance. It was the result of selection of herbicide tolerant individuals through mutation. But this does not make a difference to show the principle that there is little risk of a wild species remaining a weed for long periods, as long as agricultural practices can adapt to the new situation. In (semi-)natural habitats a herbicide tolerance does not provide an advantage to a wild relative, because there is no selection pressure in favour of herbicide resistance in natural habitats (Crawley et al, 1993). It is also unlikely that the herbicide tolerance alters the competitive ability and growth behaviour of the wild crop. A hybrid between seabiet (*Beta maritima*) and transgenic sugarbeet (*Beta vulgaris*) with a glyphosate tolerance, did not grow better or have a better competitive ability when compared to a non-transgenic parental type or a non-transgenic hybrid between sugarbeet and seabiet if the herbicide was not applied (Madsen, 1994). Similar data are available by the experiments of Bartsch et al. (1996), Brown (1999) and Pohl-Orf et al. (in press). This suggests that only during herbicide application the selection pressure privileges the herbicide tolerant types.

### **Herbicide tolerance and the weediness of the crop itself**

Herbicide tolerance is unlikely to turn the crop itself into a weed. However, if the crop is known to form volunteers in the subsequent culture, as for instance oilseed rape does, and this following crop carries the same herbicide tolerance (for instance basta tolerant oilseed rape followed by basta tolerant sugarbeets), then these volunteers cannot be controlled by the herbicide. To prevent this from happening it will be important to apply both crop- and herbicide rotation.

### **Herbicide tolerant plants and problems in weed control**

Herbicide tolerant plants have been made to make weed control more effective, easier, and also more environmentally friendly. The introduction of transgenic herbicide tolerant crops has led to the replacement of combinations of different herbicides by the application of only one active ingredient. Some are worried that through the use of only one active ingredient the emergence of tolerant weeds will be accelerated. The rate of development of tolerance in weeds can be estimated from the following. To date, resulting from the application of glyphosate (Roundup) during 26 years, two cases of glyphosate resistant weeds have been documented: annual rigid ryegrass (*Lolium rigidum*) and goosegrass (*Eleusine indica*) (Hartzler, 1998 and 1999). Glyphosate resistant ryegrass has been confirmed in Australia and California (wheat production), and resistant goosegrass was observed in Malaysia (oil palm production). In both cases, resistance occurred after 10-15 years of intensive glyphosate use (>2 applications per site per season).

It is expected that the use of these broadspectrum herbicides like glyphosate in first instance will lead to a shift towards weeds with limited leaf surface. The examples of ryegrass and goosegrass show that monocultures of only one type of herbicide year after year will promote the emergence of tolerant weeds. To prevent a backlash to the 'pre-herbicide tolerant crop situation' with the use of different, sometimes environmentally more problematic herbicides, it will be important to apply herbicide rotation. At this moment two transgenic herbicide tolerances are dominant: tolerance to glyphosate (Roundup) and to glufosinate (Basta, Liberty, Finale). It will be important to rotate these herbicides. Herbicide rotation will become easier if there is

enough supply of different herbicide tolerant crops. It is expected that in the near future plants with tolerances to herbicides other than glyphosate and glufonisate will be introduced on a fairly regular basis.

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## 4.6. Conclusion

Genetic engineering, like conventional breeding, is able to alter characteristics of crops resulting in a crop or its wild relative to become more weedy. In many crops, and especially the ones that have been domesticated to such an extent that they are no longer able to compete with wild species in natural habitats, the addition of a few genes is very unlikely to turn the crop into a weed. In crops that are still very close to their wild and sometimes weedy variants, the addition of one gene might be enough to trigger weediness. In such cases the risk assessment should be performed with great care. Experimental approaches will be needed to determine the actual risks of unwanted effects.

Herbicide tolerance is not a major concern from the viewpoint of weediness. Only during application of the herbicide there is a selective advantage for plants possessing this characteristic. However, to prevent acceleration of the selection of tolerant weeds and possible future problems with (herbicide tolerant) volunteers it will be important to apply the necessary crop- and herbicide rotation.

But, since there is no long term monitoring on transgenic crops existing which concentrates on weediness in all aspects, scenarios must remain speculative.

