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Gene flow from crops to wild plants and its population-ecological consequences in the context of GM-crop biosafety, including some recent experiences from lettuce

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Abstract

The public concern about the impact of genetically modified crops on the natural environment triggered a steady stream of research during the last decade. Among the possible impacts, the ‘escape’ of the transgene, either through dispersal of the crop plant outside the agricultural area or through hybridization with wild relatives, attracted a lot of attention, in particular in relation to the possibility of increasing ‘weediness’. For gene flow through hybridization to occur, pollen grains must achieve fertilization and seeds must germinate and produce sexually mature plants. Subsequently, the first generation hybrids should be sufficiently fit to survive to sexual maturity and thus produce follow-up generations by which actual introgression into wild acceptor-species genomes could occur through repeated backcrossing. All these steps are reviewed in this paper. It will become evident that, in order to estimate a transgene’s capacity to introgress and persist in wild relatives, all steps in the introgression process should be considered. Areas where still relatively little definite data has been published are i) assessing the extent to which genes, such as those conferring resistance to biotic as well as abiotic stresses, indeed enhance fitness in natural settings and the consequences of introgression of these for these environments; and ii) improving this assessment of fitness, e.g. by not only scoring relevant traits, such as those related to fecundity, but also monitoring them in realistic field situations. In this regard, more data on, for instance, the effects of the transgene insertion site on the introgression process and the importance of fitness of the intermediate stages (backcrosses) would be needed to reach a more general insight. In relation to co-existence of GMO and organic agriculture, crop-to-crop gene flow also needs to be controlled. Therefore, a wide variety of possible hybridization barriers, both physical and biological, are discussed. The technical limitations of assessing introgression from crop to wild avoiding the use of transgenic markers are discussed on the basis of work on lettuce.

Keywords: gene flow; hybrids; crops; wild relatives; fitness; GMO; biosafety; molecular markers; transgenes

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Introduction

Gene flow, the movement of genes between populations of a species and between these populations and interfertile relatives, has recently received considerable attention in relation to the introduction of genetically modified (GM) crops. Gene flow from genetically modified (GM) crops to conventional crops and/or wild relatives could occur through dispersal of pollen or seeds, or vegetative parts capable of clonal propagation.

Even though genetically engineered crops could have a number of agronomical or environmental benefits, such as an increase in yield or a decrease of the use of pesticides or fertilizers, there are serious concerns about the possible consequences of the escape of transgenes into the environment. Examples of the risks mentioned in the context of gene flow from GM plants are: i) creation of new weeds resulting from an escape by the crop itself; ii) creation of superweeds by hybridization of a (wild/weedy) species with the transgenic crop; iii) genetic erosion (loss of original diversity of wild relatives).

A massive body of publications on environmental safety of GM crops has appeared (for recent general reviews see Nap et al. 2003; Conner, Glare and Nap 2003). In this paper, a short overview of the gene-flow topic illustrated with a few examples of crops from the literature will be given. These examples include an understudied crop, lettuce (*Lactuca sativa*), which is part of a recently started EU project, acronymed 'ANGEL' (<http://www.plant.wageningen-ur.nl/projects/angel>), in which the authors participate. First, a few words will be devoted to gene flow within the crop in relation to different styles of farming.

Gene flow from crop to crop

Gene flow from one crop to the other is mainly a concern of breeders, who wish to keep their varieties genetically pure, especially seed-production crops, in order to guarantee their quality, and of gene-bank curators, who want to safeguard the identity of their accessions. With regard to GMOs, there is particular concern with organic and other farmers who strive to produce harvests completely free from products of modern biotechnology. The EU regulation EEC 2092/91 explicitly excludes the use of GMOs in organic farming. Moreover, the supplementary regulation EC 1804/1999 prohibits the presence of GMO material in organic produce, for all practical purposes setting a threshold at the analytical limit of quantification of about 0.1%. Thus, unintended transfer of transgenes to an organic crop could lead to seriously diminishing the quality of seed or produce, leading to a serious setback in their marketability (Eastham and Sweet 2002).

