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Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz

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Abstract The frequency of gene flow from *Brassica napus* L. (canola) to four wild relatives, *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L. and *Erucastrum gallicum* (Willd.) O.E. Schulz, was assessed in greenhouse and/or field experiments, and actual rates measured in commercial fields in Canada. Various marker systems were used to detect hybrid individuals: herbicide resistance traits (HR), green fluorescent protein marker (GFP), species-specific amplified fragment length polymorphisms (AFLPs) and ploidy level. Hybridization between *B. rapa* and *B. napus* occurred in two field experiments (frequency approximately 7%) and in wild populations in commercial fields (approximately 13.6%). The higher frequency in commercial fields was most likely due to greater distance between *B. rapa* plants. All F_1 hybrids were morphologically similar to *B. rapa*, had *B. napus*- and *B. rapa*-specific AFLP markers and were triploid (AAC, 2n = 29 chromosomes). They had reduced pollen viability (about 55%) and segregated for both self-incompatible and self-compatible individuals (the latter being a *B. napus* trait). In contrast, gene flow between *R. raphanistrum* and *B. napus* was very rare. A single *R. raphanistrum* × *B. napus* F_1 hybrid was detected in 32,821 seedlings from the HR *B. napus* field experiment. The hybrid was morphologically similar to *R. raphanistrum* except for the presence of valves, a *B. napus* trait, in the distorted seed pods. It had a genomic structure consistent with the fusion of an unreduced gamete of *R. raphanistrum* and a reduced gamete of *B. napus* (RrRrAC, 2n = 37), both *B. napus*- and *R. raphanistrum*-specific AFLP markers, and had <1% pollen viability. No hybrids were detected in the greenhouse experiments (1,534 seedlings), the GFP field experiment (4,059 seedlings) or in commercial fields in Québec and Alberta (22,114 seedlings). No *S. arvensis* or *E. gallicum* × *B. napus* hybrids were detected (42,828 and 21,841 seedlings, respectively) from commercial fields in Saskatchewan. These findings suggest that the probability of gene flow from transgenic *B. napus* to *R. raphanistrum, S. arvensis* or *E. gallicum* is very low ($<2–5 \times 10^{-5}$). However, transgenes can disperse in the environment via wild *B. rapa* in eastern Canada and possibly via commercial *B. rapa* volunteers in western Canada.

Keywords Transgenic canola · Rapeseed · Bird's rape · Dog mustard · Wild radish · Wild mustard · Gene flow · Commercial-scale fields

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Introduction

Transgenic crops are being increasingly produced worldwide (James 2001). A major concern about these agricultural releases is the escape of transgenes in the environment through hybridization with their wild relatives (Metz et al. 1997; Warwick et al. 1999; Snow 2002). Although crops and weeds have exchanged genes for

centuries, genetic engineering raises additional concerns. It not only introduces into ecosystem genes that confer novel or enhanced fitness-related traits, but also allows novel genes to be introduced into many diverse types of crops, each with its own specific potential to outcross (Snow 2002). *Brassica napus* L. (canola) is of particular concern, as it is partially allogamous, forms volunteer populations and has numerous wild relatives present in cultivated areas in Canada and world-wide.

B. napus (AACC, 2n = 38) and *Brassica rapa* L. (AA, 2n = 20) constitute about 90 and 10%, respectively, of the canola grown in western Canada, whereas only *B. napus* is grown in eastern Canada. Approximately 80% of the 4.0 million ha of *B. napus* grown in 2001 (R.K. Downey, personal communications) was herbicide-resistant, including transgenic glyphosate- (47%), glufosinate- (13%), bromoxynil (<1%)-resistant types and a non-transgenic imidazolinone-resistant type (20%). Weedy canola volunteer populations occur in subsequent crops as a result of seed loss due to shattering or harvesting operations. Volunteers can emerge from the seed bank for many years (Légère et al. 2001; Simard et al. 2002) and serve as a pollen source for dispersal of transgenes to wild relatives and canola crops that follow in rotation or are located in nearby fields (Beckie et al. 2001).

In Canada, there are four wild relatives that have the potential to cross with *B. napus*, including three predominantly outcrossing (i.e. self-incompatible) species: *B. rapa* (bird's rape, field mustard), *Raphanus raphanistrum* L. (wild radish) and *Sinapis arvensis* L. (wild mustard), and one predominantly selfing species *Erucastrum gallicum* (Willd.) O.E. Schulz (dog mustard). While *S. arvensis* is the most common of the four weeds in Canada (Warwick et al. 2000), gene flow from *B. napus* to *S. arvensis* has a low probability of occurrence. Under greenhouse conditions, Moyes et al. (2002) is the only study to-date to report sexual hybrids when *S. arvensis* was the maternal parent and no studies have detected gene transfer from *B. napus* to *S. arvensis* in field experiments (Bing et al. 1996; Chèvre et al. 1996; Lefol et al. 1996; Moyes et al. 2002). In contrast, *E. gallicum* occurrence in canola growing areas of Canada is more limited and found primarily in Saskatchewan. Gene flow from *B. napus* to *E. gallicum* has not been extensively studied. In one report, a single *B. napus* × *E. gallicum* hybrid has been obtained under greenhouse conditions, but no hybrids were detected when *E. gallicum* served as the maternal parent (Lefol et al. 1997). Wild *B. rapa* is a weedy volunteer in western Canada and has a limited distribution as an agricultural and/or ruderal weed in canola growing areas in Québec (Warwick et al. 2000). Numerous studies have indicated a high potential for hybridization between *B. napus* and *B. rapa*. Spontaneous hybridization (Jørgensen and Andersen 1994; Landbo et al. 1996) and introgression (Hansen et al. 2001) was reported between *B. napus* and wild *B. rapa* in Danish field studies, and between cultivated lines of *B. rapa* and *B. napus* in field experiments in Canada (Bing et al. 1996). *R. raphanistrum* is limited in canola growing regions of Canada to Québec and Alberta (Warwick et al. 2000). Studies in France and Australia have indicated that hybridization between *B. napus* and *R. raphanistrum* is very rare. Only three hybrids were detected in numerous experiments in France when *R. raphanistrum* served as the maternal parent (Eber et al. 1994; Baranger et al. 1995; Chèvre et al. 1997, 1998, 2000; Darmency et al. 1998; Darmency 2000). The hybridization rate was estimated at between 10^{-7} and 10^{-5} (Chèvre et al. 2000). In Australia, gene-flow studies between imidazolinone-resistant *B. napus* and *R. raphanistrum* growing in experimental field plots (Rieger et al. 2001) indicated even lower hybridization rates ($<4 \times 10^{-8}$), with no hybrids found when *R. raphanistrum* was the maternal parent.

Of the gene-flow studies mentioned above and others (reviewed in Metz et al. 1997; Rieger et al. 1999; Warwick et al. 2000), none have evaluated actual interspecific hybridization rates in agricultural settings of commercial-scale fields of a transgenic crop. The objective of this study was to determine gene-flow rates from *B. napus* to four wild relatives, *B. rapa, E. gallicum, R. raphanistrum* and *S. arvensis*. Potential hybridization rates were assessed in field or greenhouse experiments, whereas actual rates were measured in areas in Canada where commercial fields of canola and wild relatives were known to co-occur.

