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# FIELD RELEASE OF TRANSGENIC CROP PLANTS, ECOLOGICAL ASPECTS

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## Introduction

Genetic engineering will influence human life in all its aspects. This does not mean automatically, that genetic engineering leads to greater concentration of power. As a decentralised technology it will be harder to control on what we should do and what we better leave out. It is not wise to reject this promising technology in a fundamentalist way, since I still believe in progress, the only way we can overcome in a reasonable way future problems. Genetic engineering will speed up with its new precision the domestication of crop plants in future. This means that we are confronted with new problems in risk assessment, a challenge we simply *must* overcome. It is a long time ago since we left biological evolution in creating crops which are purely artificial products so essential for the survival of mankind.

Genetic engineering surpasses boundaries of species, orders and classes, even kingdoms in Systematics. But at the same time one should not over-estimate the potential to-day. Genetic engineers still have to learn how to place their transgenes at a precisely determined gene locality. Successful transformation has still to be screened for by selective processes among hundreds of trials. This means also that genetic engineers are still in the state of copying life, they are still far away from the creation of life. Successful transformation still must closely imitate gene structures found in natural organisms.

The elegance of the method is promising, quick and convincing results are more difficult to achieve. There are still many problems to be solved, among which there are the often found lack of stability of the transgenes, gene

flow from the crop to the wild relatives, possible allergic reactions caused by extraneous proteins, unforeseen changes in host specificity of plant pathogens etc.

There is a good chance that many of these potential problems can be solved, but still there is the question about the remaining risks. A future balance will be more positive, if genetic engineers learn to adapt to more ecological strategies in crop breeding, which can be seen more positive in a socio-economic perspective. But there is still one important obstacle on this way: Up to now it seems to be impossible to master polygenic characters, which would be so important for a more ecology-oriented genetic engineering: Only by mastering these techniques we will be able to influence drought tolerance, cold tolerance etc.

A certain transformation routine has developed in the last few years, in most cases Pest resistance, herbicide tolerance have been transformed.

Vertical gene flow is widespread in nature, in most cases it is confined to the limits of a given species, but not always: Intergeneric hybrids are known in nature among orchids and grasses and many other plant families. Hybridisation and thus vertical gene flow is a natural process without which there would be no evolution of species at all.

On the other hand there is no direct evidence of horizontal gene flow under natural conditions, although one has to realize that it might occur in nature more often than we would like to admit. Still, experimental evidence shows that horizontal gene flow cannot be reproduced under natural conditions. It needs a lot of artificial pressure to prove

some evidence of it in experiments (Schlüter et al. 1996).

One of the biggest hazards in genetically engineered crop plants could be the escape of an unwelcome gene in nature by vertical gene flow. We should do our best to avoid such a situation, but we also should bare in mind, that ecologists are confronted with much bigger dimensions of gene introgression through exotic and invasive plants.

### **Brassica napus, oilseed rape, a case study**

#### **Systematics of Brassica napus and close relatives**

The list of cultivars and their wild relatives, including with all possibilities, demonstrates that oilseed rape as a crop is still fairly close to its ancestral parents. There are also a lot of possibilities for natural hybridization.

<i>Cultivar</i>	<i>Wild relative</i>
<b>Brassica napus</b>	<b>Brassica oleracea diploid and B. rapa L. ssp. campestris diploid</b>
rape, swede	Wild rapeseeds and wild cabbages of Europe
<b>Brassica oleracea L.</b>	<b>Brassica oleracea subsp. oleracea s.str., B. montana Pourret, B. rupestris Rafin, B. villosa Biv. B. incana Ten. B.</b>
cabbage, cauliflower, kohlrabi, Brussels sprouts etc.	
<b>macrocarpa</b>	<b>Guss., B. cretica Lam., B. hilarionis Post</b>
<b>Brassica rapa L.</b>	<b>Brassica rapa L. subsp. sylvestris (Lam.) Janchen diploid (= Brassica rapa L. subsp. campestris)</b>
turnip	Weedy and ruderal forms of turnip

List adapted from Heywood et al. 1996

#### **Weediness of Oilseed Rape**

It is therefore easy to understand, that oilseed rape can become a weed problem (Schlink 1994, Top Agrar, Rapsanbau für Könner 1991).

Concerning germinating ecology, *Brassica napus* has all requirements to establish itself as a „wild plant“ in an agricultural system with tillage or in a ruderal habitat. Furthermore, in its growth behaviour and in its high

potential of reproduction, oilseed rape resembles segetal weeds and thus represents a typically competitive weed. In crop rotation systems including oilseed rape, the „unidentified“ volunteering rape is capable to pass through all developmental stages up to seed maturity. Seed loss before and during harvesting increases the seed stock in the soil even by seeds produced by volunteers. In this way, selection of enduring genotypes in the field is possible. Simultaneously, the seed stock in the soil is constantly enlarged by new genotypes due to rapid development of new strains of oilseed rape. Still, there are no indications that oilseed rape could become an invasive weed penetrating natural habitats.

Nowadays, volunteering oilseed rape (*Brassica napus*) has to be controlled by means of an adapted crop rotation system and tillage techniques, sometimes combined with herbicide applications. Furthermore, feral populations can often be observed in disturbed habitats outside agricultural systems. How long such a population can survive needs to be checked. In addition, its potential for invading natural plant communities has to be analysed. (These questions will be studied in the framework of the SPP project of Biotechnology 1996-99 by F. Felber, R. Guadagnolo, J. Keller Senften, P. Rufener Al Mazyad and D. Savova).

