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Experimental hybridisation of *Brassica* species in New Zealand

P. B. HEENAN
M. I. DAWSON
R. G. FITZJOHN
Allan Herbarium
Landcare Research
PO Box 40
Lincoln 7640, New Zealand
A. V. STEWART
PGG Wrightson Seeds
PO Box 939
Christchurch 8140, New Zealand

Abstract Field hybridisation experiments are described in which *B. juncea*, *B. napus*, and *B. oleracea* were crossed with *B. napus* (male), and *B. napus* was crossed with *B. juncea* (male). Five of the experiments used chlorsulfuron herbicide-resistant *B. napus* as the paternal parent, allowing over 98 000 seeds to be easily and efficiently screened for chlorsulfuron resistance to detect hybrid progeny. Two experiments used leaf morphological characters to identify putative hybrids. Intraspecific *B. napus* crosses produced low percentages (1.83% and 1.79%) of hybrid progeny. *Brassica juncea* × *B. napus* interspecific crosses produced on average 2.1% hybrids, and the *B. napus* × *B. juncea* cross produced 0.2% hybrids. No hybrids were detected by chlorsulfuron resistance in the *B. oleracea* × *B. napus* cross. Fecundity of the F_1 hybrid plants in all of the crosses was low compared with their parents, with hybrids having less than 28% pollen stainability and producing less than 2.4 seeds per flower pollinated when selfed or backcrossed; most of the F_1 hybrids studied produced less than one seed per flower pollinated. These results show that low levels of hybridisation and gene transfer between *B. napus* and some relatives could occur in New Zealand when grown in close proximity.

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INTRODUCTION

The introduction of genetically modified crops to New Zealand is a topical issue, particularly with regard to risks to the environment (e.g., Conner et al. 2003). These risks include, for example, crops becoming persistent weeds and introgression of transgenic traits to related weed species. It is thought that transgenes may result in the improved fitness, survival, and spread of weeds. In New Zealand, *B. napus* is an important crop for stock feed and, to a lesser extent, canola oil production, and several *Brassica* crops have been genetically engineered and field trialled in New Zealand (Christey & Woodfield 2001). Furthermore, several species of *Brassica* are naturalised in New Zealand, and these are a potential risk for the accidental escape of transgenes. Naturalised species include *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea*, and *B. rapa* (Webb et al. 1988; Heenan et al. 2004).

Interspecific hybridisation is known to occur in *Brassica* and has been particularly well documented between *B. napus* and *B. rapa* (e.g., Jørgensen & Andersen 1994; Wilkinson et al. 2000, 2003; Hansen et al. 2001). In New Zealand, hybridisation of wild turnip (*B. rapa* var. *oleifera*) and commercial *B. napus* seed crops (swede and rape) has been an issue for the seed industry for many years as hybrids show up as bolters in spring-sown crops (Calder 1937; Palmer 1962). In a series of experimental crosses, Jenkins et al. (2001) reported that *B. napus* pollinated a New Zealand wild population of *B. rapa* but that hybrids were rarely produced under field conditions. This observation is supported by data from a field survey of *Brassica* species naturalised in Canterbury, where only one putative wild hybrid between *B. rapa* and *B. napus* was detected (Heenan et al. 2004). Another study of six wild New Zealand populations of *B. rapa* var. *oleifera* crossed with *B. napus* has shown

that there is considerable variability among populations in the frequency of successful pollination and the number of seeds per fruit (Jenkins et al. 2005). At a lower taxonomic rank, wild hybrids between naturalised populations of pak choi (*B. rapa* var. *chinensis*) and wild turnip (*B. rapa* var. *oleifera*) have been described from near Ashburton, Canterbury (Heenan & Dawson 2005).

The purposes of this study are to further understand gene flow and hybridisation in *Brassica* species and varieties in New Zealand, with particular emphasis on providing information for risk assessment of transgenic plants. In the New Zealand context, this builds on the studies of Jenkins (2005), Jenkins et al. (2001, 2005), Heenan et al. (2004), and Heenan & Dawson (2005). This paper reports on intraspecific *B. napus* crosses, and interspecific crosses between *B. napus* and *B. oleracea* and reciprocal crosses between *B. napus* and *B. juncea*. Particular emphasis is given to hybridisation involving *B. juncea*, since this is a newly naturalised plant in the important *Brassica* seed certification area of Canterbury (Heenan et al. 2004), and relatively little is known about gene flow from *B. napus* to *B. juncea*.

METHODS

Natural field hybridisation

Seven experiments were undertaken to establish the level of spontaneous natural hybridisation under field conditions. Five of the experiments (Experiments 1–5) used chlorsulfuron-resistant selections of *B. napus* as a screen to identify hybrid plants. Resistance to chlorsulfuron occurs in hybrid progeny in crosses between a chlorsulfuron-susceptible maternal line and a chlorsulfuron-resistant paternal line. Three selections of *B. napus* var. *napus* that are resistant to the herbicide chlorsulfuron (Cs) were used as the pollen source. *B. napus* Cs30A is a homozygous chlorsulfuron line that was selected following seed mutagenesis with ethyl methanesulfonate (Conner et al. 1994). *B. napus* genotypes Cs1 and Cs2 were obtained from S. Gowers (Crop & Food Research, Lincoln), and are homozygous chlorsulfuron lines derived from his rape breeding programme. These three lines of *B. napus* all have the same mutation for chlorsulfuron resistance, with Cs1 and Cs2 being elite breeding lines derived from Cs30A (S. Gowers pers. comm.). Experiments 6 and 7 utilised different leaf morphological characteristics of *B. napus* (leaves grey, coriaceous, and lobed) and *B. juncea* (leaves green, membranous, and strongly dissected) to identify hybrid progeny. Experiments 1–5 were undertaken in open-ground plots at Landcare Research, Lincoln, and Experiments 6–7 were carried out in open-ground plots at Pyne Gould Guinness, Prebbleton; both locations are in Canterbury.