Materials and methods

Ten experiments will be described in this section. Previous studies on Canadian populations of *B. rapa* (Halfhill et al. 2001), *E. gallicum* (Lefol et al. 1997) and *S. arvensis* (Bing et al. 1996) assessed the potential for hybridization with *B. napus*, whereas comparable data was not available for Canadian populations of *R. raphanistrum* and is presented here for three greenhouse experiments. Four controlled field experiments (*B. rapa* and *R. raphanistrum*) were also conducted. Actual hybridization rates were measured in three commercial field experiments (*B. rapa, R. raphanistrum, S. arvensis* and *E. gallicum*). Various marker systems were used to detect hybrid individuals: herbicide resistance traits (HR), green-fluorescent protein marker (GFP), species-specific amplified fragment length polymorphisms (AFLPs) and ploidy level. All procedures concerning the characterization of hybrids that are common to most experiments, including screening for the above traits, description of morphological traits, and determination of pollen viability, are described at the end of the section.

Plant material

B. napus. Seeds of two transgenic glyphosate resistant (HR) cultivars of *B. napus*, 45A51 and 45A50, were used. Five transgenic homozygous lines of *B. napus* cv Westar, GT-2, GT-6, GT-7, GT-8 and GT-9, where GT- is a combined GFP (a *Aequorea victoria* mGFP5-ER variant green fluorescent protein) and Bt (a *Bacillus thuringiensis* synthetic Bt Cry1Ac gene) construct, and line GFP-2 are described in Halfhill et al. (2001).

B. rapa. Seeds originated from two wild populations collected in 1988, seven years prior to *B. napus* cultivation in Québec: QC-2974 collected from Milby, QC (45°19′N 71°49′W) and QC-2975 from Waterville, QC (45°16′N 71°54′W) (germplasm collection AAFC-ECORC, Ottawa). Two wild populations were collected in

2001 in canola growing areas in Québec: QC-9039 from Ste-Agathe de Lotbinère, QC (46°23′N 71°25′W) and QC-9047 from St. Henri de Lévis, QC (46°42′N 71°04′W). Collection and field details are provided below. The cultivar AC Parkland was used in the Saskatoon experiment.

R. raphanistrum. Seeds from wild populations were collected as follows: QC-2986 in 1988 at St-Robert, QC (45°58′N 73°00′W) (germplasm collection AAFC-ECORC, Ottawa); QC-3449 in 1999 at Ste-Victoire-de-Sorel, QC (45°57′N 73°05′W); QC-8730 in 1999 at St-Anselme, QC (46°38′N 70°58′W); QC-8731 in 1999 at La Baleine, Île aux Coudres, QC (47°24′N 70°21′W); PEI-8710 in 1999 at Harrington, PEI (46°21′N 63°10′W); AB-8712 in 1999 at Ponoka, AB (52°42′N 113°35′W); and AB-8737 in 1999 at Fort Saskatchewan, AB (53°43′N 113°13′W). Seeds from 19 wild populations of *R. raphanistrum* were collected in 2000 and 2001 in or near commercial HR *B. napus* fields in Québec and Alberta. Locations are available from the senior author upon request. A wild population FR-8716 from Rennes, France, was used. Additional European collections, Austria-3466, Austria-3618, Italy-3565, Russia-3423 and Russia-3429, were obtained from various European genebanks in 1988–90 (germplasm collection AAFC-ECORC, Ottawa).

S. arvensis and *E. gallicum*. Seeds from 79 populations of *S. arvensis* and 38 populations of *E. gallicum* were collected in commercial fields in southeastern Saskatchewan in 1999 and 2000. Collection and field details are provided below.

B. rapa (♀) × B. napus (♂)

Field experiments

A HR *B. napus* field experiment was conducted on the Central Experimental Farm, Ottawa, Ontario, Canada (45° 23′N 75°43′W) in summer 2000. One 10 m × 10 m field plot of HR *B. napus* (cv 45A51) was planted at 160 seeds m^{-2} in 30-cm rows, which approximates commercial sowing rates. At the same time, these plots were interplanted with seeds from two Québec populations of *B. rapa* (QC-2974, QC-2975) in a 1 m × 1 m grid system. Plots were seeded by hand on 6 June 2000. Three seeds of *B. rapa* were sown at each grid location and thinned to one plant per location after the seedlings had been established. A total of 100 *B. rapa* plants were grown within the plot (centre density 1 plant m^{-2}), and an additional 40 plants grown in the margin of the plot, i.e., ten plants on each side, with plants located 0.5 m from the plot and 1 m from each other. Plants of each of the two *B. rapa* populations were randomly arranged, with 50 centre-plants of each population and 20 plants in the margin. Centre and marginal plants simulated situations where *B. rapa* was more isolated from other *B. rapa* plants and in competition with the crop (all other weeds were hand removed) versus a field edge where *B. rapa* plants were separated from each other by more open space, with reduced competition as other weeds were removed from the immediate area. Time of flowering was recorded for *B. napus* and for each *B. rapa* plant. Seeds from all 140 *B. rapa* plants were harvested twice between 25–28 August, and bagged separately. From each individual plant, about 250 seeds were sown in separate trays in the greenhouse and screened for glyphosate resistance (Table 1A). Most *B. rapa* plants produced several thousand seeds, and therefore only a subset could be screened. All putative F_1 hybrids that survived the herbicide spray were tested with the glyphosate resistance test strips and evaluated for species-specific morphological traits. Ploidy level, pollen viability and the presence of *B. napus*- and *B. rapa*-specific AFLP molecular markers were determined.

A GFP *B. napus* field-experiment was conducted at the AAFC Research farm at Saskatoon, Saskatchewan, Canada (52°07′N 106°38′W), in summer 2001. A 5 m × 5 m plot of GT-6 *B. napus* was seeded on 23 May using a Hege small plot seeder (Wintersteiger, Division Seedmech, Hege USA, Colwich, Kansas) at 128 seeds m^{-2} in 30-cm rows. All seed was treated with Vitavax RS Flowable (Gustafson Canada, Calgary, Alberta) at 0.562 l/25 kg seed to control flea beetles. Plants of *B. rapa* cv AC Parkland were started in the greenhouse, and when the *B. napus* plot was flowering (10 July), 25 pots each containing a single-flowering *B. rapa* plant were buried in the plot at a density of 1 pot per m^2 on a 1 m × 1 m grid. After flowering was complete (1 August), the 25 *B. rapa* pots were removed from the field, and placed in a greenhouse for seed ripening (a requirement of the Canadian Food and Inspection Agency regulations for a confined field trial). From each individual plant, about 200 seeds (or all seeds if less than 200) were sown in separate trays in the greenhouse and screened for the GFP marker (Table 1B). All putative F_1 hybrids having the GFP trait were evaluated for species-specific morphological traits and three hybrids from each *B. rapa* plant (a total of 39 plants) were evaluated for pollen viability and ploidy level.

