Annotated Bibliography on Environmental and Ecological Impacts from Transgenic Plants I: Transgene Persistence and Gene Flow

Compiled by L. LaReesa Wolfenbarger

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Arnaud, J. F., Viard, F., Delescluse, M. and Cuguen, J. 2003. Evidence for gene flow via seed dispersal from crop to wild relatives in Beta vulgaris (Chenopodiaceae): consequences for the release of genetically modified crop species with weedy lineages. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**: 1565-1571.

Gene flow and introgression from cultivated to wild plant populations have important evolutionary and ecological consequences and require detailed investigations for risk assessments of transgene escape into natural ecosystems. Sugar beets (Beta vulgaris ssp. vulgaris) are of particular concern because: (i) they are cross-compatible with their wild relatives (the sea beet, B. vulgaris ssp. maritima); (ii) crop-to-wild gene flow is likely to occur via weedy lineages resulting from hybridization events and locally infesting fields. Using a chloroplastic marker and a set of nuclear microsatellite loci, the occurrence of crop-to-wild gene flow was investigated in the French sugar beet production area within a 'contact-zone' in between coastal wild populations and sugar beet fields. The results did not reveal large pollen dispersal from weed to wild beets. However, several pieces of evidence clearly show an escape of weedy lineages from fields via seed flow. Since most studies involving the assessment of transgene escape from crops to wild outcrossing relatives generally focused only on pollen dispersal, this last result was unexpected: it points out the key role of a long-lived seed bank and highlights support for transgene escape via man-mediated long-distance dispersal events.

De Marchis, F., Bellucci, M. and Arcioni, S. 2003. Measuring gene flow from two birdsfoot trefoil (Lotus corniculatus) field trials using transgenes as tracer markers. *Mol Ecol* **12**: 1681-1685.

Genetic engineering is becoming a useful tool in the improvement of plants but concern has been expressed about the potential environmental risks of releasing genetically modified (GM) organisms into the environment. Attention has focused on pollen dispersal as a major issue in the risk assessment of transgenic crop plants. In this study, pollen-mediated dispersal of transgenes via cross-fertilization was examined. Plants of Lotus corniculatus L. transformed with either the Escherichia coli asparagine synthetase gene asn A or the beta-glucuronidase gene uid A, were used as the pollen donor. Nontransgenic plants belonging to the species L. corniculatus L., L. tenuis Waldst. and Kit. ex Willd, and L. pedunculatus Cav., were utilized as recipients. Two experimental fields were established in two areas of central Italy. Plants carrying the uid A gene were partially sterile, therefore only the asn A gene was used as a tracer marker. No transgene flow between L. corniculatus transformants and the nontransgenic L. tenuis and L. pedunculatus plants was detected. As regards nontransgenic L. corniculatus plants, in one location flow of asn A transgene was detected up to 18 m from the 1.8 m(2) donor plot. In the other location, pollen dispersal occurred up to 120 m from the 14 m(2) pollinating plot.

Gueritaine, G., Bazot, S. and Darmency, H. 2003. Emergence and growth of hybrids between Brassica napus and Raphanus raphanistrum. *New Phytologist* **158**: 561-567.

Risk assessment studies of transgenic crops have recently brought evidence of a low spontaneous hybridization frequency of Brassica napus with Raphanus raphanistrum . The fate of the first generation hybrids is crucial to determine the initial rate of spread of transgenes.This work aims to compare the fitness components of parents and F-1 hybrids at the first step of the life cycle. The ability to emerge, establish seedling, cover the soil and develop adult plant was examined in controlled and field conditions, alone or in competition.The F-1 hybrids showed a lower seedling emergence, a significant delay of emergence, and a lower survival than for both parents. Rosette diameter and dry matter of hybrid plants were very reduced compared with those of both parents, especially when they grew in the field and under competition conditions.The relevance of these results to gene flow and possible impact to agriculture and environment is discussed.

Klein, E. K., Lavigne, C., Foueillassar, X., Gouyon, P. H. and Laredo, C. 2003. Corn pollen dispersal: Quasi-mechanistic models and field experiments. *Ecological Monographs* **73**: 131-150.

To make quantitative predictions about the pollen dispersal of a plant species under different environmental conditions, it is necessary to determine its individual pollen dispersal function. i.e., the two-dimensional density function describing the probability that a pollen grain emitted in (0, 0) fertilizes an ovule in (x, NI). This function will depend on biological and climate parameters. We present models for the individual dispersal function of corn. These models are based on Brownian motion with drift and integrate biological (difference of height between male and female flowers) and aerodynamic (settling velocity, wind speed, air turbulence) parameters. The models presented differ in the importance of vegetation in stopping the paths of pollen grains.The models were fitted to data from two large field experiments of corn using the color of kernels as a phenotypic marker for pollen dispersal. The resulting estimations for the parameters of the models and comparisons between models indicate that (1) these models can provide good predictions of the observed data, (2) vegetation is not the major obstacle that stops pollen paths, and (3) there is a benefit in considering the difference in height between male and female flowers. Furthermore, values of the parameters estimated from dispersal data appear consistent with meteorological and biological data acquired independently.

Lu, B. R., Song, Z. P. and Chen, J. K. 2003. Can transgenic rice cause ecological risks through transgene escape? *Progress in Natural Science* **13**: 17-24.

Alien transgene escape from genetically engineered rice to non-transgenic varieties or close wild relatives (including weedy rice) may lead to unpredictable ecological risks. However, for transgene escape to occur three conditions need to be met: (i) spatially, transgenic rice and its non-transgenic counterparts or wild relatives should have sympatric distributions; (ii) temporally, the flowering time of transgenic rice and the non-transgenic varieties or wild relatives should overlap; and (iii) biologically, transgenic rice and its wild relative species should have such a sufficiently close relationship that their interspecific hybrids can have normal generative reproduction. This paper presents research data on the geographic distribution, flowering habits, interspecific hybridization, and gene flow of cultivated rice (Oryza sativa) and its closely related wild relatives containing the AA genome. The objective is to estimate the possibility of transgene escape to non-transgenic rice varieties and wild relatives of rice, which may result in unpredictable ecological risks.

Meagher, T. R., Belanger, F. C. and Day, P. R. 2003. Using empirical data to model transgene dispersal. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **358**: 1157-1162.

One element of the current public debate about genetically modified crops is that gene flow from transgenic cultivars into surrounding weed populations will lead to more problematic weeds, particularly for traits such as herbicide resistance. Evolutionary biologists can inform this debate by providing accurate estimates of gene flow potential and subsequent ecological performance of resulting hybrids. We develop a model for gene flow incorporating exponential distance and directional effects to be applied to wind-pollinated species. This model is applied to previously published data on gene flow in experimental plots of Agrostis stolonifera L. (creeping bentgrass), which assessed gene flow from transgenic plants resistant to the herbicide glufosinate to surrounding non-transgenic plants. Our results show that although pollen dispersal can be limited in some sites, it may be extensive in others, depending on local conditions such as exposure to wind. Thus, hybridization under field conditions is likely to occur. Given the nature of the herbicide resistance trait, we regard this trait as unlikely to persist in the absence of herbicide, and suggest that the ecological consequences of such gene flow are likely to be minimal.

Reboud, X. 2003. Effect of a gap on gene flow between otherwise adjacent transgenic Brassica napus crops. *Theor Appl Genet* **106**: 1048-1058.

Gene flow resulting from cross pollination becomes an issue when transgenic crops are involved and the genetic modification carries a trait of ecological importance. As crop fields are often separated by a barren gap, such as an intervening roadway or unplanted area, I measured cross contamination between two herbicide-resistant transgenic fields (canola, Brassica napus) across a gap of up to 12 m. I focused on pollen exchange from the field border up to 7 m inside each field over two seasons. In the absence of a gap, I found that gene dispersal diminished rapidly with distance, with more than 40% of transgenic progeny found within the first meter from the edge of the adjacent crop. Cross contamination between fields declined more rapidly when there were intervening plants, however. Plants separated from the transgenic source by a gap of 3-4 m, yielded the same level of transgenic progeny as those separated by 1 m of crop. Both insects and wind pollinate canola, and so the explanation for my observations could involve the influence of gaps on wind patterns or on the behaviour of pollinators. The gap effect does not seem to depend only upon the variation in the density of neighbours that surrounds those plants at the crop edge versus those in the crop matrix. On the basis of this study, it is recommended that economic profit would be maximised by removing field borders after flowering rather than by leaving a surrounding gap, which would need to occupy up to threefold as much field surface to achieve the same level of containment.

Snow, A. A., Pilson, D., Rieseberg, L. H., Paulsen, M. J., Pleskac, N., Reagon, M. R., Wolf, D. E. and Selbo, S. M. 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecol Appl* **13**: 279-286.

Gene flow from transgenic crops can introduce novel traits into related species, but the ecological importance of this process is unknown. Here, we report the first empirical evidence that wild plants can benefit from a bacterial transgene under uncaged, natural conditions. Cultivated sunflower (Helianthus annuus) is known to hybridize frequently with wild sunflower (H. annuus) in the western and midwestern United States. We studied a crop-developed Bacillus thuringiensis (Bt) transgene, cry1Ac, in backcrossed wild sunflower populations. Lepidopteran damage on transgenic plants was strongly reduced relative to control plants at our two study sites, while damage by several weevil and fly species was unaffected. Our results suggest that reduced herbivory caused transgenic plants to produce an average of 55% more seeds per plant relative to nontransgenic controls at the field site in Nebraska. A similar but nonsignificant trend was seen at the site in Colorado (14% more seeds per plant).. In a greenhouse experiment the transgene had no effect on fecundity, suggesting that it was not associated with a fitness cost. If Bt sunflowers are released commercially, we expect that Bt genes will spread to wild and weedy populations, limit damage from susceptible herbivores on these plants, and increase seed production when these herbivores are common.

Soboleva, T. K., Shorten, P. R., Pleasants, A. B. and Rae, A. L. 2003. Qualitative theory of the spread of a new gene into a resident population. *Ecological Modelling* **163**: 33-44.

This work addresses the problems that arise in the estimation of the risk of invader/unwanted GMO spread and the optimal release of desired genes into a population through the release of transgenic individuals. On the basis of a general model of the propagation of an advantageous allele through a population we analyze the thresholds and critical aggregations in gene frequency necessary for the spread of new gene carriers. It is shown that if the invader appears at one place in the ecosystem then it will not spread throughout the ecosystem unless it exceeds some critical threshold, where the critical threshold is defined in terms of both the amount and distribution of the invader. The value of the critical threshold will depend on the fitness of the invader relative to the fitness of the resident organisms in the ecosystem and the mechanism of its dispersion. It is also shown that typically an invader will not spread symmetrically, even if the environment is isotropic, but rather develops clusters that form filaments within the ecosystem. We also demonstrate that if the invader aggregation is sufficiently large then after an initial period the advance of the invader into the resident population takes the form of a traveling wave. The speed of this wave tends to a speed characteristic of the relative fitness and dispersive mechanisms of the invader. (C) 2002 Elsevier Science B.V. All rights reserved.

Song, Z. P., Lu, B. R., Zhu, Y. G. and Chen, J. K. 2003. Gene flow from cultivated rice to the wild species Oryza rufipogon under experimental field conditions. *New Phytologist* **157**: 657-665.

Here, the gene flow from a cultivated rice variety (Minghui-63) to common wild rice (Oryza rufipogon) was investigated to assess the biosafety risk associated with the environmental release of transgenic varieties.Four experimental designs differing in the spatial arrangement of the Minghui-63 and O. rufipogon plants were used in experiments conducted in an isolated rice field in Hunan Province, southern China, where O. rufipogon occurs naturally.Natural hybridization events between the two species were detected by scoring a simple sequence repeat (SSR) molecular marker. A total of 296 hybrids were identified from 23 776 seedlings that were randomly germinated from > 80 000 seeds collected from O. rufipogon. The occurrence of the crop-to-wild gene flow was significantly associated with wind direction and frequencies of the gene flow, which decreased significantly with distance from the pollen sources. The maximum observed distance of gene flow was 43.2 m.The results indicated that gene flow from cultivated rice to O. rufipogon occurred at a considerable rate. Therefore, isolation measures should be considered when deploying transgenic rice in the sympatric regions of the wild rice, and when establishing in situ conservation of O. rufipogon. The experimental system in this study can be used for biosafety assessment of transgene escape of other wind-pollinated crops.

Tepfer, D., Garcia-Gonzales, R., Mansouri, H., Seruga, M., Message, B., Leach, F. and Perica, M. C. 2003. Homology-dependent DNA transfer from plants to a soil bacterium under laboratory conditions: implications in evolution and horizontal gene transfer. *Transgenic Res* **12**: 425-437.

DNA transfer was demonstrated from six species of donor plants to the soil bacterium, Acinetobacter spp. BD413, using neomycin phosphotransferase (nptII) as a marker for homologous recombination. These laboratory results are compatible with, but do not prove, DNA transfer in nature. In tobacco carrying a plastid insertion of nptII, transfer was detected with 0.1 g of disrupted leaves and in oilseed rape carrying a nuclear insertion with a similar quantity of roots. Transfer from disrupted leaves occurred in sterile soil and water, without the addition of nutrients. It was detected using intact tobacco leaves and intact tobacco and Arabidopsis plants in vitro. Transfer was dose-dependent and sensitive to DNase, and mutations in the plant nptII were recovered in receptor bacteria. DNA transfer using intact roots and plants in vitro was easily demonstrated, but with greater variability. Transfer varied with plant genome size and the number of repeats of the market DNA in the donor plant. Transfer was not detected in the absence of a homologous nptII in the receptor bacteria. We discuss these results with reference to non-coding DNA in plant genomes (e.g., introns, transposons and junk DNA) and the possibility that DNA transfer could occur in nature.

Thompson, C. J., Thompson, B. J. P., Ades, P. K., Cousens, R., Garnier-Gere, P., Landman, K., Newbigin, E. and Burgman, M. A. 2003. Model-based analysis of the likelihood of gene introgression from genetically modified crops into wild relatives. *Ecological Modelling* **162**: 199-209.

The proliferation of genetically modified crops has created a need for methods to predict the likelihood of gene introgression into related species in situ. We present a model of a modified crop and an associated unmodified plant population removed spatially from the modified crop but not completely isolated from it, reflecting standard practices for isolation of field trials. We develop models for two kinds of life histories, broadly based on Brassica and Gossypium, taxa that are targets for genetic modification. We find that current prescriptions for field trials are likely to lead to escape of transgenes into wild populations when outcrossing rates are moderate and hybrids are fertile. The results are sensitive to pollen rain within plausible bounds for model parameters, suggesting buffer widths are an important aspect of the design of field trials. When gene introgression requires the spontaneous development of a polyploid, the likelihood of gene introgression is lower but still appreciable in realistic circumstances. Events that are unlikely over periods of a few years become almost certain within scales of a few decades, emphasising the need for gene risk assessments to be set in specified time frames. The models serve to identify the parts of the system that are poorly known and that are important in determining outcomes, providing a focus for future research. There is a need for research on the consequences of changes in fitness due to the transgenes, competitive interactions between related species, and the broader ecological consequences of changes in agricultural practice resulting from the use of genetically modified crops. (C) 2002 Elsevier Science B.V. All rights reserved.

Zhang, N. Y., Linscombe, S. and Oard, J. 2003. Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice. *Euphytica* **130**: 35-45.

The potential of transferring herbicide resistance from transgenic rice (Oryza sativa L.) varieties to sexually compatible weeds is of paramount importance for development of effective weed control strategies. The objective of this research was to determine the genetic control and frequency of natural outcrossing between a transgenic, glufosinate-resistant rice line and a Louisiana biotype of red rice (Oryza sativa L.). Molecular and phenotypic data showed that outcrossing in field plots between a non-transgenic purple marker line and red rice did occur within one field season, but at a low rate of <1%. Similarly, molecular and phenotypic data demonstrated that glufosinate resistance was transferred from the transgenic line to the red rice biotype in the field within one year at a low frequency of 0.3%. Compared to parental lines, the transgenic-red rice hybrids were extremely late, tall, and never set seed during the normal field season. Genetic analyses in all F-2 populations showed glufosinate resistance behaved in a Mendelian fashion as a single, dominant gene. Presence of the bar gene for glufosinate resistance did not increase fitness or seed fecundity in hybrids or subsequent progeny. The genetic analyses and outcrossing results from this study suggest that an effective management program can be developed to prolong the usefulness of transgenic, glufosinate herbicide technology.

Cresswell, J. E., Osborne, J. L. and Bell, S. A. 2002. A model of pollinator-mediated gene flow between plant populations with numerical solutions for bumblebees pollinating oilseed rape. *Oikos* **98**: 375-384.