Experiments 1–5

Experiment 1: *Brassica napus* var. *napobrassica* 'Dominion' × *B. napus* var. *napus* Cs2; Experiment 2: *Brassica napus* var. *napobrassica* 'Winton' × *B. napus* var. *napus* Cs2; Experiment 3: *Brassica oleracea* 'Kestral' × *B. napus* var. *napus* Cs1

The field hybridisation experiments 1, 2, and 3 followed similar protocols. Fifty one-year-old plants of each of the three cultivars *B. napus* var. *napobrassica* 'Dominion', *B. napus* var. *napobrassica* 'Winton', and *B. oleracea* 'Kestral' were obtained from Pyne Gould Guinness, Prebbleton, and planted at Lincoln in three separate 2.0 × 2.0 m plots. When these plants began to flower, 10 nursery-raised flowering plants of *B. napus* var. *napus* Cs1 and *B. napus* var. *napus* Cs2 were interplanted into the plots so that they were never more than 0.3 m from plants of *B.* 'Dominion', *B.* 'Winton', and *B.* 'Kestral'. Subsequently, seeds collected from *B.* 'Dominion', *B.* 'Winton', and *B.* 'Kestral' were screened for hybrids using the chlorsulfuron resistance marker gene.

Experiment 4: *Brassica juncea* var. *juncea* (ex Tinwald) × *B. napus* var. *napus* Cs1

Plants of *B. juncea* var. *juncea* were raised from seed collected from a naturalised population near Tinwald, Ashburton, Canterbury. Twenty plants each of *B. juncea* var. *juncea* (ex Tinwald) and *B. napus* var. *napus* Cs1 were alternately planted in rows in a 1.0 × 1.5 m plot. Seeds collected from *B. juncea* var. *juncea* (ex Tinwald) were screened for hybrids using the chlorsulfuron resistance marker gene.

Experiment 5: *Brassica juncea* var. *napiformis* × *B. napus* var. *napus* Cs30A

Sixty plants of *B. juncea* var. *napiformis* and 30 plants of *B. napus* var. *napus* Cs30A were planted in a 2.0 × 3.5 m plot. Ten plants per row of *B. juncea* var. *napiformis* and five plants per row of *B. napus* var. *napus* Cs30A were planted in alternating rows. Seeds collected from *B. juncea* var. *napiformis* were screened for hybrids using the chlorsulfuron-resistance marker gene.

For Experiments 1–5, harvested seeds were screened for hybrids by utilising the chlorsulfuron-resistance marker gene. Harvested seeds from

individual plants were pooled. Seeds were surface sterilised by immersion in 1% sodium hypochlorite (plus a drop of Tween 20 surfactant) for 10 min, followed by three rinses with sterile water. Seeds were sown onto the surface of nutrient medium consisting of half-strength MS salts (Murashige & Skoog 1962) at pH 5.8 solidified with 0.8% (w/v) Gibco bacteriological agar. This medium was autoclaved for 15 min at 103 kPa, and then filter-sterilised chlorsulfuron was added to a final concentration of 10 µg/l, just prior to dispensing of 50 ml into presterilised plastic pottles. Seeds sown in each pottle were germinated at 24–26°C under light from cool white fluorescent lamps (80–100 µmol/m^2/s, 16 h photoperiod). To ensure the chlorsulfuron protocols were an effective screen we tested control samples of *B. napus* var. *napus* Cs30A, *B. napus* var. *napus* Cs1, and *B. rapa* var. *oleifera* wild type (non-chlorsulfuron) from Awatea Road, Wigram, Christchurch. Seedling plants were screened by assessing root growth. Seedling root extension into the media may be up to 50 mm after 7 days if resistance is present, and is negligible (< 10 mm) if there is no resistance to the chlorsulfuron herbicide (Conner et al. 1994). If chlorsulfuron resistance is present in the seedlings it can be inferred that hybridisation has occurred between the different maternal and paternal genotypes.

Experiments 6 and 7

Experiment 6: *Brassica juncea* var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred'); Experiment 7: *Brassica napus* var. *napobrassica* ('Melford' × 'Winfred') × *B. juncea* var. *napiformis*

Experiments 6 and 7 are reciprocal crosses. Fifty one-year-old plants of each species were transplanted to Lincoln from Pyne Gould Guiness, Prebbleton, and planted in rows of alternating plants in a 2.5 × 4.0 m plot. Seeds were harvested from both species separately. For each of the seed lots about 10 000 seeds were sown in the open ground during February (for autumn-flowering) and October (for summer-flowering) 2005. The genotype of *B. juncea* var. *napiformis* used for this experiment had green, strongly dissected leaves, whereas that of *B. napus* var. *napobrassica* had grey, shallowly lobed leaves. Putative hybrids were identified by a visual assessment of their leaf morphology.

Putative hybrids obtained from Experiment 6 were analysed for glucosinolates for further confirmation of their hybrid origin. *B. juncea* and *B. napus* are known to contain different glucosinolates, and interspecific hybrids would be expected to have glucosinolates from each of the parents. To confirm the status of putative hybrid plants raised from this experiment, leaf material from five plants was analysed for glucosinolates.

The intact glucosinolates were extracted using a procedure based on that described by Heaney & Fenwick (1980). A large and mature leaf from each of the hybrid plants was sampled and frozen for analysis. The frozen leaf samples were later freeze dried and ground to pass through a 1 mm diameter mesh. A 3.75 g subsample was extracted in heated methanol and the extract filtered using a 0.45 µ*M* syringe filter prior to analysis. Glucosinolates were also determined by HPLC with modifications including the use of a Prodogy column (Phenomenex Ltd) 5 µ ODS (250 × 4.6 mm), column heater (40°C) and automated pre-derivation of the sample. Initial isolation and identification of individual glucosinolates was made with the aid of a Photo-Diode Array (PDA; 200–350 nm and specified at 235 nm) and verified using Mass Spectrometry (VG Platform II; Fisons Instruments).