Commercial HR B. napus fields

In August 2001, seeds were collected from two Québec populations of *B. rapa* growing in or adjacent to a commercial field of HR (glyphosate-resistant) *B. napus* (QC-9039; first canola crop grown in this field) and to glyphosate-resistant *B. napus* volunteers (QC-9047; glyphosate-resistant *B. napus* crop in 2000, the first canola crop grown in this field). Seeds were collected and bagged separately from each of 16 *B. rapa* individuals in population QC-9039. The plants were located on the margin of the field (0.5 to 5 m from *B. napus*), and isolated from each other, i.e., 1–10 m apart. A bulk seed sample, from about 5 individuals, was collected from population QC-9047 which was growing in a sweet-corn field among HR *B. napus* volunteers. Both volunteer *B. napus* and *B. rapa* (QC-9047) in the corn field flowered later and had few seeds on the collection date (27 August). Seed collection occurred after the corn had been harvested and most *B. rapa* plants were still flowering. Seed was grown in trays in the greenhouse, and seedlings at the two-leaf stage screened for glyphosate resistance (Table 2). All putative F_1 hybrids that survived the herbicide spray were confirmed with the glyphosate resistance test strip and/or ploidy analysis. From population QC-9039, up to ten hybrid progeny per individual field plant, for a total of 121 plants, were retained in the greenhouse for further characterization. They were evaluated for species-specific morphological traits, ploidy level, pollen viability and the presence of *B. napus*- and *B. rapa*-specific AFLP markers. A subset of 49 plants were isolated by bagging and checked for self-compatibility.

Raphanus raphanistrum (♀) × B. napus (♂)

Greenhouse experiments

Three greenhouse crossing experiments were conducted. Single plants of *R. raphanistrum* and *B. napus*, in either a single 15-cm pot (AFLP/ploidy experiment) or separate pots (HR experiment), were paired and isolated by bagging, and allowed to flower and set seed. Each bag was shaken on a daily basis in order to facilitate the cross movement of pollen between the two plants. In a third experiment, *R. raphanistrum* plants were grown in separate pots, bagged and pollen from *B. napus* manually applied to the stigmas to ensure pollen transfer. The first experiment utilized seven Canadian [PEI-8710, AB-8712, AB-8737, QC-2986, QC-3449, QC-8730, QC-8731] and six European [Austria-3466, Austria-3618, Italy-3565, France-8716, Russia-3423,-3429] populations of *R. raphanistrum* in a total of 45 paired crosses, and used AFLP and the ploidy level to detect hybrids. In the second experiment, plants from each of four populations of *R. raphanistrum*, AB-8712, PEI-8710, QC-2986 and FR-8716, were paired with a HR (glyphosate-resistant) plant of *B. napus* (45A50) for a total of 36 paired crosses. For the third experiment, plants from the same four populations of *R. raphanistrum* received pollen from line GT-9 *B. napus*. In all three experiments, mature seed pods were collected from the *R. raphanistrum* plant. Prior to planting, seeds were treated with a 0.1% gibberellic acid solution and a 0.2% KNO_3 solution to break

dormancy. Seedlings were grown in the greenhouse to screen for hybrids using appropriate markers.

Field experiments

A HR *B. napus* field experiment, conducted on the Central Experimental Farm, Ottawa, in summer 2000, used the design described above for *B. rapa*, but included two 10 m × 10 m plots. Three Canadian (AB-8737, PEI-8710, QC-2986) and one French (FR-8716) population of *R. raphanistrum* were used. For each *R. raphanistrum* population, there were 25 centre plants and ten plants in the margin. In cases of no germination, particularly for population AB-8737, seedlings at the 4-leaf stage were transplanted to ensure one plant per m^2. Time of flowering was recorded for *B. napus* and for each *R. raphanistrum* plant. Harvested seeds were physically removed from the seed pod by manual scarification, and prior to planting were treated to break dormancy. All seeds from both plots were screened (Table 3A) and putative hybrids evaluated as for *B. rapa*.

A GFP *B. napus* field experiment was conducted on the Central Experimental Farm, Ottawa, in summer 2000. Sixteen 3 m × 3 m plots of GT-2, GT-7, GT-8 and GT-9 *B. napus* (12 plots) or GFP-2 *B. napus* (4 plots) were planted in the 2000 season. *B. napus* was sown by hand at 60 seeds per m^2. One plant from each of four populations of *R. raphanistrum*, AB-8737, PEI-8710, QC-2986 and FR-8716, was interplanted in each plot. Time of flowering was recorded for each *R. raphanistrum* plant. Pods were collected and bagged separately for each individual. The seed was scarified, treated to break dormancy, grown in trays in the greenhouse and screened for presence of the GFP marker (Table 3B).

Commercial HR B. napus fields

Seedpods of *R. raphanistrum* were collected as bulk samples from one population in 2000 and seven populations in 2001 that were growing in or adjacent to commercial fields of HR *B. napus* in Québec (Table 4). Seedpods were collected from three (2000) and eight (2001) populations of *R. raphanistrum* in or near commercial fields of HR *B. napus* in Alberta (Table 4). Seedpods were collected from individual plants from seven populations and as bulk samples in the remaining populations. Seeds were treated to break dormancy, grown in trays in a growth chamber, and seedlings screened for herbicide resistance at the two-leaf stage (Table 4).

S. arvensis or E. gallicum (♀) × B. napus (♂)

Commercial HR B. napus fields

Seeds of *S. arvensis* (1999: 42 populations; 2000: 37 populations) and *E. gallicum* (1999: 3 populations; 2000: 35 populations) were collected in Saskatchewan from fields where glyphosate-resistant *B. napus* was grown 1 or 2 years previously and in surrounding fields (Table 4). Most *S. arvensis* populations were found within fields or along field margins, whereas most *E. gallicum* populations were located along field margins or roadsides. Given the extent of HR canola grown in Western Canada, it is not possible to determine the past exposure of these weed populations to HR canola. Seed pods were sampled from 20 plants per population and at least 10 pods per plant. Seeds were treated with a 0.2% KNO_3 solution to break dormancy. Seedlings were grown in the greenhouse and screened for herbicide resistance at the two- to-four-leaf stage (Table 4).

Hybrid characterization

The hybrid status of individuals was initially determined by either a herbicide resistance HR marker (either glyphosate, glufosinate or imazethapyr resistance), a GFP marker, an intermediate ploidy level and AFLP markers, depending on the experiment. Herbicide resistance was determined by survival of seedlings sprayed with the recommended field rate for each herbicide-resistant *B. napus* line, and confirmed in surviving individuals with selective test strips which assay for proteins produced by the herbicide-resistant genes. All herbicide-resistant hybrids were further characterized for ploidy level, chromosome count (*R. raphanistrum* hybrid only), pollen viability, *B. napus*-, *B. rapa*- and *R. raphanistrum*-specific AFLP markers, and for species-specific morphological traits.

