

SESSION 4

CONSEQUENCES OF GENE FLOW BETWEEN SPECIES – I

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Gene flow and rape - the Canadian experience

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ABSTRACT

Herbicide tolerant cultivars developed by both induced mutation and gene transfer occupied 50% of Canada's 5.2 million hectares of canola (*Brassica napus* plus *Brassica rapa*) in 1998 and an expansion to 70 or 75% of the canola area is expected in 1999. Cultivars tolerant to S-triazine herbicides have been successfully grown in Canada since 1981. Early isolation studies in western Canada indicated an average outcrossing rate from large fields to small plots of 0.6 and 3.7% at 366 m for *B. napus* and *B. rapa*, respectively. However, 1998 data indicate a much lower outcrossing rate between large fields of herbicide tolerant and standard *B. rapa* and *B. napus*. Gene flow between herbicide tolerant *B. napus* cultivars and stacking of resistance genes in volunteer *B. napus* plants was observed in a commercial field when a producer did not follow agronomic and herbicide recommendations. The weeds *Raphanus raphanistrum*, *Hirschfeldia incana* and *Brassica nigra* are absent or uncommon in western Canada. However, the weed dog mustard (*Erucastrum gallicum*) is abundant and inter-generic hybrids, using *E. gallicum* as the male, have been obtained in crosses with *B. napus* and *B. rapa*. Selfed and backcross progeny are under investigation.

INTRODUCTION

The area sown to herbicide tolerant canola cultivars, resulting from both induced mutation and gene transfer has grown rapidly since their introduction and release in 1995 (Table 1). Such rapid expansion is indicative of the widespread acceptance by producers as well as consumers in North and Central America and Asia. It is predicted that some 70% or perhaps 75% of the six million ha of canola expected to be sown in western Canada in 1999 will be tolerant to one or the other of the three broad spectrum herbicides, glyphosate (Roundup), glufosinate (Liberty) or the imidazolinones, imazethapyr (Pursuit) and imazomox plus imazethapyr 1:1 (Odyssey).

Herbicide tolerant *B. napus* cultivars have been used in Canada since 1981 when OAO Triton, the first canola cultivar with tolerance to the S-triazine family of herbicides, was introduced. This herbicide tolerance results from a natural mutation (chloroplast gene *psbA*) originally identified in *B. rapa* plants growing in a field where a continuous atrazine/corn management system had been practised. The usage peaked at 145,000 ha in 1988, some 4.0% of the total canola acreage, and diminished to less than 1% by 1996. No environmental or consumer problems were encountered and since the herbicide tolerance characteristic was cytoplasmically inherited, gene flow via pollen was not a concern. The usage of triazine tolerant cultivars in Canada had limited grower acceptance because the mutated chloroplast gene, that made the plants tolerant to the herbicide, also reduced the photosynthetic efficiency of such plants, resulting in slower growth, lower seed yield and a reduced oil content. In

addition, the slow breakdown of the herbicide under western Canada conditions placed serious limitations on other crops that could be included in the rotation. On the other hand, Australian canola growers have found that triazine tolerant cultivars solved their weed problems and provided an economic return even though the triazine tolerant cultivars yielded some 20-30% less seed and produced two to three percent less actual seed oil than the corresponding non-triazine tolerant cultivars. Breeding for superior performing triazine cultivars in Canada was discontinued in the 1990's because cultivars with inserted or induced mutated genes that imparted tolerance to more environmentally friendly broad spectrum herbicides, which do not impose a physiological cost to yield and oil content, would soon be available.

Table 1. Estimated area sown ('000 ha) to herbicide tolerant canola (*B. napus*) cultivars by herbicide type and total canola production in western Canada, 1995-1999.

