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Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*

ALLISON A. SNOW,*†‡ BENTE ANDERSEN,* and RIKKE BAGGER JØRGENSEN*
Plant Biology and Biogeochemistry Department, PBK-301, Risø National Laboratory, DK-4000 Roskilde, Denmark, †Department of Plant Biology, Ohio State University 1735 Neil Avenue, Columbus, OH, 43210, USA

Abstract

Wild relatives of genetically engineered crops can acquire transgenic traits such as herbicide resistance via spontaneous crop–wild hybridization. In agricultural weeds, resistance to herbicides is often a beneficial trait, but little is known about possible costs that could affect the persistence of this trait when herbicides are not used. We tested for costs associated with transgenic resistance to glufosinate when introgressed into weedy *Brassica rapa*. Crosses were made between transgenic *B. napus* and wild *B. rapa* from Denmark. F_1 progeny were backcrossed to *B. rapa* and BC_1 plants were selected for chromosome numbers similar to *B. rapa*. Further backcrossing resulted in a BC_2 generation that was hemizygous for herbicide resistance. We quantified the reproductive success of 457 BC_3 progeny representing six full-sib families raised in growth rooms (plants were pollinated by captive bumblebees). Pollen fertility and seed production of BC_3 plants were as great as those of *B. rapa* raised in the same growth rooms. Segregation for herbicide resistance in BC_3 plants was 1:1 overall, but the frequency of resistant progeny was lower than expected in one family and higher than expected in another. There were no significant differences between transgenic and nontransgenic plants in survival or the number of seeds per plant, indicating that costs associated with the transgene are probably negligible. Results from this growth-chamber study suggest that transgenic resistance to glufosinate is capable of introgressing into populations of *B. rapa* and persisting, even in the absence of selection due to herbicide application.

Keywords: backcrosses, *Brassica campestris*, gene flow, glufosinate resistance, oilseed rape, seed production

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Introduction

A widely acknowledged drawback of genetically engineered crops is the potential for transgenes to spread to related weeds via crop–weed hybridization (e.g. Ellstrand & Hoffman 1992; Raybould & Gray 1993, 1994; Rissler & Mellon 1996). Crops that are known to interbreed with weedy relatives under field conditions include rice, sorghum, squash, oilseed rape (canola), sunflower, sugar beet, carrot, strawberry, radish, wheat, oats, and others (Raybould & Gray 1993; Snow & Morán Palma 1997; Zemetra *et al.* 1997). Most of these economically important crops have been targeted for improvement by the addition of transgenic traits, and several have already been released commercially (e.g. virus-resistant squash and herbicide-resistant oilseed rape). Useful genes from plants, bacteria, viruses, and other organisms have been inserted into crop genomes to confer resistance to herbicides, diseases, and insect pests, as well as tolerance to environmental stresses such as cold temperatures and toxic soils. Therefore, a subset of the many agronomic traits that have been developed by recombinant DNA methods could also benefit weed species capable of hybridizing with crops, and several types of fitness-related transgenes will inevitably move into weedy populations in the next few decades (Snow & Morán Palma 1997).

It is difficult to predict the extent to which beneficial transgenic traits will cause wild taxa to become more abundant, and the ecological effects of 'escaped' transgenes

Correspondence: A. A. Snow.

‡Present address: Department of Evolution, Ecology, and Organismal Biology, Ohio State University, 1635 Neil Avenue, Columbus, OH, 43210–1293, USA. E-mail: snow.1@osu.edu

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have rarely been studied empirically (e.g. Linder & Schmitt 1995; Stewart *et al.* 1997). In the short term, the most obvious problem cases will probably be weeds that acquire transgenic resistance to widely used, broad-spectrum herbicides such as glyphosate (e.g. 'Round-up') and glufosinate (e.g. Basta, Buster, Radicale, etc.; Dyer 1994). With the exception of *Lolium rigidum* in Australia (Powles *et al.* 1998), repeated use of these particular herbicides has not yet led to the evolution of resistant weed genotypes (Bradshaw *et al.* 1997; Heap 1997), as often occurs with other types of herbicides (e.g. Holt *et al.* 1993; Warwick & Black 1994). Now, however, weedy taxa that hybridize with transgenic crops will be able to acquire resistance to glyphosate and glufosinate following crop-to-wild gene flow.