Oilseed rape germinates like a weed. Under favourable conditions, it appears four or five days after sowing

Volunteering oilseed rape is problematic in the subsequent culture, whereby lost seeds may germinate over a long period of time and therefore may cause problems. Due to application of growth hormones, control of volunteering oilseed rape in cereals is no problem [per se]. But volunteering oilseed rape can become a leading weed, especially in dry zones. Because of its long germination period, its high competitiveness and difficulties in harvesting, herbicide application after germination will be necessary even if no weeds are present. Control of volunteering oilseed rape in culture of oilseed rape is very

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difficult. Therefore stands may vary greatly in their density which is negative for survival in wintertime and for quality, favours pest growth and depression of ripe plants.

**Soil seed bank:**

The ability of seeds to survive in the soil for a long period of time is caused by dormancy, germination biology and ecology, even under changing environmental conditions.

Following Zohary (1992), the spread of seed germination over two or more years is a common adaptation particularly of annual species. Partitioning of seed germination yield over two or more years is an effective device to buffer the otherwise crippling effects brought in by climatic fluctuations. But such allocation does not serve just to evade disasters. It also acts as a balance which buffers the selection in any particular year, and prevents extreme annual shifts in the genetic composition of the population.

According to Schlink (1994), crop breeding generally selects well-germinating genotypes. Especially in the breeding of winter oilseed rape, which can be sown just after harvesting, high germination rates of fresh seeds might have been a secondary breeding goal. In contrast, seeds of oilseed rape are able to survive for a long time in the soil what has been proven by model experiments using four different strains. They have shown surviving rates of over 70 % for a period of 1,5 years and of almost 60 % after five years of exposition in the soil. These rates usually are met only by weeds. The surviving seeds of oilseed rape were sensitive to light which is characteristic for wild species that are adapted to segetal and ruderal habitats. Furthermore, the surviving seeds in the soil showed changes in their germination readiness due to a dormancy cycle induced by seasonal shifting of soil temperature. Such a survival strategy is well-adapted to ecological conditions in temperate zones. It is a typical characteristic of wild plants.

The strategy of eradicating volunteering crops in agricultural systems, as it was followed some decades ago, is not reasonable,

due to the fact that the supply of fresh seeds to the soil seed stock is guaranteed by the cultivation of the crop itself.

Even if seed loss during harvesting could be prevented, not all sown seeds would germinate under particular conditions as certain genotypes would develop a secondary dormancy and would therefore be added to the bank of dormant seeds.

Crop breeding generally selects well-germinating genotypes. Especially in the breeding of winter oilseed rape, which can be sown just after harvesting, high germination rates of fresh seeds might have been a secondary breeding goal. In contrast, seeds of oilseed rape are able to survive for a long time in the soil what has been proven by model experiments using four different strains. They have shown surviving rates of over 70 % for a period of 1,5 years and of almost 60 % after five years of exposition in the soil. These rates usually are met only by weeds. The surviving seeds of oilseed rape were sensitive to light which is characteristic for wild species that are adapted to segetal and ruderal habitats. Furthermore, the surviving seeds in the soil showed changes in their germination readiness due to a dormancy cycle induced by seasonal shifting of soil temperature. Such a survival strategy is well-adapted to ecological conditions in temperate zones. It is a typical characteristic of wild plants.

**The case of transgenic *Brassica napus*, oilseed rape**

A widespread crop which still is very close to its wild relatives is e.g. *Brassica napus*, oilseed rape:

Crawley et al. (1993) were unable to show significant differences between non-transformed and transgenic oilseed (kanamycin resistance and glufosinate tolerance) in their field experiments. They argued, that non-transgenic rape plants are slightly outcompeting their transgenic cultivars. This result shows that there will not be any obvious and short term invasion possible in case of an escaped gene, as long as the transgenes do

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not cause a major change in the ecological behaviour of the novel oilseed rape. Still, the ultimate proof that transgenic oilseed rape is harmless compared to its non-transgenic relatives is not yet delivered, although Crawley's experiments are quite laborious and ingeniously planned: 12 different habitats where chosen to test a mixture of transgenic and non-transgenic oilseed rape. It has been criticised repeatedly that the discussion of the results should not be based on mean values solely, since we must also take into account founder populations with unusual ecological behaviour, which could make those plants more vigorous than others, cf. Weber (1995), see also Sukopp and Sukopp 1993.

Poulsen (1995) argues that differences in yield between cultivars are often very small, and in order to get significant results, cultivar testing normally requires larger numbers of replicates and larger plot sizes than used there.