Year	Glyphosate	Glufosinate	Imidazolinone	Total canola area sown	% herbicide tolerance
1995	0	14	10	5,273	<1%
1996	18	103	227	3,451	10%
1997	182	404	688	4,869	26%
1998	1,214	610	850*	5,260	51%
1999**	2,000	1,226	1,226*	6,070	73%

* Significant usage of non-certified seed included

** Projected production and usage

WITHIN SPECIES GENE FLOW

Prior to the introduction of the new herbicide tolerant forms of *B. napus*, background information on outcrossing within and among species, regardless of whether the tolerance resulted from mutation breeding or gene transfer, was a primary point of interest. Data on gene flow from large commercial fields (approximately 65 ha) to small (46 m²) plots of *B. napus* and *B. rapa* using a chlorophyll deficient marker were obtained in 1974 and 1977 (Stringam & Downey, 1978 & 1982). These data indicated that within species, pollen outflow resulted in an average of 0.6% and 3.7% outcrossing in *B. napus* and *B. rapa*, respectively, even at 366 m isolation (Figure 1). More recently, Raney and Falk (1998) found that the amount of outcrossing from pollen outflow from a small (0.4 ha) high erucic acid contaminant *B. rapa* source into a large (64 ha) low erucic acid recipient field was observed up to 260 m and that a 100 m buffer would be sufficient to contain more than 99% of the pollen flowing from the contaminating source.

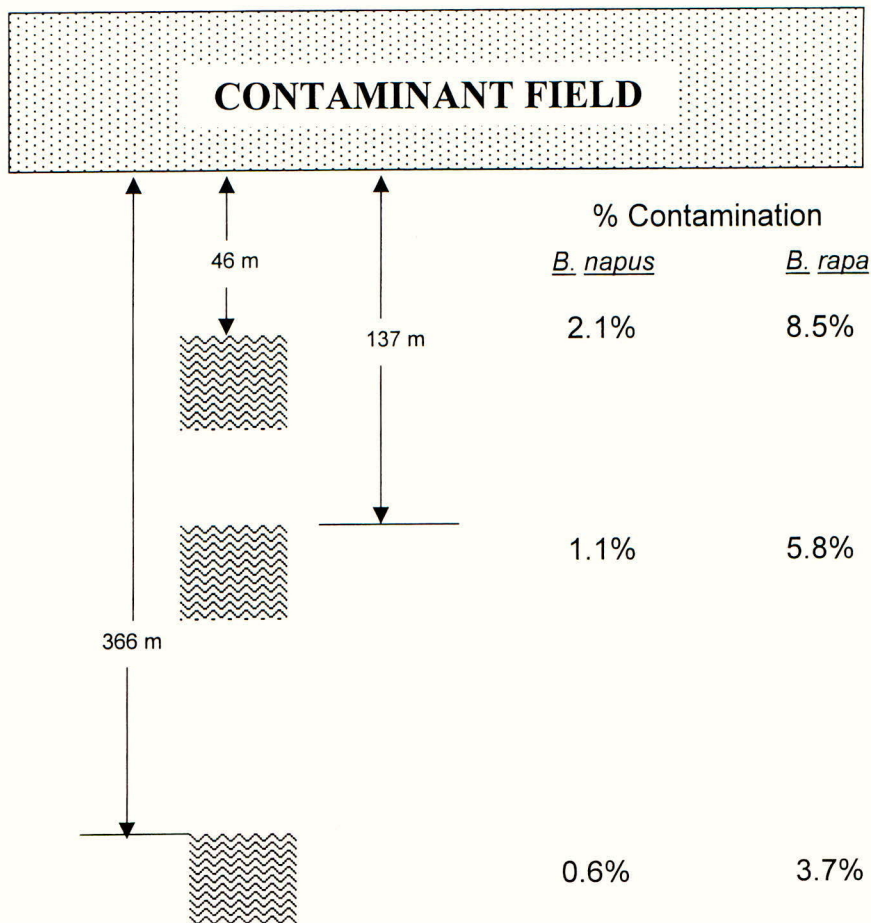


Figure. 1. Typical arrangement of 46m² isolation blocks and the average level of outcrossing from six fields of *Brassica napus* and of *B. rapa* (after Stringam & Downey 1978 & 1982).