Once transgenes conferring herbicide resistance move into weeds, their frequency within local populations will be influenced by three major processes: (i) continued immigration due to gene flow from cultivated plants; (ii) selection favouring herbicide-tolerant genotypes during periods of exposure to the herbicide; and (iii) in the absence of the herbicide, the possibility of selection against these genotypes if the transgene is associated with reduced fitness. Such costs could be caused by pleiotropy, physiological costs of the resistance trait, or effects of particular insertion sites within the genome (such as linkage to deleterious alleles or disruption of coding regions; Bergelson *et al.* 1996). Costs of herbicide resistance in non-transgenic genotypes have been documented in some cases, but they are not ubiquitous (see references in Warwick & Black 1994; Lavigne *et al.* 1995). In commercialized transgenic crops, we expect that any costs of herbicide resistance will be minimal due to economic incentives for developing high-yielding herbicide-tolerant varieties. Plant breeders will select the highest-performing transgenic lines prior to marketing, such that there are rarely any yield penalties associated with the inserted trait. Therefore, a priori, costs incurred by weedy relatives that acquire the herbicide-resistance transgenes are expected to be undetectable, but this needs to be demonstrated empirically. These costs could be different in weeds than in crops due to factors such as different genetic backgrounds (leading to pleiotropy or epistasis), genotype-by-environment interactions, or crop genes that are tightly linked to the transgene insertion site and are deleterious to the weed but not the crop.

The purpose of this study was to test for costs associated with transgenic resistance to glufosinate in a widespread weed, *Brassica rapa* L. (= *B. campestris*). A secondary goal was to characterize the seed production of selected backcrossed plants (the BC_3 generation with *B. rapa* as the recurrent parent) as compared to the seed production of pure *B. rapa* to detect major abnormalities, if any, resulting from introgression. Glufosinate herbicides inhibit glutamine synthetase, an enzyme involved in preventing ammonia from accumulating in plant cells (e.g. Wild & Mandersheid 1984; Wild *et al.* 1987). Glufosinate acts within a few days after contact with the plant, resulting in localized cell death, but it is not transported systemically or absorbed by roots and it does not persist in the soil. Resistance to glufosinate can be conferred by a *bar* gene from *Streptomyces* that has been inserted into many crop species (de Block *et al.* 1987) and does not seem to be associated with a yield penalty (e.g. de Greef *et al.* 1989; Botterman *et al.* 1991; Fredshavn *et al.* 1995). Therefore, this resistance gene has the potential to persist in weedy populations that interbreed with crops, even in the absence of strong selection from herbicide use.

B. rapa hybridizes spontaneously with *B. napus*, as described in more detail below, and backcrossing to weedy populations has been demonstrated under field conditions (e.g. Mikkelsen *et al.* 1996a). *B. napus* lines with genetically engineered resistance to herbicides, lepidopteran pests, and fungal diseases have been grown in field trials in several countries (Snow & Morán Palma 1997; for searchable databases, see www.nbiap.vt.edu and www.oecd.org/ehs/projects.htm). Furthermore, transgenic glufosinate-resistant varieties of *B. napus* are already being grown commercially in Canada and the USA and are close to being approved for marketing in the European Union. We chose to focus on glufosinate-resistant oilseed rape as one of the first crops that could affect the abundance of an agricultural weed as a result of crop–wild hybridization followed by the use of this type of herbicide.

B. rapa (L.) includes cultivated forms such as oilseed rape (*B. rapa* type), turnip, Pak choi, and Chinese cabbage, as well as wild forms that occur as weeds in agricultural fields, fallow land, along roadsides, and in other disturbed sites. Commonly known as field mustard or bird rape, wild *B. rapa* is an economically important weed in temperate regions of Eurasia, North America, South Africa, Australia, and New Zealand (Prakash & Hinata 1980; Hultén & Fries 1986; Stace 1991; Holm *et al.* 1997) and is often seen in fields of oilseed rape (e.g. Stace 1975; Jørgensen & Andersen 1994). In Denmark, cultivated *B. rapa* has not been grown commercially for many decades, so we refer to weedy populations as 'wild' rather than 'feral'. Furthermore, analyses of molecular markers show that the wild populations are different from *B. rapa* that was cultivated in former times (R. B. Jørgensen, unpublished data).