Recently, IPTS (Sevilla) and the EC's Joint Research Centre produced a synthesis report on the co-existence of genetically modified, conventional and organic crops in European agriculture (Bock et al. 2002). They studied three examples, oilseed rape for seed production, maize for feed and potatoes for consumption. Even though there are already well established segregation schemes in place, e.g. for high-erucic-acid oilseed rape and starch potatoes, co-existence will be extremely difficult with a contamination limit of 0.1% as necessitated by the demands of organic farming. This even applies to the potato, where the only problems encountered are in the occurrence of groundkeepers and the possibility of post-harvest mixing. Suggested less stringent thresholds of 0.3% for allogamous seed-production crops like oilseed rape or 1% for feed use (maize) will lead to additional costs due to necessary changes in farming

practice and the need for monitoring systems as well as additional insurance. These costs are in the order of 1-10% of the gross margin, but may go up to 41% in particularly difficult cases, such as occur in oilseed rape. Problems in oilseed rape not only relate to substantial insect-mediated pollen flow (20-30% outcrossing level on average), but also to the occurrence of volunteers in and outside of agricultural fields due to seed spillage. Volunteers are particularly problematic in the organic system with its less intensive weed control. In maize, problems are limited to wind-dispersed pollen and seed impurities. However, the difficulties in predicting long-distance pollen flow (see below) will at least necessitate co-operation between neighbouring farms. For a discussion of measures preventing gene flow, see below.

The problems with cross-pollination also apply to many other crops. Even in the as basically selfing regarded lettuce, outcrossing between varieties grown in each other's vicinity was shown up to a level of about 3% by Thompson et al. (1958).

In relation to contamination of genetic resources, a notorious case is the report of the occurrence of transgenic sequences in traditional land races of maize in the Oaxaca region of Mexico (Quist and Chapela 2001). The results were heavily criticized (Christou 2002; Metz and Fütterer 2002; Kaplinsky et al. 2002), and as a consequence, the Nature editor admitted in an editorial note that publication of the original paper was unwarranted on the basis of the evidence presented therein. In a reaction, CIMMYT (cf. <http://www.cimmyt.org>) stated that it had taken measures to keep its important maize gene-bank collection free of transgene contamination and that in line with this, no transgenes were found upon testing of accessions. As a wind-pollinated, outcrossing species, gene flow into land races of maize would come as no surprise. However, the whole affair highlighted the danger of premature publicizing in such a controversial area.

A consequence of crop-to-crop gene flow could be transgene stacking. For example, three-herbicide-resistant oilseed rape volunteers have been reported as a consequence of cross-pollination between single-resistant lines cultivated in the Alberta region of Canada (Hall et al. 2000). The appearance of such volunteers may lead to adaptation of herbicide treatments depending on the type of crop rotation used, e.g. whether there is a follow-up by other herbicide-tolerant crops, such as beet. On the other hand, weedy populations can also develop herbicide tolerance under selective pressure spontaneously, a phenomenon with which the agricultural industry is not unfamiliar (Conner, Glare and Nap 2003).

Gene flow from crop to wild

Gene flow basics

Ellstrand, Prentice and Hancock (1999) showed that 12 out of the 13 most important food crops of the world hybridize with wild relatives in some part of their agricultural distribution. Also other reviews suggest that gene flow from crop to wild relatives is a common phenomenon. In The Netherlands for instance, about one quarter of 42 reviewed species spontaneously hybridize with one or more species from the local flora (De Vries, Van der Meijden and Brandenburg 1992). De Vries, Van der Meijden and Brandenburg presented their results in the form of so-called botanical files, in which a D_{pdf} code (p for dispersal by pollen, d for dispersal by diaspores, f for frequency of dispersal) is given with each crop species for the benefit of regulatory authorities. According to Ellstrand, Prentice and Hancock (1999) the results on the universality of gene flow can be explained in an evolutionary context, because domesticated plants represent lineages that diverged from their progenitors

only a few thousand years ago. Therefore, complete reproductive isolation would not be very likely to have occurred yet.