In 1998, it was possible for the first time to investigate large field to large field (>16 ha) pollen movement with the first commercial production of the Roundup Ready (RR) *B. rapa* cultivar 41A51 from Pioneer Hi-Bred. Seed samples were harvested from herbicide susceptible canola in three fields that were paired with fields sown to RR 41A51. These paired fields were located at widely different locations in the province of Alberta and the distance between the paired fields differed with location (Table 2). Seed samples were harvested from the herbicide susceptible fields at 0, 33, 66 and 100 m from the edge of the fields closest to their corresponding RR field. Random samples of about 900 seeds from each distance and each susceptible field were sown in the greenhouse and the seedlings sprayed

twice with a 2x concentration of glyphosate herbicide. Seedlings of a susceptible check cultivar, Tobin were all killed while 11% of the RR 41A51 seedlings were also herbicide susceptible. There was no clear evidence of reduced outcrossing with increasing distance from the field edge to the 100 m distance sampled. However, as might be expected, fields at the greater distance from the herbicide tolerant RR 41A51 pollen source had a lower level of outcrossing (Table 2). The level of outcrossing under these actual farm conditions was lower than anticipated given previous observations and that *B. rapa* is an obligatory outcrossing species.

Table 2. Percent outcrossing between large fields* of herbicide susceptible and glyphosate tolerant *B. rapa* grown at three Alberta, Canada locations in 1998.

Location	Distance (metres)		Percent outcrossing**
	Between fields	From edge of susceptible crop	
A	4	0	0.2
		33	0.4
		66	0.3
		100	0.2
		Average	0.28
B	250	0	0.0
		33	0.1
		66	0.0
		100	0.0
		Average	0.02
C	600	0	0.0
		33	0.0
		66	0.0
		100	0.1
		Average	0.02

* Field size ranged from 16 to 64 ha

** Based on herbicide reaction of 900 seedlings per sampled distance

Seed from two herbicide susceptible *B. napus* fields were sampled by Monsanto at 20, 50 and 100 m from adjacent RR *B. napus* fields. Greenhouse grow outs of between 991 and 1009 seedlings per sampling site confirmed 1.5, 0.4, and 0.1% were RR survivors from Field 1 at 20, 50, and 100 m, respectively and 0.01, 0.00 and 0.40% from Field 2, for an average outcrossing rate of 0.6 and 0.16% for Field 1 and 2, respectively. Thus in 1998 the degree of cultivar outcrossing on a large field to large field basis was substantially lower for both *B. rapa* and *B. napus* than that recorded for large field to small plots, as reported by Stringam and Downey (1978 & 1982).

It was anticipated that useful information on *B. napus* gene movement could be obtained from an unusual agronomic situation that occurred on a farm in the Peace River region of northern Alberta. In 1997 a farmer, apparently wishing to compare the performance and weed control benefits of the three different herbicide tolerant *B. napus* cultivars, sowed a Roundup Ready (RR) cultivar in one field and 20 m to the east, across a road and parallel to the RR field sowed 8 ha of a Liberty Link (LL) cultivar. In the remainder of the 40 ha LL field he sowed a Pursuit Smart (PS) cultivar. The RR field was sown, swathed and combined two weeks earlier than the LL-PS field. In 1998 severe drought prevented the farmer from sowing a crop into his canola stubble in the LL-PS field. He therefore decided to chemically fallow the field, using glyphosate herbicide and noted that many of the volunteer canola plants did not die. Later the same season he twice more sprayed the same field with glyphosate with little or no control of the canola volunteers. The press then learned of the weeds that would not die resulting in much publicity and wrong conclusions. A field plot of the number and location of the volunteer plants was made by Alberta Agriculture. However, no clear or meaningful distribution pattern was evident, probably due to the swathing and combining process which almost certainly distributed seed from the area adjacent to the RR field to the far side of the LL-PS field. Nevertheless there was evidence of a significant pollen flow from the RR field to plants in the LL-PS field 20 m away. Seed harvested from the surviving volunteer plants was grown in the greenhouse by Alberta Agriculture and sprayed with glyphosate. Those plants that survived were also sprayed either with glufosinate or imidazolinone that resulted in clear evidence of multiple herbicide tolerance or gene stacking. However, it is important to note that when the 1998 field of barley, sown into the stubble of the RR canola field, was surveyed, no canola plants were found, except a few on the field edge where the spray boom had missed. From these observations we can conclude:

- That a significant amount of pollen flowed across the 20 m roadway and effected fertilization, resulting in gene stacking.
- The volunteer plants that survived the glyphosate treatment were not "super weeds" as the press and Greenpeace are wont to call them for had the producer followed recommended practices for chemical fallow and tank-mixed glyphosate with 2, 4-D or MCPA, he would have controlled the volunteers just as he controlled them in the barley that was grown on RR canola stubble.
- Growers should avoid sowing cultivars with different herbicide tolerances in the same or adjacent fields.
- All segments of the industry and particularly Canadian seed and chemical companies need to take a much greater effort to inform producers as to the management do's and don'ts of growing herbicide tolerant canola.

BETWEEN SPECIES GENE FLOW

Between crop species gene flow

Gene flow among related *Brassica* oilseed species is of minor concern. Canadian oilseed *Brassica* breeders have long known that inter-specific crossing among *B. napus*, *B. rapa* and *Brassica juncea* can and does occur in nature as recently documented by Bing *et al.* (1991). However, since these species are all major commercial crops one or another of the herbicide tolerant genes will almost certainly be deliberately introduced. Indeed limited commercial production of RR and LL *B. rapa* cultivars occurred in western Canada in 1998. If gene

transfer of the herbicide tolerant trait were to occur among these species, the agronomic and environmental consequences would be essentially nil since the presence of such genes do not make the recipient oilseed *Brassica* plants more competitive or invasive of cultivated or natural ecosystems in the absence of the specific herbicide (MacDonald, 1994; Belyk & MacDonald, 1994 & 1995 a, b, c).

Gene flow to weed species

Of greater importance is the possibility of herbicide tolerance genes escaping to closely related weedy species (Table 3). From the data available to date there appears to be general agreement that no natural gene flow would occur between the rapeseed species *B. napus* and *B. rapa* to *Sinapis arvensis*. French researchers have also found significant barriers to the introgression of *B. napus* marker genes as well as the herbicide tolerance gene into the genomes of *Raphanus raphanistrum* and *Hirschfeldia incana* (Darmency *et al.* 1998; Darmency, personal communication; Chèvre *et al.* 1997; Chèvre, personal communication). Fortunately these two weeds and *B. nigra* are either absent or uncommon in western Canada. Thus of greatest interest is the possibility of natural outcrossing of herbicide tolerant *B. napus* and *B. rapa* with *Erucastrum gallicum*. Studies by Lefol *et al.* (1997) produced no hybrids when *E. gallicum* was used as the female in crosses with *B. rapa*, *B. napus* and *B. juncea*. However, one hybrid was obtained from each of the crosses, *B. napus* x *E. gallicum* and *B. rapa* x *E. gallicum*.

Table 3. Closely related weeds to the *Brassica* oilseed species, *B. napus*, *B. rapa* and *B. juncea*.

Common name	Scientific name
Wild mustard	<i>Sinapis arvensis</i>
Black mustard	<i>Brassica nigra</i>
Wild radish	<i>Raphanus raphanistrum</i>
Hoary mustard	<i>Hirschfeldia incana</i>
Dog mustard	<i>Erucastrum gallicum</i>

Observations on the *B. napus* x *E. gallicum* hybrid strongly suggested that such a plant would not compete with *B. napus* plants and would have a very limited opportunity to backcross to *B. napus*. On the other hand, the hybrid readily backcrossed when *E. gallicum* was the male parent. The progeny of the backcross were poor competitors and all their progeny appeared to have completely reverted to *E. gallicum*, suggesting that the *B. napus* chromosomes were lost. The 13 seeds obtained from the backcross *B. napus* x [*B. napus* x *E. gallicum*] are being grown out to determine if they are true hybrids. In any case, a backcross to *B. napus* would result in retaining the gene of interest in the putative parent. These data indicated that the possibility of gene transfer from *B. napus* to *E. gallicum* exists but the probability for it to occur is very low.