## 5. Selective advantage and improved fitness of transgenic crops

### 5.1. Definition of selective advantage and fitness

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#### **Selective advantage**

In contrast to where weediness points to the undesired – sometimes economical - effects of the presence of a plant in (mostly) the agronomic environment or in habitats disturbed by man, the term selective advantage is used in risk-assessment to point to a qualitative characteristic of a newly introduced trait. A selective advantage means that after the outcrossing of the trait to a wild relative it has a good chance of being accumulated in the wild population. Selection results in the trait being preferentially attained. If a trait has no selective advantage, or also a selective disadvantage, then the outcrossing does not result in the accumulation of the new trait. It will disappear or diminish to a very low level. The term selective advantage is therefore more than the term weediness linked to the ecological effects of outcrossing of transgenes to plants in natural habitats.

#### **Fitness**

The fitness of a genotype is commonly measured by the number of successful offspring compared to the number of offspring of other genotypes (i.e., relative fitness). To this end, fitness of outcrossing species is necessarily connected to hybridisation with sexual compatible relatives. Fitness should always be defined in relation to environmental variables, e.g. habitat and climate.

The concepts of selective advantage and fitness are therefore mostly used to help to determine the risks connected to a transgene. This transgene is either part of the crop and can play a role in determining the invasiveness of the crop itself, or it is transferred to sexually compatible wild species and can play a role in determining the population size in natural habitats.

### 5.2. Risky transgenes?

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There are many different transgenes transferred to plants using genetic engineering. The genetically engineered plants now on the market and in field trials, contain genes that provide the following characteristics:

1. Tolerance to herbicides.
2. Resistance to biotic stress (virus, fungus, insect, parasite diseases)
3. Resistance to abiotic stress (salt tolerance, drought resistance, tolerance to heavy metals etc.)
4. Quality characters (better processing, improved starch quality, improved oil profile, improved nutritional value, improved vase life, etc.)
5. Altered flower colour
6. Male sterility and restorer
7. Molecular farming (production of vaccines, pharmaceuticals, biopolymers, etc.)

In laboratory research many other types of traits are being used, not only with the goal to develop a genetically engineered crop, even more with the goal of understanding plant growth and behaviour.

The question is whether the genes responsible for the abovementioned characteristics are risky and whether or not they provide a selective advantage or improve the fitness of the transgenic plants containing these characteristics. Although escaped transgenes are basically not retrievable, it should be noted that there are also considerable chances that an escaped gene will not persist in nature beyond several decades as a result of outcompeting and backcrossing. Escaped transgenes will only survive in nature for long periods of time under special circumstances (Harrison 1993). Below some considerations are given with regard to the risks of a number of categories of transgenes in terms of selective advantage or improved fitness.

### **Tolerance to herbicides**

Herbicide tolerance provides a selective advantage only when the particular herbicide is used. In other circumstances this trait does not provide an advantage. It is also very unlikely to alter the fitness of the plants containing this type of alterations in a positive sense (see chapter 4 for more detail).

### **Traits related to biotic stress**

Resistance to bacterial, viral or fungal disease and insect infestation are very important traits, especially for crops grown in the tropical and subtropical regions. Virus and insect resistance are already available on the market (virus resistant papaya, Bt maize), bacterial and fungal resistance are being developed. Resistance to biotic stress is thought to be able to lead to a selective or a fitness advantage, but only in the cases where in the wild population the viral disease or the insect infestation is a determining limiting factor in the population size. If disease plays an important role in the wild populations, then this should be taken into account very seriously in the risk assessment. However, often the wild plants are more disease resistant than the related crops. For virus resistant sugarbeets it is known that the virus is not present in the European wild beet population. Virus resistance in sugarbeets is therefore not expected to lead to a selective advantage of the beets.