In field experiments assessing competitive ability of transgenic oilseed rape (Fredshavn et al. 1995, Poulsen 1995), the competitiveness of two transgenic oilseed rape lines and their fertile transgenic hybrid was tested in field trials in Belgium and Denmark. The lines contained genes for male sterility, restoration of fertility, kanamycin resistance and tolerance to the herbicide glufosinate. The competitiveness of these three transgenic lines was related to three non-transformed commercially-grown oilseed rape varieties. As a reference to a more aggressive crucifer, white mustard (*Sinapis alba*) was used in the experiments. All crucifers were grown in monocultures and mixtures with barley (*Hordeum vulgare*). The results show that the transgenic lines behaved similarly to the non-transformed cultivars. Despite the different growth conditions in Denmark and Belgium, the monoculture yield on the two locations is not remarkably different. In their field experiments, the authors observed a change in growth behaviour towards that of the reference weed *Sinapis alba* which could be an indication of increased weediness of one of the transgenic oilseed rape lines. For

Poulsen (1995), the exact consequences of a release are impossible to predict. To detect any basic change in growth behaviour, she proposes to test the transgenic plants in critical phases of the life cycle and to compare it with a range of nontransformed well-known cultivars.

It may be appropriate in cases of novel transgenes to apply a method to assess competitiveness proposed by Fredshavn and Poulsen (1993). It allows a scientifically sound comparison between transgenic and non-transgenic crop with regard to their competitiveness. According to the authors even this refined experiments cannot solve all questions totally. Only a field release in reality will take into account most important factors determining competitiveness. The last open questions will anyway be solved in the course of mass releases over many years to come. This is a harsh but realistic statement, which has become true in many cases of classical breeding as well.

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

In the case of oilseed rape there has been recently a publication by Mikkelsen et al. (1996) showing evidence of transgene spread. 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*). It seems that this species (according to Danish and Swiss experience) coexists with *Brassica napus*, oilseed rape. It will hardly be able to occur outside the range of *B. napus*. Consequently, 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. In the case of the transgenic weed obtained by their field experiments they cannot see any dramatic effects in the future.

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This is also the opinion of Timmons et al. 1996: The research group of the Scottish Crop Research Institute found in their own study area of Angus, Scotland, intercultural hybrids at a distance of 360m of a transgenic oilseed rape field. They also were not able to detect. They conclude that attention should now focus on the probable effects of any introduced transgenes. The group infers, that in the area surveyed, the possession of glufosinate-tolerance is unlikely to affect the survival of feral populations significantly, since at present time there is no extensive use of glufosinate in non-agricultural situations. This assertion takes, according to the authors, no account of future changes in glufosinate application in non-agricultural situations, of pleiotropic effects of the transgene, or of effects on agricultural volunteers. Their work demonstrates once more the need for a careful, case-by-case approach to the risk assessment of genetically modified organisms.

#### **Transgene spread of Tobacco**

Since Tobacco has no relatives in Europe, there is no danger of transgene spread to wild relatives. Still, there is a possibility that transgenes can be spread from cultivar to cultivar or from *Nicotiana tabacum* to e.g. *Nicotiana rustica* (which is a hybrid between two Peruvian wild species).

In a case studied by Paul et al. 1995 there have been introduced two marker genes (kanamycin resistance and  $\beta$  glucuronidase into tobacco). These plants were grown in a series of field trials each consisting of a small plot of modified plants surrounded at various distances (1m, 10m, 20m) by non-modified receptor plants. Large quantities of seeds have been screened for vertical gene flow, which could be easily detected by selection against kanamycin resistance. Outcrossing over relatively short distances with a regular pattern of donor plants shows highly variable patterns for the of the progeny having received the above mentioned marker genes. (Figs. 2-5 in Paul et al. 1995).

Palauqui et al. 1995 did a field trial analysis of nitrate reductase co-suppression com-

paring 38 combinations of transgene loci. The trial was carried out with 8160 tobacco plants.

The phenomenon of co-suppression of host genes and transgenes expressing the same coding sequence has been reported for several plant genes. Results suggest, that suppression is occurring at a post-transcriptional level.

It has been shown in several cases that the phenomenon of co-suppression is connected to the frequency of the transcription of the transgene locus.

In this trial shows that co-suppression frequency is depending on:

1. Number of copies of the transgene and/or some particular structure of the locus
2. Number of transgene loci involved in a given combination
3. Growth conditions

Caligari et al. 1993 have tested the field performance of derived generations of transgenic tobacco. Two inbred cultivars of *Nicotiana tabacum* 'Samsun' and 'Xanthi' were transformed with *Agrobacterium tumefaciens*, expressing kanamycin resistance and encoding GUS. Progeny were identified and selfed over several generations.

Two major conclusions can be drawn from this experiment:

1. 'Samsun' and 'Xanthi' performed differently, since homozygous 'Samsun' showed GUS levels twice as high compared to the hemizygous ones, whereas 'Xanthi' did not show any difference. For other transgenes this has also been shown by Dean et al. 1988.
2. The two cultivars did not show the same agricultural performance after the same transformation: Whereas the changes in 'Xanthi' tended to favour the modified line (more and longer leaves), those in 'Samsun' tended to favour the unmodified line (the modified line being shorter in height, having shorter leaves, later flowering and reduced fertility).

The authors discuss several possible reasons without naming a specific one:

- 'Carry-over' effects from the treatments

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applied (*less likely*)

- Variation present in the original inbreds (*less likely, since the lines used appeared to be true-breeding.*)
- The direct effect of the inserted genes (pleiotropy). (*possible, needs more experimentation*)
- Somaclonal variation (*possible, needs more experimentation*)
- Insertional mutagenesis (*possible, needs more experimentation*)

Brandle et al. 1995 demonstrate clearly, that instability of herbicide resistance observed during field trials of the homozygous chlorsulfon-resistant transgenic tobacco line DAL516-13 was not anticipated from earlier greenhouse studies. Again transgene loci and the number of transgenes transcribed is influencing agricultural performance and expression level of the transgene. The authors suggest that transplacental effect may have been a contributing factor in the appearance of silencing in some of the studies.