The hybrid *B. rapa* x *E. gallicum*, when grown in a greenhouse pot, produced a strong, fertile plant which would probably have survived under field competition. This intergeneric hybrid produced 67 selfed seeds and on backcrossing 690 seeds from the cross *B. rapa* x [*B. rapa* x *E. gallicum*] as well as 14 seeds from the reciprocal backcross. No seed was produced when *E. gallicum* was the female parent. Backcrossing to the *B. rapa* parent would retain the gene(s) of interest in the putative parent and would not increase the risk of gene transfer to *E. gallicum* to any degree. However, the production of 14 backcross seeds on the *B. rapa* x *E. gallicum* hybrid, when pollinated by *E. gallicum*, indicates a strong possibility that gene flow could occur from *B. rapa* to *E. gallicum*. The observations on the 14 backcross and 67 selfed seed obtained on the *B. rapa* x *E. gallicum* hybrid are underway.

DISCUSSION

The degree of large field to large field gene flow, recorded for both *B. napus* and *B. rapa* in 1998, was substantially lower than suggested in previous studies which measured gene flow from large fields to small plots. Although field to field crossing levels were low, a considerable number of cultivar x cultivar hybrid seed could remain in a field after harvest. For example, a gene flow of 0.4% into a field yielding the Canadian average of 1400 kg/ha, with a harvest loss of 5% could result in some 70,000 outcrossed seed per ha remaining (7 seeds/m²) in the recipient field. The vast majority of such seed would germinate and be killed by frost and/or cultivation. However, the presence in a producer's field of a significant number of volunteer *B. napus* plants, tolerant to too different herbicides confirms that field to field gene flow can result in gene stacking if careful rotation and herbicide management practices are not followed. The absence of volunteer canola plants in the barley crop sown on RR canola stubble is clear evidence that such volunteers can be readily controlled with standard practices. Although distance isolation was found to be effective in reducing gene outflow between *B. rapa* fields, extremely low outcrossing levels were detected even at 250 and 600 m isolation. Thus, under commercial production conditions, complete containment using distance isolation alone becomes impractical. The possible escape of herbicide tolerant genes to other *Brassica* oilseed species or to weedy species, other than *E. gallicum*, is not a primary concern in Canada. Studies to determine the possibility of natural field crosses between *B. rapa* and *E. gallicum* were undertaken in 1998 and the self and backcross progenies of the hand crossed intergeneric hybrid obtained by Lefol *et al.* (1997) are under evaluation. *E. gallicum* is highly self-fertile and under field conditions *B. rapa* and *B. napus* pollen would have little or no opportunity to fertilise *E. gallicum* plants. However, the reciprocal cross could occur. The question then becomes what would be the consequences of herbicide tolerance genes occurring in *E. gallicum*? From what is known about the biology of this weed it is more of a nuisance than an ecological and agronomic threat. The species may continue to build up populations in infested canola fields, as it does now, but it is easily controlled in cereal and other crops with Group 4 and 6 herbicides.

In all such interspecific studies it is important to keep in mind that if a herbicide tolerant gene were to escape into a weedy relative that weed will be no more difficult to control than before the broad spectrum herbicide became available. Further, all data to date has shown that the trait does not confer an advantage to the plant unless the specific herbicide is applied and normal competition is eliminated.

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Gene flow from oilseed rape (*Brassica napus*) to related species

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ABSTRACT

The spontaneous gene transfer from oilseed rape (*Brassica napus*, $2n=38$, genomic constitution AACC) to the weedy relatives *B. rapa* ($2n = 20$; AA), *B. juncea* ($2n=36$, AABB), *Hirschfeldia incana* (*B. adpressa*, $2n=14$, AdAd), *Raphanus raphanistrum* ($2n=18$, RrRr) and *Sinapis arvensis* ($2n=18$, Sar Sar) has been studied in controlled experiments or in natural populations. The extent and possible consequences of this interspecific and intergeneric gene flow from oilseed rape are evaluated in a review of relevant European research.