Artificial interspecific hybrids

To provide a baseline with which to compare the results of the open-pollinated field experiments 4–7, hand pollinations were undertaken to determine the success of the interspecific *B. juncea* × *B. napus* crosses. For each of these crosses between 13 and 26 flowers of each maternal plant were pollinated by the paternal parent used in the natural field hybridisation experiments. These experiments were all undertaken in glasshouse conditions at Lincoln, Canterbury.

Fecundity of hybrid progeny

To assess fecundity of the *B. juncea* × *B. napus* F_1 hybrids produced in Experiments 4–7, pollen stainability and ovule fertility were measured on glasshouse-cultivated hybrids of each cross. To estimate male fertility, the pollen of each hybrid plant was obtained from one anther from each of three different flowers. This pollen was pooled and mixed into a drop of Alexander's Differential Stain (Alexander 1969) on a microscope slide. Slides were left for 60 min for the stain to intensify. The pollen of normal pollen grains (non-aborted, presumed viable) stain dark red, whereas aborted (presumably inviable) grains stain pale blue-green. The percentage of normally developed pollen was determined by counting 500 pollen grains per sample. To estimate female fertility of the hybrid plants, flowers were individually selfed or backcrossed (after emasculation), and seed set as a percentage of the total number

of ovules of each fruit was scored for the fruit formed from these pollinations.

To determine the fecundity of *B. juncea* × *B. napus* hybrid plants under field conditions, hybrids obtained from each of Experiments 5 and 6 were planted in open-ground plots at Lincoln among plants of *B. juncea* (the maternal parent). Hybrid plants were grown on and randomly planted in plots alongside 90 plants of *B. juncea*. Hybrid plants obtained from Experiment 7 were allowed to grow in the open ground at Pyne Gould Guinness, Prebbleton, alongside c. 1100 plants of *B. juncea* and c. 1100 plants of *B. napus*. Seed set was assessed on each hybrid plant by scoring the seed set as a percentage of the total number of ovules from each of 20 fruit; these seeds may be the result of selfing, crosses with other F_1 hybrids, or backcrosses to either parent. To estimate male fertility in the field, the pollen of hybrid plants was examined for stainability as described above for the glasshouse-grown hybrid plants.

RESULTS

Summary data from Experiments 1–7 are presented in Tables 1–3. Data for individual plants for each experiment are presented in Appendices 1–6.

Experiment 1: *Brassica napus* var. *napobrassica* 'Dominion' × *B. napus* var. *napus* Cs2

Hand pollination of 26 flowers resulted in 11 fruits with an average of 2.7 seeds per fruit (Table 1). Chlorsulfuron resistance was transferred to 72.7% of the plants raised from these seeds, suggesting them to be intraspecific hybrids. In the open-pollination field experiment, 1.8% of the plants had chlorsulfuron

Table 1 Hand-pollination experiments undertaken to assess compatibility in the intraspecific and interspecific *Brassica* crosses.

Experiment	Cross	Number of flowers crossed	Fruit length (mm; mean ± SD)	Fruit with seeds	Seeds total number	Seeds per fruit (of fruit that produced seeds)	Chlorsulfuron resistance				
							Seeds sown	Seeds germinated	Seeds with chlorsulfuron resistance	Chlorsulfuron resistance per flower crossed	% seeds with chlorsulfuron resistance
1	*B. napus* var. *napobrassica* 'Dominion' × *B. napus* var. *napus* Cs2	26	25.1 ± 14.1	11	30	2.7 ± 2.5	30	11	8	3.2	72.7%
2	*B. napus* var. *napobrassica* 'Winton' × *B. napus* var. *napus* Cs2	13	63.1 ± 10.2	13	145	11.2 ± 4.2	145	143	138	10.6	96.5%
3	*B. oleracea* 'Kestral' × *B. napus* var. *napus* Cs1	13	66.8 ± 7.5	0	0	0	–	–	–	–	–
4	*B. juncea* var. *juncea* (ex Tinwald) × *B. napus* var. *napus* Cs1	17	32.3 ± 8.1	16	103	6.4 ± 3.7	103	103	103	6.4	100.0%
5	*B. juncea* var. *napiformis* × *B. napus* var. *napus* Cs30A	17	42.6 ± 2.3	17	238	14.0 ± 2.2	238	189	187	11.0	98.9%
6	*B. juncea* var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred')	20	42.9 ± 3.7	20	330	16.5 ± 2.5	–	–	–	–	–
7	*B. napus* var. *napobrassica* ('Melford' × 'Winfred') × *B. juncea* var. *napiformis*	18	108.1 ± 3.9	18	472	26.2 ± 2.4	–	–	–	–	–

resistance, suggesting them to be intraspecific hybrids (Table 2). All of the control samples tested for chlorsulfuron resistance gave the expected result of being resistant (*B. napus* Cs30A and Cs1) or susceptible (wild type from Awatea Road).

Experiment 2: *Brassica napus* var. *napobrassica* 'Winton' × *B. napus* var. *napus* Cs2

Hand pollination of 13 flowers resulted in 13 fruits producing 145 seed, at an average of 11.2 seeds per fruit (Table 1). Chlorsulfuron resistance was transferred to 96.5% of the plants raised from these seeds, suggesting them to be intraspecific hybrids. In the open-pollination field experiment only 1.8% of the plants had chlorsulfuron resistance, suggesting a low frequency of intraspecific hybridisation (Table 2).

Experiment 3: *Brassica oleracea* 'Kestral' × *B. napus* var. *napus* Cs1

Hand pollination of 13 flowers resulted in no fruits producing seed (Table 1). A similar result was obtained in the open-pollination field experiment, where no plants produced seed with chlorsulfuron resistance, indicating there had been no interspecific hybridisation (Table 2). These results are consistent with previous studies of this interspecific cross, in which it has been shown seed set is usually below 0.05 % (Stewart 2002).