### **Traits related to abiotic stress**

Resistance to drought, salt or other stress can imply that crops can be introduced into areas where they were never able to be grown before. When it is possible to introduce traits that really have significant effects, then these crops could turn out to be able to invade new habitats outside the fields that they have never inhabited before. General conclusions are however impossible, and it will depend very much on the crop, the newly introduced trait, and its competitiveness compared to the natural flora in these areas. At this moment there are almost no empirical data on the selective advantage or fitness of these types of crops. Most of them still are in fundamental research phases, tested in laboratories or greenhouses. One of the few examples ready to be tested in the field are aluminum tolerant plants in Mexico. These plants are able to grow in areas polluted with aluminum. There are no data available yet on the question whether these plants have a selective advantage or an improved fitness in areas not polluted with aluminum.

### **Quality characters**

There are many examples of traits related to quality that are being engineered. Examples of plants already on the market are tomatoes with an improved shelflife and carnations with improved vase life. In both cases the introduced traits are not thought to be problematic in terms of selective advantage or fitness. In the case of the tomato in Europe there are no wild relatives with which the transgenic tomato could hybridise. The „gene-question“ (what will happen **if** the gene gets into the wild population) is therefore not relevant for tomato, and therefore also the question with regard to selective advantage and fitness.

There are no general predictions possible on what the effects in terms of selective advantage and fitness are of genes related to quality characters. The types of changes are too diverse. One might however speculate that there might be a difference between crops that are very much domesticated and no longer able to compete with the wild plants in natural habitats, and crops that are still very close to their wild relatives. In the latter case the addition of a few genes could perhaps easier lead to detrimental effects. It is therefore not only the question whether a particular trait will result in a selective advantage or an improvement of fitness, but even more on whether this selective advantage or improved fitness would lead to unwanted effects in the natural



population. To illustrate this it can be imagined that the introduction of a certain trait in a very domesticated crop like potato can improve its fitness (it is stronger, grows more tubers, etc.), but that this will not lead to detrimental effects in the natural population. Although its fitness may be improved, the crops does not have to become invasive. To be able to have a better idea on the effects additional experience with transgenic crops will have to be gained. Especially for crops that are close to their wild relatives, or that have wild relatives in their growing area with which they can successfully hybridise, data on their behaviour will have to be carefully assessed.

### **Molecular f(ph)arming**

In cases of molecular farming the traits that are added to crops are not put into plants with the goal of improving their fitness or behaviour. Transgenic plants are only used as a means to produce a certain compound. It can be speculated that such traits are more likely to be a selective disadvantage to the plant, rather than a selective advantage.

### **Traits related to hybridisation**

Genetic modification may affect hybrid formation either by changing frequency with which it occurs, or by altering the range of species with which the crop is sexually compatible. In case of an enhancement of reproduction characteristics, this could lead to selective advantages. But, increasingly the evidence suggests that modification has little impact on either factor, except for a laboratory study of Bergelson et. al. (1998), where a transgenic Arabidopsis showed an increased pollen fertility, full data are not revealed yet, so the case remains open and needs more discussion.

### **Traits related to effects on pollinators**

Another important question is whether or not the relation between pollinators and plants will be modified by transformation. First the new proteins synthesized by the transgenic plant should not be toxic for bees. The non-toxicity of chitinase has been proven (Pham-Delègue et al. 1992). Could the character of a transgenic plant modify the activity of the insect? The same group demonstrated that the foraging time was shorter on a transgenic plant than on a non-transgenic one. However, Pham-Delègue et al. concluded that transgenic oilseed rape has no negative effects on foraging bees under controlled conditions. They need to repeat the experiment in the field (Grallien et al., 1995). Similarly, Paul et al. (1991) found that there are no differences in the range of animals and the frequency of visits between modified and non-modified tobacco plants.

Insects like bees are attracted by light of a wavelength between 300 nm and 650 nm (Dumas 1984). This includes part of the ultraviolet light but excludes the long-waved red light. A change in the flower colour could disturb the attractiveness of the flower for the insects and change cross-pollination rate for insect-pollinated plants. This would imply that the changing of flower colour resulting in a lower attractiveness for pollinating insects would not result in the trait being selectively attained in a wild population and vice versa, but only when the finding of the flowers by the insects would be a limiting factor for hybridisation in nature.