B. napus ssp. *oleifera*, the more widely cultivated type of oilseed rape, is an ancient allotetraploid possessing the AA genome from *B. rapa* ($2n = 20$) as well as the CC genome from *B. oleracea* ($2n = 18$; e.g. cabbage, cauliflower, and broccoli; see U 1935, Song & Osborn 1992; Song *et al.* 1995). Thus, F_1 hybrids between *B. napus* and *B. rapa* have

a triploid AAC genome constitution of $2n = 29$. Because they have a full complement of the *B. rapa* genome, fertility of some of the F_1 progeny can be almost as high as that of pure *B. rapa* (U 1935; Jørgensen & Andersen 1994; Jørgensen *et al.* 1996; Hauser *et al.* 1997). The A and C genomes are similar enough to allow intergenomic recombination to take place, and repeated backcrossing with *B. rapa* eventually results in the loss of nonrecombined DNA from C chromosomes (Quiros *et al.* 1987; Chen *et al.* 1990, 1992; McGrath & Quiros 1990). Backcrossed progeny with high pollen fertility often have chromosome numbers of $2n = 20 - 21$ (*B. rapa* as the recurrent parent; Jørgensen *et al.* 1996; Mikkelsen *et al.* 1996a).

Crop-to-wild hybridization is facilitated by the fact that *B. rapa* is an obligate outcrosser that relies on bees, other insects, and possibly wind to effect pollen transfer (Jørgensen & Andersen 1994). Pollen from fields of *B. napus* has been detected at distances of up to 1.5 km away from the crop (e.g. Timmons *et al.* 1995, 1996; also see Scheffler *et al.* 1995), and further gene movement via human-mediated seed dispersal may be common. When *B. rapa* occurs withinfields of simultaneously flowering *B. napus*, gene flow can occur in either direction (Jørgensen & Andersen 1994) and the frequency of F_1 hybrid seeds on *B. rapa* plants can be as high as 69% (Landbo *et al.* 1996). Lower rates of hybridization are seen when the weed occurs adjacent to the crop or a short distance away (e.g. Scott & Wilkinson 1998). Spontaneous backcrossing to the weed has been demonstrated in field experiments involving both DNA markers and transgenic resistance to glufosinate (Jørgensen *et al.* 1996; Mikkelsen *et al.* 1996a). Therefore, although F_1 hybrids have an average pollen fertility of only about 50% (U 1935; Jørgensen & Andersen 1994; Jørgensen *et al.* 1996), it is very likely that crop genes can introgress into wild populations.

Materials and methods

Plant materials and crosses

We used transgenic BC_2 plants described in Mikkelsen *et al.* (1996a) to create a BC_3 generation segregating for the presence or absence of three tightly linked transgenes. The original crop parent used in these crosses was a transgenic line of Drakkar oilseed rape, serial number 93B1104, developed by Plant Genetic Systems, Belgium. The three linked transgenes are the *bar* gene for glufosinate resistance (a selectable marker as well as a commercially valuable trait), the *neo* gene coding for NPTII (conferring resistance to kanamycin, another selectable marker), and the *barstar* gene, which restores male fertility by inhibiting the function of a male-sterility transgene in a complementary line used for hybrid seed production (Mariani *et al.* 1990, 1992). These three transgenes and their respective promoters were inserted as a single construct at one locus of *B. napus*. As such, they are completely linked and are transmitted from one generation to the next as dominant alleles that follow Mendelian laws of inheritance. The *bar* and *neo* genes are expressed constituitively in green tissue, whereas the promoter of the *barstar* gene is only active in tapetal cells of developing anthers (EU Application No. C/F/95/05/01B). This multiple-gene construct has no detectable effects on the yield or competitive ability of Drakkar oilseed rape (Fredshavn *et al.* 1995).

The crosses we made to obtain the BC_3 generation involved varying numbers of plants, depending on their availability at times when crosses were conducted. First, to obtain F_1 seeds, transgenic *B. napus* was used as the maternal parent in crosses with weedy *B. rapa* collected from a wild population in southeast Zealand, Denmark (population Bc25; Fig. 1). In each of three subsequent generations, glufosinate applications were used to select transgenic progeny to serve as maternal parents, which were then crossed with weedy *B. rapa* from either population Bc25 or population P1, located at Risø National Laboratory, Roskilde, Denmark (P1 was artificially established using a mixture of seeds from three additional wild populations in southeast Zealand). Two BC_1 plants were selected for further crossing based on the fact that they had pollen fertilities > 95%, chromosome numbers of 20–21, and growth forms similar to pure *B. rapa* (these were plants FF13 and AA23 in Mikkelsen *et al.* 1996a). These two 'grandmothers', referred to as 1 and 9 in this study, were then crossed with one *B. rapa* plant to obtain the BC_2 generation. For the BC_3 generation, six BC_2 progeny were each crossed with a different *B. rapa* plant. Thus, the BC_3 progeny used in this study were divided into six full-sib families: 1–3, 1–4, 1–10, 1–12, 1–13, and 9–14, with the first number denoting the seed's 'maternal grandmother' and the second number referring to the *B. rapa* paternal parent from wild population P1 (each full-sib family had a unique maternal and paternal parent).