Co-suppression of the *csr1-1* transgenes and the endogenous tobacco AHAS genes was triggered by the common agronomic practice of seedling transplantation. The same is true for a herbicide resistant tobacco line carrying a mutant AHAS gene *ahas3R* from *Brassica napus*.

Obviously, numerous factors influence transgene stability. Also it is evident, that common agricultural practice can have a profound influence on the resulting phenotype in the field, and that the field performance cannot simply be extrapolated from greenhouse results. It is clear that many steps that are taken for granted may need to be reevaluated for successful commercialization of transgenic crops.

#### **Suggestions how to solve the problems with escaped transgenes**

As a result of discussions in the symposium at Louverain, we propose **gene flow indices** after the idea of some Dutch authors ([Frietema] De Vries et al. 1992, Frietema De Vries 1996) We are giving here an adapted version in order to spur discussion on an European level. We think it is desirable to

establish a European classification system as proposed by Frietema De Vries (1996), where some of our proposals have been adopted. It is not possible to arbitrate the crops and their wild relatives on one and the same level all over Europe: Classification work has to be done on a *regional* scale taking into account local environmental conditions, species and transgenes. This regional scale has been proposed by Frietema De Vries (1996), following the well known subdivisions of Meusel.

Critical comments for the proposals given here are welcome. Here we deal only with the three first codes, but we feel strongly the necessity of a fourth code for the future: We need to assess also the *risk of the inserted transgene itself*. For this *Dg code* we need experimental approaches on all levels from a strict containment over small scale field releases to the large scale releases over long periods. For the time being there remains only the possibility of a rough estimate of how transgenes will have side effects in the long run, some comments are built in provisionally in code Dp (vertical gene flow). The authors are well aware of the pragmatic view they take, which is blurring the logic of the three codes already defined.

These codes are presented here in order to open debate on feasibility and organisation of such codes for future risk assessment. The codes can serve as a first rough estimate, before going into more detail for a risk assessment based on field monitoring and experimental approach, where judged necessary.

### **Dpdf-codes, adapted to European needs**

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

Dispersal of pollen and hybridization 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)

**Category Dp 0:** No chance for hybridization because there are no wild relatives growing in the region. No ecological effects when the

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cultivated plants come into flower.

Monitored field releases possible, no containment experiments and no field experiments necessary.

**Category Dp 1:** No chance for hybridization with wild relatives because it is experimentally proven that wild species of the same genus in the region are not compatible with the cultivated plant: (artificial pollination methods and/or embryo rescue are necessary to produce hybrids).

No ecological effects when cultivated plants come into flower. Monitored field releases possible without containment. However, experiments should be carried out, to test there are no negative effects on the host / predator system in case of transgenes introducing new resistance and/or competition effects.

**Category Dp 2:** No chance for hybridization with wild relatives because there is no record of spontaneously formed hybrids of the cultivated plant with wild species of the same genus in The region.

However, hybridization is possible under experimental conditions and progeny is fertile without any artificial help. Chances of gene flow by hybridization is small due to various outcrossing barriers (competition, biogeographical or ecological incompatibility), but under special local or artificial conditions in agricultural systems still to be considered as possible rare events.

- a) In certain species groups there is a small chance of getting new transgenic hybrids, but no invasions are to be expected.
- b) In other species groups there is a small chance of getting new transgenic weeds which tend to be aggressive and will possibly cause invasions under unfavourable conditions.

**Category Dp 3:** Natural hybridization occurs only occasionally, backcrosses have not been observed up to now. Local situations have to be studied carefully in risk assessment of field experiments. Species to species, region by region and step by step approach required.

- a) In certain species groups and under unfavourable circumstances gene flow by pol-

len transfer will occur, but new transgenic hybrids do not tend to be invasive.

- b) In other species groups and under unfavourable circumstances gene flow by pollen transfer can influence ecosystems negatively: Local invasions of new transgenic weeds will occur.

**Category Dp 4:** Chance for natural hybridization is medium; backcrosses have been observed, successful outcrossing occurs fairly often. Natural fertile hybrids are sometimes observed, small hybrid populations can be detected in nature. Species to species, region by region and step by step approach required.

- a) Transgenic hybrids will have no ecological effects on the flora of the Region, since the new hybrid is only capable to invade small ecological niches, and therefore does not demonstrate any disturbing invasiveness, since the inserted gene itself did not show negative ecological effects in long term monitoring experiments. Experiments should also be carried through proving that there are no negative effects on the host / predator system.

- b) Transgenic hybrids will have ecological effects on the flora of the Region, since the new weed is capable to invade ecological niches, and therefore is potentially demonstrating invasiveness. There may also be negative effects (e.g. more competitive, more allelopathic) caused by the inserted gene itself.

**Category Dp 5:** Chance for natural hybridization is high; vertical gene flow occurs often, hybrids are fertile and backcross frequently. Hybrid populations are often found in nature. Species to species, region by region and step by step approach required.

- a) Transgenic weeds will have no ecological effects on the flora of The region, nevertheless the new weed is capable to invade important ecological niches and it will act as a new weed (which should by all means be avoided!), but the inserted gene itself does not show negative ecological effects.