Herbicide resistance

Seeds were planted 1 cm deep in 18 rows in a plastic flat (54 × 28 × 6 cm) containing a mixture of soil, peat and sand (1:2:1 by volume). Three *B. napus* lines, glyphosate-resistant cultivar 45A50, glufosinate-resistant cultivar 'Innovator' and herbicide-susceptible cultivar 'Excel', *B. rapa* population QC-2974 and *R. raphanistrum* population QC-2986 were planted as single rows in each flat to confirm efficacy and levels of resistance. Greenhouse conditions were 20/16 °C day/night temperature regime and a 16-h photoperiod. At the two-leaf stage, seedlings were sprayed with either glyphosate (440 g ai ha^{-1}), glufosinate (400 g ai ha^{-1}) or imazethapyr (75 g ai ha^{-1}) using a track sprayer calibrated to deliver 216 l ha^{-1} of spray solution at 200 kPa. Plants were categorized as susceptible or resistant 14 days after application.

Herbicide resistance in plants that survived spraying with glyphosate was confirmed using glyphosate test strips from a commercially available kit [Trait RUR (Roundup Ready)] from Strategic Diagnostics Inc. The RUR trait kit detects the CP4 EPSPS protein produced by the HOR gene, a gene derived from *Agrobacterium* sp. strain CP4 and incorporated into glyphosate-resistant *B. napus* canola. Following the procedure outlined in the kit, approximately 100 mg of leaf tissue is ground in 0.75 ml of buffer in a test tube, a capillary based strip is inserted in the tube and scored after 5 min for the presence of two bands on the strip, a control band that tracks the capillary action and a second band that indicates the protein.

Green fluorescent protein

Seeds were sown on trays of soil in the greenhouse and plants grown to the four-leaf stage and screened for the GFP marker using a hand-held UV light. GT-*B. napus* plants were used as positive controls and the parental accessions *B. rapa* AC Parkland or *R. raphanistrum* used as the negative controls.

Morphology

Putative hybrids were evaluated for species-specific morphological traits. Vegetative traits: the presence of hairs on the leaf and a light green color in *B. rapa* and *R. raphanistrum*, versus no hairs and waxy green-blue leaf color in *B. napus*. Reproductive traits: petal color bright yellow and petals smaller in *B. rapa*; petal color (yellow with white at the base of the petal or white or purple), dark nerves on the petals, erect sepals and closed calyx, jointed seed pod and absence of valves in *R. raphanistrum*, versus petal colour light yellow, absence of dark nerves on the petals, spreading sepals, open calyx and seed pods which open by valves in *B. napus*.

Ploidy

Ploidy levels were assessed from flow cytometric data using a Partec PA-I Flow cytometer (Partec GmbH, Münster, Germany) which measures relative amounts of DNA (Dolezel 1991). Previous studies by Eber et al. (1997) established a linear regression between the chromosome number observed from root tips of *B. napus* and *R. raphanistrum* hybrids and flow-cytometric data. About 0.5–1 cm^2 of leaf material was chopped with 0.5 ml of lysis buffer (solution A,

a high resolution plant DNA-kit, Partec) in a Petri dish using a razor blade. Following filtration through a 50 μm nylon mesh filter (Celltrics, Partec), approximately 2 mls of the staining solution (solution B, Partec), containing DAPI was added. The sample was gently stirred for 1 min and the analysis was performed using a Partec PA-I equipped for UV excitation and blue light emission (100 W mercury HBO lamp, Partec). Histograms of fluorescence were registered over 512 channels with linear amplification. At least 8,000 nuclei were analyzed in each sample and the histogram mean-value for G1 peaks evaluated using DPAC software (Partec). Calibration of the tetraploid *B. napus* at 200 resulted in diploid species-values between 85 and 90. Intermediate cytometric values were expected for putative triploid hybrids.

The chromosome number of the *R. raphanistrum* × *B. napus* hybrid was determined in root-tip cells fixed and stained according to standard procedures (Sharma and Sharma 1965). Briefly, root tips were treated with a 0.05% colchicine solution for 1.5 h at room temperature, fixed in Farmer's solution (3:1 absolute ethanol:glacial acetic acid, vol:vol) for 48 h at room temperature and stored in the fixative at 4 °C. The root tips were stained with 1% aceto-carmine overnight, washed in distilled H_2O, then digested for 45 min at 37 °C in an enzyme buffer solution [3% cellulase from *Aspergillus niger* (Sigma), 2% pectolyase from *Aspergillus japonicus* (Sigma), 4 mM citric acid, 6 mM trisodium-citrate, pH 4.8], washed in distilled H_2O, squashed on a microscope slide in 45% acetic acid, and observed under a light microscope.

Pollen viability

Pollen viability was assessed as the percentage of pollen stained by a 1% aceto-carmine solution. For *B. rapa* × *B. napus* hybrids, two to three flowers and 300–400 pollen grains were analyzed per plant. Ten flowers were collected from the *R. raphanistrum* × *B. napus* hybrid and a total of 4,855 pollen grains analysed.

Selfing

B. rapa, R. raphanistrum and *S. arvensis* are normally self-incompatible, whereas *E. gallicum* and *B. napus* are self-compatible. Forty nine *B. rapa* × *B. napus* hybrids, obtained from the HR commercial field population QC-9037, were isolated by bagging and their ability to set seed recorded. Seed set on bagged inflorescences of the*R. raphanistrum* × *B. napus* hybrid was also recorded.

AFLPs

Approximately 100 mg of lyophilized plant tissue was ground in a Fast Prep FP120 (BIO 101) grinder and the total genomic DNA extracted using a modified 2 × CTAB procedure (Doyle and Doyle 1987). AFLPs were generated based on the protocol of Vos et al. (1995) with minor modification. For each sample, about 250 ng of DNA was digested with 1.25 U of *Eco*R1 and 1.25 U of *Mse*1 (Invitrogen) in a 5 × reaction buffer (50 mM of Tris-HCl pH 7.5, 50 mM of MgAc, 250mM of KAc) in a reaction volume of 12.5 μl at 37 °C for 2.5 h, and the restriction enzymes were heat-inactivated at 65 °C for 10 min. The digested DNA samples were ligated to an adaptor-ligation solution containing 0.5 U of T4 DNA Ligase, 5 × ligation buffer (Invitrogen), 0.4 mM of ATP, 1 pMol of *Eco*R1 adapter and 10 pMol of *Mse*1 adapter (Cortec) in a reaction volume of 12.5 μl at 22 °C for 2 h. All adapter and primer sequences are given in Vos et al. (1995). The adapter-ligated DNA was diluted 10-fold with TE buffer and used as a template for pre-amplification. Pre-amplification was carried out with primers complimentary to the *Eco*RI and *Mse*I adapters, with one selective nucleotide at the 3′ end. The pre-amplification reaction included 2.5 μl of the diluted-adapter ligated DNA, 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$, 0.5 U of *Taq* (Roche), 10 × PCR buffer (Invitrogen), 7.5 ng of the *Eco*RI and *Mse*I primers in a reaction volume of 10.0 μl. Amplifications were performed in a Thermolyne Amplitron Thermocycler (Techne Inc., Princeton, N.J.) following the PCR parameters in Vos et al. (1995). The pre-amplified DNA was diluted 4-fold with TE buffer and used as a template for selective amplification with *Eco*RI and *Mse*I primers, each having three selective nucleotides on the 3′ end. The selective amplification reaction included 1.25 μl of diluted pre-amplification product, 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$, 0.5 U of *Taq* (Roche), 10 × PCR buffer (no $MgCl_2$) (Invitrogen), 7.5 ng of the *Mse*I selective primer and 0.125 μl of *Eco*RI selective primer labelled with an infrared dye IRD-700 (LI-COR) in a reaction volume of 5.0 μl. The PCR parameters for selective amplification followed Vos et al. (1995). The amplified product, mixed with an equal volume (5 μl) of loading buffer/formamide dye, was denatured at 94 °C for 3 min and held at 4 °C, and separated in a 5% polyacrylamide gel on an automated sequencer (LI-COR) for 5 h. Infrared gel images were analyzed using a GeneIR (Scanalytics) program (LI-COR).