Because our maternal plants were always hemizygous at the transgene locus, segregation ratios in the BC_3 generation were expected to approximate to 1:1 for glufosinate-resistant:glufosinate-sensitive genotypes. Transgenic progeny were always used as maternal plants in our crosses, and therefore the BC_3 generation also possessed the cytoplasmic DNA of *B. napus*. In the field, the weed can sire seeds on the crop (e.g. U 1935; Jørgensen & Andersen 1994) and these seeds could escape being harvested; therefore it is not unrealistic to focus on BC_3 plants with cytoplasmic DNA from *B. napus*. However, future studies should also consider crosses in which *B. rapa* is the maternal parent.

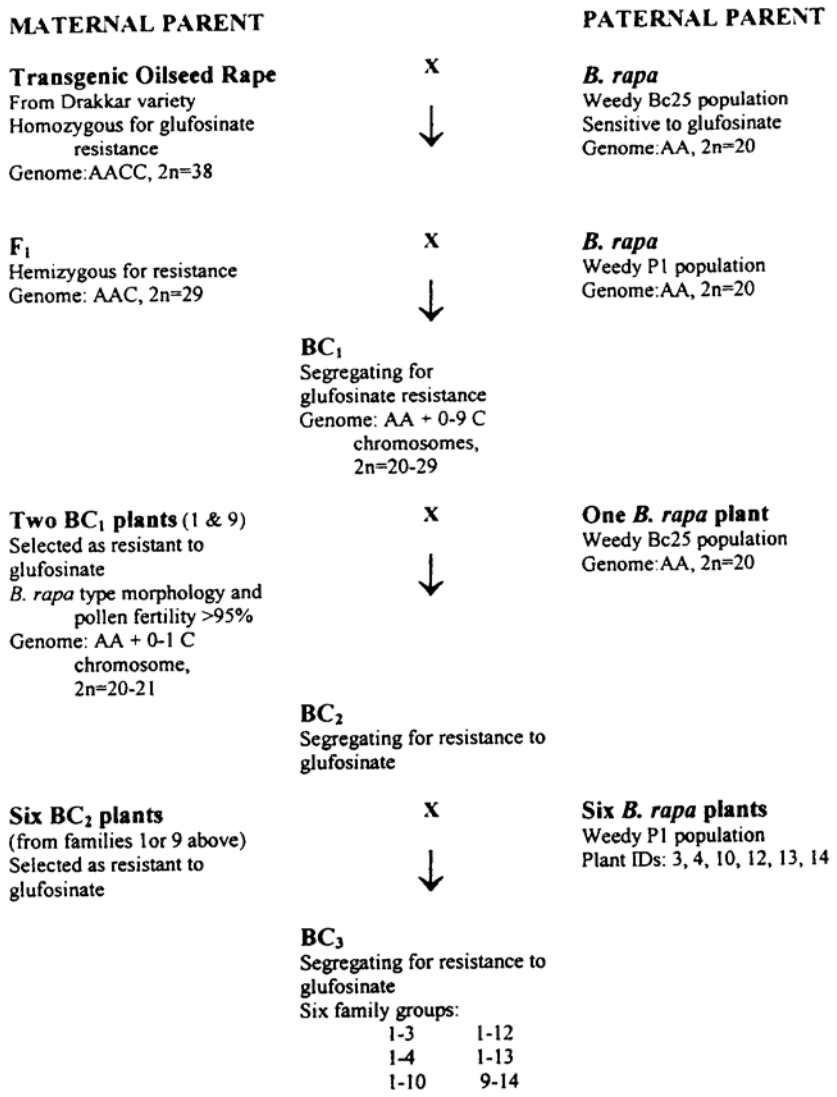

Fig. 1 Crossing design for obtaining BC_3 families.