We present a model that predicts the level of gene flow mediated by animal pollinators from a source population to a sink population. The model requires specification of three elements: (1) the paternity that originates from a single flower, the paternity shadow; (2) the mean number of flowers that pollinators visit during stays in the sink population, the residence; (3) the proportion of pollinators arriving at the sink that carry pollen from the source population. Provided that pollinators visit enough flowers in the sink to exhaust the paternity shadows from the source, the general results are that gene flow is inversely proportional to the mean pollinator residence in the sink population, and is proportional to the fraction of pollinators arriving with pollen from the source. These results are used to propose explanations for two of the widely observed patterns in gene flow among plant populations. Numerical solutions to the model are derived using experimentally determined values of elements (1) and (2) that represent bumblebees, Bombus spp., visiting agricultural fields of oilseed rape, Brassica napus L. In B. napus, the paternity shadow attenuates rapidly over approximately 20 recipient flowers. Mean bumblebee residences in the fields studied varied between 490 and 720 flowers. In the absence of a direct measurement of element (3), we calculated the maximum level of bumblebee-mediated gene flow by assuming that all bees arrived at the sink saturated with pollen from extrinsic sources. In this case, the model predicts that bumblebee-mediated gene flow accounted for between 0.1% and 0.5% of the progeny in the agricultural fields studied. A likelihood analysis Of Our observations is unable to reveal convincingly the proportion of bees arriving at the sink via a source population, but the literature suggests that bumblebees have high site fidelity, which implies that bee-mediated gene flow may be substantially less than our estimated maximum. We consider the role of various factors, including wind pollination, in accounting for the differences between the model's predictions and the generally higher levels of gene flow observed in previous studies of oilseed rape.

Cummings, C. L. and Alexander, H. M. 2002. Population ecology of wild sunflowers: effects of seed density and post-dispersal vertebrate seed predators. *Oecologia* **130**: 274-280.

Assessing the effects of seed density on the population dynamics of wild plant species with crop relatives will be vital in determining the potential effects of introducing traits into wild populations as a result of crop-to-wild gene flow. We examined experimental sunflower (Helianthus annuus) patches in eastern Kansas to determine the effects of seed density and predation on seedling recruitment and seed production in the next generation. High seed density treatment plots had significantly more seedlings and adult plants than did low seed density treatment plots. Overwinter vertebrate seed predator exclusion treatments resulted in increases in plant density compared to plots in which vertebrates were not excluded. Control patches (no seeds added) contained virtually no plants. Head production and estimated total seed production for a patch were not statistically different among treatments (excluding control plots). Although initial seed density and vertebrate post-dispersal seed predation do appear to have effects on seedling recruitment. neither appear to be limiting seed production of competing adult plants. Therefore, variation in seed densities (over the range examined) may have limited effects on local population dynamics. It is important to note that the choice of seed densities may affect the results obtained: the seed densities used in this study may, in retrospect. be higher than in the small roadside populations typical in eastern Kansas. yet other natural sites have much larger densities. Further. the effects of increased seed density at a local site may have other important effects such as altering metapopulation dynamics through increased long-distance dispersal or increased local seed bank size.

Cummings, C. L., Alexander, H. M., Snow, A. A., Rieseberg, L. H., Kim, M. J. and Culley, T. M. 2002. Fecundity selection in a sunflower crop-wild study: Can ecological data predict crop allele changes? *Ecol Appl* **12**: 1661-1671.

Genes that spread from transgenic crops to populations of weedy relatives can be a cause of concern if fitness-related, transgenic traits persist and enhance weed invasiveness. Studies of the prevalence of crop-specific genetic markers in wild populations can provide data on such introgression. We conducted a field experiment in eastern Kansas to measure changes in frequencies of crop-specific genetic markers in wild sunflower (Helianthus annuus). Three allozyme markers were monitored in three artificial populations that each initially consisted of 100 wild and 100 F, hybrid plants. Survival, flowering time, and average seed production per plant were quantified during the first year of the study (1997). Hybrid plants produced only 1-2% as many seeds per plant as wild plants but did not differ in survivorship. Simple selection models incorporating fecundity differences between wild and F, hybrids accurately predicted crop allele frequencies in the 1998 seedlings. We predicted that frequencies of crop alleles in 1998 would average similar to0.03 for the three populations. Crop-specific allele frequencies dropped from the initial level of 0.25 to a mean of 0.03 in the 1998 seedlings and averaged 0.05 in the next generation of seeds. Accounting for differences in flowering phenology and predispersal seed predation did not improve the accuracy of our predictions for 1998 seedlings. Our results suggest that ecological data can be useful for estimating the frequencies of crop genes following episodes of crop-wild hybridization in sunflowers. This approach can be applied to other study systems in which data on survivorship and fecundity are used to estimate a genotype's evolutionary fitness.

Decker-Walters, D. S., Staub, J. E., Chung, S. M., Nakata, E. and Quemada, H. D. 2002. Diversity in free-living Populations of Cucurbita pepo (Cucurbitaceae) as assessed by random amplified polymorphic DNA. *Syst Bot* **27**: 19-28.

One area of risk assessment for transgenic crops concerns cross-compatible wild and weedy relatives, In squash (Cucurbita pepo), free-living populations are diverse in their distributions, ecologies, histories, and genetic and phenotypic compositions. As part of the effort to understand this diversity random amplified polymorphic DNA (RAPD) data were collected from 37 wild or weedy populations and 16 cultivars, which together represented all infraspecific taxa of C. pepo. Twenty-six primers yielded 70 scorable and variable markers. The presence/absence of bands for these markers produced a data matrix which was analyzed using cluster analysis, The analysis confirmed the relationships among infraspecific taxa that had been revealed, in part, in previous genetic analyses (e.g., isozymes, chloroplast DNA restriction-site mutations, inter simple sequence repeats). Also supported were findings of varying degrees of gene flow from cultivars into free-living populations. Some of the RAPID variation in subsp. ovifera var, ozarkana populations was found to be correlated with the distribution of the drainage systems along which these populations are dispersed, Finally, the RAPD results support the idea that transgenic gene flow experiments with free-living populations should consider using representatives from each of the three free-living taxa, as well as from genetically or ecologically distinct populations within these taxa.

Desplanque, B., Hautekeete, N. and Van Dijk, H. 2002. Transgenic weed beets: possible, probable, avoidable? *J Appl Ecol* **39**: 561-571.

1. Weed beets pose a serious problem for sugar beet Beta vulgaris crops. Traditionally, the only efficient method of weed control has been manual removal, but the introduction of transgenic herbicide-tolerant sugar beets may provide an alternative solution because non-tolerant weed beets can be destroyed by herbicide. We evaluated the possibility that new, transgenic, weed beets may arise by gene flow between wild and crop plants. 2. In a study area in northern France, weed beets were present in variable densities in sugar beet fields of up to 80 weed beet plants m(-2) . Weed beets arise from a long- lived seed bank, with seeds germinating from depths of 5 cm or less. In addition, diploid F-1 crop-wild hybrids and triploid variety bolters (individuals with a low vernalization requirement) were present in low densities in virtually all sugar beet fields. We found gene flow to be possible between all forms, illustrated by both overlapping flowering periods in the field and successful controlled cross-pollinations. 3. The F-1 crop-wild hybrids result from pollination in the seed- production region by wild plants possessing the dominant bolting allele B for flowering without experiencing a period of cold. In the case of a transgene for herbicide tolerance incorporated into male-sterile seed-bearer plants, such hybrids will contain both the herbicide-tolerance and the bolting allele. Contamination of the fields by transgenic weed beets will be the result unless bolters are removed manually. The same will apply in the case of a cytoplasmically inherited transgene. 4. Incorporation of the transgene into the pollinator plants will prevent the immediate formation of transgenic weed beets. However, in sugar beet fields, variety bolters may successfully cross-pollinate with weed beets in neighbouring fields. The use of diploid pollinator plants instead of tetraploids will considerably enhance gene flow towards wild beets, and is not, therefore, an attractive option. 5. In conclusion, the appearance of transgenic weed beets is possible but can best be retarded if the transgene for herbicide tolerance is incorporated into the tetraploid pollinator breeding line.

Gueritaine, G., Sester, M., Eber, F., Chevre, A. M. and Darmency, H. 2002. Fitness of backcross six of hybrids between transgenic oilseed rape (Brassica napus) and wild radish (Raphanus raphanistrum). *Mol Ecol* **11**: 1419-1426.

The process of introgression between a transgenic crop modified for better agronomic characters and a wild relative could lead potentially to increased weediness and adaptation to the environment of the wild species. However, the formation of hybrid and hybrid progeny could be associated with functional imbalance and low fitness, which reduces the risk of gene escape and establishment of the wild species in the field. Our work compares the fitness components of parents and different types of backcross in the sixth generation of hybrids between transgenic oilseed rape (Brassica napus , AACC, 2n = 38) resistant to the herbicide glufosinate and wild radish (Raphanus raphanistrum, RrRr, 2n = 18). The backcross with oilseed rape cytoplasm (OBC) has a fitness value 100 times lower than that of the backcross with wild radish cytoplasm (RBC). The herbicide-resistant RBC has similar growth to the susceptible RBC, but final male and female fitness values are two times lower. In turn, susceptible RBC exhibit similar fitness to the control wild radishes. The relative fitnesses of the different types are the same whether or not they grow under competitive conditions. The consequence on fitness of the chromosome location of the transgene conferring resistance and the relevance of these results to the impact of gene flow on the environment are discussed.

Ilardi, V. and Barba, M. 2002. Assessment of functional transgene flow in tomato fields. *Mol Breed* **8**: 311-315.

As the flow of pollen-carrying transgenes is considered to be one of the main problems associated with the release of genetically modified plants, the impact on the environment of transgenic tomato plants highly resistant to cucumber mosaic virus (CMV) was evaluated. The frequency of spontaneous crossing between homozygous transgenic tomato, cv. UC82B, and untransformed controls was assessed in two Italian growing areas. Transformed plants expressed the CMV coat protein (CP), inducing resistance to the virus, and neomycin phosphotransferase II (NPTII), used as an in vitro selectable marker. For each field, a sample of at least 2000 progeny plants of the `wild-type' UC82B control was screened to search for `hybrids' expressing the transgenic proteins. Kanamycin sprayed on seedling leaves and DAS-ELISA were used to detect NPTII and CMV-CP, respectively. Plants expressing the transgenic proteins were then analysed by PCR using CMV-CP gene-specific primers. Two plants from each field were found to express the transgenes. These plants, however, proved to be homozygous for transgenes, indicating that they did not originate by cross-pollination between transgenic and `wild- type' parents, but probably derived from transgenic seeds that accidentally contaminated untransformed seed stocks during harvesting. These results show that under our conditions, no transgene flow was detected in cv. UC82B tomatoes in the two fields.

Lavigne, C., Klein, E. K. and Couvet, D. 2002. Using seed purity data to estimate an average pollen mediated gene flow from crops to wild relatives. *Theor Appl Genet* **104**: 139-145.

Gene flow from crops to wild related species has been recently under focus in risk-assessment studies of the ecological consequences of growing transgenic crops. However, experimental studies addressing this question are usually temporally or spatially limited. Indirect population-structure approaches can provide more global estimates of gene flow, but their assumptions appear inappropriate in an agricultural context. In an attempt to help the committees providing advice on the release of transgenic crops, we present a new method to estimate the quantity of genes migrating from crops to populations of related wild plants by way of pollen dispersal. This method provides an average estimate at a landscape level. Its originality is based on the measure, of the inverse gene flow, i.e. gene flow from the wild plants to the crop. Such gene flow results in an observed level of impurities from wild plants in crop seeds. This level of impurity is usually known by the seed producers and, in any case, its measure is easier than a direct screen of wild populations because crop seeds are abundant and their genetic profile is known. By assuming that wild and cultivated plants have a similar individual pollen dispersal function, we infer the level of pollen-mediated gene flow from a crop to the surrounding wild populations from this observed level of impurity. We present an example for sugar beet data. Results suggest that under conditions of seed production in France (isolation distance of 1,000 in) wild beets produce high numbers of seeds fathered by cultivated plants.

Moyes, C. L., Lilley, J. M., Casais, C. A., Cole, S. G., Haeger, P. D. and Dale, P. J. 2002. Barriers to gene flow from oilseed rape (Brassica napus) into populations of Sinapis arvensis. *Mol Ecol* **11**: 103-112.

One concern over growing herbicide-tolerant crops is that herbicide-tolerance genes may be transferred into the weeds they are designed to control. Brassica napus (oilseed rape) has a number of wild relatives that cause weed problems and the most widespread of these is Sinapis arvensis (charlock). Sinapis arvensis seed was collected from 102 populations across the UK, within and outside B. napus-growing areas. These populations were tested for sexual compatibility with B. napus and it was found that none of them hybridized readily in the glasshouse. In contrast to previous studies, we have found that hybrids can be formed naturally with S. arvensis as the maternal parent. Six diverse B. napus cultivars (Capricorn, Drakkar, Falcon, Galaxy, Hobson and Regent) were tested for their compatibility with S. arvensis but no cultivar hybridized readily in the glasshouse. We were unable to detect gene transfer from B. napus to S. arvensis in the field, confirming the extremely low probability of hybridization predicted from the glasshouse work.

Pertl, M., Hauser, T. P., Damgaard, C. and Jorgensen, R. B. 2002. Male fitness of oilseed rape (Brassica napus), weedy B-rapa and their F-1 hybrids when pollinating B-rapa seeds. *Heredity* **89**: 212-218.

The likelihood that two species hybridise and backcross may depend strongly on environmental conditions, and possibly on competitive interactions between parents and hybrids. We studied the paternity of seeds produced by weedy Brassica rapa growing in mixtures with oilseed rape (B. napus) and their F-1 hybrids at different frequencies and densities. Paternity was determined by the presence of a transgene, morphology, and AFLP markers. In addition, observations of flower and pollen production, and published data on pollen fertilisation success, zygote survival, and seed germination, allowed us to estimate an expected paternity. The frequency and density of B. napus, B. rapa, and F-1 plants had a strong influence on flower, pollen, and seed production, and on the paternity of B. rapa seeds. Hybridisation and backcrossing mostly occurred at low densities and at high frequencies of B. napus and F-1, respectively. F-1 and backcross offspring were produced mainly by a few B. rapa mother plants. The observed hybridisation and backcrossing frequencies were much lower than expected from our compilation of fitness components. Our results show that the male fitness of B. rapa, B. napus, and F-1 hybrids is strongly influenced by their local frequencies, and that male fitness of F-1 hybrids, when pollinating B. rapa seeds, is low even when their female fitness (seed set) is high.

Ritala, A., Nuutila, A. M., Aikasalo, R., Kauppinen, V. and Tammisola, J. 2002. Measuring gene flow in the cultivation of transgenic barley. *Crop Sci* **42**: 278-285.

Genetic engineering is becoming a useful tool in the improvement of plants and plant-based raw materials. Varieties with value-added traits are developed for nonfood use in industrial and medical production, and different production lines must be kept separate. For good management practices, knowledge of relevant gene flow parameters is required. In the present study, pollen-mediated dispersal of transgenes via cross-fertilization was examined. A transgenic barley (Hordeum vulgare L.) line carrying a marker gene coding for neomycin phosphotransferase 11 (nptII) was used as a pollen donor. For maximum resolution, a cytoplasmically male-sterile barley line was utilized as recipient and the flow of nptII transgene was monitored at distances of 1, 2, 3, 6, 12, 25, 50, and 100 m from the donor plots of 225 and 2000 m(2). Male-fertile plots at a distance of I m were included to measure the transgene flow in normal barley. The number of seeds obtained from male- sterile heads diminished rapidly with distance and only a few seeds ss ere found at distances of 50 and 100 m. Molecular genetic analysis (polymerase chain reaction-PCR) revealed that all seeds obtained from male-sterile heads at a distance of I m were transgenic, as anticipated. However, only 3% of the distant seeds (50 m) actually carried the transgene, whereas most of them resulted from fertilization with nontransgenic background pollen. This background pollen was mainly due to pollen leakage in some male-sterile heads. In normal male- fertile barley, the cross-fertilization frequency with transgenic pollen varied from 0 to 7% at a distance of I m. 0 depending on weather condition, on the heading day. We conclude that, because of competing self-produced and nontransgenic background pollen, the possibility of cross-pollination is very low between a transgenic barley field and an adjacent field cultivated with normal barley. However, adequate isolation distances and best management practices are needed for cultivation of transgenic barley.

Shaw, J. F., Chen, H. H., Tsai, M. F., Kuo, C. I. and Huang, L. C. 2002. Extended flower longevity of Petunia hybrida plants transformed with boers, a mutated ERS gene of Brassica oleracea. *Mol Breed* **9**: 211-216.

Petunia x hybrida Hort.Vilm.-Andr. was transformed with boers, a mutated allele of BOERS, an ethylene receptor sensor gene of Brassica oleracea. boers was obtained by removing an EcoRI cutting site with a silent mutation at Gly-521 and introducing a point mutation at Ile-62, replacing isoleucine with phenylalanine. Transformation was Agrobacterium tumefaciens mediated. Hygromycin resistant regenerants were tentatively confirmed as transformants by PCR's for HPH and boers and more definitively by Southern hybridization of genomic DNA with pBOERS4421. Flowers of transgenic plants retained turgidity and pigmentation considerably longer than those of untransformed controls, whether left undisturbed on plants or excised and placed in water. Furthermore, flowers were unaffected by exposure to exogenous ethylene. Excised shoots of transgenic plants released considerably more ethylene than those of untransformed plants. Transformed plants also produced apparently larger flowers. Unexpectedly higher mortality was observed, suggesting that the ethylene insensitive petunia plants were also lower in disease resistance.