The selective primer, *Eco*R1 + AGG/*Mse*1 + CAC, was used with all *B. rapa* × *B. napus* hybrids (Table 5), to screen seedlings from *R. raphanistrum* in greenhouse experiment 1, and five primer pairs were used to analyze the *R. raphanistrum* × *B. napus* hybrid obtained in the Ottawa 2000 HR field trial experiment (Table 6). For each primer pair, AFLP markers were designated as *B. napus*-specific if they were absent in five individuals from each of the parental lines of *B. rapa* (QC-2974, QC-2975) and *R. raphanistrum* (PEI-8710), and present in all five individuals of *B. napus* (cv 45A51) screened, or present, in the hybrid and at least one of the *B. napus* individuals. The same criteria were used to define *B. rapa*- or *R. raphanistrum*-specific markers. The presence/absence of each species-specific marker was recorded in all hybrids.

Results

Hybridization rates/frequency

B. rapa (♀) × B. napus (♂)

In the *B. rapa* × HR *B. napus* field experiment, *B. napus* flowered for about 1 month starting 10 July. Both *B. rapa* populations (QC-2974, QC-2975) had typical summer annual habits, with phenologies similar to that of *B. napus*. *B. rapa* plants initiated flowering on 10–14 July and produced mature seed pods by mid-to-late August. *B. rapa* plants within the *B. napus* stand stopped flowering, whereas those grown on the edge of the plot continued to flower after *B. napus* flowering had ended. *B. rapa* plants reached the same size as *B. napus* (or slightly taller) in the crop and, as expected, were larger and more branched on the margin. Bee pollinators were very abundant in the Ottawa field experiment.

A total of 13,423 and 15,850 seedlings were screened from *B. rapa* populations QC-2974 and QC-2975, respectively, in the *B. rapa* × HR *B. napus* field experiment (Table 1A). Hybridization rates for both populations were similar with means of 6.8% and 6.5% for QC-2974 and QC-2975, respectively. Average hybridization frequencies were slightly higher ($P < 0.05$) in the center of the plot (7.7 to 8.3%) compared with plants in the margin (3.4%). Although glyphosate-resistant *B. napus* cultivar 45A51 was assumed to be phenotypically pure for resistance when the experiment was sown, later use of this cultivar as a positive control in greenhouse herbicide spray tests indicated, and further analyses

Table 1 Number of seedlings screened and hybrids detected: A. *B. rapa* × HR *B. napus* field experiment (Ottawa 2000) with two populations of *B. rapa* (QC-2974, QC-2975) based on detection of an HR marker; and B. *B. rapa* × GFP *B. napus* field experiment (Saskatoon 2001) with *B. rapa* cultivar AC Parkland based on detection of a GFP marker

B. rapa population	Plot area	No. of plants	No. of seedlings	No. of hybrids	% of hybrids	
					Mean ± SE	Range
A. HR *B. napus* field experiment – Ottawa 2000						
QC-2974	Centre	47	9,380	775	8.3 ± 1.1	0–26.6
	Margin	19	4,043	136	3.4 ± 0.8	0–15.2
	Total	66	13,423	911	6.8 ± 0.8	0–26.6
QC-2975	Centre	50	11,337	875	7.7 ± 1.2	0–18.4
	Margin	20	4,513	153	3.4 ± 0.2	0–36.2
	Total	70	15,850	1,028	6.5 ± 0.9	0–36.1
B. GFP *B. napus* field experiment – Saskatoon 2001						
AC Parkland	Total	22	2,881	176	7.1 ± 2.1	0–32.7

Table 2 Number of seedlings screened and hybrids detected from two populations of *B. rapa* (QC-9039, QC-9047) collected near the margin of a commercial field (QC-9039) or among volunteer HR (glyphosate-resistant) *B. napus* (QC-9047) in Québec, 2001

Population- individual or bulk	No. of seedlings	No. of hybrids	% of hybrids
B. rapa QC-9039			
1-1	377	25	6.6
1-2	544	12	2.2
1-3	190	92	48.4
1-4	503	28	5.6
1-5	515	74	14.4
1-6	123	0	0
1-7	87	0	0
1-8	177	0	0
1-9	276	38	13.8
1-10	365	41	11.2
1-11	7	1	14.2
1-12	284	10	3.5
1-13	493	263	53.3
1-14	587	158	26.9
1-15	584	52	8.9
1-16	196	16	8.2
Total	5,308	810	13.6 ± 4.0 (SE)
B. rapa QC-9047			
Bulk	4,259	1	0.023

confirmed, that only 78% of 2,207 of seeds tested had a glyphosate-resistant phenotype. This obviously would have resulted in a lower measured rate of hybridization in these plots.

A total of 2,881 seedlings were screened for the GFP trait in the *B. rapa* AC Parkland × GFP *B. napus* field experiment (Table 1B). An average hybridization frequency of 7.1% was detected, with a range of 0 to 32.7%.

Based on a total screening of 5,308 seedlings, hybridization rates in the wild population *B. rapa* QC-9039 from a commercial HR *B. napus* field in Québec averaged 13.6% and ranged from 0 to 53.3% per plant (Table 2). In population *B. rapa* QC-9047, which was growing among volunteer HR *B. napus* in a corn field in Québec, only one hybrid was detected in the 4,259 seedlings (hybridization frequency of 0.023%) (Table 2).