Experiment 4: *Brassica juncea* var. *juncea* × *B. napus* var. *napus* Cs1

Hand pollination of 17 flowers resulted in 16 fruits producing 103 seed, at an average of 6.4 seeds per fruit (Table 1). Chlorsulfuron resistance was

Table 2 Spontaneous open-pollination field experiments undertaken to assess geneflow in intraspecific and interspecific crosses from chlorsulfuron-resistant *B. napus* to *B. juncea*, *B. napus*, and *B. oleracea*.

Experiment	Cross	Ratio female: male	Seeds sown	Plants grown	Hybrid plants as indicated by chlorsulfuron resistance or morphology (*)	% hybrid plants
1	*B. napus* var. *napobrassica* 'Dominion' × *B. napus* var. *napus* Cs2	5:1	20000	–	367	1.83%
2	*B. napus* var. *napobrassica* 'Winton' × *B. napus* var. *napus* Cs2	5:1	20000	–	358	1.79%
3	*B. oleracea* 'Kestral' × *B. napus* var. *napus* Cs1	5:1	20000	–	0	0
4	*B. juncea* var. *juncea* (ex Tinwald) × *B. napus* var. *napus* Cs1	1:1	18333	–	1083	5.91%
5	*B. juncea* var. *napiformis* × *B. napus* var. *napus* Cs30A	2:1	20000	–	28	0.14%
6	*B. juncea* var. *napiformis* × *B. napus* ('Melford' × 'Winfred') Summer-flowering	1:1	–	2168	21*	0.97%
	Autumn-flowering	1:1	–	c. 4000	48*	1.20%
7	*B. napus* ('Melford' × 'Winfred') × *B. juncea* var. *napiformis* Summer-flowering	1:1	–	c. 2000	0*	0
	Autumn-flowering	1:1	–	c. 4000	8*	0.20%
Control	*B. napus* var. *napus* Cs30A	–	100	100	100	–
Control	*B. napus* var. *napus* Cs1	–	100	100	100	–
Control	*B. rapa* var. *oleifera* wild type from Awatea Road, Wigram, Christchurch	–	100	0	–	–

Table 3 Summary of data obtained from the *B. juncea* and *B. napus* F_1 hybrid pollination experiments. Experiments 4–7.

Exp.	Parents and crosses	Phenotype	Treatment	Sample number	Pollen stainability (%) Mean	Pollen stainability (%) Range	Fruit length (mm) Mean	Fruit length (mm) Range	Seed per fruit Mean	Seed per fruit Range	App. no.
4	*B. juncea* var. *juncea* (ex Tinwald)	Parent	-	2	80.0 ± 12.1	71.4–88.6	31.5 ± 0.1	31.5–31.6	9.7 ± 0.5	9.4–10.1	1
	B. juncea var. *juncea* (ex Tinwald) × *B. napus* var. *napus* Cs1	F_1 hybrid	hand pollination	5	13.3 ± 4.0	7.9–19.1	15.8 ± 1.6	13.9–18.1	0.1 ± 1.5	0.0–0.2	1
	B. juncea var. *juncea* (ex Tinwald) × *B. napus* var. *napus* Cs1	F_1 hybrid	spontaneous field pollination	8	12.4 ± 6.7	2.3–17.9	19.1 ± 3.4	14.4–26.2	0.1 ± 0.1	0.0–0.3	1
5	*B. juncea* var. *napiformis*	Parent	-	2	91.7 ± 7.8	88.3–95.2	32.7 ± 1.5	31.7–33.8	11.9 ± 0.7	11.4–12.4	3
	B. juncea var. *napiformis* × *B. napus* var. *napus* Cs30A	F_1 hybrid	hand pollination	3	27.0 ± 5.5	22.9–33.2	11.1 ± 0.1	10.4–11.8	0	0	2
	B. juncea var. *napiformis* × *B. napus* var. *napus* Cs30A	F_1 hybrid	spontaneous field pollination	3	27.0 ± 5.5	22.9–33.2	11.9 ± 1.8	9.9–13.5	0.04 ± 0.1	0.0–0.14	2
	(*B. juncea* var. *napiformis* × *B. napus* var. *napus* Cs30A) × *B. napus* var. *napus* Cs 30A	Backcross hybrid	hand pollination	3	27.0 ± 5.5	22.9–33.2	15.1 ± 2.8	12.1–16.6	0.1 ± 1.8	0.0–0.18	2
	B. juncea var. *napiformis* × *B. napus* var. *napus* Cs30A	F_1 hybrid	hand pollination	pollen 4; fruit/seed 1	25.5 ± 5.4	21.1–33.2	25.4 ± 5.2	–	0.2 ± 0.4	–	3
	B. juncea var. *napiformis* × *B. napus* var. *napus* Cs30A	Patromorph *B. napus* var. *napus*	hand pollination	pollen 11; fruit/seed 5	78.0 ± 14.5	49.8–93.2	34.7 ± 6.3	25.7–41.9	4.7 ± 3.0	0.5–8.8	3
6	*B. juncea* var. *napiformis*	Parent	-	4	90.2 ± 9.0	76.6–94.8	36.4 ± 2.7	32.4–38.3	17.2 ± 2.3	15.2–19.6	4
	B. napus var. *napobrassica* ('Melford' × 'Winfred')	Parent	-	2	73.1 ± 7.0	68.1–78.0	63.5 ± 7.6	58.1–68.9	25.1 ± 1.7	26.3–23.9	4
	B. juncea var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred')	F_1 hybrid	spontaneous field pollination	5	20.3 ± 4.5	15.4–26.4	15.3 ± 4.7	9.1–19.9	1.2 ± 1.1	0.0–2.2	4
	B. juncea var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred')	F_1 hybrid	hand pollination	3	27.9 ± 8.3	19.2–35.6	20.8 ± 5.9	15.2–27.0	1.6 ± 1.5	0.4–3.2	5
	(*B. juncea* var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred') × *B. juncea* var. *napiformis*	Backcross hybrid	hand pollination	3	27.9 ± 8.3	19.2–35.6	27.3 ± 3.1	23.8–29.4	1.3 ± 0.7	0.8–2.1	5
	(*B. juncea* var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred')) × *B. napus* ('Melford' × 'Winfred')	Backcross hybrid	hand pollination	3	27.9 ± 8.3	19.2–35.6	27.1 ± 4.8	21.6–30.7	2.4 ± 2.0	0.05–3.6	5
7	*B. juncea* var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred')	F_1 hybrid	hand pollination	4	23.8 ± 10.1	14.4–33.9	–	–	–	–	6
	B. napus var. *napobrassica* ('Melford' × 'Winfred') × *B. juncea* var. *napiformis*	Matromorph *B. napus* var. *napobrassica*	hand pollination	4	95.4 ± 5.4	87.4–99.4	–	–	–	–	6