- b) Transgenic weeds will have negative ecological effects on the flora of The re-

gion since it is capable of invading many ecological niches as a major new weed and/or since the inserted gene itself may have characters demonstrating negative ecological effects.

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

## 2. Classification of the codes for the dispersal of diaspores (Dd)

**Category Dd 0:** No chance for dispersal of diaspores to the wild: Seeds are sterile or otherwise deficient, they have lost reproductive function. No ecological effects are expected from fruiting of the cultivated plants.

**Category Dd 1:** Dd to the wild occurs only occasionally and under very favourable conditions, plants usually survive only for one season (advena), they are not adapted for survival in our climate. No ecological effects are to be expected regarding the Swiss ecosystem.

**Category Dd 2:** Chance for dispersal of diaspores to the wild is small, but under favourable and exceptional conditions possible. Further research on population dynamics seems necessary. For risk assessment the standing of the plants in the Swiss ecosystem can be of importance.

**Category Dd 3:** Chance for dispersal of diaspores (by spontaneous vegetative reproduction) is real; fruiting of the cultivated plant is essentially undesirable and will normally be suppressed by various methods. Further research on population dynamics is necessary. For risk assessment the standing of the plants in the Swiss ecosystem can be of importance.

**Category Dd 4:** Chance for dispersal of diaspores to the wild real. Fruiting of the cultivated plant occurs normally during cultivation. Ecological effects can be expected from fruiting of the cultivated plant. For risk assessment the standing of the plants in the Swiss ecosystem will be of importance.

**Category Dd 5:** Dispersal of diaspores to the wild will be the rule. Fruiting occurs very frequently and also extremely abundant. Ecological effects can be expected from fruiting of cultivated plant. For risk assessment the standing of the plants in the Swiss

ecosystem will be of importance.

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

## 3. Classification of the codes for Df (frequency of distribution)

**Category Df 0:** No plants of this species or of a wild relative, no feral populations found in nature; no ecological effects are expected from the introduction of the cultivated transgenic plant.

**Category Df 1:** Plants of this species or of wild relatives are extremely rare in the wild and have their stable place in the Swiss ecosystem in specific associations. No feral populations are found in The region. Chances for hybridising with the wild or feral populations are negligible. Locations to grow transgenic plants should be appropriately chosen in order to avoid hybridisation and any ecological effect.

**Category Df 2:** Plants of this species or of wild relatives are rare, but occur sporadically, distribution difficult to predict and essentially uncontrollable. Feral populations may exist in certain regions. Chances for hybridising with wild populations are scanty but unpredictable. Ecological effects from the introduction of the cultivated plant may be expected, but in most cases on a local scale only. Locations to grow transgenic plants should be appropriately chosen in order to avoid hybridisation and any ecological effect.

**Category Df 3:** Plants of this species or of wild relatives are not very common in the wild and have their stable place in Swiss ecosystem. Feral populations are known from The region, but not frequent. Chances of hybridising with the wild populations exist

TABLE 1

### Summing up the codes

#### Dp: Hybridisation and pollen dispersal index

Dp0: No wild relatives in The region

Dp1: No compatible wild relatives in The region

Dp2: No records of spontaneous hybrids in The region

Dp3: Occasional natural hybridisation, no backcrosses observed in The region

Dp4: Natural hybridisation occur and hybrids are fertile and do backcross.

Dp5: Natural hybridisation occurs fairly often, hybrids are fertile and do backcross frequently.

**Dd: diaspora dispersal index**

Dd0: No chance for diaspora dispersal (seeds are sterile or deficient)

Dd1: Diaspora dispersal possible occasionally under very favourable and exceptional conditions

Dd2: Diaspora dispersal possible under favourable conditions

Dd3: Diaspora dispersal occurs, fruiting is usually undesirable and is normally suppressed by various methods.

Dd4: Diaspora dispersal is important, fruiting occurs normally during cultivation

Dd5: Diaspora dispersal is the rule, fruiting occurs very frequently and is very abundant

**Df: Dispersal frequency**

Df0: Wild relatives not known in the wild or as feral populations in The region

Df1: Wild relatives extremely rare in the wild and do not occur as feral populations in The region

Df2: Wild relatives very rare in the wild and/or they occur sporadically as feral populations in The region

Df3: Wild relatives and/or their feral populations not very common in the wild in The region.

Df4: Wild relatives and/or their feral populations not frequent in the wild but well distributed over the whole plateau in The region

Df5: Wild relatives and/or their feral populations common in the wild and well distributed over the whole Swiss plateau in The region

but are small. Some ecological effect from the introduction of the cultivated plant may be expected under unfavourable conditions when cultivated plants and wild relatives are not sufficiently separated. Locations to grow transgenic plants should be carefully chosen in order to avoid hybridisation and any ecological effect.

**Category Df 4:** Plants of this species and their wild relatives are not frequent but well distributed over the whole Swiss plateau, chances for hybridising with wild populations are considerable, but under very favourable conditions it can still be safely prevented. Feral populations are known and distributed over an important part of The region. Locations to grow transgenic plants should be carefully chosen in order to avoid hybridisation and any ecological effect. De-

tailed biogeographical studies are necessary to reach this goal.