Experimental procedures

To test for costs associated with glufosinate resistance, we grew plants that segregated for the presence or absence of glufosinate resistance and recorded the total number of fruits and seeds produced by each plant. The BC_3 material consisted of plants from full-sib families in order to minimize genetic variability and thereby maximize the chance of detecting subtle effects due to the transgenes. Progeny from each family were grown under the same environmental conditions, but due to lack of space and temporary technical problems with two growth rooms, it was not possible to put all of the families in the same growth room or to distribute them equally among rooms (Table 1). Therefore, seed production differences among families cannot be distinguished from differences due to environmental conditions of the growth rooms because these two factors were confounded. All plants within each family were treated identically, however, such that the effects of being transgenic were tested within each family and the entire data set was analysed by nesting families within growth rooms (see below). We also grew pure *B. rapa* plants from the P1 population to serve as a reference for general comparisons with the BC_3 generation (Table 2).

Seeds from each BC_3 family and the pure *B. rapa* group were germinated in Petri dishes using standard methods for breaking seed dormancy in *B. rapa* (Landbo *et al.* 1997). Seedlings were transferred to small pots filled with standard potting soil and maintained in a growth chamber with 16 h of daylight and temperatures averaging 18 °C by day and 15 °C by night. When each plant produced its third true leaf, about 2 weeks after germination, it was transplanted to a larger pot (10 cm diameter) and moved into one of three large growth rooms. Environmental

Table 1 Experimental protocol, initial sample sizes, and survivorship of BC_3 plants. Leaf dot test refers to topical applications of glufosinate (see text)

	Family 1–3	Family 1–10	Family 1–4	Family 1–12	Family 1–13	Family 9–14
Growth room	1	1	2	2	3	3
Genotype screening method	Leaf dot test	Leaf dot test	Leaf dot test and PCR marker	Leaf dot test dot and PCR marker	PCR marker	PCR marker
No. of seeds started	73	134	110	160	130	90
No. of seeds germinated	73 (100%)	92 (70%)	93 (85%)	90 (56%)	80 (62%)	77 (865)
Proportion of plants with ≥ 1 fruit	1.00	0.99	0.91	1.00	0.94	0.95

conditions in the growth rooms were the same as those described above, but the halogen lamps in room 3 were somewhat brighter than those in rooms 1 and 2. Plants in each family were numbered in the order in which they were transplanted and the wild *B. rapa* plants were equally divided between room 2 and room 3, with odd-numbered plants going to one room and even-numbered to the other (see Tables 1 and 2 for sample sizes; space limitations prevented us from using room 1 for *B. rapa*). From past experience, we found that *B. rapa* raised in cool-temperature growth rooms are healthier and larger than those raised in greenhouses, where temperature control is more difficult. Although light levels under halogen lamps are lower than full sunlight, plants raised in growth rooms show no symptoms of light deprivation and attain sizes similar to those of field-grown plants.

Plants were watered once a day, and every 2 weeks they were moved to a randomly chosen position within the growth room to minimize effects of variable light or water availability within rooms. The pots were placed close together at a density of about 64 plants/m^2, such that after they bolted and produced side-branches they competed for light to some extent. When flowering began approximately 5 weeks after germination, we placed a hive of bumblebees in each room to allow seminatural pollination of these obligately outcrossing plants (Biobest greenhouse pollination kits, Westerlo, Belgium; hives were replaced every 2–3 weeks). During 'daylight' hours,

Table 2 Reproductive characteristics of BC_3 plants (transgenic vs. not transgenic) and pure *Brassica rapa*. Differences between pairs of means in the same family were analysed using *t*-tests. SE and sample sizes are given in parentheses

	BC_3 Family						Pure *B. rapa*	
	1–3	1–10	1–4	1–12	1–13	9–14	P1 population	
Growth room	1	1	2	2	3	3	2,3	
Pollen fertility	95% (0.01, 20)	93% (0.02, 20)	88% (0.02, 20)	90% (0.01, 20)	88% (0.03, 20)	88% (0.02, 20)	92% (0.01, 20)	
Seeds per fruit								
Transgenic	10.4 (0.8, 35)	11.4 (0.7,47)	10.3 (0.8, 29)	14.0 (0.6, 44)	9.1 (0.8, 46)	15.7 (0.7, 30)	Rm 2	13.0 (0.59, 42)
Nontransgenic	10.0 (0.6, 34)	11.8 (0.7, 40)	9.4 (0.7, 51)	14.0 (0.7, 44)	8.7 (0.8, 26)	16.3 (0.8, 31)	Rm 3	15.1 (0.58, 41)
	NS	NS	NS	NS	NS	NS		$P < 0.05$
Fruits per plant								
Transgenic	64.2 (4.6, 35)	50.3 (2.9, 47)	104.5 (6.8, 29)	70.0 (2.6, 44)	121.0 (53,46)	80.7 (3.0, 30)	Rm 2	57.1 (3.8, 42)
Nontransgenic	80.9 (6.2, 34)	50.4 (4.0, 40)	126.5 (5.8, 51)	67.4 (4.0, 44)	120.3 (8.6, 26)	84.1 (3.0, 31)	Rm 3	68.2 (2.8, 41)
	$P < 0.05$	NS	$P < 0.05$	NS	NS	NS		$P < 0.05$
Seeds per plant								
Transgenic	653 (63, 35)	584 (46, 47)	986 (71, 29)	967 (50, 44)	991 (78, 46)	1256 (70, 30)	Rm 2	762 (56, 41)
Nontransgenic	767 (55, 34)	593 (46, 40)	1111 (63, 51)	894 (46, 44)	972 (86, 26)	1351 (70, 31)	Rm 3	1041 (59, 41)
	NS	NS	NS	NS	NS	NS		$P < 0.001$