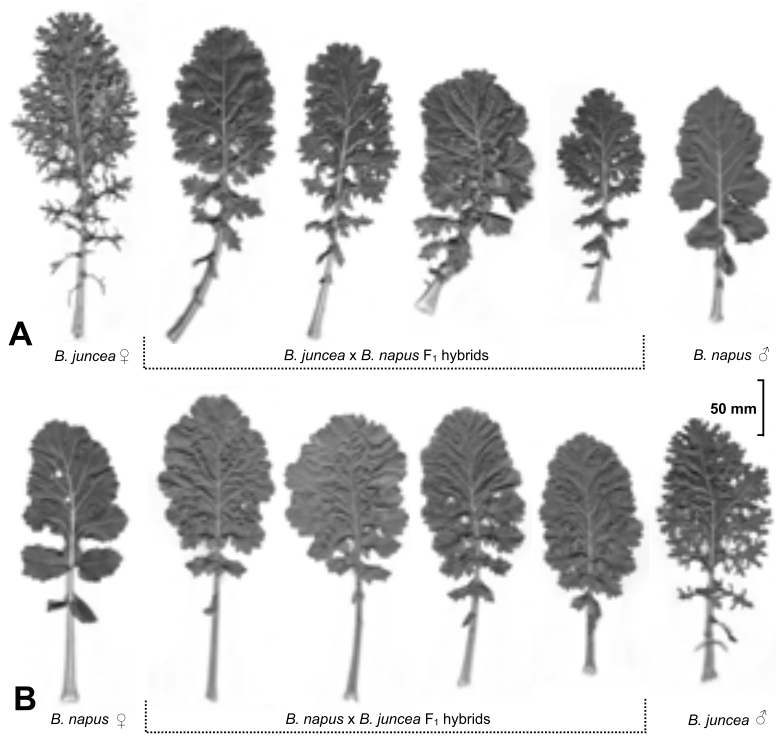

Fig. 1 Leaf silhouettes of *B. juncea* var. *napiformis* × *B. napus* ('Melford' × 'Winfred') putative hybrid plants and their parents. The hybrids were raised from controlled hand pollinations. **A**, *B. juncea* (♀) × *B. napus*; **B**, *B. napus* (♀) × *B. juncea*.

transferred to 100% of the plants raised from these seeds, suggesting them to be interspecific hybrids. In the open-pollination field experiment, 5.91% of the plants had chlorsulfuron resistance, suggesting them to be interspecific hybrids (Table 2). The F_1 hybrids had low pollen stainability (12.4–13.3%), and set few seeds (0.1) per fruit (Appendix 1).

Experiment 5: *Brassica juncea* var. *napiformis* × *B. napus* var. *napus* Cs30A

Hand pollination of 17 flowers resulted in 17 fruits producing 238 seed, at an average of 14.0 seeds per fruit (Table 1). Chlorsulfuron resistance was transferred to 98.9% of the plants raised from these seeds, suggesting them to be interspecific hybrids. Fifteen putative F_1 hybrid plants were grown on from seed produced by controlled hand pollinations. These included four F_1 hybrids that were identified by their intermediate leaf morphology, and these had relatively low pollen stainability (25.5%) and very low numbers of seeds per fruit (0.2) (Table 3). However, 11 of the putative F_1 hybrids had the vegetative and floral morphology of *B. napus*, high pollen stainability (78.0%), and moderate numbers of seeds per fruit (4.7) (Table 3). These plants are most likely to be patromorphs. In addition to the plants described above that were examined in some detail, we also raised 10 other putative hybrid plants. Based on vegetative and floral morphology, seven of these were patromorphs (*B. napus* var. *napus* Cs30A) and three were F_1 hybrids.

In the open-pollination field experiment, 0.14% of the plants had chlorsulfuron resistance, suggesting them to be interspecific hybrids (Table 2). The F_1 hybrids had relatively low pollen stainability (27.0%), and when selfed or backcrossed with *B. napus* var. *napus* produced very few seeds per fruit (0.0–0.2) (Table 3).

Experiment 6: *Brassica juncea* var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred') (Fig. 1)

Hand pollination of 20 flowers resulted in 20 fruits producing 330 seed, at an average of 16.5 seeds per fruit (Table 1). In the open-pollination field experiment some plants had leaf morphology intermediate between the two parents, suggesting these to be interspecific hybrids (Table 2). From the summer-flowering seedlot 21 (0.97%) of 2168 plants were determined to be hybrids, and from the autumn-flowering seedlot 48 (1.20%) of c. 4000 plants were considered to be hybrids. These F_1 hybrids

Table 4 Distribution and quantity (µg/g dry weight) of glucosinolates in *B. juncea* var. *napiformis*, *B. napus* var. *napobrassica* ('Melford' × 'Winfred'), and the putative hybrid *B. juncea* var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred'). Experiment 6.

Glucosinolate	*B. juncea* var. *napiformis*	Hybrid glasshouse	Hybrid field	*B. napus* var. *napobrassica* ('Melford' × 'Winfred')
Sinigrin	33.5	4.8	9.4	–
Progoitrin	–	10.1	9.1	5.9
Gluconapin	–	3.1	0.7	0.6
Glucobrassicanapin	–	2.5	1.3	1.5
Total glucosinolates	35.8	24.2	22.5	15.8

had relatively low pollen stainability (20.3–27.9%), and when selfed or backcrossed with *B. napus* var. *napobrassica* produced low numbers of seeds per fruit (1.2–2.4) (Table 3).