INTRODUCTION

For many common traditionally bred crops the gene flow to their wild relatives has resulted in increased weediness as well as crop gene flow has been a factor responsible for extinction of some wild crop relatives (Ellstrand et al., 1999). Recently hybridisation as a way for the escape of crop transgenes has put extra focus on the spontaneous gene flow from cultivated plants to their wild relatives. As genetic modification differs from traditional breeding in that totally new characteristics can be added to crop plants at a high rate - i.e. many at a time as precisely designed gene pyramids with regulating sequences - the spread of transgenes by introgression represent a new potential of increasing weediness. Oilseed rape, *Brassica napus*, is easily genetically modified and related *Brassica* species or species from related genera are known to be potential recipients of new oilseed rape genes. The extent and consequences of spontaneous introgression of *B. napus* genes are evaluated.

Spontaneous hybridisation between oilseed rape and related species demands that some basic conditions are met: Oilseed rape and the wild recipient must have overlapping flowering periods and the distance between the crop and recipient must allow transfer of pollen between them by the aid of appropriate vectors. There must be genetic cross compatibility so that the heterospecific pollen can germinate and fertilise the egg cell. Subsequently a hybrid embryo must develop from the germinating seed and produce a viable plant. If the hybrid survives to the adult stage, and shows at least some fertility the introgression process may continue with backcrossing, or a combination of backcrossing and selfing. Many other factors have an impact on hybridisation, i.e. the agricultural management, the environmental conditions, the direction of the cross and the breeding system.

In 1994 Scheffler & Dale reviewed the literature to access the ease with which *Brassica napus* crossed with related species and formed hybrids, F_2 and backcross progeny. They ranked the different wild recipients as to their cross compatibility with oilseed rape in open pollinations, hand pollinations and by embryo rescue. Four species - *B. rapa*, *B.*

juncea, *Hirschfeldia incana* (*B. adpressa*) and *Raphanus raphanistrum* - were reported to hybridise spontaneously with *B. napus*. In the light of new experimental data on spontaneous hybridisation, also *Sinapis arvensis* is now added to this list of species (Lefol et al., 1996a), however, hybridisation with this species is rare.

INTERSPECIFIC GENE FLOW

Brassica rapa

B. rapa ($2n=20$, genomes AA) is one of the parental species of oilseed rape. *B. rapa* is a common annual weed in agricultural fields worldwide (in the temperate zone) particularly in oilseed rape fields with a potential for introgression of oilseed rape genes from the crop to the weed. Outside the field *B. rapa* populations are ephemeral, as seeds will only germinate when the soil is turned. Harberd (1975) reported the spontaneous occurrence of the *B. napus* x *B. rapa* hybrid (*B. x harmsiana*) in oilseed rape fields. Frequencies of hybridisation between oilseed rape and the wild *B. rapa* have been reported from field experiments and survey of natural populations of the wild species. Jørgensen & Andersen (1994), Jørgensen et al. (1996, 1998), Landbo et al. (1996) and Scott & Wilkinson (1998) found hybrid frequencies between 0-69 % of the seeds depending of i.e. parental genotypes, experimental design, agricultural practise, population size and site. Generally *B. rapa* produces more hybrids than oilseed rape (Jørgensen & Andersen, 1994; Jørgensen et al., 1998; Hauser et al., 1997).

The interspecific hybrids have reduced fertility (Jørgensen & Andersen, 1994), but spontaneous backcrossing can take place. *B. rapa* and interspecific hybrids with a gene providing Basta resistance were sown together in field experiments to assess the extent of backcrossing (Mikkelsen 1996, Mikkelsen et al., 1996a). Seed set per pod on interspecific hybrids was low (approximately 2.5) compared to seed set on the parental species (normally 16-23). The reciprocal cross *B. rapa* x hybrid was not observed among more than 2000 offspring from 30 *B. rapa* plants. A large number of herbicide resistant plants developed from seeds harvested on the interspecific hybrids, and herbicide resistant individuals with a *B. rapa*-like morphology were selected for further analysis. Most of the selected plants were clearly backcross plants and a few (0.5%) were almost identical to *B. rapa* (chromosome number $2n=20$, high pollen fertility) and set a normal number of seeds in crosses with genuine *B. rapa* (Mikkelsen et al., 1996a).

The field experiments quantifying introgression are supplemented by descriptive analysis of introgression in natural populations. A natural Danish population of *B. rapa* showing introgression beyond the stage of F_1 hybrids (more advanced generations) is presently being characterised by AFLP.