On the other hand, genetic engineering may be favourable to the hybridisation process. Studies regarding alfalfa (*Medicago sativa*) treated by a pesticide (dimethoate) clearly demonstrated that this pesticide is found in the pollen and the nectar at a very low level but even at a low level this pesticide is toxic for the bees (Dumas 1984). This has an effect on the entomophilous pollination. Plants resistant to diseases will not be treated with pesticides, so that the efficiency of pollination for entomophilous plants could be enhanced.

### **Traits related to seed production**

Seed production may also be influenced by transformation. In the case of an experiment comparing mutant and transgenic herbicide tolerant Arabidopsis, Purrington et al. (1997) found that herbicide-resistant individuals produced 26% fewer seeds than their susceptible counterparts. In another of many more experiments Linder (1998) results suggest that high-laurate wild-crop hybrids lack germination cuing mechanisms and will germinate primarily at inappropriate times. It is becoming gradually clear, that fitness

differences produced by transgenic traits up to now are readily comparable with differences of traits produced by classic breeding methods.

### **Experience from field trials**

In various review papers the impacts of gene transfer have been evaluated (Ahl Goy et al. 1994, 1995 and 1996, see also the regular accounts of Clive James from ISAAA). Reviews of hundreds of field trials with male sterility in oilseed rape, herbicide tolerance in oilseed rape, sugarbeet and maize and insect resistance in potato and maize has not revealed any hints that one of these transgenes would enhance competitiveness and therefore cause invasions of crop plants into natural habitats, resulting in negative effects (Crawley 1992, Crawley et al. 1993, Fredshavn and Poulsen, 1993, Crawley, personal communication). Crops like oilseed rape, potatoes and maize with these kinds of traits have the same competitiveness outside agrosystems as the non-transgenic counterparts. They hardly can persist more than one generation. In no case sexual reproduction has been observed in natural ecosystems (Crawley personal communication and Crawley et al. 1993).

From the experience with transgenic crops it is postulated by some ecologists that selective advantage, fitness, but also weediness through addition of a certain characteristic should be opposed to the additional 'genetic load' accompanied by the addition of that characteristic. It is believed that in many cases this additional genetic load will be the cause that the characteristic will disappear from a population.

## **5.3. Lessons learned from traditional breeding**

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Many of the above mentioned traits, like for instance tolerances to (a)biotic stress, quality characters, and others can also be achieved through conventional breeding, although it may require more time or they may be altered to a somewhat lesser extent. But the fact that the traits can be similar in a qualitative sense makes that experience from conventional breeding is relevant for a number of transgenic crops.

Traditional breeding has so far focussed on yield improvement. In comparison to wild relatives, cultivars are in general genetically less diverse and therefore less adapted to natural environments. Reports of fitness advancement for hybrids in natural ecosystems are rare. In opposite, hybridisation and escape of genes of conventional cultivars has led to disadvantages for wild plant populations in some documented cases. Hybridisation with domesticated species has also been implicated in the extinction of certain wild crop relatives (e.g. Ellstrand & Elam 1993, Small 1984). This proves that the exchange of genetic material between crops and wild plants can influence the natural flora. It also suggests that the pressure from (genes from) domesticated crops until now mostly has had an impact in the sense that the outcrossed domesticated genes have made the wild plant less competitive. This is also confirmed by recent experiments by Keller et. al. (2000), discussed in Moore, P.D. (2000). In these experiments individual plants from the same species but from different regions in Europe were cross-hybridised. The first generation offspring showed greater biomass, but in the second generation the plants showed a decreased biomass yield, survivorship and seed mass. The overall message from these experiments is that introduction of genes from distant plant populations is more likely to do harm to the native flora of an area than to have positive effects in terms of biodiversity.

Pressure from gene flow from a crop to a wild relative does not necessarily result in a decrease in the genetic diversity of the wild plant, as shown by Bartsch et. al. (1999) in their study of gene flow from traditionally bred beets into the wild sea beet populations of north-eastern Italy. In this case the cultivated beets were far less diverse and outnumbered the wild relatives by the factor 10.000 to 1.