R. raphanistrum (♀) × *B. napus* (♂)

No hybrids were detected in the three greenhouse experiments. A total of 94 and 119 seedlings were screened from seven Canadian and European populations of *R. raphanistrum*, respectively, with AFLP and ploidy markers. None of the seedlings had *B. napus* AFLP markers and all were diploid, and therefore not of hybrid origin. Similarly, no *B. napus* marker was detected in a total of 619 (HR marker) and 702 (GFP marker) seedlings screened from four *R. raphanistrum* populations (AB-8712, PEI-8710, QC-2986 and FR-8716). Because *R. raphanistrum* is normally self-incompatible, seed formation from selfing was not expected. Seeds were most-likely produced from unreduced gametes, although seed production as a result of selfing cannot be completely ruled out. There were considerable differences between individual *R. raphanistrum* plants in their ability to

Table 3 Number of seedlings screened and hybrids detected from two Ottawa 2000 field experiments with four populations of *R. raphanistrum* (AB, PEI, QC, FR) and based on an HR (glyphosate resistance) marker or a GFP marker

R. raphanistrum population	Plot area	No. of plants	No. of seedlings	No. of hybrids	% of hybrids
A. HR marker					
AB-8737	Centre	12	329	0	
	Margin	3	1,225	0	
	Total	15	1,554	0	
PEI-8710	Centre	50	4,942	1	
	Margin	18	6,169	0	
	Total	68	11,111	1	0.009%
QC-2986	Centre	48	6,949	0	
	Margin	20	4,883	0	
	Total	68	11,832	0	
FR-8716	Centre	30	4,614	0	
	Margin	13	3,710	0	
	Total	43	8,324	0	
Total		194	32,821	1	0.003%
B. GFP marker					
AB-8737		11	527	0	
PEI-8710		14	1,107	0	
QC-2986		16	1,687	0	
FR-8716		8	738	0	
Total		49	4,059	0	0

produce such seed, ranging from no seed production to those producing over 200 seeds.

In the two *R. raphanistrum* × *B. napus* field experiments, Canadian populations of *R. raphanistrum* behaved as typical summer annuals, coming into flower at the same time as *B. napus* (10–14 July), but continuing to flower well into the fall (2 months after *B. napus* had finished). Plants were usually 10–20 cm shorter than *B. napus*. In contrast, the population from France (FR) was clearly a winter annual and showed poor overlap in flowering time with spring canola, with several plants flowering well after *B. napus* had finished. FR plants most likely required vernalization as rosette sizes reached over 1 m before bolting; flowering plants were as tall as *B. napus*. As in the parallel *B. rapa* experiment, bee pollinators were abundant. Higher dormancy levels were observed for the AB population even after treatment to break dormancy, resulting in fewer plants in the experiment.

Only one *R. raphanistrum* × *B. napus* hybrid, from the *R. raphanistrum* population PEI-8710, was detected among the 32,821 seedlings screened from the four *R. raphanistrum* populations (AB, PEI, QC and FR) in the *R. raphanistrum* × HR *B. napus* field experiment (Table 3A). The frequency of hybridization in the PEI population was 0.009%. No hybrids were detected among the 4,059 seedlings screened from the same four *R. raphanistrum* populations in the *R. raphanistrum* × GFP *B. napus* field experiment (Table 3B).

No hybrids were detected in the 17,202 (Québec) and 4,912 (Alberta) seedlings screened from wild populations of *R. raphanistrum* collected next to the commercial HR *B. napus* fields (Table 4).

S. arvensis or *E. gallicum* (♀) × *B. napus* (♂)

No hybrids were detected among the nearly 43,000 seedlings of *S. arvensis* or 22,000 seedlings of *E. gallicum* screened for glyphosate resistance (Table 4). Thus, the hybridization frequency between *B. napus* and *S. arvensis* is less than 2×10^{-5} and that of *B. napus* by *E. gallicum* is less than 5×10^{-5}.

Hybrid characterization

B. rapa ♀ × *B. napus* ♂ *hybrids*

All F_1 hybrids tested from the HR *B. napus* field experiment were glyphosate-resistant, as determined from spray survival and positive test-strip results. All F_1 hybrids were similar to *B. rapa* in vegetative morphological traits, i.e., light green leaves with hairs. All 822 of the *B. rapa* F_1 hybrids tested (417 and 405 plants from QC-2974 and QC-2975, respectively) had intermediate flow cytometry values, compared with the values for *B. rapa* and *B. napus*, which is consistent with a triploid genomic structure (2n = 29, AAC). All 154 and 156 F_1 hybrid plants from QC-2974 and QC-2975, respectively, showed reduced pollen viability, with an average of 54% for both populations and ranges of 20–77% and 10–86% for QC-2974 and QC-2975, respectively, compared with values of 80–95% for both parental species. Both *B. napus*- and *B. rapa*-specific AFLP markers were detected in the hybrids, with means of 16.2–16.4 out of 17 *B. napus*-specific AFLP markers and 3.8–4.1 out of ten *B. rapa*-specific markers (Table 5).

All 39 F_1 hybrids tested from the GFP *B. napus* field experiment also exhibited a triploid ploidy level (AAC)

Table 4 Number of seedlings screened from wild populations of *R. raphanistrum*, *S. arvensis* and *E. gallicum* collected between 1999 and 2001 in or near commercial HR *B. napus* fields in Alberta, Québec and Saskatchewan, and the number of hybrids detected based on an HR marker

Species location/year collected[a]	No. of populations	No. of plants	No. of seedlings	No. of hybrids
Raphanus raphanistrum				
Québec 2000	1	445	9,386	0
Québec 2001	7	–[b]	7,816	0
Total 2000 and 2001	8	–	17,202	0
Alberta 2000	3	–[b]	1,443	0
Alberta 2001	8	–[b]	3,469	0
Total 2000 and 2001	11	–	4,912	0
Sinapis arvensis				
Saskatchewan 1999 (1998)	42	760	20,672	0
Saskatchewan 2000 (1998)	19	360	12,204	0
Saskatchewan 2000 (1999)	18	342	9,952	0
Total 1999 and 2000	79	1,462	42,828	0
Erucastrum gallicum				
Saskatchewan 1999 (1998)	3	52	1,544	0
Saskatchewan 2000 (1998)	14	278	8,507	0
Saskatchewan 2000 (1999)	21	365	11,790	0
Total 1999 and 2000	38	695	21,841	0

[a] Year that the adjacent HR-resistant canola field was sown is indicated in brackets, if different from the year of seed collection
[b] Sample was either individual plants (from 6 to 20 per population) or a bulk collection

Table 5 Numbers of *B. napus*- and *B. rapa*-specific AFLP markers detected in hybrids between *B. rapa* (♀) × HR *B. napus* (♂) F_1 hybrids for the primer pair *Eco*R1 + AGG/*Mse*1 + CAC[a]

Experiment	No. of species-specific markers				
	B. napus (17 markers)		*B. rapa* (10 markers)		Unique
	Mean ± SE	Range	Mean ± SE	Range	
HR Field experiment – Ottawa 2000					
QC-2974 (n = 119)	16.4 ± 0.12	10–17	3.8 ± 0.14	1–7	0
QC-2975 (n = 98)	16.2 ± 0.10	11–17	4.1 ± 0.18	1–10	0
Commercial HR *B. napus* field, Québec					
QC-9039 (n = 102)	15.3 ± 0.15	9–17	4.3 ± 0.18	1–8	0

[a] The core sequences of primers for the selective amplification were as follows: 5′GACTGCGTACCAATTC3′ for the *Eco*R1 primer and 5′GATGAGTCCTGAGTAA3′ for the *Mse*1 primer. Each primer contained three selective nucleotides at the 3′ end (*Eco*R1 + AGG contained the core sequence plus AGG at the end)

and a reduced pollen viability averaging 32% and range of 0 to 97.2%.