The *B. juncea* × *B. napus* plants obtained from this experiment express glucosinolates that are typical of both putative parent species, confirming their hybrid origin (Table 4). They also express an intermediate level of total glucosinolates. *B. juncea* has a high concentration of sinigrin and *B. napus* has a high concentration of progoitrin, glucobrassicanapin, and gluconapin, and each of these compounds is specific to the individual species (Table 4).

Experiment 7: *Brassica napus* var. *napobrassica* ('Melford' × 'Winfred') × *B. juncea* var. *napiformis* (Fig. 1)

Hand pollination of 18 flowers resulted in 18 fruits producing 472 seed, at an average of 26.2 seeds per fruit (Table 1). Four of the putative F_1 hybrids had high pollen stainability (95.4%) and the vegetative and floral morphology of *B. napus* var. *napobrassica* (Table 3); these plants are most likely to be matromorphs. In the open-pollination field experiment, none (0.0%) of the c. 2000 plants examined from the summer-flowering seedlot had leaf morphology intermediate between the two parents (Table 2). However, 8 (0.20%) of c. 4000 plants examined from the autumn-flowering seedlot had leaf morphology intermediate between the two parents (Table 2).

DISCUSSION

The herbicide-resistant chlorsulfuron marker used in five of the seven experiments undertaken here has provided a very effective screen for studying hybridisation in *Brassica*. In this study, over 98 000 seeds were easily and quickly screened on agar growth media. This is an ideal method for screening large-progeny populations, particularly for intraspecific crosses or when the morphological traits of species used in interspecific crosses may be similar.

Brassica napus intraspecific hybrids

The level of intraspecific geneflow reported here (1.8% and 1.7%) in our mixed-plot field experiments is lower than experimental plant-to-plant outcrossing rates reported in Europe (20–40%; Becker et al. 1992) and Canada (5–75%; Lewis & Woods 1994), and also lower than the outcrossing rate in a series of plot-to-plot (4%), row-to-row (rows various distances apart: 3.9%, 5.6%, and 9.5%), and plant-to-plant (21%) experiments undertaken in Canada (Cuthbert & McVetty 2001). However, the level of hybridisation in our study is greater than that from a study of genetically modified *B. napus* crops to nearby conventional crops in Australia (< 0.2%; Rieger et al. 2002) and Canada (0.03%; Staniland et al. 2000). Distances between plants in these studies vary and this will affect the results. Since *B. napus* is self-compatible it is to be expected that selfing rates would be relatively high and outcrossing rates, even for intraspecific crosses, relatively low. It is also difficult to extrapolate from an outcrossing rate to expected levels of geneflow since there will be local differences in pollinators and the amounts of related and foreign pollen available.

The hand-pollination experiments reported here for these intraspecific *B. napus* crosses gave very different results, with the cultivar 'Winton' producing only 2.7 seeds per pollination and the cultivar 'Dominion' producing 11.2 seeds per pollination. The male parent in each of the crosses was *B. napus* var. *napus* Cs2, and so the difference in seed set suggests there is a strong maternal genotype effect.

Brassica juncea and *B. napus* interspecific hybrids

Spontaneous hybrids between *B. juncea* × *B. napus* were identified in three field experiments, with these crosses producing 0.14%, 0.97%, 1.20%, and 5.91% hybrids (Table 2), at an average of 2.05% hybrids. These percentages are comparable to the range of results (0.3–3.0% hybrids) reported elsewhere for spontaneous hybrids (Frello et al. 1995; Bing et al. 1996; Jørgensen et al. 1998). Other studies of crosses between *B. juncea* and *B. napus* have shown hybrids to occur at an average of 3.74 ± 2.0 (range 0.23–6.99) seeds per pollination (data summarised from hand pollination experiments by Roy 1980; Wahiduzzaman 1987; Sharma & Singh 1992; Frello et al. 1995; Choudhary & Joshi 1999; Ghosh Dastidar & Varma 1999). The controlled hand-pollination experiments reported here for *B. juncea* × *B. napus* produced reasonably high numbers of seeds per pollination (6.4, 14.0, and 16.5; Table 1), and these are comparable to other hand-pollination experiments (Davey 1959; Sharma & Singh 1992; Subudhi & Raut 1994; Bing et al. 1996). The difference between the high numbers of hybrids produced by controlled hand pollinations and the lower number of hybrids produced by spontaneous pollinations in the field is similar to results in other studies.

The reciprocal field cross *B. napus* × *B. juncea* (Experiment 7) produced very few hybrids from summer (0.0%) and autumn (0.2%) flowerings of the same seedlot, significantly less than the number (1.0–1.3%) of spontaneous hybrids reported by Bing et al. (1996) and Jørgensen et al. (1998). This cross does not appear to be particularly easy to accomplish, as other studies of *B. napus* × *B. juncea* have also produced very low numbers of seeds per pollination (0.38 ± 1.0; range 0.0–4.02) (data summarised from Heyn 1977; Roy 1980; Yamagishi & Takayanagi 1982; Dhillon et al. 1985; Wahiduzzaman 1987; Prakash & Chopra 1990; Rashid et al. 1994; Frello et al. 1995; Choudhary & Joshi 1999; Ghosh Dastidar & Varma 1999). The low number of *B. napus* × *B. juncea* hybrids identified by leaf morphology from among the *B. napus* population may in part be due to differential segregation of leaf morphological characters and the associated difficulty of recognising F_1 hybrid plants. Sabharwal & Dolezel (1993), for example, reported that hybrids of *B. napus* × *B. juncea* had leaf morphological characters more like *B. napus*. Furthermore, as shown in our controlled hand-pollination study, matromorphy may be a feature of some crosses (Table 3), and if foreign pollen is required as a stimulus for matromorphy this may reduce the number of hybrids that result from interspecific pollination. The occurrence here of patromorphy also seems to be unusual in *Brassica*, and to our knowledge has not been reported before.