There is also a number of examples of cultivars or genes of cultivars escaped into natural ecosystems (Williamson 1993, Bartsch et al. 1993, Bartsch and Ellstrand 1999), one being carrot (*Daucus carota*) where some wild Northwest European *Daucus* populations have almost certainly been derived from once cultivated populations. Some of the changes caused by man have been regretted, other changes like in the case of carrot

not. These new carrot populations are now appreciated as added value to the biodiversity. Oilseed rape escaped to ruderal places all over the world, but does not take over in natural habitats.

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#### 5.4. Lessons from exotic species and exotic genomes

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According to Sukopp and Sukopp (1993) there are hundreds if not thousands of new and foreign genomes introduced with trees, shrubs, herbs, microbes and higher and lower animals each year. Many of those survive and can, after years and even many decades of adaptation, begin to be invasive. This trend, overlooked by most and realised and judged to be a true hazard by only a few ecologists, is not evident, since it works slowly but steadily all over the world thanks to human activity in transport and tourism, which so often go hand in hand with the destruction of habitats. The dynamics of this trend is not yet sufficiently known. There are already many cases known where virulent new weeds invade ecosystems. Insular ecosystems are especially fragile and need to get much more attention regarding introduced new genomes in future. Examples are Guava on Mauritius and *Pittosporum undulatum* or *Goldfussia (Acanthaceae)* in Jamaica.

The knowledge of the introduction of wild genomes is often referred to as the „*exotic species model*“ and therefore a close comparison has its pitfalls as is pointed out by Scholz (1993), because all the examples in the exotic species model are wild species or cultivated wild species, and not crops. Crop plants, even in the phase of escaping from their agrosystems are not wild species and cultigeneous species cannot be compared in their genotype and phenotype with truly wild species.

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#### 5.5. Conclusion

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Selective advantage and fitness are important concepts that can help in the determination of the risks of genetically engineered crops. They provide a measure for the hazards posed by a genetically engineered crop containing a certain genetically modified trait. But if a genetically engineered crop has obtained a certain selective or fitness advantage through genetic engineering, one cannot conclude that the newly introduced gene will have a selective or fitness advantage in all plants. It is always the combination of the plant, the trait and the environment together that determine a selective or fitness advantage. Information on the selective advantage of a certain trait in a certain crop can therefore not be transferred to other plants. Selective or fitness advantage will have to be determined on a case-by-case basis.

For the transgenic crops on the market now like herbicide tolerant maize there are no indications that these crops have an improved competitiveness or that gene flow from these organisms has a negative impact on the wild flora. But it should be stated that many of the field trials with transgenic plants have not been monitored as they could have been, and many of them lack a longterm perspective.

Classical breeding, and the introduction of foreign genomes where gene flow has been implicated in negative events like the extinction of certain wild crop relatives (e.g. Ellstrand & Elam 1993, Small 1984). This evidence from conventional crops and from the introduction of foreign genes through the introduction of foreign plants shows that negative effects from gene flow from transgenic crops are a realistic possibility. For future releases of transgenic crops the risks should not be underestimated, because possibilities of gene flow as proven by the experience with conventional crops, are rather realistic for crops like carrot, sugarbeets, oilseed rape or wild grasses (Ammann, 1995). Introductions of such crops should be done with great care.

## 6. Ecological view and consequences for risk assessment

### 6.1. Ecology and safety research

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In considering the ecological risks of a crop it should be avoided to focus strictly only on transgenic crops. Many genes (or combinations of genes) responsible for various kinds of traits including pest resistances have been brought back to crops by classic breeding methods and subsequently released in large number into the field.

On the other hand we should not jump to preliminary conclusions or generalisations which are based on a relatively short experience with transgenic crops when compared to non-transgenic crops (Gabriel 1993, Regal 1994). In genetic engineering only *genes* are moved in contrast to classical breeding where usually *alleles* are moved around. Still Regal (1994) does conclude that many transgenic crops *will* be non-competitive because, (1) the parent organisms were highly modified forms such as extensively domesticated maize to begin with, (2) there may be cases in which the genetic engineering process itself does demonstrably incapacitate the transgenic form ecologically and (3) if the host is the sort of foreign wild species that simply cannot persist without human help under local conditions of inappropriate weather, soil, etc., biotechnology is unlikely to turn it into a locally ecologically vigorous organism. Still, domestication should not be the miracle key word for safety of any transgenic crop.