The key condition for gene flow through hybridization and consequent introgression is, of course, the presence of wild or weedy relatives in the vicinity of the crop. Generally, pollen flow from a specific source follows a leptokurtic distribution, implying that the great majority occurs over a short distance. Long-distance dispersal is rare, but also quite variable and thus it is, for instance, hard to predict what the maximum distance of occurrence is. For instance, in a re-analysis of previous data, Klinger (2002) showed that even for the obligatory outcrossing radish (*Raphanus sativus*), it is difficult to predict the actual rate of gene flow in the field; variability in the rate of pollination was high between experiments, particularly so at larger distances from the source. In wind-pollinated species, particularly trees (e.g. poplar, DiFazio et al. 1999), the picture might even be more complicated, depending on prevailing wind direction etc.

Furthermore, additional pre- and post-zygotic barriers can be found in a number of species and/or varieties. Lefol, Fleury and Darmency (1996) showed that, for example, between genetically modified *Brassica napus* and hoary mustard (*Hirschfeldia incana*) two types of pre-zygotic barriers occurred; the first barrier is pollen germination and growth on the papilla of the foreign species, and the second barrier is the weak attraction of foreign pollen tubes to the micropyles of ovules. With oilseed rape, an example of a post-zygotic barrier was apparent in a cross with wild radish (*Raphanus raphanistrum*): there was low seedling survival when the hybrid plants received their cytoplasm from oilseed rape as mother plant (Guéritaine et al. 2002).

Further introgression of crop genes (transgenes) will depend on the chances of survival of the F₁ hybrids and their fecundity. This will be discussed in the next section.

Fitness of first generation hybrids

Chances of introgression of crop traits into wild relatives will especially be high if fertile hybrids occur frequently (Ellstrand, Prentice and Hancock 1999). In this regard, it is sometimes assumed that crop genes, particularly the ones associated with domestication (and therefore also transgenes), will only be disadvantageous to wild populations or will have certain detrimental/fatal fitness effects on hybrid populations (Small 1984), and therefore they will not be able to spread. However, alleles encoding domestication traits often show a recessive character (cf. Doebley et al. 1990). As a consequence, first-generation hybrids will show more similarity to the wild parent, which will increase their chances of survival away from cultivation.

Nevertheless, examples of maladaptation occur, such as described by Hauser (2002) in hybrids between cultivated carrot (*Daucus carota* ssp. *sativus*) and wild *Daucus carota* ssp. *carota*: these hybrids inherited the sensitivity to frost from their cultivar parent and therefore had a survival rate which was significantly lower than that of the wild carrot and only slightly better than that of the cultivar.

However, in many taxa interspecific hybrids are found that are sometimes as fit as or even fitter than their parents. For example, Hauser, Shaw and Østergård (1998) found that hybrids between weedy *Brassica rapa* and cultivated *Brassica napus* were intermediate in fitness between their parents and significantly fitter than weedy *Brassica rapa* as determined by seed characteristics and survival in the field.

Generally, the transfer of (trans)genes from a crop to wild relatives is more likely to succeed if the trait itself is neutral or beneficial to the hybrid population. In

Cucurbita pepo, the F₁ generation produced from a doubly virus-resistant crop and wild *C. pepo* ssp. *ovifera* was vigorous enough to contribute to the gene pool of subsequent generations (Spencer and Snow 2001), but there was a lot of variation in fecundity between experiments. Whether the trait for virus resistance had any significant effect on hybrid fitness could not be established.

In some crop–wild combinations F₁ hybrids are assumed to be generally rare, and they can be sterile or have a very low fecundity. For example, fertilization of wild radish by oilseed rape was assessed using a transgenic herbicide-resistance marker under agronomical conditions by several groups and low, but widely varying success rates were reported: from none (Rieger et al. 2001), to 10⁻⁷ to 3.10⁻⁵ (Chèvre et al. 2000) or up to 6.10⁻⁵ to 2.10⁻³ (Darmency, Lefol and Fleury 1998). The varying results may be due to variation in experimental set-up, environmental conditions, or to genotypic differences; e.g., Guéritaine and Darmency (2001) showed wide individual variation in effectiveness of ovule fertilization in wild radish by oilseed-rape pollen under controlled conditions. In case of low hybrid fecundity, persistence of hybrid populations will depend on whether they are able to reproduce vegetatively or to recover fertility by repeated backcrossing to the wild progenitor. The possibilities of further introgression are the subject of the next section.