A successful introgression of crop genes to the wild relatives is very dependent on the fitness of the hybrids and subsequent backcross generations. In this respect the reproductive fitness is very important but also other stages of the life cycle, i.e. establishment of introgressed plants in the field and longevity of the seeds in the soil. As all known relatives capable of receiving genes from oilseed rape are weedy species or crops, the fitness of the introgressed plants should undergo long time monitoring in the agro-ecosystem with the appropriate management strategy. This is a demanding task, and therefore up till now the fitness data available were generated in controlled experiments.

Hauser et al. (1998b) intercrossed three weedy *B. rapa* populations and three *B. napus* varieties and estimated the fruit and seed set. The offspring plants were grown in the field, and monitored for survival and reproduction. Combining these fitness components into a multiplicative fitness estimate, hybrids were intermediate to *B. rapa* and *B. napus* (Hauser et al., 1998b; Figure 1).

Hybrids, *B. rapa* and *B. napus* plants, originating from crossings between two populations and two varieties in the first generation, were intercrossed to obtain F₂, backcross (on both *B. rapa* and hybrids), and pure *B. rapa* and *B. napus* seeds. The same fitness components as described above were estimated in the field for these offspring plants. On an average offspring from backcrossings and F₂ matings had a reduced fitness relative to offspring from intraspecific matings (pure species) (Hauser et al., 1998a).

A third backcross generation (BC₃) produced from backcrossing *B. rapa*-like BC₁ plants for another two generations were quantified as to their seed production and pollen fertility. With respect to these two fitness parameters there were no differences between the BC₃ plants and genuine *B. rapa* (Snow et al., 1999).

All the weedy species known to hybridize with oilseed rape have seed dormancy, a beneficial trait in an agro-ecosystem with efficient weed control. Therefore the seed germination pattern of hybrids and backcross plants is probably a key fitness parameter with a large effect on survival. Differences in seed germination between oilseed rape, wild *B. rapa* and their hybrids were investigated on seeds from controlled crosses (to get well characterised seeds of the two species and their reciprocal hybrids) as well as on seeds from a number of natural populations of weedy *B. rapa*. As expected all *B. napus* seeds germinated showing no sign of dormancy. *B. rapa* on the contrary, showed a very high degree of dormancy. As to the hybrids, both types (*napus* × *rapa*) and (*rapa* × *napus*) seeds showed almost no sign of dormancy (Landbo & Jørgensen, 1997). Lack of dormancy could be disadvantageous, as the number of hybrids reaching the flowering state could be limited due to germination under unfavorable conditions (e.g. with effective weed management) thereby limiting interspecific gene flow. The seed dormancy was restored in seeds from the first backcross with *B. rapa*.

In conclusion spontaneous hybrids and backcross plants are produced in field experiments and found in natural populations, and flow of transgenes from oilseed rape to *B. rapa* seems to be inevitable. Detailed information about transfer of oilseed rape markers is therefore valuable to identify segments of the oilseed rape genome rarely or never transferred to the wild recipient. Such segments could serve as "safe integration sites" for transgenes. For this purpose hybrids were obtained between different maternal *B. napus* cultivars/transgenic lines and *B. rapa* individuals from cultivars or a wild Danish population (Mikkelsen, 1996, Mikkelsen et al., 1996b). The hybrids were backcrossed to *B. rapa* individuals from the same population. In two different crossing combinations all 30 oilseed rape DNA markers – most of them specific to the C-genome – were transferred to the first backcross generation. The majority of the markers were transferred in a ratio that were not significantly different from 50% (Mikkelsen et al., 1996b); only one marker had a low transfer frequency (26%). Plants with 20 chromosomes and a high pollen fertility, both characters of weedy *B. rapa* were identified among backcross plants with oilseed rape markers (Mikkelsen, 1996). Metz et al. (1997) analysed the transfer of a transgene encoding phosphinothricin-tolerance in backcrosses with *B. rapa*. They found

different transmission frequencies of the transgene for two *B. napus* lines, and ascribed an unexpected low transmission (26 % of the BC₁ progeny) to a position in the C-genome. In conclusion oilseed rape markers with a low transmission rate can be found, however, all markers studied were inherited to the next generation.