The sound use of existing knowledge from plant ecology and from the use of conventional crops, together with carefully planned and monitored field trials should give good enough scientific data for taking good risk management decisions. For determining gene flow use can be made of harmless tracer genes which can be screened on their pathway. Bartsch et al. (1993) postulate experiments in confined and open systems within the framework of long term monitoring on the basis of scientific criteria. Also from ecological monitoring one can learn a lot, even though findings often remain inconclusive. Andow (1994) suggests that mathematical models of resource competition might be useful for identifying categories of plants that either are unlikely to alter community structure or that have the potential for altering community structure.

### 6.2. Consequences for risk assessment

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When we take the knowledge and experience into account from classical breeding, from the introduction of foreign genomes, and from the transgenic crops in the field until now, a rational approach to the risk assessment of genetically engineered crops would have to:

- list up all related species having possible gene flow with the transgenic cultivar,
- to define a given region (and thus a given biogeographical situation),
- to deal with specific transgenes,
- follow a step by step procedure.

One example of a scheme that can help to structure this step-by-step procedure is the one proposed by Rissler and Mellon (1993) (cited in Snow et al. 1995). The disadvantage of such procedures is that they suggest it is always 'either' 'or'. Bearing the complex interactions between fitness factors in mind, it is often not that

simple. This means that the scheme is an aid, but results from one step will have to be looked at carefully before stepping blind into a next step of a procedure.

### 6.3. Testing scheme for risky crops

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For all transgenic crops the approach mentioned above should be followed. The first two steps could be made easier when for all relevant regions listings were given of the Dpdf-codes for the different crops and that on the basis of these codes the crops were divided into one of the five risk categories as given in chapter three (ranging from 'no gene flow effect' to 'substantial and widespread effect'). The question then is what type of testing to require for risky crops. It is postulated here that for all crops and related wild species, which belong to the two highest risk categories (substantial but local effect, substantial and widespread effect), the following testing scheme should be performed in a medium to long term monitoring using an experimental approach.

Crops belonging to the highest category of risk should be treated according to a test procedure proposed by Fredshavn et al. 1993, which can be carried through in greenhouses. Competition experiments in confinements will reveal data in the influence of plant size on competitiveness, on substitution rates as a measure of competitiveness and on the interaction between habitat and gene expression. But even with these preliminary greenhouse experiments it will be impossible to predict the exact ecological consequences of a release. It is, however, possible to test a transgenic plant in a confinement in critical phases of the life cycle and compare it with a range of non-transformed well-known varieties, and thus detect any principal changes in growth behavior. A set of standard growth conditions is proposed. If these experiments do not reveal any major change in the competitiveness of the transgenic crop, then the field experiment procedures according to Rissler and Melon (1993) can be started.

This approach involves a threestep analysis to evaluate both crop weediness and gene flow. The steps are designed to identify non-risky plants early in the analysis and to require extensive field testing only for plants that appear to pose substantial risks. The evaluation proceeds under the assumption that crops on the lower end of the spectrum of weediness potential are sufficiently unlikely to be converted to weeds by the addition of transgenes that they can be subject to simplified population replacement experiments.

The first step assesses:

1. The potential for weediness. It separates crops into two risk categories. The lower-risk category contains crops that are not weedy and do not have close weedy relatives in Switzerland. By contrast, the higher-risk group is weedy or has close weedy relatives. The higher-risk crops are subject to a standard set of experiments, while the ones with lower-risk undergo an abbreviated procedure.
2. Experimental assessment of the potential for transgene flow determines whether transgenic hybrids will form between transgenic crops and their wild/weedy relatives. Where hybrids are not formed, the transgenic crop is deemed to pose low risk in terms of gene flow and no further tests are required. Where hybrids are formed, the analysis moves to step 2. Once gene transfer occurs, the assessment of potential adverse impacts is the same as for the transgenic crop itself.

The second step analysis relies on relatively simple experiments, which can be conducted along with efficacy tests, to evaluate the performance of transgenic crops relative to non transgenic ones.