Introgression

In the case of poor compatibility between crop and wild species, fertility can be restored in hybrid offspring when the hybrids backcross to the wild relative. Thus, in hybrids between oilseed rape and wild radish, fertility could be restored by repeated backcrossing to the wild radish (Guéritaine et al. 2002). However, no stable integration of oilseed rape's transgenic herbicide resistance into the wild radish genome was found (Chèvre et al. 1997). It could not be excluded that this was a consequence of the position on the oilseed-rape genome of the herbicide-resistance transgene (Chèvre et al. 1998). Hauser, Jørgensen and Østergård (1998) found that backcrosses and F₂ hybrids between weedy *Brassica rapa* and oilseed rape (*Brassica napus*) had a reduced fitness relative to their parents for most of the fitness components measured, but there was a lot of variation related to genotypes used, and some of the hybrids were as fit as their parents. Therefore, in spite of the low fitness of second-generation hybrids, introgression of transgenes will not be completely hindered.

The other way around, backcrossing will also bring in fitness characters stemming from the wild parent, such as seed dormancy. Resulting progeny can adopt these traits from the wild relative, resulting in plants that are more adapted to the variable environmental conditions of wild populations. Landbo and Jørgensen (1997) found backcross seeds harvested from *Brassica napus* x *Brassica rapa* hybrids to show more weedy *B. rapa*-like behaviour, that is, more seeds were dormant. This result indicated that (trans)gene escape in time, through the persistence of dormant seeds in the soil, is likely to happen.

The chances of transfer of transgenes could be influenced by the integration site of the construct on the genome, as was implied by Metz et al. (1997). They found large differences in the transmission frequency of the transgene between the offspring of two transgenic lines of the amphidiploid *Brassica napus* (containing both A and C genomes) and non-transgenic *Brassica rapa* (containing only the A genome) during backcrossing. The authors suggested this to be due to the construct being situated on the C genome, which might limit the transfer to *Brassica rapa*. On the other hand, related species containing the C genome, e.g. *Brassica oleracea*, would then have an

increased chance of introgression of the transgene. Tomiuk, Hauser and Bagger-Jorgensen (2000), however, using a simple population-genetic model, claimed that the results of Metz et al. (1997) could also be explained by selection against transgenic A chromosomes during backcrossing. In the end, the safety of using specific integration sites would depend on the amount of homologous and homeologous (between A and C genomes) recombination events.

In relation to the relevance of the position of the transgene on the parental genome, interesting observations came from studies of hybridization between wild species from the sunflower genus *Helianthus*. Rieseberg et al. (1996) experimentally resynthesized a known ancient hybrid between *H. annuus* and *H. petiolaris*, *H. anomalus*. The three different hybrid lineages created conformed well in their genomic composition to the hybrid species *H. anomalus*, as evidenced by a comparison of linkage maps made using molecular markers; so, only particular combinations of the parental genomes appeared to lead to viable hybrid offspring. This would mean that chances for a transgene to end up in introgressed wild populations could depend on the insertion site. On the other hand, it appears doubtful whether this would be a feasible approach to containment of transgenes in crops.

In conclusion, it is clear that, in order to estimate whether a transgene is capable of introgressing and persisting in wild relatives, all steps in the introgression process should be considered (Landbo and Jørgensen 1997). Furthermore, environmental consequences of introgression in the long run need to be taken into consideration, which is discussed in the next section.

Population-ecological effects

As Ellstrand, Prentice and Hancock (1999) already pointed out, gene flow between a crop and its wild relatives can have two potentially harmful ecological/evolutionary consequences: enhanced weediness in the wild/weedy relatives by introgression of certain crop traits and extinction of wild relatives through genetic swamping or outbreeding depression. For example, the evolution of enhanced weediness in one of the world's worst weeds, *Sorghum halepense*, is assumed to be a result of introgression from the crop *Sorghum bicolor*. Transgenes may enhance these chances, depending on the specific traits encoded.