Indeed, in this study we have identified matromorphy as occurring in one, and possibly another three, of the controlled hand-pollination experiments. The morphology of four plants raised from the cross *B. napus* var. *napobrassica* ('Melford' × 'Winfred') × *B. juncea* var. *napiformis* (Experiment 7; Table 3) is consistent with the female parent. These four plants also had high pollen stainability (95.4%), which is much greater than four putative hybrids obtained from the same cross (23.8%) (Fig. 1). Results from the chlorsulfuron-resistant marker gene experiments also infer matromorphy since three of the hand-pollination chlorsulfuron experiments did not give 100% chlorsulfuron resistance (Table 1); the few plants in the three experiments that did not have chlorsulfuron resistance are most likely to be matromorphs, although some could also be weak hybrid plants.

The success of F_1 hybrids and their potential environmental impact can be assessed by their ability to produce additional generations of plants through F_2 and backcross progeny. Male fecundity for the *B. juncea* × *B. napus* F_1 hybrids raised from controlled hand pollination and spontaneous field pollination was measured by pollen stainability, and in all crosses this was much lower in the F_1 hybrids than in the putative parents (Table 3). Typically, F_1 hybrid plants had between 20.3% and 27.9% stainable pollen, although hybrids from Experiment 4 had 12.4% and 13.3% stainable pollen. These figures are similar to a median of 22.05% stainable pollen from a range of studies (data summarised from Anand et al. 1985; Wahiduzzaman 1987; Prakash & Chopra 1988; Frello et al. 1995; Choudhary & Joshi 1999). In our study, parent plants usually had between 73.1% and 91.7% stainable pollen (Table 3). Female fecundity was measured by seed set from controlled hand pollinations (self and backcross) and spontaneous open pollination, and this was very low (range 0.0–2.4 seeds per pollination) in comparison with parent plants (9.7–25.1 seeds per pollination) (Table 3). There appeared to be some genotype effect with F_1 hybrids from Experiment 6 producing more seed per pollination (1.2–2.4) than F_1 hybrids from Experiments 4 and 5 (0.0–0.2).

Conclusions

This study provides useful baseline information for the assessment of risk posed by the introduction of

transgenic plants to New Zealand. The hand-pollination and spontaneous field-pollination experiments undertaken here for *B. juncea* × *B. napus*, and for the intraspecific crosses in *B. napus*, have given similar results to those for *B. rapa* × *B. napus* previously reported from New Zealand (Jenkins et al. 2001). That is, while hand pollinations show the ease with which crosses can usually be made, spontaneous field pollinations produce only low numbers of F_1 hybrids. Furthermore, examination of the F_1 hybrids has shown they are not particularly fecund in regard to both male and female fertility and they do not easily produce F_2 or backcross progeny. Within the context of this study the low incidence of intraspecific and interspecific hybrids makes potential risk of gene transfer relatively low, although not totally negated. Furthermore, we observed some genotypic variation (cf. Jenkins et al. 2005), and future studies should examine this in more detail.

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Appendix 1 Fecundity of *B. juncea* var. *juncea* (ex Tinwald) × *B. napus* var. *napus* F_1 hybrids. Fruit length and seeds per fruit were obtained by spontaneous open pollinations in the field and hand self-pollinations in a shadehouse. Hybrids derived from experiment 4.

Experiment type	Phenotype	Plant number	Pollen stainability (%)	Fruit length (mm) (n = 10)	Seeds per fruit (n = 10)
B. juncea var. *juncea* parent	*B. juncea* var. *juncea*	J1	88.6	31.5 ± 2.8	10.1 ± 4.0
	B. juncea var. *juncea*	J2	71.4	31.6 ± 4.4	9.4 ± 2.3
B. juncea var. *juncea* × *B. napus* var. *napus* Cs1; spontaneous open pollination in field among *B. juncea* var. *juncea*	F_1 hybrid	1	10.0	26.2 ± 4.7	0.1 ± 0.3
	F_1 hybrid	2	17.9	16.5 ± 2.1	0
	F_1 hybrid	3	13.5	17.9 ± 5.4	0.1 ± 0.3
	F_1 hybrid	4	14.0	19.5 ± 2.9	0
	F_1 hybrid	5	10.1	18.6 ± 4.4	0.1 ± 0.3
	F_1 hybrid	6	13.7	19.4 ± 5.6	0
	F_1 hybrid	7	17.9	20.6 ± 4.7	0.3 ± 0.5
	F_1 hybrid	8	2.3	14.4 ± 2.2	0
B. juncea var. *juncea* × *B. napus* var. *napus* Cs1; hand self-pollination in a shadehouse	F_1 hybrid	1	13.9	14.7 ± 3.0	0.2 ± 0.4
	F_1 hybrid	2	12.8	13.9 ± 3.5	0.2 ± 0.6
	F_1 hybrid	3	12.9	18.1 ± 4.1	0
	F_1 hybrid	4	19.1	16.6 ± 3.8	0
	F_1 hybrid	5	7.9	15.9 ± 2.9	0

Appendix 2 Fecundity of *B. juncea* var. *napiformis* × *B. napus* var. *napus* Cs30A F_1 hybrids. Fruit length and seeds per fruit were obtained by hand self-pollinations of the F_1 hybrids. Hybrids derived from Experiment 5.

B. juncea var. *napiformis* × *B. napus* var. *napus* Cs30A F1 hybrids	Pollen stainability (%)	Hand self-pollination		Spontaneous self-pollination		Hand backcross-pollination to *B. napus* Cl 30A	
		Fruit length (mm)	Seeds per fruit	Fruit length (mm)	Seeds per fruit	Fruit length (mm)	Seeds per fruit
Plant 8	24.8	10.4 ± 1.5	0	12.4 ± 2.6	0.14 ± 0.4	12.1 ± 2.2	0
Plant 10	22.9	11.8 ± 0.9	0	9.9 ± 0.7	0	16.6 ± 2.7	0.15 ± 0.4
Plant 006/4	33.2	–	–	13.5 ± 2.8	0	16.5 ± 3.9	0.18 ± 0.4

Appendix 3 Fecundity of *B. juncea* var. *napiformis* × *B. napus* var. *napus* Cs30A F_1 hybrids. Fruit length and seeds per fruit were obtained by spontaneous self-pollination of the F_1 hybrids. Hybrids derived from Experiment 5.