The third step analysis allows developers an opportunity to demonstrate that transgenic crops that outperform non-transgenics in the ecological performance tests do not pose risks as weeds under conditions of commercial use.

ASSESSING THE POTENTIAL FOR TRANSGENIC CROPS TO BECOME WEEDS

ASSESSING THE POTENTIAL FOR TRANSGENE FLOW TO PRODUCE WEEDS.

**Is the parent crop weedy or does the crop have close relatives in ?**

**Do viable, fertile hybrids form between the crop and wild/weedy relatives ? (See scheme 2)**

No

Yes or insufficient information

Yes

No

Simplified ecological performance evaluation      ecological performance evaluation

**Does the transgenic plant outperform the nontransgenic plant in population replacement experiments ?**

**LOWER RISK**  
End of analysis

3 years replacement experiments in 3-5 growing areas and/or where wild relatives occur:  
1. Net replacement rate  
2. Seed bank persistence

3 years population replacement experiments in the full range of growing environment including field margins and/or where wild relatives occur:  
1. Net replacement rate  
2. Seed bank persistence.

No

Yes

No

**LOWER RISK**  
End of analysis

**WEEDINESS**  
**Is weediness increased in transgenic plants exhibiting enhanced performance ?**

**LOWER RISK**  
End of analysis

Weediness field experiments : Multiyear confined small-scale field tests in several environments.

Yes

No

**HIGHER RISK**  
reconsider commercialisation

**LOWER RISK**  
End of analysis

There are a number of ways to evaluate the replacement capacity of a genetic type in a population of plants. Option A describes one alternative. N.Ellstrand, J.Hancock, P.Kareiva, R.Linder, R.Manasse and M.L.Roush were especially helpful in developing this experimental approach given by Rissler and Mellon (1993). Here we cite it from Snow et al. (1995), appendix 3:

**Do viable fertile hybrids form between the crop and wild/weedy relatives? Does the crop reproduce sexually?**

Yes or insufficient information

No

Does the crop have outbreeding potential with relatives in the region where the plants will be introduced ?

LOWER RISK  
End of analysis

Yes or insufficient information

No

Do the crop-relative breeding systems permit gene flow in and out ?

LOWER RISK  
End of analysis

Yes or insufficient information

No

Does the flowering period of the crop and weed/weedy relatives overlap, or nearly so ?

LOWER RISK  
End of analysis

Yes or insufficient information

No

Do crop and wild/weedy relatives share the same means of pollination ?

LOWER RISK  
End of analysis

Yes or insufficient information

No

Do crop and wild/weedy relatives naturally cross-pollinate, fertilize, and set viable , fertile seeds under field condition ?

LOWER RISK  
End of analysis

Yes or insufficient information

No

Go to the ecological performance of transgenic wild/weedy plants. (go to scheme 1)

LOWER RISK  
End of analysis

## 7. Discussion and Conclusion

The risks associated with vertical gene flow from genetically engineered plants form a major concern in the discussions on the safe use of transgenic crops. How invasive are transgenic crops themselves and how invasive are the transgenes that are present in these crops? Transgenes can only invade wild populations if wild sexually compatible relatives are present. But what will the effect of such gene flow be? In this paper the concepts of weediness, selective advantage and fitness have been discussed to see whether they can help in determining the risks associated with particular transgenes.

From this paper it will be clear that sexual reproduction among crops, weeds and wild plants can occur but depends on the specific crop and the region where the crop is cultivated. Maize, potato, rice, wheat, beans (*Phaseolus*) and tomato for instance have no wild relatives in Europe with which successful hybridisation can occur. For these crops only the invasiveness of the crop itself is a concern. Successful hybridisation is determined by many factors, some of which can also be altered using breeding methods or genetic engineering. The alteration of crops can therefore also lead to the alteration of the success with which a particular crop can hybridise with its wild relatives. There is quite some knowledge on the sexual compatibility of crops with wild relatives, but on a worldwide scale additional studies are necessary to determine whether escaped transgenes are likely to persist in wild populations.