≈ 10–15 bees foraged for pollen and nectar at any given time in each room. Bumblebees proved to be very active and efficient pollinators, and periodic checks indicated that fruit set was close to 100%. To characterize pollen fertility, we collected anthers from two flowers on 20 randomly chosen plants from each BC_3 family and from the *B. rapa* plants. Fertility was scored as in Jørgensen & Andersen (1994) by staining the pollen with cotton blue and counting the numbers of nonviable and normal pollen grains in a sample of 400 grains per plant.

Transgenic resistance to glufosinate was scored in two ways. First, when each plant had several leaves longer than 6 cm, a 9-mm diameter leaf disc was removed and frozen for later DNA extraction and screening with a PCR-based marker for the transgenic construct (personal communication from Plant Genetic Systems; methods as in Mikkelsen 1996). Two more discs were sampled from each plant at the time of flowering to provide back-up DNA. In order to standardize leaf damage, all plants had leaf discs removed, including the pure *B. rapa*. To save time and expense, the PCR method was used for only a portion of the BC_3 families, as shown in Table 1.

The second method for identifying transgenic plants was to determine whether they were sensitive to glufosinate by means of a leaf dot test. A 4 × 8 mm piece of filter paper was dipped in a 1% vol. solution of glufosinate and placed on the distal portion of a healthy leaf for ≈ 2 h. The leaf was marked with permanent ink and checked 5–7 days later for damage. Sensitive plants exhibited an obvious area of localized cell death, which sometimes covered 2–5-times the area of the original exposure site, whereas resistant plants showed no leaf damage (these plants were checked again 10 days after exposure). To minimize damage to nontransgenic plants, the dot test was not used until the end of the flowering period, when most plants were at least 50 cm tall and the majority of their fruits were fully formed. Also, to equalize damage on both sensitive and resistant plants, we removed the distal half of each labelled leaf. This was also done with the *B. rapa* plants in room 2 but not those in room 3, where the dot test was not used to identify transgenic plants. For a sample of 74 BC_3 plants from room 2, equally divided between families 1–4 and 1–12, we used both the PCR marker and the leaf dot test to determine how closely the two sets of results agreed. The same result was obtained in 99% of these samples (all but one), leading us to conclude that both methods give the same results.

Survival and lifetime seed production were quantified to compare the performance of transgenic vs. nontransgenic plants. When the plants stopped flowering 5–6 weeks after the first flowers opened, we counted the number of fruits per plant and collected 10 mature, undehisced fruits from each plant for seed counts (fruits were randomly selected from two to three branches per plant). In a few cases, it was not possible to retrieve 10 fruits, so a smaller sample was used. We then calculated the average number of seeds per fruit and the number of seeds per plant (number of fruits × average number of seeds per fruit).

Statistical analyses

Frequency data were analysed using chi-square tests. The statistical software program SAS (SAS 1994) was used for ANOVAs (PROC GLM) and comparisons between pairs of means (*t*-tests). Two-tailed *t*-tests were used because it was possible that the transgene could result in either a cost or a benefit due to linkage with other genes from the crop. Type III sums of squares were used in all ANOVAs. In the ANOVAs, families were nested within growth rooms to account for each of these sources of variation.