Bartsch, D., Brand, U., Morak, C., Pohl-Orf, M., Schuphan, I. and Ellstrand, N. C. 2001. Biosafety of hybrids between transgenic virus-resistant sugar beet and Swiss chard. *Ecol Appl* **11**: 142-147.

One important issue of biosafety research is whether gene flow from transgenic crops to nontransgenic relatives causes unwanted effects. We carried out field trials with hybrids between transgenic sugar beets, and a close cultivated relative, Swiss chard. This hybrid also acts as a model for "weed beet" hybrids between sugar beet and wild/weed beet (Beta vulgaris ssp. maritima). Transgenic beets with beet necrotic yellow vein virus (BNYVV) coat protein (cp), phosphinothricin- acetyl-transferase (bar), and neomycin-phospho-transferase (nptII) genes were hand-crossed to Swiss chard. The resulting FI plants and controls were grown at two different BNYVV infestation levels and three different competitive conditions with Chenopodium album. Transgenic hybrids had consistently higher biomass than controls under high background BNYVV infestation, and consistently lower biomass than controls under low background infestation. The transgenic hybrids had a significantly lower rate of bolting than controls at all sites. Competition with Ch. album always had a strong negative influence on the performance of all genotypes. We conclude that ecological implications due to the introduction and spread of virus-resistant transgenic hybrids will be observed only in those feral Swiss chard and wild beet populations where fitness is significantly influenced by high infestations of BNYVV.

Burns, M. J., Edwards, K. J., Newbury, H. J., Ford-Lloyd, B. V. and Baggott, C. D. 2001. Development of simple sequence repeat (SSR) markers for the assessment of gene flow and genetic diversity in pigeonpea (Cajanus cajan). *Mol Ecol Notes* **1**: 283-285.

Pigeonpea (Cajanus cajan) is an important subsistence crop in India where traditional landraces and improved hybrids are grown alongside each other. Gene flow may result in genetic erosion of these landraces and their wild relatives, whilst transgene escape from future genetically engineered varieties is another potential hazard. To assess the impact of these factors gene flow needs to be measured. A set of 10 simple sequence repeat markers have been developed, which exhibit polymorphism across a range of pigeonpea varieties. Use of these markers also offers an efficient system for the assessment of genetic diversity within populations of pigeonpea.

Colbach, N., Clermont-Dauphin, C. and Meynard, J. M. 2001. GENESYS: a model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers - II. Genetic exchanges among volunteer and cropped populations in a small region. *Agric Ecosyst Environ* **83**: 255-270.

The aim of the model GENESYS is to rank cropping systems according to their risk of gene escape from genetically modified. herbicide tolerant winter oilseed rape cultivars to rapeseed volunteers. The model integrates the effects of crop succession and crop management at the level of a region. The first part of the model presented by Colbach et al. [Colbach, N., Clermont-Dauphin, C., Meynard, J.M., 2001. GENESYS: a model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers. I. Temporal evolution of a population of rapeseed volunteers in a field. Agric. Ecosyst. Environ.. in press], describes the temporal evolution of rapeseed volunteers in a field. In this paper, the temporal sub-model was extended to include the evolution of genotype proportions with time and the effect of genotype on herbicide efficacy, on seed and pollen production. The spatial dimension was introduced by modelling demographic and genetic evolution of rapeseed volunteers in all the plots of a region, whether they are cropped fields or uncultivated field margins and waysides. Each year, pollen and seed are exchanged between plots and the importance of these exchanges depends on plot areas and distances. and, in the case of pollen, on flowering dates. (C) 2001 Elsevier Science B.V. All rights reserved.

Driessen, S., Pohl, M. and Bartsch, D. 2001. RAPD-PCR analysis of the genetic origin of sea beet (Beta vulgaris ssp maritima) at Germany's Baltic Sea coast. *Basic Appl Ecol* **2**: 341-349.

The spread of plant populations into new habitats and their establishment there is an important object of ecological research, particularly for those plant species consisting of cultivated, weedy and wild forms. One of these species is Beta vulgaris L., which is taxonomically divided into the subspecies vulgaris (sugar beet, Swiss chard, red beet, some weedy beet forms) and maritima (sea beet). In northeastern Europe, the distribution range of the different Beta subspecies is not well known. The aim of this study was to investigate the origin of sea beet populations at the Baltic Sea coast of Germany by collecting seed samples and performing RAPD-PCR analysis. Theoretically, the spread and the establishment of beet populations in the wild could be the result of various different processes: (1) introduction of seeds from wild populations, (2) naturalization of cultivated or weedy types after introduction into wild habitats, and (3) a combination of (1) and (2) with subsequent hybridisation and evolution into an intermediate genotype. Genetic analysis suggests that the Baltic Sea coast populations are pure wild beets (1) and not weedy or escaped sugar beets (2 or 3). In a UPGMA phenogram, the wild and the cultivated beets could be clearly distinguished from one another. In addition to the strong evidence that German sea beet originated from Danish sea beet, the possibility of seed disperal between these groups via seawater was demonstrated. Beta vulgaris seeds survived a long period in seawater and up to 2% of the seeds still germinated after 28 weeks. The fact that sea beet populations can now be found sympatrically to flowering sugar beets and weed beets in coastal agricultural areas offers new possibilities of gene flow. This is important fundamental knowledge for the risk assessment of gene flow from transgenic sugar beet.

Guadagnuolo, R., Savova-Bianchi, D., Keller-Senften, J. and Felber, F. 2001. Search for evidence of introgression of wheat (Triticum aestivum L.) traits into sea barley (Hordeum marinum s.str. Huds.) and bearded wheatgrass (Elymus caninus L.) in central and northern Europe, using isozymes, RAPD and microsatellite markers. *Theor Appl Genet* **103**: 191-196.

Seeds of English and Austrian populations of bearded wheatgrass (Elymus caninus L.) and sea barley (Hordeum marinum Huds.) growing in the vicinity of wheat (Triticum aestivum L.) fields were collected in order to search for evidence of the introgression of wheat traits into these wild relatives. Seeds were sown and plants grown for subsequent analyses using morphological and genetic (isozymes, RAPD and wheat microsatellites) markers. No F-1 hybrids were found within the individuals of the two species grown, neither with morphological nor with genetic markers. Also, no evidence of introgression of wheat traits into E. caninus was observed. However, in one individual of H. marinum which had the typical morphology of this species, numerous species-specific DNA markers of wheat were amplified, thereby demonstrating previous hybridization. Consequently, the hybridization between wheat and H. marinum under natural conditions and the introgression of wheat traits into this wild relative seems to be possible. Our results contribute to the risk assessment of transgenic wheat cultivation.

Gueritaine, G. and Darmency, H. 2001. Polymorphism for interspecific hybridisation within a population of wild radish (Raphanus raphanistrum) pollinated by oilseed rape (Brassica napus). *Sex Plant Reprod* **14**: 169-172.

The within-population polymorphism of wild radish (Raphanus raphanistrum) for interspecific hybridisation with two cultivars of oilseed rape (Brassica napus) was investigated by hand crossing experiments and fluorescence microscopy. Wide variability among plants was observed in the ability of oilseed rape pollen to germinate on the wild radish stigma; the frequency of pistils showing pollen tubes ranged from 0 to 1, depending on the female plant. The ratio of fertilised ovules to the total number of ovules in ovaries where pollen tubes arrived ranged from 0.02 to 0.51. Overall, the results provide evidence for the presence of different phenotypes. In 40% of the plants, pistils had no or very few pollen tubes and few fertilised ovules. In 23%, the foreign pollen tubes grew through the style towards the ovary, but had low ovule fertilisation efficiency. The remaining 37% showed a large number of pollen tubes in the style and frequent ovule fertilisation, and two plants showed no difference between foreign and conspecific pollen. With regard to post-zygotic barriers, pollen germination and ovule fertilisation represent minor barriers to interspecific hybridisation between oilseed rape and wild radish. It is suggested that the effectiveness of these barriers could be improved through plant breeding; this could reduce the risk of gene flow from transgenic oilseed rape to populations of wild relatives.

Hansen, L. B., Siegismund, H. R. and Jorgensen, R. B. 2001. Introgression between oilseed rape (Brassica napus L.) and its weedy relative B-rapa L. in a natural population. *Genet Resour Crop Evol* **48**: 621-627.

We investigated introgression in a mixed weedy population of oilseed rape (Brassica napus) and its relative B. rapa using species-specific AFLP-markers. The population was situated in a field relayed from conventional to organic cultivation 11 years ago. One-hundred-and-two B. napus or B. rapa-like plants were collected in a 3 m(2) plot. Of these, one was a first generation hybrid (F-1) and nearly half (44 plants) were introgressed, having both B. napus and B. rapa specific markers. The remaining plants apparently corresponded to pure species, with 50 having only B. rapa- and seven having only B. napus-specific markers. We compared the number of markers in the plants from the weedy population with the numbers in controlled backcross generations (BC1 and BC2). The marker distribution in the weedy population resembled the distribution in the second backcross generation most closely. Together with the cultivation history of the field. this suggests that the introgression process in the weedy mixed population has been in progress for some time. This study is the first to show introgression between B. napus and B. rapa under natural conditions.

Hudson, L. C., Chamberlain, D. and Stewart, C. N. 2001. GFP-tagged pollen to monitor pollen flow of transgenic plants. *Mol Ecol Notes* **1**: 321-324.

In this study, the pollen-active LAT59 promoter from tomato was used to express a green fluorescent protein (GFP) encoding gene in Nicotiana tabacum (tobacco) pollen. This promoter is preferentially expressed in anthers and pollen. Pollen in transgenic plants segregated in a 1 : 1 Mendelian ratio, and the plants were polymerase chain reaction (PCR)-positive. GFP- tagged pollen was developed as a tool for tracking the movement of transgenic plant pollen in the environment. Specifically, it should be a useful tool for characterizing the spatial distribution patterns of transgenic pollen, to determine pollination mechanisms, to monitor the effects on nontarget organisms, and to monitor gene flow in field conditions.

James, C. M., Barrett, J. A., Russell, S. J. and Gibby, M. 2001. A rapid PCR based method to establish the potential for paternal inheritance of chloroplasts in Pelargonium. *Plant Mol Biol Rep* **19**: 163-167.

A simple procedure for the amplification of chloroplast DNA from pollen is described. This allows the potential for biparental or paternal inheritance of chloroplasts in angiosperms to be established rapidly and reliably. Such information is important when chloroplast DNA, which is generally assumed to be inherited maternally, is used to study gene flow or reconstruct phylogenies. The technique could also prove valuable in assessing the risks of transgene escape via pollen of genetically modified plants, in which the chloroplast genome rather than the nucleus has been transformed.

Kuvshinov, V., Koivu, K., Kanerva, A. and Pehu, E. 2001. Molecular control of transgene escape from genetically modified plants. *Plant Sci* **160**: 517-522.

Potential risks of gene escape from transgenic crops through pollen and seed dispersal are being actively discussed and have slowed down full utilization of gene technology in crop improvement. To ban the transgene flow, barren zones and 'terminator' technology were developed as GMO risk management technologies in transgenic crops. Unfortunately, the technologies have not protected reliably the transgene migration to wild relatives. The present study offers a novel molecular technique to eliminate gene flow from transgenic plants to wild relatives by recoverable block of function (RBF). The RBF consists of a blocking sequence linked to the gene of interest and a recovering sequence, all in one transformable construct. The blocking sequence blocks a certain molecular or physiological function of the host plant. Action of the blocking sequence leads to the death of the host plant or to an alteration in its phenotype resulting in inability for sexual reproduction in nature. The recovering construct recovers the blocked function of the host plant. The recovering construct is regulated externally by a specific chemical or physical treatment of the plants and does not act under natural conditions. In nature, hybrids of the transgenic plants with its wild relatives carrying the RBF will die or be unable to reproduce because of the blocking construct action. A wet-king model of RBF is described in this report as one example of the RBF concept. This RBF example is based on ba, nase (the blocking construct) and barstar (the recovering construct) gene expression in tobacco under sulfhydryl endopeptidase (SH-EP) and a heat shock (HS) promoter, respectively. (C) 2001 Elsevier Science ireland Ltd. All rights reserved.

Libiakova, G., Jorgensen, B., Palmgren, G., Ulvskov, P. and Johansen, E. 2001. Efficacy of an intron-containing kanamycin resistance gene as a selectable marker in plant transformation. *Plant Cell Reports* **20**: 610-615.

In this project we have analysed the use of an intron- containing neomycin phosphotransferase II nptII - gene. The advantage of this construct is that only eukaryotic organisms will be able to process this gene. Accordingly, the theoretical risk of horizontal gene flow of antibiotic resistance genes from transgenic plants to enteric bacteria is eliminated. The ST-LS1 intron IV2 from potato was inserted into the coding region of nptII Transformation of Solanum tuberosum (potato) and Nicotiana tabacum (tobacco) with constructs containing the intron nptII showed similar transformation frequencies to transformation with constructs containing the normal nptII. Analysis of total DNA and RNA confirmed that the intron- containing nptII gene was present in the plants and that the mRNA was processed correctly.

Messeguer, J., Fogher, C., Guiderdoni, E., Marfa, V., Catala, M. M., Baldi, G. and Mele, E. 2001. Field assessments of gene flow from transgenic to cultivated rice (Oryza sativa L.) using a herbicide resistance gene as tracer marker. *Theor Appl Genet* **103**: 1151-1159.

Development of plant genetic engineering has led to the deployment of transgenic crops and, simultaneously, to the need for a thorough assessment of the risks associated with their environmental release. This study investigated the occurrence of gene flow from transgenic rice to non-transgenic rice plants under agronomic conditions using a herbicide resistance gene as a tracer marker. Two field experiments were established in the paddy fields of two main Mediterranean rice-growing areas of Spain and Italy. In both locations analyses of phenotypic, molecular and segregation data showed that pollination of recipient plants with pollen of the transgenic source occurred at a significant frequency. A gene flow slightly lower than 0.1% was detected in a normal side-by-side plot design. Similar results were found in a circular plot when the plants were placed at 1-m distance from the transgenic central nucleus. A strong asymmetric distribution of the gene flow was detected among this circle and highest values (0.53%) were recorded following the direction of the dominant Wind. A significant lowest value (0.01%) was found in the other circle (5 m from the transgenic plants) as was expected according to the characteristics of rice pollen. Such circular-field trial designs could also prove to be very useful in studying the gene flow to other commercial cultivars of rice with the aim of establishing strategies to prevent pollen dispersal from commercial transgenic fields to the neighbouring conventional fields.

Snow, A. A., Uthus, K. L. and Culley, T. M. 2001. Fitness of hybrids between weedy and cultivated radish: Implications for weed evolution. *Ecol Appl* **11**: 934-943.

Weed species are known to evolve rapidly with their associated crops. A better understanding of the mechanisms and rates of weed evolution could aid in limiting or at least anticipating this process. Spontaneous hybridization between crops and related weed species can transfer crop genes coding for fitness-enhancing traits to wild populations, but little is known about how easily this takes place in various weed-crop complexes. We studied interspecific hybrids between wild and cultivated radishes (Raphanus raphanistrum X R. sativus), which often co-occur and share pollinators. To determine whether the F, generation represents a strong barrier to subsequent introgression, we compared the fitness of wild plants and wild- crop hybrids. Two experiments were carried out in Michigan, USA, one with potted plants and the other involving four artificially established populations. In the artificial populations, we used white flower color, a dominant, crop- specific allele, to document the persistence of crop genes over time. Wild plants had yellow flowers, which is a recessive trait. F. hybrids had lower fitness than wild plants due to lower pollen fertility, fewer seeds per plant, and delayed flowering. Despite these disadvantages, hybrids contributed substantially,to each population's gene pool. After 3 yr, frequencies of white-flowered plants in the artificial populations ranged from 8% to 22%, demonstrating that crop genes persisted. Other studies of flower color variation in wild populations of R. raphanistrum provide circumstantial evidence for frequent crop-to-wild gene flow. We predict that, if cultivated radish is engineered to possess transgenes coding for traits such as resistance to insect herbivores, disease, herbicides, or environmental stress, these fitness-related crop genes will easily spread to R. raphanistrum.

Spencer, L. J. and Snow, A. A. 2001. Fecundity of transgenic wild-crop hybrids of Cucurbita pepo (Cucurbitaceae): implications for crop-to-wild gene flow. *Heredity* **86**: 694-702.

Hybridization between crops and their weedy or wild relatives is an area of concern because the widespread use of genetically engineered crops may allow novel, beneficial transgenes to enter nearby populations. We compared fitness components of wild Cucurbita pepo from Arkansas, USA, with wild-crop hybrids derived from yellow squash (a cultivar of C. pepo with transgenic resistance to two viruses). Wild and hybrid progeny were grown in agricultural fields in Arkansas (1996-98) and Ohio (1996) in six similar experiments. Cross types (wild and hybrid) did not differ significantly in seedling survival, which exceeded 85% in all cases. In Ohio, where more detailed observations were made, hybrid plants produced 41% as many mate flowers, 21% as many female flowers, and 28% as many seeds as wild plants. At all sites, flowering periods of the two cross types overlapped extensively. Putative virus symptoms were more common in wild plants than in hybrids. Lifetime fecundity varied considerably among sites and years. The average fecundity of hybrids ranged from 453 to 4497 seeds per plant and represented 15% - 53% of the numbers of seeds produced by wild plants in the same experiments. These results suggest that the F-1 generation does not represent a strong barrier to the introgression of neutral or beneficial crop genes into free- living populations of C. pepo.