**Category Df 5:** Plants of this species and their wild relatives are common and well distributed over the whole Swiss plateau, chances for hybridising with wild populations must be expected and cannot be prevented in field experiments. Feral populations are frequent and distributed over the whole Region. In exceptional cases locations to grow transgenic plants can still be carefully chosen in order to avoid hybridisation and any ecological effect. Detailed biogeographical studies are necessary to reach this goal

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

### Classification by combination of the three codes

The goal of this study was to develop a convenient classification of gene dispersal probability from transgenic crop to the wild flora, adapted for The region. After an evaluation of the three single factors (see above, dispersal codes), the combination of these codes enables us to estimate impact on the wild flora. Five categories of risk probability have been developed:

After an evaluation of the three single factors, their combination enables us to estimate the impact of a transgenic species on the environment. Six categories of risk probability have been developed:

**1 No effect** 

- No related species or no compatible related species of the crop are known in The region. Field releases of species belonging to this category are possible without any containment or short term monitoring.

TABLE 2

Dispersal indices for some important crops and risk categories

species	English name	dispersal index Df.Dd.Dp	risk category
Festuca arundinacea	Fescue	5.5.5	Substantial and widespread
Festuca pratensis	Meadow fescue	5.5.5	Substantial and widespread
Lolium multiflorum	Italian ryegrass	5.5.5	Substantial and widespread
Lolium perenne	Perennial ryegrass	5.5.5	Substantial and widespread
Medicago sativa	Alfalfa	5.4.5	Substantial and widespread
Lactuca sativa	Lettuce	2.5.5	Substantial but local
Daucus carota spp. sativus	Carrot	4.2.4	Substantial but local
Brassica napus	Oilseed rape	2.5.3	Low but local
Brassica rapa	Turnip	2.4.3	Low but local
Raphanus sativus	Radish	3.3.3	Low but local
Cichorium intybus	Chicory	4.3.3	Low but local
Secale cereale	Rye	4.3.2	Minimal effect
Cichorium endivia	Endive	2.2.3	Minimal effect
Brassica oleracea	Cabbage	3.3.3	Minimal effect
Trifolium pratense	Red clover	5.3.1	No effect
Trifolium repens	White clover	5.3.1	No effect
Beta vulgaris	Sugar beet	1.2.1	No effect
Solanum tuberosum	Potato	5.1.0	No effect
Lycopersicon esculentum	Tomato	0.1.0	No effect
Triticum aestivum	Wheat	4.2.2	No effect
Hordeum vulgare	Barley	4.2.2	No effect
Zea mays	Maize	4.0.0	No effect

- 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 effects

- No records of spontaneous hybridization between the crop and the wild relatives are known in The 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 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 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 effects

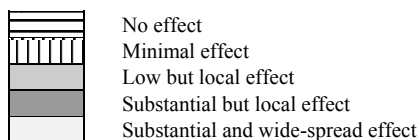
- Gene flow is high, substantial, and wide-spread and will not be controllable by any means.

- No field releases of species belonging to this fifth category are possible.

TABLE 3

Risk categories for 22 important crops

Df code	Dd code	Dp code								
		0	1	2	3	4	5			
0	0									
	1	tomato								
	2	tobacco								
	3	o								
	4									
	5									
1	0									
	1									
	2		beet							
	3									
	4									
	5									
2	0									
	1									
	2									
	3									
	4									
	5									ettuce
3	0									
	1									
	2									
	3									
	4									
	5									
4	0	maize								
	1									
	2									
	3									
	4									
	5									
5	0									
	1	potato								
	2									
	3									
	4									
	5									alfalfa grasses



- Medium term monitoring under strict con-

finement 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.

**Unknown** (one of the three indices is unknown) More studies are needed before any field releases are done.

The following combinations of the three Codes are possible, each one applicable to a given crop:

**Preliminary examples of risk assessment**

As an illustration, several examples are given here (a full risk assessment with these codes will be given in the publication of the final report about the above mentioned Priority Programme Project)

**Alfalfa (*Medicago sativa*) with transgene causing rapid spread under agricultural conditions:**

Categories Df 5, Dd 4, Dp 5, which means no field release of transgenic alfalfa as described above, is possible. Medium term monitoring under strict confinement conditions is necessary in order to find out about competitiveness of the transgenic variety and in order to experiment with less risky transgenes having the same favourable effect desired. No field releases to be permitted until case is solved in such a way, that transgenic alfalfa varieties has been proved to be in lower risk categories. Although we judge field trials to be premature in Europe, there have been given in the USA as many as 15 permits for insect resistance, herbicide tolerance and virus resistance (Snow et al. 1995).

**Barley (*Hordeum vulgare*)**

Categories Df 4, Dd 2, Dp 2, which means no effect with field releases of transgenic barley.

Field releases of transgenic barley possible. Certain transgenes have to be tested in medium term field experiments regarding their secondary effects on ecosystems: Insect resistance genes could well be designed in such a way, that pest insect species develop themselves too rapidly resistant populations. Field

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experiments should be carried out with less effective expression of the gene or with a mixture of different transgenic varieties expressing various resistances. Another strategy would be to introduce non-transgenic populations of the crop in order to produce refugial non-resistant pest insect populations. Number of permits in the USA for herbicide tolerance and virus resistance: 2 (Snow et al. 1995)

#### **Oilseed rape (*Brassica napus*)**

Categories Df 2, Dd 5, Dp 3, which means low but local effect with field releases of transgenic oilseed rape, since gene flow occurs towards a wild (feral ?) species which does not occur outside agricultural environment and control. Number of permits in the USA: 35 for herbicide tolerance, industrial enzymes produced, insect resistance, product quality (Snow et al. 1995).