Results

Per cent seed germination varied from 56% to 100% among families (Table 1), indicating possible genetic differences in the degree of seed dormancy or viability. Overall, the proportion of BC_3 seedlings that were herbicide resistant was 0.51, which is very close to the expected Mendelian proportion of 0.50 ($N = 457$). This pattern was also seen within four of the six full-sib families (Fig. 2) but in Family 1–4 there were significantly fewer transgenic progeny than expected (36%, $P < 0.025$), and in Family 1–13 there were significantly more transgenic progeny (64%, $P < 0.025$). These differences could be due to chance, however, because they were not statistically significant in a sequential Bonferroni test, which is more appropriate when several chi-square tests are reported together (Rice 1989). Other deviations from the expected proportion of 0.50 were not statistically significant.

The proportion of seedlings that survived to maturity and produced at least one fruit was greater than 0.90 in all families (Table 1) and was not affected by whether the plant was transgenic (chi-square tests). In addition to this small amount of mortality, we inadvertently damaged a few plants during pot rotations, and several plants could not be used because of problems with scoring for glufosinate resistance by means of the DNA marker (due to poor quality of the DNA). Thus, we started with 505 BC_3 seedlings, 457 of which could be used for analysis, and little of this difference was attributed to survivorship of BC_3 plants. Survivorship of pure *B. rapa* was > 95% (data not shown). All plants grew vigorously under the environmental conditions of this experiment and attained final sizes that were comparable to those of *B. rapa* plants in the field.

Analysis of variance showed that the total number of seeds per plant was affected by family and growth room, but was not influenced by whether the plants were

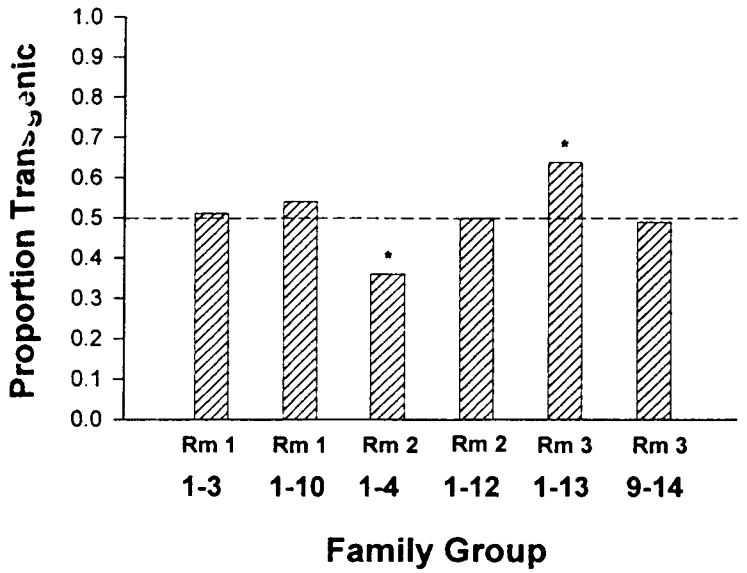

Fig. 2 Frequency of transgenic BC_3 progeny. The dotted line illustrates the expectation for equal frequencies. Chi-square tests were used to compare frequencies of transgenic vs. nontransgenic progeny in each family; sample sizes as in Table 2. * indicates $P < 0.025$ (see text for results of sequential Bonferroni test).

transgenic (Tables 2 and 3; Fig. 3). Therefore, based on this measure of overall performance, no cost of transgenic resistance to glufosinate was detected. Similar conclusions can be drawn from comparisons of the number of seeds per fruit, which varied among families and growth rooms but did not differ between transgenic vs. nontransgenic plants. For the number of fruits per plant, a marginally significant ($P < 0.07$) main effect of the transgenic construct was detected, as well as a marginally significant family-by-genotype interaction ($P < 0.10$, Table 3). These effects were due to Families 1–3 and 1–4, in which the number of fruits per plant was about 20% lower in transgenic plants as compared to those that were not transgenic ($P < 0.05$ in both cases; Fig. 3).