Thalmann, C., Guadagnuolo, R. and Felber, F. 2001. Search for spontaneous hybridization between oilseed rape (Brassica napus L.) and wild radish (Raphanus raphanistrum L.) in agricultural zones and evaluation of the genetic diversity of the wild species. *Bot Helv* **111**: 107-119.

Possible spontaneous gene flow under agricultural conditions was studied between the crop Brassica napus subsp. napus (oilseed rape), as pollen donor, and the common weed Raphanus raphanistrum (wild radish), as mother plant. Conventional cultivars were used. Around 750 individuals of R. raphanistrum were screened by flow cytometry or chromosome counts, and further genetic analysis was performed with random amplified polymorphic DNA (RAPD) on 98 selected plants. No hybrids were detected. The genetic structure of B. napus was uniform over the area investigated, which was expected for a cultivated species. In contrast, R. raphanistrum was genetically variable within populations, while geographical genetic variation among populations within the regions and even among regions was small. It is interpreted as an indication of gene flow sensu lato over large areas caused by seed dispersal by man.

Wang, Z. N., Zemetra, R. S., Hansen, J. and Mallory-Smith, C. A. 2001. The fertility of wheat x jointed goatgrass hybrid and its backcross progenies. *Weed Sci* **49**: 340-345.

The spontaneous flow of genes from wheat to jointed goatgrass is of great concern to breeders intending to release herbicide- resistant wheat. The objectives of this research were to study how genes could flow from wheat to jointed goatgrass through crossing and backcrossing between these two species and, based on this knowledge, to propose possible ways to minimize the chance of gene flow between them. Results showed that the wheat X jointed goargrass hybrid can only serve as a female parent to produce the BC1 generation. The BC1 generation was found to have 1.8% male fertility and 4.4% female fertility, indicating that it could serve as either the male or female parent to produce a BC2 generation. The fertility of the resultant BC2 generation further increased. The average male, female, an self-fertility was 8.9, 18.0, and 6.9%, respectively. After the BC2 generation, the backcross progeny has three possible ways to reproduce: to pollinate jointed goatgrass, to be pollinated by jointed goatgrass, or to pollinate itself. Restoration of the chromosome number of jointed goatgrass continues as the BC2 generation is selfed, but some plants can contain an a-lien chromosome over generations. The possible ways to reduce the chance of gene flow between these two species are (1) prevent the production of hybrids, (2) prevent the production of the BC1 generation, and (3) put a herbicide-resistant gene on the A- or B-genome of wheat.

Amand, P. C. S., Skinner, D. Z. and Peaden, R. N. 2000. Risk of alfalfa transgene dissemination and scale-dependent effects. *Theor Appl Genet* **101**: 107-114.

Pollen can function as a vehicle to disseminate introduced, genetically engineered genes throughout a plant population or into a related species. The measurement of the risk of inadvertent dispersal of transgenes must include the assessment of accidental dispersion of pollen. Factors to be considered include the rate of pollen spread, the maximal dispersion distance of pollen, and the spatial dynamics of pollen movement within seed production fields; none of which are known for alfalfa (Medicago sativa L.), an insect-pollinated crop species. Using a rare, naturally occurring molecular marker, alfalfa pollen movement was tracked from seed and hay production fields. Results indicated that leafcutter bees ((Megachile spp.)

used in commercial seed production show a directional, non-random bias when pollinating within fields, primarily resulting in the movement of pollen directly towards and away from the bee domicile. Within-field pollen movement was detected only over distances of 4 m or less. Dispersal of pollen from alfalfa hay and seed production fields occurs at distances up to 1000 m. By examining widely dispersed, individual escaped alfalfa plants and their progeny using RAPD markers, gene movement among escaped alfalfa plants has been confirmed for distances up to 230 m. The outcrossing frequency for large fields was nearly 10-times greater than that of research-sized plots. A minimum isolation distance of 1557 m may be required to prevent gene flow in alfalfa. Data suggest that complete containment of transgenes within alfalfa seed or hay production fields would be highly unlikely using current production practices.

Astoin, M. F., Champolivier, J. and Messean, A. 2000. Evaluation of the impact of flows of tolerant transgenic organisms to various broad-spectrum herbicides. *OCL-Ol Corps Gras Lipides* **7**: 345-349.

Given the characterisation and evaluation of the gene flow from oilseed rape done by INRA, CNRS and Cetiom, the present paper aims to investigate the possible agro-environmental impacts of gene flow from herbicide-tolerant genetically-modified (CM) oilseed rape (seed loss outside and inside the field, pollen dispersal, interspecific hybridization). We identified two main impacts of the release of such varieties in French agricultural systems: the possible questioning of CM content norms for the harvest of non-GM rape fields, and of the long-term effectiveness of such weed control strategies through the development of tolerant weeds (oilseed rape and hybrids). This text is an extract from a report that summarises French research on gene flow carried out by Cetiom towards the end of the two-year French moratorium on genetically-modified oilseed rape and sugar beet.

Colbach, N., Meynard, J. M., Clermont-Dauphin, C. and Champolivier, J. 2000. GeneSys-Colza: a model for the medium-term and long-term effects of cultivation systems on the flows of genes between colza fields and new growth in a given agricultural area. *OCL-Ol Corps Gras Lipides* **7**: 329-340.

The aim of the model is to evaluate the influence of cropping systems on transgene escape from rapeseed crops to rapeseed volunteers in time and space. The model input variables are the regional field pattern, crop succession and cultivation techniques. The main output variables are, for each year and plot, the number of individuals per m(2) and the genotype proportions of the adult rapeseed plants, the newly produced seeds and the seedbank. The model comprises an annual life- cycle for volunteer and cropped rapeseed plants simulated for each plot and year; the relationships between the various life- stages depend on crop type and management. Pollen and grain exchanges between plots depend on distance between plots and cropping system. With the help of the simulations performed with the model, it is possible to identify low-gene-flow cropping systems or the minimum distance between rape plots to avoid contamination of the harvest product, to estimate the consequences if one farmer does not manage his rope volunteers correctly etc.

Darmency, H. and Fleury, A. 2000. Mating system in Hirschfeldia incana and hybridization to oilseed rape. *Weed Res* **40**: 231-238.

Concerns have been raised about the possibility of sexual transfer of herbicide resistance genes from transgenic crops towards weedy relatives. The average rate of spontaneous hybridization between Hirschfeldia incana (L.) Lagreze-Fossat and oilseed rape (Brassica napus L.) was 0.6 hybrids per plant over 3 years of field experiments using herbicide-resistant oilseed rape as a pollen donor. Self-incompatibility was shown to be the mating system of most individuals within a population of H. incana, although some plants had some ability to self- fertilize, which could mitigate gene flow. Back-crossing interspecific hybrids to H. incana over five generations showed that introgression was not successful in our experiment.

Davenport, I. J., Wilkinson, M. J., Mason, D. C., Charters, Y. M., Jones, A. E., Allainguillaume, J., Butler, H. T. and Raybould, A. F. 2000. Quantifying gene movement from oilseed rape to its wild relatives using remote sensing. *Int J Remote Sens* **21**: 3567-3573.

The potential environmental risks due to gene flow from genetically modified (GM) crops into wild plant populations are currently of great concern. Of particular interest is gene flow from GM oilseed rape (Brassica napus) into its wild parental species B. rapa and B. oleracea, with which it forms spontaneous hybrids. The rate of hybridization is best determined empirically under a wide variety of field conditions. A procedure for quantifying hybrid formation using remote sensing over a large area of southern England is described. Satellite sensor images were used to identify localities where gene flow was most likely by searching for oilseed rape fields adjacent to regions where the parent plants occur, namely rivers and clifftops. These sites were visited and screened for hybrids. A single hybrid was observed in an area of over 16 000 km(2). We use these data to suggest a strategy to minimise gene flow following commercial scale release of GM B. napus across Europe and propose a procedure that may allow post-release containment.

Genissel, A., Viard, F. and Bourguet, D. 2000. Population genetics of Chrysomela tremulae: a first step towards management of transgenic Bacillus thuringiensis poplars Populus tremula X P-tremuloides. *Hereditas* **133**: 85-93.

Many strategies have been proposed for delaying the development of insect resistance to Bacillus thuringiensis (Bt). The current paradigm for Bt resistance management is the high dose- refuge strategy. For this strategy to be successful: (i) heterozygotes must be killed in treated areas, (ii) resistant alleles must be rare (frequency < 10-3), and (iii) there must be a high level of gene flow between populations to ensure random mating. We studied gene flow within and between populations with a view to managing the resistance of Chrysomela tremulae (Coleoptera: Chrysomelidae) to new transgenic, highly toxic poplars expressing a synthetic Br gene. In this study, we assessed the extent of gene flow in C. tremulae within and between 16 sites in France and Belgium, using allozyme markers. We found a high level of genetic variability in C, tremulae, with a mean of 0.206<plus/minus>0.16. There were no obvious limitations to gene flow between populations of C. tremulae over large geographical distances (several hundreds of kilometres). Nevertheless, a very low level of genetic differentiation was observed between a site located in the south of France and the sampled sites from the Centre region.

Giddings, G. 2000. Modelling the spread of pollen from Lolium perenne. The implications for the release of wind-pollinated transgenics. *Theor Appl Genet* **100**: 971-974.

The dispersal of pollen from a Lolium perenne source has previously been described using various Gaussian plume models which take distance and wind direction into account. One of these models is used here to calculate, using integration, possible pollen deposition onto small conspecific populations a kilometer from the source. The percentage of immigrant pollen is compared for six different sets of parameter values previously estimated from pollen-dispersal experiments. The source size is then scaled up to simulate what might happen if transgenic ryegrass was grown on a large scale. In this case it is seen that small conspecific populations might, in some conditions, be swamped by immigrant pollen, even if they are not directly downwind of the source. The implications of this are discussed in terms of assessing and managing the risks of releasing wind-pollinated transgenic crops.

Pierre, J. and Pham-Delegue, M. H. 2000. How to study the impact of genetically modified colza on bees? *OCL-Ol Corps Gras Lipides* **7**: 341-344.

Different methods are used to study the incidence of genetically modified oilseed rape on honeybees, that depend on the type of transgene and on the transformation induced in the plant. Two examples are chosen to present risk assessment procedures. One deals with oilseed rape resistant to pest insects (by expressing protease inhibitors PI), the other concerns oilseed rape tolerant to an herbicide (glufosinate). In the first case, the aim is to ensure the safety of honeybees. First, the occurrence of the transgene product in nectar and pollen is checked and the foraging behaviour on the plants is observed Second, the effects of various concentrations, equal or higher than those expressed in the plant, is evaluated under laboratory conditions. Thus, acute and chronic toxicity, individual learning behaviour and life span of workers exposed to the PI is investigated. Experiments are also carried out to examine the effects on the colony activity and development. In addition, indirect effect related to secondary changes in plant signals cueing bees visits are examined in the second case, the aim is to assess the risk for transgene dispersal in the environment, mediated by honeybees, It is unlikely that tolerance to herbicide has toxic effects, but a pleitropic effect of the transgene could effect the value and attractiveness of the plants. So, pollen and nectar production, flower size and density are evaluated and experiments are carried out in the field to study the foraging behaviour In particular, the ability of honeybees to cross- visit transgenic and traditional oilseed rape or oilseed rape and weedy relatives is observed. Both types of studies show how complementary methods and collaborations between teams have been designed to study various aspects of the impact of genetic engineered oilseed rape on honeybees.

Rognli, O. A., Nilsson, N. O. and Nurminiemi, M. 2000. Effects of distance and pollen competition on gene flow in the wind-pollinated grass Festuca pratensis Huds. *Heredity* **85**: 550-560.

Pollen dispersal and gene flow in the grass meadow fescue (Festuca pratensis Huds.) were studied using two populations which were homozygous for different allozymes at the Gpi-2 locus. The populations were established in a concentric donor- acceptor field experiment. Gene flow was found mainly to be affected by the distance between the donor and acceptor plants. Analysing 21 132 acceptor plant progenies, gene flow was shown to decrease rapidly with distance to the donor field up to 75 m, and beyond this distance much more slowly. The ability of donor pollen to fertilize acceptor plants depended very much on the density of the acceptor plants. Pairs of acceptor plants produced more compatible pollen locally, and captured significantly less donor pollen than single-plants. Despite the higher seed production of acceptor plants planted in pairs, the absolute number of heterozygous seeds carrying the donor allele was always lower than for single-plants, Wind direction had only a slight effect upon the type of pollen captured. Because of pollen production within the two plant populations being continuous and overlapping, the time when anthesis occurred had little effect on gene flow between the populations. Vigorous and tall acceptor plants with many panicles, high seed yield and high 1000-seed weight were able to capture more donor pollen than shorter plants. The results may be used to assess the risk of gene flow and to develop strategies for monitoring the spread of transgenes from genetically modified grasses.

Saeglitz, C., Pohl, M. and Bartsch, D. 2000. Monitoring gene flow from transgenic sugar beet using cytoplasmic male-sterile bait plants. *Mol Ecol* **9**: 2035-2040.

One of the most discussed environmental effects associated with the use of transgenic plants is the flow of genes to plants in the environment. The flow of genes may occur through pollen since it is the reproductive system that is designed for gene movement. Pollen-mediated gene escape is hard to control in mating plants. Pollen from a wind pollinator can move over distances of more than 1000 m. To investigate the efficiency of transgenic pollen movement under realistic environmental conditions, the use of bait plants might be an effective tool. In this study, cytoplasmic male-sterile (CMS) sugar beets were tested with regard to their potential for monitoring transgene now. As the pollen source, transgenic sugar beets were used that express recombinant DNA encoding viral (beet necrotic yellow vein virus) resistance, and antibiotic (kanamycin) and herbicide (glufosinate) tolerance genes. In a field trial, the effectiveness of a hemp (Cannabis sativa) stripe containment strategy was tested by measuring the frequency of pollinated CMS bait plants placed at different distances and directions from a transgenic pollen source. The results demonstrated the ineffectiveness of the containment strategy. Physiological and molecular tests confirmed the escape and production of transgenic offspring more than 200 m behind the hemp containment. Since absolute containment is unlikely to be effective, the CMS-bait plant detection system is a useful tool for other monitoring: purposes.

Staniland, B. K., McVetty, P. B. E., Friesen, L. F., Yarrow, S., Freyssinet, G. and Freyssinet, M. 2000. Effectiveness of border areas in confining the spread of transgenic Brassica napus pollen. *Can J Plant Sci* **80**: 521-526.

The development of transgenic Brassica napus L. cultivars requires field trials in agricultural settings. For field testing of transgenic constructs that have not been granted full environmental release, current Canadian Government regulations require either large isolation zones (200 m) or 10 m wide borders of synchronously flowering, non-transgenic B. Napus to contain transgenic pollen. To investigate the effectiveness of border areas in containing transgenic B. napus pollen, border areas 15 to 30 m wide were planted around 30 m x 60 m central plots of bromoxynil-herbicide-resistant transgenic B. napus strains. Four field trials were conducted at Carman and Winnipeg, Manitoba, Canada in 1994 and 1995. Seed samples were harvested from the border area at 0, 2.5, 5, 10, and 15 m for the four cardinal directions and additionally at 20, 25, and 30 m for two cardinal directions. These seed samples were planted in the field in 1995 and 1996 and the seedlings screened for the presence of bromoxynil-resistant plants (i.e., from outcrossed seeds). Distance from the central plot significantly affected outcrossing rates while environment (site-year) and direction effects were non-significant. Outcrossing rates averaged 0.70% at 0 m and declined exponentially to 0.02% at 30 m. More than four-fifths of the total outcross events detected occurred in the first 10 m of border area indicating that border areas effectively reduce pollen-mediated gene flow in B. napus but cannot completely eliminate it.

Walther-Hellwig, K. and Frankl, R. 2000. Foraging habitats and foraging distances of bumblebees, Bombus spp. (Hym., apidae), in an agricultural landscape. *J Appl Entomol-Z Angew Entomol* **124**: 299-306.

In selected foraging habitats of an agricultural landscape flower visits of bumblebees and community structure of foraging bumblebees were studied, with special regard to the role of crops as super-abundant resources. Most crops represent temporal foraging habitats with high abundance of bumblebees but mainly with low diversity in the bumblebee forage community, in contrast to permanent foraging habitats such as, for example, a hedgerow. The high numbers of bumblebees in the monoculture of crop plantations consisted mainly of short- tongued bumblebee species. The role of foraging distances for the visitation rate of foraging habitats was studied by performing capture-recapture experiments with natural nests of Bombus terrestris, Bombus lapidarius and Bombus muscorum. Differences were found on the species as well as the individual level. The foraging distances of B. muscorum were more restricted to the neighbourhood of the nesting habitat than the foraging activity of B. terrestris and B. lapidarius. High percentages of B. terrestris workers were recaptured while foraging on super-abundant resources in distances up to 1750 m from the nest. Isolated patches of highly rewarding forage crops, in agricultural landscapes, are probably only accessed by bumblebee species with large mean foraging distances, such as the short-tongued B. terrestris. Species like the rare, long-tongued B. muscorum depend on a close connection between nesting and foraging habitat. A restricted foraging radius might be one important factor of bumblebee species loss and potential pollinator limitation in modern agricultural landscapes. Furthermore, long-distance flights of bumblebee pollinators have to be considered in the present discussion on gene flow from transgenic plant species on a landscape scale.