#### **Tobacco (*Nicotiana tabacum*)**

Categories Df 0, Dd 2, Dp 0, which means no effect with field releases of transgenic tobacco, since gene flow cannot occur towards a wild or feral species. Numerous permits all over the world.

However, breeders should carefully assess the risk of transgene spread amongst cultivars.

These examples are restricted to transgenes which cause favourable effects regarding herbicide treatment (herbicide tolerance) and which do not cause enhanced competitiveness outside agricultural environment. Any other transgenes should be carefully studied regarding their effect on the competitiveness of the transgenic oilseed rape. Such experiments should be done in confinement.

#### **How to proceed in risk assessment**

There is no great hope to solve problems in risk assessment with consensus technique, since these techniques are successful in legislation on a political level. Risk assessment in field release of transgenic plants has to cope with concrete problems in a concrete environment. Here planning methodology of the second generation will be the solution.

There are several ground rules to be followed:

- Symmetry of knowledge (or ignorance): Experts and lay persons have different kinds of knowledge. If both parts learn to respect this rule, they will also start to listen to each other.
- All key role players should be invited to the discussions, they should all feel free to play their role and to speak out about their genuine interests.
- Only a step to step approach will be successful, the discursive process should be accompanied by professional moderators and also computer aid has to be considered seriously in order to keep track to the argumentative process, to document the pro's and the con's and to be able to reduce and enlarge the catalogue of critical questions and arguments.
- Most important is the circumscription of the problem: The periphery of the problematic case has to be defined precisely, this does not mean to describe the problem precisely, which would mean a major step towards problem solution already.

In our case of problems to be solved this would mean to

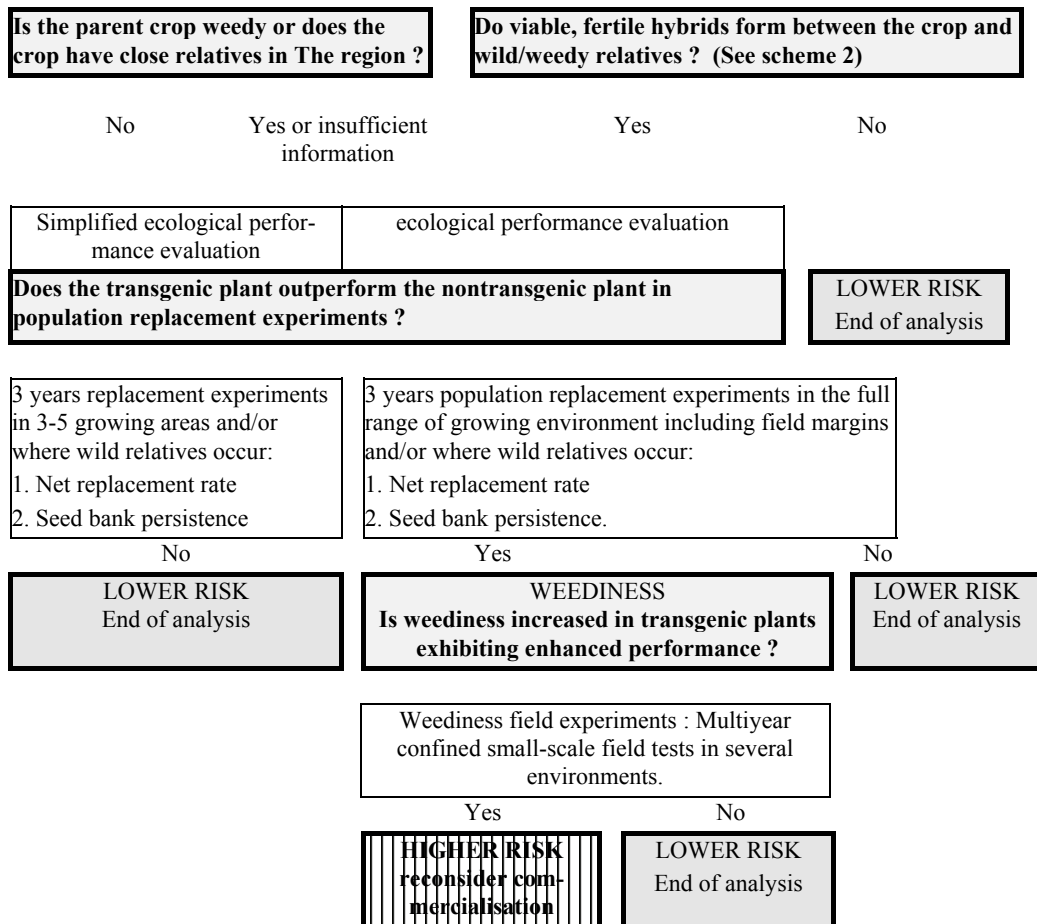
- list up all related species having possible gene flow with the transgenic cultivar
- to define a given region (and thus a given biogeographical situation).
- to deal with specific transgenes
- to invite all partners affected by future field releases.
- follow a step by step procedure, which could be structured following the scheme proposed by Rissler and Mellon (1993) (cited in Snow et al. 1995).