Brassica juncea

Spontaneous hybridisation between oilseed rape and *B. juncea* has also been reported (Frello et al., 1995, Jørgensen et al., 1998). Depending on the proportions between the parental species up till 3% of the offspring harvested on *B. juncea* were hybrids. Production of hybrids with *B. napus* as female was less successful (Jørgensen et al., 1998). Pollen fertility of the hybrids was rather low, 0-28%, and 20 *B. napus* specific RAPD markers were all transferred to the first backcross generation with *B. juncea* (Frello et al., 1995).

INTERGENERIC GENE FLOW

Hirschfeldia incana

Field experiments also showed that spontaneous hybrids could be formed between oilseed rape and wild cruciferous species from other genera. When herbicide resistant oilseed rape was mixed with hoary mustard (*Hirschfeldia incana* (synonym *Brassica adpressa*)) in a density of 1:625, hoary mustard:oilseed rape, 1.5% of the seeds germinated to be hybrids, on an average one intergeneric hybrid was produced per mustard plant (Lefol et al., 1996b). When male sterile oilseed rape was used as female parent in a 1:1 proportion of oilseed rape:hoary mustard, 70% of the seeds were hybrids (Lefol et al., 1996b). The hybrids plants developed better vegetatively than hoary mustard but their reproductive fitness was very low (Lefol et al., 1996b). They only produced 0.1 viable seed per plant. For the hybrids between oilseed rape and hoary mustard seed dormancy was in between that of the parents (Chadoeuf et al., 1998).

Raphanus raphanistrum

In a similar experiment the herbicide resistant oilseed rape was mixed with wild radish (*Raphanus raphanistrum*) in different densities. Hybridisation was detected at a density of 1:625, wild radish:oilseed rape, as 0.2% of the seeds derived from wild radish were intergeneric hybrids (Darmency et al., 1995). When male sterile oilseed rape functioned as the mother, 45 intergeneric hybrids were obtained from each *B. napus* plant. Grown in mixtures with wild radish each hybrid produced less than one backcross plant, but fertility improved in subsequent backcross generations with the wild parent. However, no stable integration of oilseed rape genetic material into the genome of wild radish was observed. In the third backcross generation none of the transgenic plants had the same chromosome number as the weedy parent possibly indicating that the transgene was not recombined into the weed-genome (Chevre et al., 1997, 1998). The hybrids between oilseed rape and wild radish had a seed dormancy in between that of the parents (Chadoeuf et al., 1998).

Sinapis arvensis

Successful hybridisations between oilseed rape and wild mustard (*Sinapis arvensis*) were only detected when male sterile oilseed rape was used as the female. Six hybrids were obtained from 50.000 flowers (Lefol et al., 1996a). With wild mustard as the female and a transgenic herbicide resistant *B. napus* as pollen donor no hybrids were found among 2.9 million seeds harvested.

CONCLUSION

At present we know that five relatives of oilseed rape may receive transgenes by spontaneous gene flow. However, the cross compatibility of many other wild relatives has never been thoroughly analysed. The fitness of offspring between oilseed rape and a wild relative will of course depend on the genes introgressed to the offspring and the recipient ecosystem. Except for herbicide resistance the fitness advantage provided by transgenic traits introgressed from oilseed rape is poorly known. In a non-selective environment Snow et al. (1998) analysed the fitness of herbicide tolerant BC₃ plants from backcrossing to *B. rapa*. The BC₃ plants also expressed a NPTII gene and a gene for restoration of fertility. As expected there was no cost of expression compared to non-transgenic segregants. Therefore, a transgene providing the wild plants with a selective advantage is likely to increase fitness and speed up the introgression process. With commercialisation of new transgenic oilseed rape lines, spontaneous gene flow may pyramide several advantageous transgenes in the wild recipients and create weedier weeds.

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Figure 1. Multiplicative fitness estimate of F₁ hybrids and pure parental species (*B. rapa* and *B. napus*). Modified from Hauser et al. (1998b).