Wilkinson, M. J., Davenport, I. J., Charters, Y. M., Jones, A. E., Allainguillaume, J., Butler, H. T., Mason, D. C. and Raybould, A. F. 2000. A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. *Mol Ecol* **9**: 983-991.

One of the major environmental concerns over genetically modified (GM) crops relates to transgene movement into wild relatives. The pattern of hybridization ultimately affects the scale and rapidity of ecological change and the feasibility of containment. A new procedure for quantifying hybrid formation over large areas is described. Remote sensing was used to identify possible sites of sympatry between Brassica napus and ifs progenitor species across 15 000 km(2) of south-east England in 1998. Two sympatric populations with B. rapa and one with B. oleracea were found over the entire survey area. Every newly recruited plant in these populations in 1999 was screened for hybrid status using now cytometry and molecular analyses. One hybrid was observed from the 505 plants screened in the B. rapa populations but none of the nine B. oleracea recruits were hybrids. Measures to minimize gene now are suggested, and a procedure for the post-release evaluation and containment of GM cultivars is proposed.

Assuncao, R. and Jacobi, C. M. 1999. Optimal sampling designs for studies of gene flow - A challenging trade-off: A reply to Klein and Laredo. *Evolution* **53**: 2005-2007.

Bartsch, D., Lehnen, M., Clegg, J., Pohl-Orf, M., Schuphan, I. and Ellstrand, N. C. 1999. Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations. *Mol Ecol* **8**: 1733-1741.

Gene flow and introgression from cultivated plants may have important consequences for the conservation of wild plant populations. Cultivated beets (sugar beet, red beet and Swiss chard: Beta vulgaris ssp. vulgaris) are of particular concern because they are cross-compatible with the wild taxon, sea beet (B.vs. ssp. maritima). Cultivated beet seed production areas are sometimes adjacent to sea beet populations; the numbers of flowering individuals in the former typically outnumber those in the populations of the latter. In such situations, gene flow from cultivated beets has the potential to alter the genetic composition of the nearby wild populations. In this study we measured isozyme allele frequencies of 11 polymorphic loci in 26 accessions of cultivated beet, in 20 sea beet accessions growing near a cultivated beet seed production region in northeastern Italy, and 19 wild beet accessions growing far from seed production areas. We found one allele that is specific to sugar beet, relative to other cultivated types, and a second that has a much higher frequency in Swiss chard and red beet than in sugar beet. Both alleles are typically rare in sea beet populations that are distant from seed production areas, but both are common in those that are near the Italian cultivated beet seed production region, supporting the contention that gene flow from the crop to the wild species can be substantial when both grow in proximity. Interestingly the introgressed populations have higher genetic diversity than those that are isolated from the crop. The crop-to-wild gene flow rates are unknown, as are the fitness consequences of such alleles in the wild. Thus, we are unable to assess the long- term impact of such introgression. However, it is clear that gene now from a crop to a wild taxon does not necessarily result in a decrease in the genetic diversity of the native plant.

Desplanque, B., Boudry, P., Broomberg, K., Saumitou-Laprade, P., Cuguen, J. and Van Dijk, H. 1999. Genetic diversity and gene flow between wild, cultivated and weedy forms of Beta vulgaris L-(Chenopodiaceae), assessed by RFLP and microsatellite markers. *Theor Appl Genet* **98**: 1194-1201.

Beets belonging to the species Beta vulgaris L. can be found in crop, wild and weedy forms, all of which are interfertile. We studied the intra-specific genetic relationships of about 300 individuals from 54 populations of various French geographic origins using nuclear molecular markers (five single-copy RFLP loci and one microsatellite locus). The patterns of diversity were congruent for both types of markers. Genetic diversity in wild beets appeared to be high, both in term of allele number and observed heterozygosity, whereas the narrowness of the cultivated-beet gene pool was confirmed. Genetic distances between all forms showed that weed beets in northern France are intermediates between sugar beet and inland wild beets in south-western France. This analysis allowed us to infer the paternal origin of weed beets and furthermore, is in agreement with a previous study which focused on their maternal origin: weed beet infesting sugar-beet fields originated from accidental and recurrent hybridization between cultivated lines and ruderal inland wild beets during the production of commercial seeds in southwestern France. Inland wild beets are genetically close to Mediterranean coastal wild beets, but differ from other coastal forms (from Biscay, Brittany and northern France). The study of gene flow in the beet complex contributes to the risk assessment of transgenic beets.

Jenczewski, E., Prosperi, J. M. and Ronfort, J. 1999. Evidence for gene flow between wild and cultivated Medicago sativa (Leguminosae) based on allozyme markers and quantitative traits. *Am J Bot* **86**: 677-687.

Genetic differentiation between co-occurring crops and their wild relatives will be greatly modified by crop-to-weed gene flow and variation between human and natural selective pressures. The maintenance of original morphological features in most natural populations of Medicago sativa in Spain questions the relative extent of these antagonistic forces. In this paper, we measured and compared the pattern of population differentiation within and among the wild and cultivated gene pool with respect to both allozymes and quantitative traits. Patterns of diversity defined three kinds of natural populations. First, some populations were intermediate with respect to both allozymes and quantitative traits. This suggests that crop-to-weed gene flow may have created hybrid populations in some locations. Second, some populations were different from all the cultivated landraces with respect to both allozymes and quantitative traits. This probably results from variable gene Row in space and in time, due to demographic stochasticity in either natural or cultivated populations. Third. differentiation from cultivated landraces was only achieved for the quantitative traits but not for allozymes in two populations. This suggests that natural selection in some locations may oppose gene flow to establish cultivated traits into the natural introgressed populations.

Klein, E. K. and Laredo, C. 1999. Optimal sampling designs for studies of gene flow: A comment on Assuncao and Jacobi. *Evolution* **53**: 2002-2005.

Maskell, L. C., Raybould, A. F., Cooper, J. I., Edwards, M. L. and Gray, A. J. 1999. Effects of turnip mosaic virus and turnip yellow mosaic virus on the survival, growth and reproduction of wild cabbage (Brassica oleracea). *Ann Appl Biol* **135**: 401-407.

Wild plants of Brassica oleracea (wild cabbage) are commonly infected with turnip mosaic potyvirus (TuMV), turnip yellow mosaic tymovirus (TYMV) and several other viruses. A field experiment in which plants were inoculated either with TuMV or TYMV showed that virus infection significantly reduced survival, growth and reproduction. Relative to water inoculated-controls, plants infected with TYMV had greater mortality, were shorter, had a smaller leaf area and number, showed a greater amount of damage from herbivory and chlorosis, were less likely to flower and produced fewer pods and lower total seed output. Plants infected with TuMV did not appear to be adversely affected at first; however, mortality after 18 months was higher than control plants. Although TuMV infection had no effect on the number of plants flowering, the infected plants did produce fewer pods and a lower total seed output. We conclude that both viruses can significantly affect vegetative and reproductive performance of wild cabbage and hence that introgression of virus resistance (particularly when conferred by a major gene or a transgene) from a crop might increase plant fitness in natural populations of this species. Ecological risk assessments of virus resistance transgenes must do more than survey adult plants in natural populations for the presence of the target virus. Failure to detect the virus could be due to high mortality on infection with the virus.

Pohl-Orf, M., Brand, U., Driessen, S., Hesse, P. R., Lehnen, M., Morak, C., Mucher, T., Saeglitz, C., von Soosten, C. and Bartsch, D. 1999. Overwintering of genetically modified sugar beet, Beta vulgaris L. subsp vulgaris, as a source for dispersal of transgenic pollen. *Euphytica* **108**: 181-186.

The potential impact of transgenic crops on community ecology will depend on the distribution and establishment of the new transgenic traits, on the sexual transfer of their new genes to the environment (Bartsch & Pohl-Orf, 1996) and on the potential ecological impact of the transgenic trait. Flowering and pollen dispersal is important for outcrossing of the genetically engineered trait. For a biennial plant, like the cultivars of Beta vulgaris L., overwintering is normally necessary to become generative and to produce pollen and seeds (Abe et al., 1997), which usually does not happen with sugar beet as a field crop harvested in autumn (Longden 1989). The starting point for the project was a transgenic sugar beet, Beta vulgaris L. subsp. vulgaris (Lange et al., 1998), with rhizomania and herbicide (Basta (R), Liberty (R)) resistance. Cold tolerance is one of the most important factors for survival of sugar beet in Central- and North-Europe. Among other ways, spreading of transgenic traits into weed beet (Boudry et al., 1993) or wild beet can occur if genetically engineered - biennial - plants survive the winter, flower in spring and spread their pollen. Field experiments were performed with transgenic breeding lines and their hybrids, transgenic and non-transgenic hybrids with Swiss chard and three conventional beet cultivars to evaluate winter survival rates at seven different field sites. We could show that survival of sugar beet - transgenic as well as conventional ones - in Germany and at the Dutch border is possible. Survival rates were well correlated with temperature data and were unexpectedly high. Differences between sugar beet hybrids and breeding lines could be detected but not within different breeding lines or hybrids. There were no differences detectable between transgenic and non-transgenic plants. The data are crucial for the risk assessment of the release of transgenic sugar beet and are the basis for further experiments towards outcrossing and establishment.

Rieseberg, L. H., Kim, M. J. and Seiler, G. J. 1999. Introgression between the cultivated sunflower and a sympatric wild relative, Helianthus petiolaris (Asteraceae). *Int J Plant Sci* **160**: 102-108.

High rates of hybridization and introgression have been reported between the cultivated sunflower and its wild progenitor (both Helianthus annuus), raising concerns that neutral or favorable transgenes might escape and persist in wild H. annuus populations. However, little consideration has been given to the possibility that other wild sunflower species may hybridize with the cultivated sunflower. Here, we estimate the frequency of hybridization and introgression between the cultivated sunflower and Helianthus petiolaris, a closely related wild species whose range almost completely overlaps that of the cultivated sunflower in the central and northern plains and which hybridizes extensively with wild H. annuus. Assays of 21 cultivar-specific AFLP (amplified fragment length polymorphism) markers in 159 individuals from four sympatric populations of H. petiolaris revealed low rates of introgression, with average marker frequencies per population ranging from 0.006 to 0.026. Twenty-seven individuals had a hybrid ancestry, and all hybrids appeared to represent later- generation backcrosses. There was significant heterogeneity in rates of introgression for different markers, and eight of the 21 markers failed to introgress into any of the four populations. This and other evidence indicate that the H. petiolaris genome may be differentially permeable to introgression. These data indicate that (1) beneficial and neutral transgenes will take much longer to move into H. petiolaris populations than into wild H. annuus; (2) the risk of escape may depend on the genomic location of the transgene; and (3) escape is likely to be sporadic, occurring in some populations and not others and at different times. Thus, risk assessment in wild H. annuus is of more immediate concern than in H. petiolaris.

Snow, A. A., Andersen, B. and Jorgensen, R. B. 1999. Costs of transgenic herbicide resistance introgressed from Brassica napus into weedy B-rapa. *Mol Ecol* **8**: 605-615.

Wild relatives of genetically engineered crops can acquire transgenic traits such as herbicide resistance via spontaneous crop-wild hybridization. In agricultural weeds, resistance to herbicides is often a beneficial trait, but little is known about possible costs that could affect the persistence of this trait when herbicides are not used. We tested for costs associated with transgenic resistance to glufosinate when introgressed into weedy Brassica rapa. Crosses were made between transgenic B. napus and wild B. rapa from Denmark. F-1 progeny were backcrossed to B. rapa and BC1 plants were selected for chromosome numbers similar to B. rapa. Further backcrossing resulted in a BC2 generation that was hemizygous for herbicide resistance. We quantified the reproductive success of 457 BC3 progeny representing six full-sib families raised in growth rooms (plants were pollinated by captive bumblebees). Pollen fertility and seed production of BC3 plants were as great as those of B. rapa raised in the same growth rooms. Segregation for herbicide resistance in BC3 plants was 1:1 overall, but the frequency of resistant progeny was lower than expected in one family and higher than expected in another. There were no significant differences between transgenic and nontransgenic plants in survival or the number of seeds per plant, indicating that costs associated with the transgene are probably negligible. Results from this growth- chamber study suggest that transgenic resistance to glufosinate is capable of introgressing into populations of B. rapa and persisting, even in the absence of selection due to herbicide application.

Tatout, C., Warwick, S., Lenoir, A. and Deragon, J. M. 1999. SINE insertions as clade markers for wild crucifer species. *Mol Biol Evol* **16**: 1614-1621.

Short interspersed nuclear elements (SINEs) are small noncoding transposable elements that are widespread in most eukaryotic genomes. Plant S1 SINEs are present in crucifers, especially in species of the Brassiceae tribe, and were generated by several waves of amplification of different evolutionary ages, suggesting that S1 insertional variability (presence/absence of an S1 at a given genomic site) could be used as a classification criterion to evaluate phylogenetic relationships. We applied this strategy on closely related species from the Brassica oleracea species complex using 21 independent S1 genomic sites. The microsatellite-like variation of S1 3' poly(A) tails was also used as a complementary classification criterion to obtain internal resolution in two different clades. The phylogenetic tree obtained by this approach is in general agreement with the classification made from chloroplast DNA restriction site polymorphisms and differs significantly from two other (nonequivalent) classifications made using nuclear restriction fragment length polymorphisms. Brassica incana Brassica montana, and Brassica hilarionis are confirmed as the closest relatives of B. oleracea. From our data, we suggest that Brassica drepanensis emerged recently from a B. incana/Brassica villosa hybrid (with B. villosa as the maternal patent) following backcrosses to B. incana. We also detected several introgressions, confirming that these highly related species are capable of genetic exchange in their natural habitat. S1 markers are therefore very useful in understanding the detailed evolutionary history of wild Brassica species and could also be used to identify potential gene flow between cultivated (including transgenic) Brassica and their wild relatives.

Chevre, A. M., Eber, F., Baranger, A., Hureau, G., Barret, P., Picault, H. and Renard, M. 1998. Characterization of backcross generations obtained under field conditions from oilseed rape wild radish F-1 interspecific hybrids: an assessment of transgene dispersal. *Theor Appl Genet* **97**: 90-98.

Gene flow from glufosinate-resistant transgenic oilseed rape to wild radish was studied over two backcross generations. Under field conditions,seed production from oilseed rape-wild radish F-1 hybrids due to pollination by wild radish was always low: on average 0.12 and 0.78 seeds per 100 flowers and per plant, respectively. The cytogenetics of the resulting "BC1" plants can be explained in the main by three different genomic constitutions: either ACRrRr, 2n = 37, ACRr, 2n = 28 (the same chromosome number as the mother plant), or by the amphidiploid AACCRrRr, 2n = 56. The probability of gene exchange through chromosome pairing was high only in plants with Zn = 28 or 37 chromosomes. Due to the viability of unreduced or partially reduced female gametes, most of the "BC1" plants (81.9%) were Basta resistant whereas the analysis of oilseed rape specific loci indicated that their transmission varied with the locus. In spite of low male fertility (8.7%), an improvement of the female fertility over the F-1 hybrids was observed with an average production of 1.4 and 11 seeds per 100 flowers and per plant, respectively. At the following "BC2" generation, the bar gene transmission (57.2% of Basta-resistant plants) decreased as did the chromosome number, with a majority of plants having between 24 and 27 chromosomes, with 10.5% similar to wild radish (2n = 18). The lower the chromosome number, the better the fertility of the "BC2" plants. On average, 7.9 and 229.3 seeds per 100 flowers and per plant were produced. Gene-flow assessment is discussed based on these data.

Daniell, H., Datta, R., Varma, S., Gray, S. and Lee, S. B. 1998. Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nat Biotechnol* **16**: 345-348.

Glyphosate is a potent herbicide. It works by competitive inhibition of the enzyme 5-enol-pyruvyl shikimate-3-phosphate synthase (EPSPS), which catalyzes an essential step in the aromatic amino acid biosynthetic pathway. We report the genetic engineering of herbicide resistance by stable integration of the petunia EPSPS gene into the tobacco chloroplast genome using the tobacco or universal vector. Southern blot analysis confirms stable integration of the EPSPS gene into all of the chloroplast genomes (5000-10,000 copies per cell) of transgenic plants. Seeds obtained after the first self-cross of transgenic plants germinated and grew normally in the presence of the selectable marker, whereas the control seedlings were bleached. While control plants were extremely sensitive to glyphosate, transgenic plants survived sprays of high concentrations of glyphosate. Chloroplast transformation provides containment of foreign genes because plastid transgenes are not transmitted by pollen. The escape of foreign genes via pollen is a serious environmental concern in nuclear transgenic plants because of the high rates of gene flow from crops to wild weedy relatives.

Garcia, M., Figueroa, J., Gomez, R., Townsend, R. and Schoper, J. 1998. Pollen control during transgenic hybrid maize development in Mexico. *Crop Sci* **38**: 1597-1602.