Experiment type	Plant type	Plant number	Pollen stainability (%)	Fruit length (mm)	Seeds per fruit
B. juncea var. *napiformis*	parent	1	88.3	33.8 ± 2.1	12.4 ± 2.4
	parent	2	95.2	31.7 ± 6.1	11.4 ± 3.2
B. juncea var. *napiformis* × *B. napus* var. *napus* Cs30A	F_1 hybrid	6	21.1	25.4 ± 5.2	0.2 ± 0.4
	F_1 hybrid	8	24.8	–	–
	F_1 hybrid	10	22.9	–	–
	F_1 hybrid	006/4	33.2	–	–
	Patromorph - *B. napus* var. *napus* Cs30A	1	88.8	–	–
	Patromorph - *B. napus* var. *napus* Cs30A	2	87.1	31.4 ± 3.7	4.1 ± 2.3
	Patromorph - *B. napus* var. *napus* Cs30A	3	82.7	36.5 ± 4.7	4.4 ± 2.3
	Patromorph - *B. napus* var. *napus* Cs30A	4	93.1	41.9 ± 3.7	8.8 ± 2.3
	Patromorph - *B. napus* var. *napus* Cs30A	5	49.8	37.9 ± 4.0	5.5 ± 2.2
	Patromorph - *B. napus* var. *napus* Cs30A	7	69.9	25.7 ± 4.1	0.5 ± 0.9
	Patromorph - *B. napus* var. *napus* Cs30A	9	54.7	–	–
	Patromorph - *B. napus* var. *napus* Cs30A	006/1	76.4	–	–
	Patromorph - *B. napus* var. *napus* Cs30A	006/2	81.4	–	–
	Patromorph - *B. napus* var. *napus* Cs30A	006/3	93.2	–	–
	Patromorph - *B. napus* var. *napus* Cs30A	006/5	80.9	–	–

Appendix 4 Fecundity of *B. juncea* var. *napiformis* × *B. napus* ('Melford' × 'Winfred') F_1 hybrids. Fruit length and seeds per fruit were obtained by spontaneous open pollination of the F_1 hybrids. Hybrids derived from Experiment 6.

				Fruit length (mm)		Seeds per fruit	
Experiment type	Plant type	Plant number	Pollen stainability (%)	Mean	Range	Mean	Range
B. juncea var. *napiformis*	parent	1	94.8	38.3 ± 4.9	24–45	15.2 ± 2.6	8–19
		2	76.6	37.2 ± 2.1	31–40	19.6 ± 1.4	17–22
		3	94.8	37.6 ± 3.4	32–48	18.7 ± 1.8	16–22
		4	94.4	32.4 ± 3.5	23–37	15.3 ± 2.4	8–18
B. napus var. *napobrassica* ('Melford' × 'Winfred')	parent	1	68.1	68.9 ± 4.6	62–79	26.3 ± 3.6	18–33
		2	78.0	58.1 ± 11.8	25–73	23.9 ± 6.9	0–33
B. juncea var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred')	F_1 hybrids	3	22.8	19.9 ± 5.6	13–32	2.2 ± 2.4	0–9
		4	16.8	17.1 ± 4.4	10–27	1.5 ± 1.7	0–5
		5	15.4	18.7 ± 6.7	7–33	2.1 ± 1.8	0–7
		6	26.4	9.1 ± 1.0	7–11	0	0
		7	20.0	11.5 ± 2.3	9–20	0	0

Appendix 5 Fecundity of *B. juncea* var. *napiformis* × *B. napus* (Melford × Winfred) F_1 hybrids. Fruit length and seeds per fruit were obtained by hand self-pollination and hand backcross-pollination. Hybrids derived from Experiment 7.

B. juncea var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred') F_1 hybrids	Pollen stainability (%)*	Hand self-pollination		Hand backcross-pollination to *B. juncea* var. *napiformis*		Hand backcross-pollination to *B. napus* var. *napobrassica* ('Melford' × 'Winfred')	
		Fruit length	Seeds per fruit	Fruit length	Seed set	Fruit length	Seeds per fruit
PGG1	29.0	20.2 ± 8.5	1.1 ± 1.5	28.7 ± 9.7	1.0 ± 0.9	29.0 ± 10.1	3.4 ± 2.3
PGG2	19.2	27.0 ± 5.3	3.2 ± 1.6	23.8 ± 5.9	2.1 ± 1.6	21.6 ± 6.6	0.05 ± 0.2
PGG3	35.6	15.2 ± 5.9	0.4 ± 0.9	29.4 ± 5.5	0.8 ± 0.8	30.7 ± 5.3	3.6 ± 1.8

*Two additional hybrids had 32.4% and 19.8% pollen stainability.

Appendix 6 Fertility and morphology of *B. napus* (Melford × Winfred) × *B. juncea* var. *napiformis* hybrids. Hybrids derived from Experiments 6 and 7.

Experiment type	Plant number	Plant type	Pollen stainability (%)
B. juncea var. *napiformis* × *B. napus* var. *napobrassica* ('Melford' × 'Winfred'), hand self-pollination	6/1	F1 hybrid	33.9
	6/2	F1 hybrid	31.1
	6/3	F1 hybrid	15.9
	6/4	F1 hybrid	14.4
B. napus var. *napobrassica* ('Melford' × 'Winfred') × *B. juncea* var. *napiformis*, hand self-pollination	7/1	Matromorph *B. napus* (Melford × Winfred)	97.4
	7/2	Matromorph *B. napus* (Melford × Winfred)	87.4
	7/3	Matromorph *B. napus* (Melford × Winfred)	97.2
	7/4	Matromorph *B. napus* (Melford × Winfred)	99.4