All F_1 hybrids tested from the two Québec commercial HR *B. napus* fields (QC-9039 and QC-9047) were similar to those described above. All were more similar to *B. rapa* morphologically. A total of 121 plants from QC-9039 and one plant from QC-9047, had intermediate DNA values consistent with a triploid level, AAC. All 121 F_1 hybrids from population QC-9039 showed reduced pollen viability, with an average of 55.6% and range of 32 to 84%. The F_1 hybrids from population QC-9039 had an average of 15.3 out of 17 *B. napus*-specific markers and 4.3 out of ten *B. rapa*-specific AFLP markers (Table 5). The majority of F_1 hybrids from population QC-9039 were self-incompatible (*B. rapa* trait); however, 11 out of the 49 (22%) plants appeared to be fully self-compatible (defined here as the production of >20 siliques per bagged plant) as in *B. napus*. The F_1 hybrids crossed readily with each other. Eight groups of three hybrids each were bagged and found to produce large numbers of seed pods per plant, as did 50 hybrids grown together in a separate greenhouse.

R. raphanistrum ♀ × *B. napus* ♂ *hybrid*

The *R. raphanistrum* × *B. napus* F_1 hybrid from the HR *B. napus* field experiment was glyphosate-resistant, based on spray survival and a positive test strip result. A chromosome count of approximately 34–35 and a Partec value close to that of *B. napus* were consistent with a genomic structure of RrRrAC, 2n = 37, i.e., the fusion of an unreduced gamete of *R. raphanistrum* (RrRr) and a reduced gamete of *B. napus* (AC). The hybrid was almost male-sterile, with a pollen viability of 0.12% based on six stained grains out of 4,855 and did not set seed when selfed. The hybrid was similar to *R. raphanistrum* in vegetative morphology (presence of hairs, leaf shape) and floral morphology (yellow petals with white at the base, distinct petal venation and erect, closed calyx). However,

Table 6 Numbers of *B. napus*- and *R. raphanistrum*-specific AFLP markers detected in the PEI *R. raphanistrum* (♀) × HR *B. napus* (♂) F_1 hybrid for five primer pairs (see footnote Table 5)

Primer	*B. napus* markers		*R. raphanistrum* markers		Unique hybrid
	B. napus	Hybrid	*R. raphanistrum*	Hybrid	
*Eco*R1 + AGG/*Mse*1 + CAC	17	16	8	2	2
*Eco*R1 + AAC/*Mse*1 + CAA	43	21	15	0	4
*Eco*R1 + AAC/*Mse*1 + CAT	50	34	20	6	5
*Eco*R1 + AAG/*Mse*1 + CAA	50	33	30	7	5
*Eco*R1 + AAG/*Mse*1 + CAT	53	36	27	6	6
*Eco*R1 + AAC/*Mse*1 + CAG	41	23	22	3	9
Total	254	163	122	24	31

the seed pods were distorted but had valves as in *B. napus*. The hybrid had 64% of 254 *B. napus*-specific AFLP markers, 20% of 122 *R. raphanistrum*-specific markers and 31 unique markers (Table 6).

Discussion

Brassica rapa × *B. napus*

Gene flow from *B. napus* into *B. rapa* was detected in both field experiments and in wild populations in commercial fields. Successful hybridization and gene flow was expected as a high potential for hybridization and was previously reported (Jørgensen and Andersen 1994; Bing et al. 1996; Warwick et al. 2000). Hybridization rates were similar for both the weedy *B. rapa* populations and the *B. rapa* cultivar, and comparable to those obtained from studies with *B. rapa* and GFP *B. napus* (Halfhill et al. 2002). The hybridization values of 7% obtained in the HR *B. napus* field experiment in Ottawa was most likely an underestimate since only 78% of the *B. napus* cv 45A51 had a glyphosate-resistant phenotype and therefore some hybrids would have been killed with the spray.

The mean hybridization rate for the wild population of *B. rapa* from a commercial HR *B. napus* field (QC-9039) was higher (13%) and showed greater plant-to-plant variability (0 to 53%) than in field trials. Differences in *B. rapa* × *B. napus* hybridization frequencies between the field trials and samples collected adjacent to a *B. napus* crop or among *B. napus* volunteers are probably explained by differences in the degree of isolation of the *B. rapa* plants from con-specific plants and *B. napus* plants, as was found in Danish fields (Jørgensen and Andersen 1994; Landbo et al. 1996).

In terms of risk, a 7–8% rate of hybridization essentially means that where *B. rapa* and *B. napus* co-occur, there will be hybridization between them, the degree of which depends on the density and spatial distribution of the weed species relative to *B. napus*. High pressure of canola pollen on isolated plants of self-incompatible species, such as *B. rapa*, are the most favourable situations for inter-specific hybridization (Darmency et al. 1998). The role of volunteer canola as a pollen source in inter-specific hybridization between *B. napus* canola and wild relatives was also demonstrated in a population of *B. rapa* growing among HR (glyphosate-resistant) *B. napus* volunteers in a corn field. Proximity between the two species and relative isolation between *B. rapa* plants were sufficient to create favorable hybridization conditions. *B. rapa* plants do not need to be exposed to large volumes of pollen from a *B. napus* crop in order for hybridization to occur.

Fewer *B. rapa*-specific AFLP markers were found than *B. napus*-specific markers, due to extensive homology of shared A genomes in *B. rapa* and *B. napus*. *B. napus*-specific AFLP markers were situated in the C genome. *B. napus* can pass its genes to *B. rapa* even when these genes are carried on non-homologous C chromosomes (Halfhill et al. 2001). Hansen et al. (2001) found all monomorphic *B. rapa*- and *B. napus*-specific AFLP markers in the *B. rapa* × *B. napus* F_1 hybrids tested, and assumed that individuals with less than a fully additive pattern of species-specific AFLP markers were the result of backcrossing. Most of our *B. rapa* × *B. napus* F_1 hybrids, from the HR experiment and the wild population, had all 17 monomorphic *B. napus*-specific markers, but not all individuals showed completely additive patterns. Means and ranges were similar in the hybrids for the wild population QC-9039. The fact that all hybrids were triploid, is consistent with their being F_1s. Introgressed individuals were not expected as it was the first time that *B. napus* had been grown in that field. The assumption by Hansen et al. (2001) that an F_1 hybrid always contains all *B. napus*- and *B. rapa*-specific AFLP markers may not be true in all cases.