#### **Summary of scheme to assess two environmental risks**

All crops and related wild species, which belong to the two highest risk categories (substantial but local effect, substantial and widespread effect), should be treated according to the following scheme in a medium to long term monitoring with an experimental approach.

ASSESSING THE POTENTIAL FOR  
TRANSGENIC CROPS TO BECOME  
WEEDS

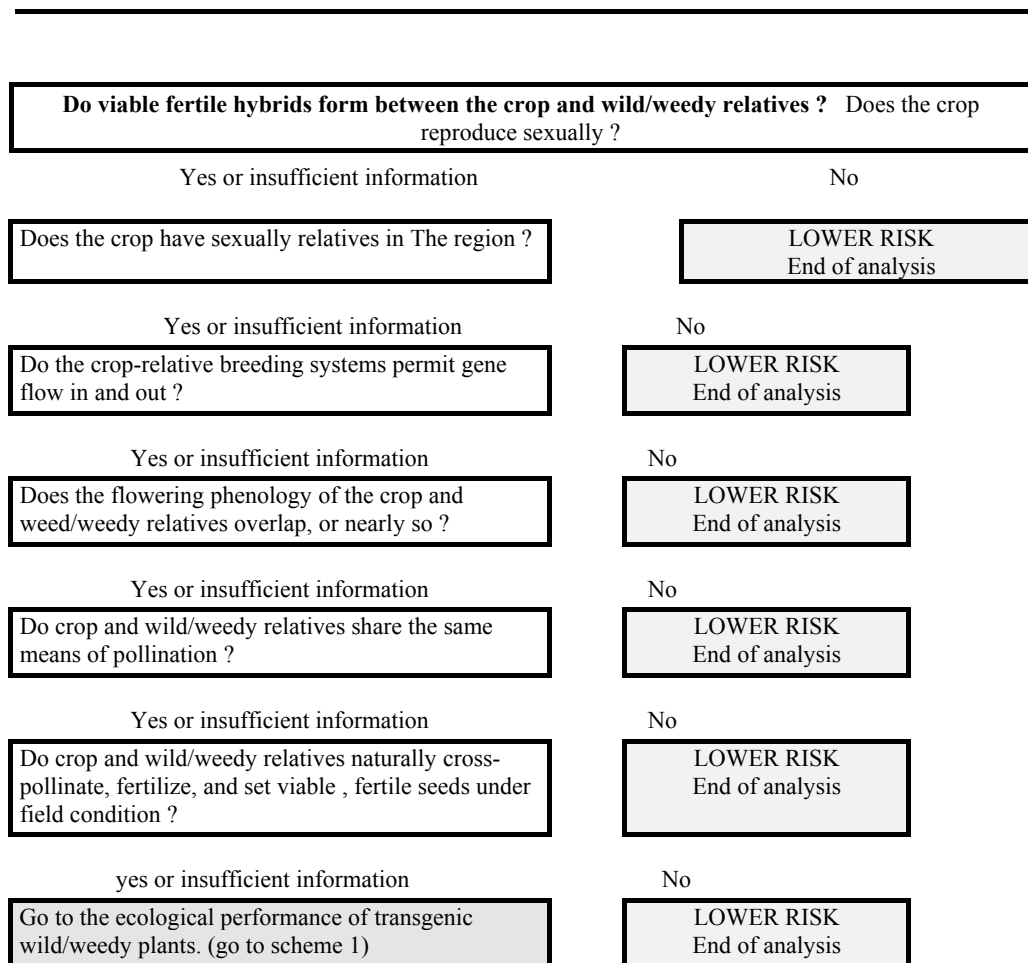
ASSESSING THE POTENTIAL FOR TRANSGENE FLOW  
TO PRODUCE WEEDS.



Scheme 1.

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 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



**Scheme 2.**

Rissler and Melon (1993) can be started.

This approach involves a three step analysis to evaluate both crop weediness and gene flow. The step 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 The region. 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,

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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.

The proposal of Rissler and Mellon (1993) are summarized in scheme 1 and 2 below.

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:

### Conclusions

The hundreds of small-scale field tests in order to evaluate the performance of genetically engineered crop varieties are up to now not designed to investigate the ecological risks of widespread commercialization (1994 International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms in Monterey, CA, USA).

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. To assess invasiveness, the transgenic plant's capacity to disperse and establish in adjacent and nearby habitats should be investigated.

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. Recent evolution of weed beets in France [Boudry et al., 1993] demonstrate the novelty and effectiveness of certain fitness-related transgenes. Closer attention should be paid to possible effects on free-living wild relatives.

Rigorous studies of the sexual compatibility of crops and wild relatives are clearly needed to determine whether escaped transgenes are likely to persist in wild populations. Further research is also needed to predict how escaped transgenes are likely to affect the abundance and invasiveness of the transgenic hybrids.

### Concluding remarks

Genetic engineering could go together with organic farming and there are a whole range of good possibilities for this new technique, which should be developed without ideological bias.

It sounds reasonable to design future research in genetic engineering of crop plants by means of iterative and argumentative methods, where problem solution is the ultimate target. Modern planning methods of the second generation has done this for many years already and it is about time to consider design of science as an important issue. This second generation planning will also lead from time to time to a zero-solution.

This would leave no room for fundamentalist views, which often tend to generalise risk assessment prematurely.

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