With regard to the possibility of 'swamping' of wild relatives, Rufener Al Mazyad and Ammann (1999) showed that the wild *Medicago falcata* is overtaken by the cultivated *Medicago sativa* and the hybrid between *M. sativa* and *M. falcata* (*Medicago x varia*) in Switzerland. This was in line with the observations that backcrosses of hybrids to cultivated *M. sativa* were considerably more vigorous than backcrosses to *M. falcata*. In some former populations of *Medicago falcata*, nowadays only introgressed forms with weakly represented traits of *M. falcata* are found. However, this phenomenon was limited to tetraploid forms of *M. falcata*, since these cross most readily to tetraploid *M. sativa*. Diploid *M. falcata* populations occurring in the eastern part of Switzerland were essentially free from introgression by *M. sativa*.

In contrast, the genetic variation of sea-beet populations (*Beta vulgaris* ssp. *maritima*) was found to increase slightly, for most parameters, by gene flow from sugar beet as well as red beet/Swiss chard (*Beta vulgaris* ssp. *vulgaris*) (Bartsch et al. 1999). In this regard, beet may be a special case, since beet cultivars show an equivalent level of genetic diversity as compared to their wild progenitors, which is highly unusual for a crop/wild combination (Bartsch et al. 1999). Beet also shows interesting complications as a consequence of the possibility of gene flow from wild to crop in the seed multiplication areas in Southern Europe. In Northern Europe, the

biennial beet is usually harvested before the onset of flowering, except for the occasional occurrence of bolters. However, hybridization in the seed-production areas between sugar beet and annual/weedy forms of wild sea beet (*Beta vulgaris* ssp. *maritima*) can lead to contamination of seed lots. Through this route, annual weed beets have established in beet cultivation areas in Northern Europe and these weed beets may facilitate gene flow from the crop to wild sea beet where beet cultivation occurs close to the coastal habitat of sea beet in Northern Europe (Boudry et al. 1993). As far as has been ascertained up to now, exchange rates between weed beet and coastal sea beet were below detection level in French cultivation areas (Desplanque et al. 1999).

Recent preliminary analyses of ecological consequences of transgenes in wild populations of *Helianthus annuus* for the first time showed that backcrossed plants transgenic for the *Bacillus thuringiensis* (*Bt*) gene cry1Ac can have a considerably higher seed production (due to decreased herbivory) in a realistic field situation (Snow et al. 2003). This could lead to a high frequency of the *Bt* gene in wild populations and consequently influence the population structure of certain native herbivores.

Barriers to gene flow

Even though pollen flow quickly falls off with distance, distance alone will not completely prevent gene flow and hybridization between crop and wild relatives. In a specific set-up, Reboud (2003) showed that a gap between crops is an inefficient way of reducing pollination: an unplanted distance of 3-4 m had no more effect than one of 1m. Removing the field-border plants of the acceptor plot would be the most efficient way of removing undesired cross-pollinated seeds. Likewise, a non-transgenic barrier crop could act as an 'absorber' of GM pollen by planting it on the area immediately surrounding the crop and subsequently destroying it before seed set, as suggested by Morris, Kareiva and Raymer (1994). Yet, this would be a rather costly practice. Dense stands of shrubs or tree-sized vegetation will also act as traps for airborne particles, including pollen. Jones and Brooks (1952) showed for maize that a tree barrier was effective only for plants growing near to the barrier, but it was much less effective at greater distances.

In addition, engineering biological barriers to prevent gene flow has been widely suggested. Possibilities include genetic engineering the inhibition of flowering, male sterility or seed sterility, and inserting the transgene into the chloroplast genome. Since the chloroplasts are usually only maternally inherited (with notable exceptions, such as in pines), the latter would seriously impede gene flow through pollen dispersal. Seed sterility has not yet been adopted as a technique for genetic isolation because several aspects of these so-called GURTs (Genetic Use Restriction Technologies) or, popularly, 'terminator' technology are not yet totally reliable (Eastham and Sweet 2002). For this purpose, Kuvshinov et al. (2001) suggested a newly developed method called Recoverable Block of Function (RBF) to overcome this unreliability. Their construct will prevent sexual reproduction completely (thus pollen and seed flow is stopped) unless a trigger is applied, which then restores the ability to reproduce. Most recently, a repressible seed-lethal system was proposed, in which the repressor is inserted at the same locus as the seed-lethal system (to which the desired transgene can be linked) itself, but on a different chromosome. At outcrossing, the seed-lethal system (together with any other transgene linked to it) will become segregated from its repressor, thus leading to non-viable offspring