Our *B. rapa* × *B. napus* F_1 hybrids showed reduced but still high levels of fertility, as assessed by pollen viability, and were able to backcross with *B. rapa* (Warwick and Voloaca, studies in progress). Similar results were reported in Danish studies (Landbo et al. 1996). The fact that a large proportion of the F_1 hybrids were able to self as in *B. napus* is significant, as it permits the establishment/perpetuation of crop genes in the related species, allowing them to spread widely even if the hybrid had reduced fertility or if the hybridization event is rare.

Raphanus raphanistrum × *B. napus*

Hybridization between *R. raphanistrum* and *B. napus* was extremely rare. Only one hybrid was detected in >32,000 seedlings, giving a likelihood of 3×10^{-5}. This value is

similar to estimates of 10^{-7} to 10^{-5} obtained in a field experiment in France (Chèvre et al. 2000) which utilized a glufosinate-resistant marker, but higher than that estimated ($<4 \times 10^{-8}$) in an Australian field experiment (Rieger et al. 2001) using a triazine resistance marker, in which no hybrids were obtained when *R. raphanistrum* was the maternal parent. No hybrids were detected in all other greenhouse and field experiments. Similarly, no hybrids between *R. raphanistrum* and *B. napus* were reported in field studies of Thalmann et al. (2001).

Given the low probability of this hybridization event, the lack of detection of hybrids in commercial fields in Québec and Alberta may simply be due to insufficient sampling. Variation among individuals and populations of *R. raphanistrum*, and their ability for selfing and ability to outcross, is also an important factor (Darmency et al. 1998; Gueritaine et al. 2000). Very low hybridization frequencies have also been observed between *B. napus* and *R. raphanistrum* when *B. napus* is the maternal parent (Chèvre et al. 2000; Rieger et al. 2001), but this direction was not studied in this paper. This may cause a potential problem in the field if such hybrid seed from *B. napus* is not removed at harvest and remains in the seed bank to form part of a subsequent volunteer population.

Our *R. raphanistrum* × *B. napus* F_1 hybrid is similar to that described in French studies (Chèvre et al. 2000). Both hybrids had a chromosome number of 2n = 37 (RrRrAC), and were produced from the fusion of an unreduced gamete of *R. raphanistrum* (RrRr, 2n = 18) and a reduced gamete of *B. napus* (AC, n = 19). Production of unreduced gametes is common in the Brassicaceae (Heyn 1977; Moyes et al. 2002). An in situ hybridization study of the F_1 hybrid has confirmed the presence of 18 *R. raphanistrum* and 19 *B. napus* chromosomes (S. Warwick and C. Voloaca, unpublished data). Given the presence of the full complement of the wild radish genome, it was surprising that only 20% of the *R. raphanistrum* AFLP markers were detected in the hybrid. The reason for the discrepancy is not obvious and again demonstrates the difficulty in using AFLP molecular data by itself to distinguish hybrid versus introgressed generations. Such a genomic structure is clearly unstable. Both hybrids have low pollen viability, with <1% and 6.5% for the Canadian and French hybrids, respectively. Given the low pollen viability, it was not possible to evaluate self-compatibility in the Canadian hybrid. Chèvre et al. (1997) reported low fertility of the *R. raphanistrum* × *B. napus* hybrids under field conditions (an average less than one seed per plant), although recovery of fertility was observed under repeated generations of backcrossing to *R. raphanistrum* (Chèvre et al. 1998). However, the herbicide resistance gene as well as other marker genes from *B. napus* were not integrated into the *R. raphanistrum* genome after four generations of backcrossing (Chèvre et al. 1998).

S. arvensis or *E. gallicum* × *B. napus*

Our results indicate that gene flow from commercial fields of *B. napus* to *S. arvensis* or *E. gallicum* has a very low probability of occurrence. These findings are in agreement with previous studies that have failed to detect gene transfer from *B. napus* to *S. arvensis* in field experiments (Bing et al. 1996; Chèvre et al. 1996; Lefol et al. 1996; Moyes et al. 2002). Lefol et al. (1996) screened 3.8 million seeds from field-grown *S. arvensis* and found no hybrids with *B. napus*. Hybridization potential between *B. napus* and *E. gallicum* had not previously been investigated in commercial fields.

Risks and implications of gene flow in Canada

Gene-flow rates in commercial fields supported the experimental results. The escape of transgenes to *R. raphanistrum, E. gallicum* or *S. arvensis* is unlikely. However, transgenes can disperse in the environment via wild *B. rapa* which is present at low frequencies in eastern Canada. In western Canada, gene flow is also possible between *B. napus* and commercial *B. rapa*, and presumably *B. rapa* volunteer populations. Persistence is likely as *B. napus* and *B. rapa* are estimated to have long-lived seed banks (Légère et al. 2001; Simard et al. 2002). Studies evaluating the occurrence/persistence of the herbicide resistance trait and its introgression in wild *B. rapa* populations are currently in progress.

Epidemiological evidence (e.g., Heap 2002) suggests that the probability of evolved glyphosate resistance in *B. rapa* would be much less than resistance acquired via hybridization through gene flow. To-date, glyphosate-resistant weed biotypes have been described in only four weed species (Heap 2002). Currently, herbicide-resistant weeds in Canadian cropping systems (Warwick et al. 1999), whether selected by herbicide use or as the result of gene flow, can generally be controlled in subsequent crops by using other weed-control practices, or herbicides with different modes of action, assuming of course that there has not been accidental stacking of traits conferring resistance to multiple modes of action. Also, we may assume no fitness-cost in the acquisition of herbicide resistance in *B. rapa*, as there is no evidence of reduced fitness in glyphosate-resistant *B. napus* in greenhouse fitness experiments (Simard and Légère, unpublished data). Similarly, no fitness-cost was associated with glufosinate resistance *B. napus* lines (Kumar et al. 1998) or when introgressed from *B. napus* into wild *B. rapa* (Snow et al. 1999). Looking ahead, we may find that the presence of weeds with novel traits other than herbicide resistance, acquired through gene flow, will have different, more complex agronomic and environmental implications. Traits such as insect or disease resistance, or resistance to environmental stress, are likely to result in increased overall weed fitness and competitiveness in cropping systems, as well as in other habitats.

Most cultivated plants mate with one or more wild relatives in some portion of their geographic range, and many crops are known to naturalize and persist as feral-weed populations (Ellstrand et al. 1999). It is impossible to prevent gene flow between sexually compatible species in the same area, as pollen and seeds disperse too easily, and too far to make containment practical. This reality makes the need for environmental studies even more urgent (Snow 2002). When novel genes spread to wild plant populations, they have the potential to create or exacerbate weed problems by providing novel traits that may allow these plants to compete better, produce more seeds, and become more abundant. Enhanced understanding of this process requires interdisciplinary research on the ecological and agronomic effects of gene flow – and more importantly of the impact of crop gene introgression into populations growing on roadsides, field margins or uncultivated areas. This knowledge is essential for prudent decision-making concerning the adoption of transgenic crops, if major agronomic and environmental problems are to be avoided.

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