Pollen containment may be necessary to prevent the dissemination of novel genes from transgenic crops into sexually compatible land races or wild relatives in locations where these are grown or occur naturally in the same vicinity. Routine maize (Zea mays L.) breeding activities employ controlled pollinations and are sometimes done in areas where land races or wild relatives are known to occur. The ability of researchers to control pollen movement and to thereby control the potential pow of novel genes from transgenic maize to land races or wild relatives was investigated. Using white- and yellow-seeded inbreds, pollen control was measured in two mating designs. The ability to control pollen was assessed by observing seed color in pollinations on adjacent plantings intended to trap uncontrolled pollen. In one experiment, the yellow-seeded maize contained a transgene. In this experiment contaminant seeds observed in the white maize were analyzed for the presence of recombinant DNA and the gene expression product. The results from these experiments indicated that routine plant breeding activities can be conducted with completely effective pollen containment if the transgenic line is detasseled and serves as the female for pollination with a nontransgenic male inbred. However, precautions in addition to those used in these experiments are necessary to provide complete control of pollen dissemination if a transgenic male is used to make crosses and approximate to 0.1% outcrossing to adjacent rows is deemed unacceptable.

Lavigne, C., Klein, E. K., Vallee, P., Pierre, J., Godelle, B. and Renard, M. 1998. A pollen-dispersal experiment with transgenic oilseed rape. Estimation of the average pollen dispersal of an individual plant within a field. *Theor Appl Genet* **96**: 886-896.

In order to help establish a basis for the assessment of gene flow associated with the large-scale release of transgenic oilseed rape, we previously designed a method which makes it possible to retrieve the average pollen dispersal of a single plant from that of a large source plot. The 'individual' pollen distribution thus obtained is less dependent on the experimental design than pollen distributions usually published and could therefore be used to model the possible escape of a transgene from commercial transgenic crops. In this study we report on a field experiment set up to study the pollen dispersal from an herbicide-resistant transgenic variety of oilseed rape and to test the applicability of the method on the experimental data. Two techniques were used to determine the individual pollen dispersal, and their outcomes are compared. The results suggest that approximately half of the pollen produced by an individual plant fell within 3m and that the probability of fertilisation afterwards decreased slowly along a negative exponential of the distance. Comparison with the global pollen distribution from the source plot indicates that pollen-dispersal distributions based on dispersal from whole plots instead of individual plants would have underestimated the proportion of pollen that was dispersed over average or long distances.

Linder, C. R. 1998. Potential persistence of transgenes: Seed performance of transgenic canola and wild x canola hybrids. *Ecol Appl* **8**: 1180-1195.

Because wild, weedy-ephemeral species take advantage of periodic disturbance For growth and reproduction, many have considerable seed dormancy and longevity, coupled with germination cued on environmental changes correlated with disturbance. Brassica napus canola is derived from a weedy ephemeral and has sexually compatible wild relatives that are weedy ephemerals, most notably B. rapa. Hence, if transgenes introduced into B. napus canola alter its seed bank dynamics to be more similar to its wild relatives, the chance of transgene escape and persistence is increased. Further, if introgression of the transgene into a wild relative enhances or does not disrupt the seed bank dynamics of wild plants, the transgene could persist in wild populations. I conducted experiments with transgenic, oil-modified B. napus canola (high-stearate and high-laurate types) and wild B. rapa x B. napus canola hybrids (high-laurate type) to determine whether they possessed seed dormancy and germination cuing characteristics favoring persistence of escaped transgenes. Seeds of high-stearate B. napus canola and its untransformed parental type were germinated in growth chambers in a complete factorial design of light (full light, darkness, simulated foliage shade), nutrient concentration (high, low), and temperature (10 degrees, 20 degrees, 35 degrees C). Most combinations of factors did not alter high-stearate canola's timing of germination or total proportion of seeds germinated relative to its nonpersistent parental control. Where effects were seen, they were disadvantageous, with high-stearate seeds germinating more slowly than controls (35 degrees C:full light:high nutrient) and in lower proportions (35 degrees C:darkness:high nutrient and 35 degrees C:full Light:high nutrient). Ungerminated high- stearate seeds in treatment combinations having reduced germination were primarily dormant rather than dead. Although most of their dormancy was enforced by high temperature, under some conditions (35 degrees C:full light:high nutrients and 35 degrees C:darkness:low nutrients), I detected significantly higher levels of induced dormancy for high-stearate seeds relative to controls. Hence, persistent high-stearate canola seed banks could form under some field conditions. For high- laurate B. napus canola and high-laurate wild B. rapa x B. napus canola hybrids and their controls, I germinated seeds in growth chambers, varying light and nutrients using the same treatments as above in a complete factorial design. Temperature was maintained at 10 degrees C. High-laurate canola germinated later than its parental control, with the difference enhanced by low nutrients, In low nutrients, high-laurate canola also germinated at a lower proportion than its control. Both of these altered germination characteristics do not favor population persistence of high-laurate canola. Under some conditions (darkness:high nutrients), high-laurate canola had higher overall dormancy and induced dormancy than its control, suggesting the possibility of increased seed bank persistence. Finally, in spite of expectations that, the high-laurate wild x crop hybrid would show strong maternal effects, this hybrid and the hybrid control always germinated at proportions at or near 1.0, whereas the wild parent had very low germination proportions and high levels of dormancy, especially in simulated foliage shade. These results suggest that high- laurate wild-crop hybrids lack germination cuing mechanisms and will germinate primarily at inappropriate times. However, when they do germinate with wild B. rapa, they are likely to compete well with it: because the high-laurate hybrids germinated and grew as fast or faster than their wild parental control. This should provide opportunities for backcrossing to wild B. rapa.

Linder, C. R., Taha, I., Seiler, G. J., Snow, A. A. and Rieseberg, L. H. 1998. Long-term introgression of crop genes into wild sunflower populations. *Theor Appl Genet* **96**: 339-347.

Hybrids between cultivated and wild sunflowers (Helianthus annuus) are frequently reported. As much as 42% of progeny from wild plants near cultivar fields are hybrids, and cultivar genes have been shown to persist in wild populations at least five generations. We report the effects of up to 40 years of persistent cultivar gene flow on the genetic structure of three wild H. annuus populations that are adjacent to cultivated fields, Eighteen cultivar-specific markers were surveyed in a total of 115 individuals. We also developed cultivar-based genetic maps for the markers and used them to see if marker patterns in the wild populations were consistent with introgression and if selection was acting jointly on unlinked loci. High levels of crop specific markers were detected in all three wild populations: ranging from 0.315 to 0.382, on average. All 115 plants had at least 1 cultivar marker. Introgression is the most likely explanation for the presence of crop-specific markers in the sympatric populations because (1) the markers were either not present or found at much lower levels in four allopatric populations, (2) markers that were genetically linked in the cultivar invariably showed linkage disequilibrium patterns in the sympatric populations consistent with introgression, and (3) homology tests ruled out convergent evolution as an alternate explanation. Hence, introgression of cultivar loci was widespread, and the sympatric wild populations have been replaced by advanced generation hybrids, Unlinked, introgressed markers did not show significant linkage disequilibria, indicating a lack of epistatic associations among introgressed markers. Hence, transgenes in cultivated sunflowers should readily introgress into sympatric wild populations, and their fate will be determined primarily by their fitness effects on the wild plants.

Nurminiemi, M., Tufto, J., Nilsson, N. O. and Rognli, O. A. 1998. Spatial models of pollen dispersal in the forage grass meadow fescue. *Evol Ecol* **12**: 487-502.

Several bivariate probability distributions, generated by different underlying dispersal mechanisms, are fitted to the observed frequencies of an isozyme marker gene using a maximum likelihood approach. The pollen dispersal data were generated using two experimental populations of meadow fescue (Festuca pratensis Huds.), homozygous for different allozymes at the (Pgi-2) locus, arranged in a circular donor-acceptor field design. The contribution of a plant depends on plant position, fecundity and flowering time, factors which are taken into account when fitting the different models. Several approximate likelihood-ratio tests are done between alternative nested models, and a wind threshold model with bimodality in the wind direction is selected. The evolutionarily important variances and expectations of gene displacement under the selected model are calculated. It is also shown that the underlying probability distribution is significantly more than exponentially leptokurtic. By fitting a distribution of deposition in all three dimensions to the data, taking into account differences in plant height, separate estimates of additional physical parameters are obtained, showing that gravity and vertical random movements are more important than intervening vegetation in limiting pollen dispersal in meadow fescue. According to the model, plants with a high seed yield contribute pollen over-proportionally to neighbouring plants.

Snow, A. A., Moran-Palma, P., Rieseberg, L. H., Wszelaki, A. and Seiler, G. J. 1998. Fecundity, phenology, and seed dormancy of F-1 wild-crop hybrids in sunflower (Helianthus annuus, Asteraceae). *Am J Bot* **85**: 794-801.

Crop-to-wild hybridization has the potential to introduce beneficial traits into wild populations. Gene flow from genetically engineered crops, in particular, can transfer genes coding for traits such as resistance to herbicides, insect herbivores, disease, and environmental stress into wild plants. Cultivated sunflower (Helianthus annuus) hybridizes spontaneously with wild/weedy populations (also H. annuus), but little is known about the relative fitness of F-1 hybrids. In order to assess the ease with which crop-to-wild introgression can proceed, we compared characteristics of F-1 wild-crop progeny with those of purely wild genotypes. Two nontransgenic, cultivated varieties were crossed with wild plants from three different regions-Texas, Kansas, and North Dakota. Seed burial experiments in the region of origin showed that wild-crop seeds had somewhat higher germination rates (less dormancy) than wild seeds from Kansas and North Dakota, while no differences were seen in seeds from Texas. Progeny from each type of cross were grown in outdoor pots in Ohio and in a weedy field in Kansas to quantify lifetime fecundity and flowering phenology. Flowering periods of hybrid and wild progeny overlapped considerably, especially in plants from North Dakota and Texas, suggesting that these hybrids are very likely to backcross with wild plants. In general, hybrid plants had fewer branches, flower heads, and seeds than wild plants, but in two crosses the fecundity of hybrids was not significantly different from that of purely wild plants. In Ohio, wild-crop hybrids from North Dakota appeared to be resistant to a rust that infected 53 % of the purely wild progeny, indicating a possible benefit of "traditional" crop genes. In summary, our results suggest that F-1 wild-crop hybrids had lower fitness than wild genotypes, especially when grown under favorable conditions, but the F-1 barrier to the introgression of crop genes is quite permeable.

Van Gaal, T. M., Galatowitsch, S. M. and Strefeler, M. S. 1998. Ecological consequences of hybridization between a wild species (Echinacea purpurea) and related cultivar (E-purpurea 'White Swan'). *Sci Hortic* **76**: 73-88.

In the first such study of its kind, we examined gene flow potential between a valued native plant and a popular cultivar. We studied outcomes of controlled hybridization between Echinacea purpurea and a conspecific cultivar, E. purpurea 'White Swan', by comparing levels of competitive ability and reproductive potential. Differences in plant performance of wild-types and Fl's were studied at three densities (20.3, 45.7, and 182.9 plants/m(2)) using a field competition experiment. Wild-types were slightly larger but F(1)s had higher reproductive output. These differences can be attributed to floricultural breeding of the cultivar parent. Based on the results of this research, if the initial hybridization between the wild-type and cultivar were to occur, it is anticipated that the resulting Fl generation would survive and reproduce, creating the potential for continued gene flow. This methodology has potential for use in risk assessment of plant introductions, including transgenic crops. (C) 1998 Elsevier Science B.V. All rights reserved.

Bartsch, D. and Schmidt, M. 1997. Influence of sugar beet breeding on populations of Beta vulgaris ssp. maritima in Italy. *J Veg Sci* **8**: 81-84.

It is highly probable that transgenic cultivars of sugar beet may influence wild beets in the seed-production-area of northern Italy. For this reason a survey of the local wild beet populations and their habitat characteristics was conducted in 1994/1995, i.e. before transgenic beets and their offspring could have become established. Wild beets (Beta vulgaris ssp. maritima) were found at 21 locations between Trieste and Cesenatico, as part of the natural littoral vegetation classified as Atriplicetum tatarici (Cakiletea maritimae) and Crithmetum (Crithmo-Staticetea). The analysis of phenotypic attributes leads to a division into three different subpopulations. Greenhouse studies on the morphology and life- cycle attributes demonstrated actual gene flow between conventional seed beet and the examined wild beet population.

Giddings, G. D., Hamilton, N. R. S. and Hayward, M. D. 1997. The release of genetically modified grasses .1. Pollen dispersal to traps in Lolium perenne. *Theor Appl Genet* **94**: 1000-1006.

As part of a series of experiments on determining the risk of introducing genetically modified wind-pollinated forage grasses an experiment on pollen dispersal was conducted and-the use of theoretical descriptions to predict dispersal in model systems investigated. Pollen traps were placed around a central source of Lolium perenne. Traps were exposed with their sticky surfaces towards and away from the pollen source and also facing skywards during four stages of anthesis (early, mid 1, mid 2 and late). There was a great deal of variation in dispersal over time and to traps of different orientations. Twelve datasets were collected and used to comprehensively test Bateman's equations for the wind dispersal of pollen. The equations were not particularly useful for describing dispersal over distance and clearly need to be modified to take factors such as wind direction into account.

Metz, P. L. J., Jacobsen, E., Nap, J. P., Pereira, A. and Stiekema, W. J. 1997. The impact on biosafety of the phosphinothricin-tolerance transgene in inter-specific B-rapa x B-napus hybrids and their successive backcrosses. *Theor Appl Genet* **95**: 442-450.

There is strong evidence indicating that gene flow from transgenic B. napus into weedy wild relatives is inevitable following commercial release. Research should now focus on the transmission, stability, and impact of transgene expression after the initial hybridization event. The present study investigated the transfer of a phosphinothricin-tolerance transgene by inter-specific hybridization between B. rapa and two transgenic B. napus lines. The expression of the transgene was monitored in the F-1 hybrids and in subsequent backcross generations. The transgene was transmitted relatively easily into the F-1 hybrids and retained activity. Large differences in the transmission frequency of the transgene were noted between offspring of the two transgenic lines during backcrossing. The most plausible explanation of these results is that the line showing least transmission during backcrossing contains a transgene integrated into a C-genome chromosome. Approximately 10% of offspring retained the tolerant trait in the BC, and BC, generations. The implications of these findings for the stable introgression of transgenes carried on one of thp chromosomes of the C-genome from B. napus and into B. rapa are briefly discussed.

VanRaamsdonk, L. W. D. and Schouten, H. J. 1997. Gene flow and establishment of transgenes in natural plant populations. *Acta Bot Neerl* **46**: 69-84.

In this paper the possibilities are indicated to model the introgression of a domestication gene, especially a transgene, into a wild plant population. The entire process of introgression consists of two phases, i.e. gene flow by pollen (hybridization) or by seed (escape), and establishment. A literature survey demonstrated that a lot of information is available on the first phase (gene flow), although seed dispersal is less studied than pollen dispersal. The second phase of introgression (establishment) is hardly studied in a quantitative way. It is important to focus further research on fitness effects of establishment, because even at low levels of gene flow a (trans)gene will finally succeed in entering a wild population. In this paper several parts of a simulation model are described. In case of small wild populations a stochastic model should be used in order to account fbr genetic drift.

Wang, T. Y., Chen, H. B., Reboud, X. and Darmency, H. 1997. Pollen-mediated gene flow in an autogamous crop: Foxtail millet (Setaria italica). *Plant Breed* **116**: 579-583.

Gene how is a key concern associated with the contamination of seed multiplication fields and the use of transgenic crops. The release of herbicide-resistant germplasms and the use of male- sterile varieties make foxtail millet (Setaria italica) an appropriate material to investigate this concern. Pollen dispersal from pollen donor sources and gene flow in fertile and male-sterile varieties of foxtail millet were investigated in experiments in China and France. Although foxtail millet is mainly autogamous (less than 2% outcrossing), pollen from a 5 m diameter plot could fertilize a fertile variety up to 24 m away, and a male-sterile variety up to 40 m. Negative exponential and Weibull functions were used to fit the relationships between percentage and number of hybrids per cm of ear with distance from the pollen source. Wind velocity and direction resulted in variations of gene how intensity, but did not change the slope of the regression equations. Pollen competition for available target ovules was observed up to 2 m away from a Im diameter pollen source. The percentage of pollen grains that fertilized ovules outside the pollen donor source was 1.4% of the total pollen grain production. For every 100 pollen grains released under field conditions 1.5 seeds were produced. The probability function of pollen dispersal for one donor plant was calculated. It allowed predictions of gene flow according to field shapes, estimations of isolation distances to preserve variety purity against pollen contamination, and the formulation of efficient male/female ratios for hybrid seed production.

Whitton, J., Wolf, D. E., Arias, D. M., Snow, A. A. and Rieseberg, L. H. 1997. The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization. *Theor Appl Genet* **95**: 33-40.