(Schernthaner et al. 2003). However, all these terminator technologies have also been challenged because of possible disadvantageous effects to biological diversity by keeping other breeders from further improvement of this germplasm and by harming subsistence farming by preventing farmers from multiplying the crop themselves for future croppings (Visser et al. 2001)

Assessing gene flow

In outcrossing crops with cross-compatible wild relatives, studying gene flow may be relatively straightforward using standard population-genetic methods of analysis (cf. Raybould and Clarke 1999). This will also be the case with well-circumscribed populations. Thus, Whitton et al. (1997) could assess persistence of crop alleles over five generations following a single hybridization event with the crop in a naturally occurring population of wild *Helianthus annuus* using cultivar-specific RAPD markers. However, ascertaining the occurrence of gene flow will be more difficult where outcrossing events might be expected to be relatively rare or in broader field surveys in which origins of crop alleles are not directly assignable. In an example of the latter on strawberry (*Fragaria x ananassa*), Westman et al. (2001) showed the presence of crop-specific AFLP markers, both from cultivars used in the past and in the present, in wild populations of *F. virginiana* growing in the vicinity of areas of strawberry cultivation. However, the reliability of such observations depends strongly on the degree to which the crop-specificity of the AFLP markers can be ascertained.

In lettuce, Frietema-De Vries, Van der Meijden and Brandenburg (1994) performed an additional field study, because the botanical files study by De Vries, Van der Meijden and Brandenburg (1992) indicated uncertainty on the relationship to its closest wild relative, prickly lettuce (*Lactuca serriola*). They were shown to be essentially conspecific, based on morphology and crossability. Nevertheless, it remained unclear whether any gene flow occurred between crop and wild, for both are regarded as self-pollinating crops, with very limited knowledge available on any amount of cross-pollination. If there were any exchange, this would make it a potentially interesting case for studying the involvement of gene flow from the crop in increased invasiveness, since the wild *L. serriola* expanded its occurrence in Northwestern Europe enormously during the last decades.

One of the aims of the afore-mentioned EU project is filling in this knowledge gap by the use of molecular marker systems. When tracing evidence for past introgression from crop to wild by such methods, several problems arise. Even though markers can be selected that are specific for a panel of varieties of the cultivated form, rare occurrences of these seemingly crop-specific markers in the wild form may still be attributable to common ancestry at a considerably earlier stage of domestication, and not to recent introgression. On the other hand, wild relatives are widely used in breeding, e.g. for the introduction of disease-resistance genes, and thus, specific genomic segments from particular wild accessions have recently been introgressed into the cultivated form. In turn, these segments may also end up in other wild populations in the vicinity of lettuce cultivation.

To tackle these problems, three molecular methods are tested: in the first place, two 'neutral' multi-locus marker systems (AFLP and the retrotransposon-based SSAP). Markers will be selected by linkage mapping, to achieve a good coverage of the genome and thus to obtain maximum informativeness. In this way, it should, in principle, be possible to identify specific chromosomal segments shared by wild and cultivated populations, which could be taken as better evidence for recent

introgression than random marker data. For the AFLP technique, this method was indicated to be workable in principle (Van de Wiel et al. 2003). The relatively novel system, SSAP, which is based on retrotransposons occurring ubiquitously in eukaryotic genomes, was also successfully developed for lettuce (Van de Wiel et al. 2003). It has the additional advantage of, in principle, providing markers of which the direction of change can be inferred from the way they move through the genome. Thus, shared unique insertions can be taken as strong evidence for a common origin of the chromosomal parts of the plants/populations in which they are found (for a review see Kumar and Hirochika 2001). In the second place, in order to be able to trace genomic regions most likely involved in breeding efforts, the third 'functional' multi-locus marker system, NBS-profiling, was implemented (Van de Wiel et al. 2003). NBS profiling is screening for variation in and around disease-resistance genes containing the conserved NBS (Nucleotide Binding Site) region, which make up the great majority of resistance genes known so far (Van Tienderen et al. 2002; Van der Linden et al. 2004). In lettuce, a considerable amount of breeding effort has been put into introducing resistance against pathotypes of the downy mildew, *Bremia lactucae*. At least one of these resistance factors, Dm3, has recently been shown to be encoded by a gene belonging to the NBS-LRR type of resistance genes (Meyers et al. 1998).