To give a measure to the possibilities of gene flow to wild relatives and to how widespread the effects of gene flow will be, gene flow indices have been developed which are based on data concerning: (1) the dispersal of pollen, (2) the dispersal of diaspores, and (3) the frequency of distribution of wild relatives in a given region. These gene flow indices can be a tool to divide crops into gene flow risk categories. The higher the risk class, the more careful one should be with introducing 'risky' genes. However, it should be borne in mind the gene flow indices are not completely static measures, but that genetic engineering, like classical breeding has the potential to change reproductive isolation barriers thereby changing the success of gene flow. Changing these types of properties of plants also changes the gene flow codes and the risk classes. The risk classes may be important in two different ways: (1) by guiding the measures necessary to be able to perform field trials in a safe manner, and (2) by guiding the testing procedure for the marketing of a crop; the higher the risk class the more stringent testing schemes will be necessary.

Even more important than determining the chances of gene flow to wild relatives is the question how risky the transgene itself is, especially in those cases where the crop is expected to be easily reverted or transformed into a weedy form. The closer a crop is to weedy or wild variant, the higher the chances that the addition of one or a few genes can make a crop invasive. Crops that are very much domesticated, and no longer capable of competing with wild plants in natural habitats, are less likely to become invasive after the addition of one or a few genes.

The addition of genes, either through conventional breeding or genetic engineering has the potential to make crops more weedy. It can also make wild plants to which the trait has been transferred more weedy. If genetic exchange between transgenic crops and wild relatives has weediness potential, there should nearly always be evidence of this process with non-transgenic crop/weed complexes. Weediness as such is a multiform characteristic that will not be easily obtained by the addition of one transgene, or only in cases where the crop is close to the weed. Herbicide tolerance is - based on the experience from traditional breeding - from a weediness point of view not a concern, if there are enough possibilities for crop and herbicide rotation. In the case of transgenic herbicide tolerant beets, this herbicide tolerance can help to control weedy beets, but only



in the case that transgenic bolters are controlled. If not, it can be expected that the herbicide tolerance will be transferred to the weedy beets, making them again as difficult to control as they are today.

Selective advantage and fitness are important concepts for specifying the risk of a transgene. It is very difficult to give general conclusions, but for instance traits related to the success of gene flow, resistance to biotic or abiotic stress might result in selective advantages or serious fitness improvements, if the absence of the trait in nature is an important determining factor in the existing ecological balances. Whether in specific cases there is a real risk can only be determined through thoughtful analysis and experimentation. It should be kept in mind that many of the above mentioned types of traits can also be obtained through classical breeding methods and that their possible ecological effects should be assessed in the same way. Further research is also needed to predict how escaped transgenes are likely to affect the abundance and invasiveness of the transgenic hybrids.

Experience from conventional breeding and the introduction of exotic genes and genomes forms the proof of the fact that the introduction of new varieties and new crops has the potential to influence the natural flora. However, reports of fitness advancement for hybrids in natural ecosystems are rare. In opposite, gene flow from domesticated crops has mostly made the wild plants less competitive. There are few recorded examples of the opposite, for instance in the case of carrot where some wild populations have most certainly been derived from once cultivated variants. In contrast to the known effects of these conventional crops, there are no indications that the transgenic crops currently on the market have such unwanted effects. However, it should be stated that these crops have not been monitored as they could have been. This is also no proof whatsoever for the transgenic crops now being developed. The experience from non-transgenic practice directs us to take great care.

For the future risk assessment a rational stepwise approach is necessary taking into account the knowledge of the crop and its wild relatives, knowledge on the biogeographical situation, and knowledge on the transgene. The testing of the transgenic crop should follow a step-by-step procedure evaluating data from a first step before stepping into a next phase. The more risky the crop and/or the transgene, the more stringent the testing scheme will have to be before the transgenic crop can be allowed to be grown commercially on a large scale. But in the end one dilemma will remain: even after the most careful risk assessment process, only a mass release will bring to the surface all effects. The small-scale field trials do not allow to investigate the ecological risks of widespread commercialization. Therefore in order to achieve sustainability in cultivating transgenic crops, the focus should be on long term monitoring of several years in the same field where the transgenic crop was planted.

## 8. Literature

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