The development of transgenic plants has heightened concern about the possible escape of genetically engineered material into the wild. Hybridization between crops and their wild relatives provides a mechanism by which this could occur. While hybridization has been documented between several crops and wild or weedy relatives, little is known about the persistence of cultivar genes in wild populations in the generations following hybridization. Wild and weedy sunflowers occur sympatrically with cultivated sunflowers throughout much of the cultivation range, and hybridization is known to occur. We surveyed two cultivar-specific RAPD markers in 2700 progeny in a naturally occurring population of wild Helianthus annuus over five generations following a single generation of hybridization with the cultivar. Moderate levels of gene flow were detected in the first generation (42% hybrids at the crop margin) and cultivar allele frequencies did not significantly decline over four subsequent generations. These results indicate that gene flow from cultivated into wild populations of sunflowers can result in the long-term establishment of cultivar alleles in wild populations. Furthermore, we conclude that neutral or favorable transgenes have the potential to escape and persist in wild sunflower populations.

Widmer, F., Seidler, R. J., Donegan, K. K. and Reed, G. L. 1997. Quantification of transgenic plant marker gene persistence in the field. *Mol Ecol* **6**: 1-7.

Methods were developed to monitor persistence of genomic DNA in decaying plants in the field. As a model, we used recombinant neomycin phosphotransferase II (rNPT-II) marker genes present in genetically engineered plants. Polymerase chain reaction (PCR) primers were designed, complementary to 20-bp sequences of the nopaline synthase promoter in a transgenic tobacco and the cauliflower mosaic virus 35S promoter in a transgenic potato. The PCR reverse primer was complementary to a 20-bp sequence of the N-terminal NPT-II coding region. The PCR protocol allowed for quantification of as few as 10 rNPT-II genes per reaction. We analysed rNPT-II marker gene amounts in samples obtained from two field experiments performed at different locations in Oregon. In transgenic tobacco leaves, buried at 10 cm depth in a field plot in Corvallis, marker DNA amount dropped to 0.36% during the first 14 days and was detectable for 77 days at a final level of 0.06% of the initial amount. Monitoring of residual potato plant litter, from the soil surface of a test field in Hermiston, was performed for 137 days. After 84 days marker gene amounts dropped to 2.74% (leaf and stem) and 0.50% (tuber) of the initially detected amount. At the final sample date 1.98% (leaf and stem) and 0.19% (tuber) were detectable. These results represent the first quantitative analysis of plant DNA stability under field conditions and indicate that a proportion of the plant genomic DNA may persist in the field for several months.

Arriola, P. E. and Ellstrand, N. C. 1996. Crop-to-weed gene flow in the genus Sorghum (Poaceae): Spontaneous interspecific hybridization between johnsongrass, Sorghum halepense, and crop sorghum, S-bicolor. *Am J Bot* **83**: 1153-1159.

The role of crop-to-weed gene flow is often controversial and overlooked. As a consequence, the likelihood of spontaneous crop-to-weed hybridization in most crop/weed systems is generally unknown. The lack of data relating to the formation of crop/weed hybrids has particular contemporary significance when considering the wide scale commercial release of transgenic crop plants and the potential for escape of engineered genes via crop-to-weed hybridization. We created an experimental system whereby we could examine the incidence and rate of spontaneous crop-to-weed hybridization between Sorghum bicolor and S, halepense, johnsongrass. An isozyme marker was used to identify hybrid plants through progeny testing. Incidence and rate of hybridization were highly variable with respect to weed distance from the crop, location of the study site, and year the study was performed. Crop/weed hybrids were detected at distances of 0.5-100 m from the crop. Interspecific hybridization can and does occur in this system at a substantial and measurable rate. Transgenes introduced into crop sorghum can be expected to have the opportunity to escape cultivation through interspecific hybridization with johnsongrass. Traits that prove to be beneficial to weeds possessing them can be expected to persist and spread. This is an issue that needs to be addressed when developing biosafety guidelines for the commercial release of transgenic sorghums.

Bartsch, D. and PohlOrf, M. 1996. Ecological aspects of transgenic sugar beet: Transfer and expression herbicide resistance in hybrids with wild beets. *Euphytica* **91**: 55-58.

An increasing number of genetically engineered cultivars of several crops is being experimentally released into the environment. In future, crops with new transgenic traits will probably play an important role in agricultural practice. The long-term effect of transgenes on community ecology will depend on the distribution and establishment of transgenic plants in the wild, on the sexual transfer of their new genes to the environment and on the potential ecological impact of the transgenic trait. The starting point was the use of transgenic sugar beet lines, Beta vulgaris subspec. vulgaris var. altissima DOLL (Helm 1957), with transgenes coding for rhizomania and herbicide (BASTA(R)) resistance. The first two questions to answer were: Can the transgenes be transferred via pollen to wild beets, Beta vulgaris subspec. maritima (L.) ARCANG. or cultivated relatives such as red beet or spinach beet and are they expressed in the hybrids? Can transgenes be monitored in young Beta vulgaris-hybrids? The experimental transfer of transgenes was conducted in 1993 at a field location in northern Germany. The beets were hand-pollinated with transgenic pollen. In a non destructive biotest, the hybrid seedlings were tested for herbicide resistance. Transgenic plants showed no noxious phenotypic effects whereas control plants developed leaf necroses. All herbicide resistant hybrids within the biotest were assumed to be transgenic.

Lefol, E., Fleury, A. and Darmency, H. 1996. Gene dispersal from transgenic crops .2. Hybridization between oilseed rape and the wild heavy mustard. *Sex Plant Reprod* **9**: 189-196.

The risk of release of genetically modified oilseed rape (Brassica napus) was investigated in relation to interspecific gene flow with hoary mustard (Hirschfeldia incana). Microscopic studies showed polymorphism within the population of hoary mustard for pollen germination on oilseed rape flowers. The transgenic herbicide-resistant and a commercial cultivar of oilseed rape were not different for pollen behaviour and ovule fertilization. Pollen tube growth was slow and erratic in interspecific crosses. Fertilization efficiency of oilseed rape and hoary mustard pollen in interspecific crosses was 15% and 1.3%, respectively, of that in intraspecific crosses. This unequal efficiency in reciprocal crosses was confirmed by hybrid seed set in pods. There was no post-zygotic barrier to the development of hybrid embryos in hoary mustard pods. Up to 26 spontaneous hybrids per male sterile oilseed rape plant, and one per hoary mustard plant, were obtained in field experiments. Hybrids were identified by isozyme electrophoresis, morphology and cytology. All hybrids were triploid with 26 chromosomes, and had low fertility. They produced 0.5 seeds per plant after spontaneous backcrossing with hoary mustard. Some of these descendants were produced from unreduced gametes. Our results suggest that gene flow is likely to occur, but its actual frequency under crop growing conditions remains to be estimated.

Mikkelsen, T. R., Jensen, J. and Jorgensen, R. B. 1996. Inheritance of oilseed rape (Brassica napus) RAPD markers in a backcross progeny with Brassica campestris. *Theor Appl Genet* **92**: 492-497.

Different cultivars/transgenic lines of oilseed rape (Brassica napus) were crossed (as females) with different cultivars/populations of Brassica campestris. All cross combinations produced seed, with an average seed set per pollination of 9.8. Backcrossing of selected interspecific hybrids (as females) to B. campestris resulted in a much lower seed set, average 0.7 seed per pollination. In the single backcross progeny where a large enough population (92 plants) was obtained for analysis, 33 B. napus specific RAPD markers were investigated to determine the extent of transfer of oilseed rape genetic material into this population. Markers were transferred to the backcross generation with frequencies ranging from 26% to 91%. Almost all of the markers (30/33) were transferred in a frequency not significantly different from 50%. Analysis of the pairwise segregation of markers revealed that 23 markers could be assigned to six linkage groups, most probably reflecting six B. napus C-chromosomes. The presence of backcross plants with recombinant genotypes suggests that complex genetic processes can take place during the interspecific hybridisation and backcrossing in these Brassica species. The implications of our results for the possible choice nf integration sites of transgenes in oilseed rape are discussed.

Raybould, A. F., Mogg, R. J. and Clarke, R. T. 1996. The genetic structure of Beta vulgaris ssp maritima (sea beet) populations: RFLPs and isozymes show different patterns of gene flow. *Heredity* **77**: 245-250.

Genetic variation in 12 populations of sea beet was assessed at nine isozyme and seven RFLP loci. Mean observed heterozygosity, diversity index and number of alleles per locus were not significantly different between the two classes of marker. The genetic structure of 10 of the populations was analysed using seven of the isozymes and six of the RFLPs. F-ST values between all pairs of populations were calculated separately for the isozymes and RFLPs. F-ST values were converted to amounts of gene flow (Nm) between populations under the assumptions of an island model. A regression of log Nm against log distance was used to test for isolation by distance. Mantel tests showed a highly significant decrease in Nm with distance for RFLPs but not for isozymes. It is suggested that uniform balancing selection may operate to maintain approximately equal allele frequencies among populations at the isozyme loci. If this is true then isozymes may be unsuitable for modelling the spread of neutral transgenes.

Stewart, C. N., Adang, M. J., All, J. N., Raymer, P. L., Ramachandran, S. and Parrott, W. A. 1996. Insect control and dosage effects in transgenic canola containing a synthetic Bacillus thuringiensis crylAc gene. *Plant Physiol* **112**: 115-120.

Zygotic hypocotyls of canola (Brassica napus L.) cv Oscar, cv Westar, and the breeding line UCA1 88-208 were transformed with a truncated synthetic Bacillus thuringiensis insecticidal crystal protein gene (Bt crylAc) under the control of the cauliflower mosaic virus 35S promoter using Agrobacterium tumefaciens-mediated transformation. Fifty-seven independently transformed lines were produced, containing 1 to 12 copies of the transgenes. A range of cry expressors was produced from 0 to 0.4% Cry as a percentage of total extractable protein. The Brassica specialists, the diamondback moth (Plutella xylostella L.) and the cabbage looper (Trichoplusia ni Hubner), were completely controlled by low-, medium-, and high-expressing lines. Whereas control of the generalist lepidopteran, the corn earworm (Helicoverpa tea Boddie), was nearly complete, the other generalist caterpillar tested, the beet armyworm (Spodoptera exigua Hubner), showed a dose response that had a negative association between defoliation and cry expression. These plants were produced as models for an ecological research assessment of the risk involved in the field release of naturalized transgenic plants harboring a gene (Bt) that confers higher relative fitness under herbivore-feeding pressure.

Luby, J. J. and McNicol, R. J. 1995. Gene Flow from Cultivated to Wild Raspberries in Scotland - Developing a Basis for Risk Assessment for Testing and Deployment of Transgenic Cultivars. *Theor Appl Genet* **90**: 1133-1137.

In order to help establish a basis for assessing the risk associated with the testing and large-scale deployment of transgenic raspberries, wild and feral raspberry populations in Scotland were surveyed for evidence of the escape of genes introduced into raspberry cultivars by traditional breeding. The genes concerned were introduced into cultivars using traditional breeding techniques and were deployed at known times 20 to 30 years prior to the present survey. Escape of the semidominant L(1) gene, affecting fruit size and plant morphology, could not be detected after 30 years in test plots at the Scottish Crop Research Institute near Dundee. The recessive gene s, conferring spinelessness, was detected at very low frequencies (estimated at 0.004) in wild populations within the commercial production locales where cultivars carrying this gene had been introduced on a large scale beginning 21-years prior to this survey. This gene was not, however, found in any areas remote from the commercial production locales. The results of the survey indicate that escape does occur following large-scale deployment but that gene flow events are probably infrequent and spread is localized for genes having probable neutral selective value.

Paul, E. M., Capiau, K., Jacobs, M. and Dunwell, J. M. 1995. A study of gene dispersal via pollen in Nicotiana tabacum using introduced genetic markers. *J Appl Ecol* **32**: 875-882.

1. Agrobacterium tumefaciens was used to introduce two marker genes (kanamycin resistance and

beta glucuronidase) into tobacco. 2. These plants were grown in a series of field trials each consisting of a small plot of modified plants surrounded at various distances (1 m, 10 m, 20 m) by non-modified receptor plants. 3. Capsules from these receptor plants were harvested and samples of the seed were germinated on kanamycin-containing medium in laboratory conditions, as a screen for the presence of the resistance gene. Large populations of seed could be screened in this way. 4. Using these techniques, gene flow from the 'marked' plants could easily be detected. Although there were some differences in the absolute degree of gene dispersal in the different trials, there was an overall decline in transfer as distance from the marked plants increased. 5. The potential for the use of introduced genetic markers in the study of gene flow is discussed.

Scheffler, J. A., Parkinson, R. and Dale, P. J. 1995. Evaluating the Effectiveness of Isolation Distances for Field Plots of Oilseed Rape (Brassica-Napus) Using a Herbicide- Resistance Transgene as a Selectable Marker. *Plant Breed* **114**: 317-321.

The effectiveness of 200- and 400-m isolation distances were evaluated for small-scale trials of oilseed rape (Brassica napus L.). These distances have been used for previous transgenic release experiments and are commonly adopted for production of basic and certified breeders seed. A 400-m(2) donor plot contained plants with a dominant transgene conferring resistance to the herbicide glufosinate-ammonium. This character was used as a selectable marker to measure the frequency of hybrid formation in 400-m(2) target plots of non- transgenic rape. Seeds produced by the non-transgenic plants were permitted to fall onto the plots and allowed to germinate. At the first true-leaf stage, the plants ware sprayed with glufosinate-ammonium. Surviving plants were resprayed, and a subsample assayed for the presence of the transgene using a colorimetric assay and Southern-blot analysis. The average frequency of hybridization over two replicates was 0.0156% at 200 m and 0.0038% at 400 m. These estimates are within the limits established for the production of basic seed (0.1%). Results indicated that bees were the most likely agent for long-distance pollen dispersal.

Timmons, A. M., Obrien, E. T., Charters, Y. M., Dubbels, S. J. and Wilkinson, M. J. 1995. Assessing the Risks of Wind Pollination from Fields of Genetically-Modified Brassica-Napus Ssp Oleifera. *Euphytica* **85**: 417-423.

Intensive research over the past 10 years has produced many genetically-modified lines of oilseed rape with market potential. Assessment of these lines in statutory trials prior to their release as cultivars is necessary, owing to concern over the likelihood of transgene escape from such crops. Here, we examine the movement of airborne pollen grains from oilseed rape fields and assess their capacity for long-range geneflow. Pollen dispersal from isolated rape fields was monitored over two seasons and related to the distribution of fields and 'feral' (domesticated plants growing outside cultivations) populations of the crop in Tayside and North East Fife regions of Scotland. Airborne pollen density declined with distance and at 360 m was 10% of that at the field margin. Pollen counts of 0-22 pollen grains m(3) were observed 1.5 km from source fields and apparently were sufficient in number to allow seed set on emasculated bait plants. Oilseed rape pollen has greater capacity for long-range dispersal than had been suggested by small-scale field trials. Mean separation of oilseed rape fields in the survey area was 410 m and the mean distance from 'feral' populations to commercial fields was 700 m. Sixty percent of 'feral' populations with more than 10 plants occurred downwind and within 2 km of an oilseed rape field. Provided that the flowering biology of genetically-modified oilseed rape does not differ from the conventional crop, these data suggest that transgene movement to non genetically- modified fields or 'feral' populations is likely following commercial release.

Arias, D. M. and Rieseberg, L. H. 1994. Gene Flow between Cultivated and Wild Sunflowers. *Theor Appl Genet* **89**: 655-660.

With the development of transgenic crops, concern has been expressed regarding the possible escape of genetically- engineered genes via hybridization with wild relatives. This is a potential hazard for sunflowers because wild sunflowers occur as weeds in fields where cultivated sunflowers are grown and hybridization between them has been reported. In order to quantify the potential for gene escape, two experimental stands of sunflower cultivars were planted at two sites with different rainfall and altitude profiles. Populations of wild plants were planted at different distances from each cultivar stand. An allele homozygous in the cultivar (6Pgd-3-a), but absent in the wild populations, was used as a molecular marker to document the incidence and rate of gene escape from the cultivar into the wild populations of sunflowers. Three-thousand achenes were surveyed to determine the amount of gene flow from the cultivated to the wild populations. The marginal wild populations (3 m from the cultivar) showed the highest percentage (27%) of gene flow. Gene flow was found to decrease with distance; however, gene flow occurred up to distances of 1000 m from the source population. These data suggest that physical distance alone will be unlikely to prevent gene flow between cultivated and wild populations of sunflowers.

Cresswell, J. E. 1994. A Method for Quantifying the Gene Flow That Results from a Single Bumblebee Visit Using Transgenic Oilseed Rape, Brassica- Napus L Cv Westar. *Transgenic Res* **3**: 134-137.

Genetically modified plants containing selectable markers offer a unique opportunity for pollination biologists to investigate some of the major, but intractable questions about paternity distributions and their causes. Here, a method is reported that uses transgenic plants to enable the quantification of the outcrossed fertilizations that result from a single pollinator visit. Gene flow mediated by worker bumblebees (Bombus terrestris) was studied among plants of oilseed rape (Brassica napus L. cv. Westar) where transgenic paternity in seeds of a non-transgenic plant was manifested as herbicide resistance. Overall, 91% of the resistant seeds resulted from the first four flowers that were visited after the bumblebee left the transgenic plant, and none was found beyond the 14th successively visited newer. The possibilities for developing the method to address various questions in pollination biology are discussed.