Conclusions

In recent years, many new data have been generated on the whole gene-flow process as relevant to the GMO debate. Nevertheless, knowledge gaps remain in the following areas: i) actual outcrossing and introgression rates in understudied crops, particularly the ones that have a self-pollination reputation, like lettuce; ii) persistence of crop (trans)genes in wild populations in relation to the extent to which they confer a fitness advantage; and iii) the population-ecological and evolutionary effects of the persistence of introgressed genes. Most important in this regard is the establishment of baseline data, that is, the impact that gene flow from crops already had on their wild congeners, to which the effects of GM crops could be weighed. The great majority of transgenes will confer characteristics not essentially different from their conventional counterparts. Because of the high variability in gene-flow results and the diversity of constructs used, it is often stressed that biosafety should be assessed using a case-by-case approach. This is exemplified by the observations of Linder and Schmitt (1995) that two oil-modification transgenes had a different effect on persistence of both feral *Brassica napus* canola and hybrids of *Brassica napus* and wild *Brassica rapa*. Thus, even transgenes with similar functions could lead to different environmental effects.

With regard to i) it is evident that gene-flow assessment by molecular marker technology can be technically demanding, wherever the use of transgene markers needs to be avoided, as is shown by the lettuce case. In relation to ii) it should be emphasized that still very little evidence was generated on the extent to which genes, such as those conferring resistance to biotic as well as abiotic stresses, enhance fitness in natural settings. Potential threats of the use of certain types of traits (e.g. apomixis genes or stress-tolerance genes) have hardly (or not at all) been studied yet. In addition, there is also a need for improving assessment of fitness, e.g. not only by scoring relevant traits, such as those related to fecundity, but also by demographic monitoring in realistic field situations. Demographic monitoring could establish the life-cycle stages most relevant to population survival and growth, and so put any of the fecundity traits measured in the proper perspective, that is, to what extent the pertaining traits are of critical importance in a taxon's life cycle and therefore, for its

establishment, survival and spread in the field (Oostermeijer 2000; Luijten et al. 2002).

Furthermore, evolutionary effects of (trans)gene flow can not be evaluated for a particular region only, because the whole life history of crop and wild relatives has to be examined, in which not only regional variation may occur but which can also be spread out over different areas. This is exemplified by the occurrence of gene flow from wild to crop in seed-producing areas far removed from the ultimate cultivation areas, confounding any gene flow from crop to wild occurring in the latter, such as described for beet. Also, regional differences in the presence of crop relatives may cause a given crop introduction to have very different potential effects. In this respect, export of genetically modified (transgenic) crops to other countries could be a point. For instance, a genetically modified potato cultivar bred in Europe will not show any gene flow there, because of the lack of cross-compatible wild relatives. However, when exported to the potato's region of origin in South America hybridization partners will abound.

With the present uncertainties, the EU Directive 2001/18/EC on the deliberate release into the environment of GM organisms includes a set of outlines for monitoring after introduction in order to establish any unforeseen harmful effects. Since such effects, by definition, are difficult to predict, these claims might very well prove so costly that they seriously interfere with marketability of crop varieties, with a possible exception for the largest and most profitable crops, such as maize or soybean.

Acknowledgements

Part of the reviewing work presented in this article was performed in the framework of a report on gene flow assigned by COGEM (Committee on Genetic Modification), advisory body for VROM (Netherlands Ministry of Housing, Spatial Planning and the Environment). The lettuce work was performed in a project co-funded by the EU FP5 Quality of Life Programme, contract no. QLK3-2001-01657.

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