Darmency, H. 1994. The Impact of Hybrids between Genetically-Modified Crop Plants and Their Related Species - Introgression and Weediness. *Mol Ecol* **3**: 37-40.

Assessing the impact of hybrids between transgenic plants and nontarget wild species involves answering several questions such as: (i) what are the hybridization and introgression rates; (ii) what is the behaviour of a transgene in a wild population; and (iii) what will be the consequences of the expression of a transgene in a wild population? These issues are discussed using results from experiments on oilseed rape and wild related Brassiceae. Evidence is given of large variations in the estimates of cross-fertilization probabilities. The first stage of introgression into wild populations is demonstrated to occur spontaneously through back-crossing. Population analysis may also be valuable to detect traces of past introgression. Data from the literature on weed biology, and especially herbicide resistance, are used to illustrate the behaviour of a new gene in weed populations. The need for computer models simulating the introgression process is stressed.

Eijlander, R. and Stiekema, W. J. 1994. Biological Containment of Potato (Solanum-Tuberosum) - Outcrossing to the Related Wild-Species Black Nightshade (Solanum-Nigrum) and Bittersweet (Solanum-Dulcamara). *Sex Plant Reprod* **7**: 29-40.

The biological containment of the potato (Solanum tuberosum) was assessed by establishing the crossability of this tuberous crop with the related wild non-tuberous species in The Netherlands, black nightshade (S. nigrum) and bittersweet (S. dulcamara). To circumvent crossability barriers, genotypes with different ploidy number were employed and crosses were performed under different environmental conditions. S. dulcamara was shown to be incongruent with potato at all ploidy levels, while S. nigrum displayed unilateral incompatibility. If S. nigrum was emasculated and used as female, fertilization by potato pollen resulted in berry set and seed development. Emasculation of S. nigrum was essential in this cross, because analysis of the fertilization process demonstrated that this species is highly self-compatible and potato pollen was outcompeted by pollen of S. nigrum. The hybrid seeds derived from this cross did not mature and appeared not to be viable. By application of the technique of embryo rescue of immature embryos, hybrid plants could be obtained. However, these hybrid plants proved to be sterile. These data demonstrate that gene flow by pollen dispersal from potato to its most common wild relatives in Western Europe is highly unlikely. The potato is thus a naturally contained species in this part of the world.

Klinger, T. and Ellstrand, N. C. 1994. Engineered Genes in Wild Populations - Fitness of Weed-Crop Hybrids of Raphanus-Sativus. *Ecol Appl* **4**: 117-120.

The transfer of engineered genes (transgenes) from crops to natural populations will depend first on mating between the crop and related weeds and then upon the relative fitness of the weed-crop hybrid. While weed-crop hybridization is known to occur readily under agricultural conditions, almost nothing is known of the fitness of the hybrids produced. Therefore, we measured the relative fitness of weedy radishes and their sibling weed-crop hybrids under field conditions. Specifically, we compared germination success, time to first flowering, fruit production, seed production, and frequency of transmission of the crop allele to seed progeny. Hybrids showed significantly greater fruit and seed production, and equaled weeds in all other measured characters. Thus, in this experiment, the fitness of hybrids exceeded that of their wild siblings. These results suggest that, in at least this system, neutral or advantageous transgenes introduced into natural populations will tend to persist.

Linder, C. R. and Schmitt, J. 1994. Assessing the Risks of Transgene Escape through Time and

Crop- Wild Hybrid Persistence. *Mol Ecol* **3**: 23-30.

Transgenes introduced into crops can escape in time, as well as space, via the seed bank. For annual plants, especially ruderals, seed bank behaviour may be the most important factor determining population persistence. Crop seeds may exhibit some dormancy and germination cueing in the soil but are expected to be less able to persist than their wild relatives, which often have considerable dormancy and longevity, as well as effective germination cueing responses. Crop-wild hybrids may have seed bank characteristics more suited to persistence, and maternal effects may favour persistence of hybrids having wild plants for their female parent. Escape of transgenes via crop-wild hybrids presents unique concerns not present for crops. Hybrids can undergo natural selection and may back-cross with wild plants. We suggest methods that can be used in conjunction with evaluation of the relative fitness of crop-wild hybrids that will determine the likelihood of back-crossing. Accurate assessment of escape in time and transgene persistence via crop-wild hybrids requires proper plant materials. We emphasize the use of null segregants as controls for transgenic crops and for generating crop-wild hybrid controls for transgenic hybrids. Since good empirical and theoretical understanding of how individual genes influence the fate of plants in different environments is lacking, evaluation of escape in time and the persistence of transgenes via crop-wild hybrids should be on a case-by-case basis.

Morris, W. F., Kareiva, P. M. and Raymer, P. L. 1994. Do Barren Zones and Pollen Traps Reduce Gene Escape from Transgenic Crops. *Ecol Appl* **4**: 157-165.

As genetically engineered crop varieties near widespread cultivation, both agronomic and environmental concerns mandate the development of effective strategies for isolating transgenic varieties from related non-transgenic varieties or cross-fertile weeds. We present the results of the first field experiment designed to test the effectiveness of two containment strategies that are commonly used in field trials of transgenic crops: (1) an isolation zone devoid of vegetation to discourage emigration of insect pollinators from transgenic plots; and (2) trap crops (non-transgenic varieties of the same crop planted adjacent to the transgenic plot that can "cleanse" emigrating pollinators of transgenic pollen). In conjunction with field trials of genetically engineered canola (Brassica napus) conducted by Calgene, Inc., in California and Georgia, we varied both the width of the barren zone and the presence or absence of a trap crop, and measured the effects on gene escape. Escape was easily detected since the genetic construct inserted into the transgenic canola contained a gene that rendered seedlings resistant to the normally lethal antibiotic kanamycin. Our results suggest that barren zones 4-8 m in width may actually increase seed contamination over what would be expected if the intervening ground were instead planted entirely with a trap crop. When trap crops occupied a limited portion of the isolation zone separating transgenic and non-transgenic varieties, the effectiveness of the trap depended on the width of the isolation zone: they reduced gene escape when the two varieties were separated by 8 m, but increased escape across a 4-m isolation zone. We conclude that, for the relatively short isolation distances we examined, the most effective strategy for reducing the escape of transgenic pollen is to devote the entire region between transgenic and non-transgenic varieties to a trap crop.

Tabei, Y., Oosawa, K., Nishimura, S., Hanada, K., Yoshioka, K., Fujisawa, I. and Nakajima, K. 1994. Environmental Risk-Evaluation of the Transgenic Melon with Coat Protein Gene of Cucumber Mosaic-Virus in a Closed and a Semiclosed Greenhouses .1. *Breed Sci* **44**: 101-105.

Environmental risk evaluation of transgenic melon plants introduced with coat protein gene of cucumber mosaic virus was carried out in a closed and a semiclosed greenhouse. The following evaluation items were; compared between transgenic melon plants and non-transgenic melon plants. 1. Morphological characteristics was compared in a closed greenhouse. Fruits maturation period was examined in a semi-closed greenhouse. Morphological characteristics of transgenic melon plants were not different from those of non-transgenic melon plants. Maturation period of the transgenic melon plants was 44.7 days in average and the non-transgenic melon plants was 43.0 days in average. This result demonstrated that transgenic melon plants did not differ from the non-transgenic plants in fruit maturation period. 2. Pollen form and fertility were not different between transgenic and non-transgenic melon plants. Longevity of the pollen from transgenic and non-transgenic melon were examined on fine days in May 1992. Pollen was collected at 9 : 30, 11 : 30, 13 : 30, 15 : 30, 17 : 30 from both transgenic and non-transgenic melon plants, then sowed onto pollen germination medium. Most of the pollen collected at 9 : 30 germinated. Few collected at 13 : 30 germinated, and pollen collected at 15 : 30 did not germinate. Therefore, longevity of the pollen in both transgenic and non-transgenic melon plants seems to be within about 13 : 30 in a closed greenhouse on fine day. 3. Wind pollination of transgenic and non-transgenic melon plants were investigated under artificial wind, generated by an electric fan, in a closed greenhouse. Pollen germination medium were placed at various distance, 0, 5, 10, 15, 50, 100, 200 and 300 cm from the plants. The wind was blown from 10 : 00 to 15 : 30, at a velocity of 0.5-4.0 m/sec. Pollen from transgenic or non-transgenic melon plants were not detected on germination medium at all distance from the plants. We concluded that pollen of melon, an entmophilous plant, dose not disperse by wind, and is generally dispersed only by insects. 4. Seed fertilities of transgenic and non- transgenic plants were 75.5 % and 58.4 %, respectively. We considered that this difference was due to the affection of weakness of non-transgenic seedling. Germination ratios of seeds stored for 0, 6 and 12 months were not different between transgenic and non-transgenic melon. 5. Cross compatibility of melon (Cucumis melo) in Cucurbitaceae was investigated. Pollen of melon were pollinated to female flowers of cucumber, C. angria, C. metuliferus, C. africanus, watermelon, pumpkin, bottle gourd and bitter gourd. Twenty-four hrs after pollination, elongation of pollen tubes in the styles was investigated by staining with aniline blue. Degrees of elongation of pollen tubes were dependent on the female plants. However, the pollen from the non-transgenic melon plants did not fertilize any related species. These results indicated that melon can not fertilize other species than melon. From these results, it is reveled that there was no difference between transgenic melon and non-transgenic melon plants for characteristics of morphology and gene flow.

Asakawa, Y., Fukumoto, F., Hamaya, E., Hasebe, A., Ichikawa, H., Matsuda, I., Matsumura, T., Motoyoshi, F., Noguchi, K., Ohashi, Y., Okada, M., Sato, M., Shiyomi, M., Ugaki, M., Ukai, Y. and Yokoyama, K. 1993. Evaluation of the Impact of the Release of Transgenic Tomato Plants with Tmv Resistance on the Environment. *Jarq - Jpn Agric Res Q* **27**: 126-136.

Studies on the evaluation of the impact of the release of the tomato plants with an introduced gene of TMV resistance on the environment were carried out from January, 1989, to January, 1992, mainly at the National Institute of Agro-Environmental Sciences (NIAES) in collaboration with the National Institute of Agrobiological Resources (NIAR) and the National Agriculture Research Center (NARC). Experiments were carried out according to the guidelines enacted by the Science and Technology Agency and the Ministry of Agriculture, Forestry and Fisheries. The following characteristics were compared between the original plants and transgenic plants: (1) growth characteristics such as plant height and vigor, (2) pollen dispersion based on fruit set on emasculated flowers, (3) kinds of chemical substances produced by plants such as allelochemicals in plant tissues, soil and air, (4) microorganism flora in soil, (5) overwintering ability, (6) ability to become a weed, (7) the amount of Agrobacterium on plants, and (8) kinds of flower- visiting-insects. Cultivation was safely carried out. Since no harmful impact on the environment where the transgenic tomato plants had been cultivated was detected, it is suggested that this tomato strain can be cultivated in an open field. The TMV resistance was maintained throughout generations. These transgenic tomato plants were cultivated in an ordinary field in the summer of 1992 in the campus of the NIAES.

Boudry, P., Morchen, M., Saumitoulaprade, P., Vernet, P. and Vandijk, H. 1993. The Origin and Evolution of Weed Beets - Consequences for the Breeding and Release of Herbicide-Resistant Transgenic Sugar- Beets. *Theor Appl Genet* **87**: 471-478.

Populations of weed beets have expanded into European sugar beet production areas since the 1970s, thereby forming a serious new weed problem for this crop. We sampled seeds in different French populations and studied mitochondrial DNA, chloroplast DNA and life-cycle variability. Given the maternal inheritance of the mitochondrial and chloroplastic genomes and the nuclear determinism of the annual habit, we were able to determine the maternal origin and evolution of these weed beet populations. Our study shows that they carry the dominant allele "B" for annual habit at high frequency. The main cytoplasmic DNA type found in northern weed beet populations is the cytoplasmic male-sterile type characteristic of sugar beets. We were able to determine that these populations arise from seeds originating from the accidental pollinations of cultivated beets by adventitious beets in the seed production area, which have been transported to the regions where sugar beets are cultivated. These seeds are supposedly the origin of the weed forms and a frequently disturbed cultivated environment has selected for annual habit and early flowering genotypes. We discuss the consequences of the weed beet populations for the breeding, seed production and release of herbicide-resistant transgenic sugar beets.

Crawley, M. J., Hails, R. S., Rees, M., Kohn, D. and Buxton, J. 1993. Ecology of Transgenic Oilseed Rape in Natural Habitats. *Nature* **363**: 620-623.

CONCERNS about genetically engineered crop plants centre on three conjectural risks: that transgenic crop plants will become weeds of agriculture or invasive of natural habitats; that their engineered genes will be transferred by pollen to wild relatives whose hybrid offspring will then become more weedy or more invasive; or that the engineered plants will be a direct hazard to humans, domestic animals or beneficial wild organisms (toxic or allergenic, for example). Here we describe an experimental protocol for assessing the invasiveness of plants. The object is to determine whether genetic engineering for herbicide tolerance affects the likelihood of oilseed rape becoming invasive of natural habitats. By estimating the demographic parameters of transgenic and conventional oilseed rape growing in a variety of habitats and under a range of climatic conditions, we obtain a direct comparison of the ecological performance of three different genetic lines (control, kanamycin-tolerant transgenics and herbicide-tolerant transgenic lines). Despite substantial variation in seed survival, lines were less invasive and less persistent than their conventional counter arts.

Kerlan, M. C., Chevre, A. M. and Eber, F. 1993. Interspecific Hybrids between a Transgenic Rapeseed (Brassica- Napus) and Related Species - Cytogenetical Characterization and Detection of the Transgene. *Genome* **36**: 1099-1106.

In interspecific hybrids produced between a transgenic rapeseed, an allotetraploid species, resistant to herbicide, phosphinotricin, and five diploid related species, the risk for gene introgression in weed genomes was explored through cytogenetic and bar gene characterizations. Among the 75 hybrids studied, most had the expected triploid structure, with the exception of B. napus - B. oleracea amphidiploid plants and one B. napus - S. arvensis amphidiploid plant. In triploid hybrid plants, the reciprocal hybrids did not exhibit any difference in their meiotic behavior. The comparison of the percentage of chromosome pairing in the hybrids with that of haploid rapeseed permit to conclude that allosyndesis between AC genomes and related species genomes took place. This possibility of recombination was confirmed by the presence of multivalent associations in all the interspecific hybrids. Nevertheless, in B. napus - B. adpressa hybrids a control of chromosome pairing seemed to exist. The possibility of amphidiploid plant production directly obtained in the F-1 generation increased the risk of gene dispersal. The B. napus B. oleracea amphidiploid plant presented a meiotic behavior more regular than that of the B. napus - S. arvensis amphidiploid plant. Concerning the herbicide bar gene characterization, the presence of the gene detected by DNA amplification was correlated with herbicide resistance, except for two plants. Different hypotheses were proposed to explain these results. A classification of the diploid species was established regarding their gene dispersal risk based on the rate of allosyndesis between chromosomes of AC genomes of rapeseed and the genomes of the related species.

Manasse, R. S. 1992. Ecological Risks of Transgenic Plants - Effects of Spatial- Dispersion on Gene Flow. *Ecol Appl* **2**: 431-438.

The spread of advantageous transgenic genes from crop plants into wild and weedy relatives is a potential ecological problem. The available theory indicates that the spread of a gene, over space and time, will depend in part on the relative selective advantage of that gene, and in part on gene flow, or the probability distribution of gene movement from source to target plants within a single generation. Risk assessment will require knowledge of both. This paper describes an empirical investigation of the effect of spatial dispersion on gene flow in Brassica campestris, utilizing a system of wild genotypes for target plants, and agronomic genotypes with a marker gene for source plants. I have found that gene flow can be reliably modeled with an exponential probability density function, and that the mean of that distribution can vary with the spatial dispersion of plants. Two-dimensional arrays of plants had lower mean gene dispersal distance than did one-dimensional arrays. In contrast, the degree of clumping and the size of patches of plants in my experiments had no effect on gene flow. However, I found an increase in mean gene dispersal distance with increased distance between clumps of plants, or between individual plants in one year. These results imply that large isolation distances accompanied by a rare long-distance pollination event will ultimately increase the risk of gene spread.

Klinger, T., Elam, D. R. and Ellstrand, N. C. 1991. Radish as a Model System for the Study of Engineered Gene Escape Rates Via Crop-Weed Mating. *Conserv Biol* **5**: 531-535.

Engineered genes in transgenic crops may escape into the ambient environment via crop-weed hybridization. However, natural crop-weed mating rates (gene flow) are largely unknown. We measured mating between wild and cultivated radishes in an experiment that simulated natural stands around seed multiplication plots. We used a genetic marker to identify crop-weed mating events. Although weeds at the cultivar plot margin (1 m distance) received much more gene flow than distant plants, detectable gene flow occurred at our most distant site (1000 m). For insect-pollinated, outcrossing crops like radish, strategies other than distance must be employed to ensure complete isolation.

Doebley, J. 1990. Molecular Evidence for Gene Flow among Zea Species - Genes Transformed into Maize through Genetic-Engineering Could Be Transferred to Its Wild Relatives, the Teosintes. *Bioscience* **40**: 443-448.