In comparing the fecundity of the BC_3 generation, whose fathers were from the P1 population, with that of pure *B. rapa*, it is important to note that the latter represents a more diverse mixture of genotypes from the P1 population, and may have differed from the relatively small group of the *B. rapa* plants involved in our crosses (Fig. 1). Also, the BC_3 plants were derived from BC_1 plants that were selected to have pollen fertility, chromosome

Table 3 ANOVAs for the effects of the transgene construct, growth room, and family (nested within growth rooms) on seed and fruit production of BC_3 plants

	Model R^2	df	Mean square	P
Seeds per fruit	0.20			
Transgene		1	0.39	NS
Room		2	68.42	0.04
Family		3	644.08	0.0001
Transgene × Family		5	9.12	NS
Error		445		
Fruits per plant	0.44			
Transgene		1	3208	0.07
Room		2	59109	0.001
Family		3	49357	0.001
Transgene × Family		5	17852	0.10
Error		445		
Seeds per plant	0.23			
Transgene		1	243112	0.21
Room		2	7728868	0.0001
Family		3	969299	0.0004
Transgene × Family		5	127891	0.52
Error		445		

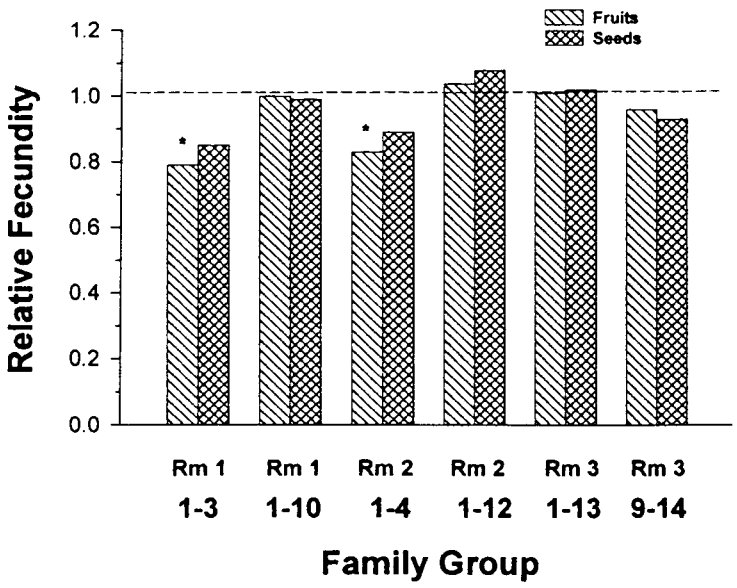

Fig. 3 Relative fecundity of transgenic BC_3 progeny, calculated as the average number of seeds or fruits of transgenic plants divided by the corresponding mean for nontransgenic plants. The dotted line illustrates the expectation for equal fecundity. *t*-tests were used to compare fruit or seed production of transgenic vs. nontransgenic plants within families (as in Table 2). * indicates $P < 0.05$.

numbers, and growth forms similar to those of pure *B. rapa* (Fig. 1). With these considerations in mind, we can compare the pollen fertility and seed production of BC_3 plants with those of wild plants that were raised in the same growth room. Pollen fertility of plants in the BC_3 families averaged 88–95% and was not significantly different from the pollen fertility of *B. rapa* (92%, Table 2; ANOVA, details not shown). There were no detectable effects of glufosinate resistance on pollen fertility of BC_3 plants, nor did differences between growth rooms affect these variables for *B. rapa* (analyses not shown). The number of seeds per plant varied among BC_3 families, as described above, and was higher than or similar to that of *B. rapa* plants from the same growth room (Table 2). Based on these comparisons involving four BC_3 families, we conclude that the lifetime fecundity of BC_3 plants was similar to that of a mixture of pure *B. rapa* genotypes.

Discussion

This study confirmed that transgenic herbicide resistance was transmitted to the BC_3 generation at an average frequency of ≈ 0.50, as expected for a dominant Mendelian trait. Predictable expression of transgenic herbicide resistance has also been documented in other studies with *B. napus* hybrids (e.g. Baranger *et al.* 1995; Brown & Brown 1996; Mikkelsen 1996; Metz *et al.* 1997), including fifth generation backcrosses with *Raphanus raphanistrum* (Chevre *et al.* 1997), but segregation ratios sometimes deviate from expected frequencies (e.g. Mikkelsen *et al.* 1996a; Metz *et al.* 1997). The present experiment showed that the frequency of transgenic offspring varied somewhat among families, perhaps due to differential fertilization, abortion, or seed germination. In one family (1–13), 64% of the BC_3 progeny were transgenic, indicating an unexpected advantage associated with the transgene construct. It is noteworthy that the family with only 36% transgenic BC_3 plants (Family 1–4) was also one of two families in which the transgene construct was associated with a significant reduction in the number of fruits per plant and a smaller, nonsignificant reduction in the number of seeds per plant (Fig. 3). The combined effects of reduced transmission and lower fecundity suggest that transgenic progeny were less fit than those lacking the transgene in this particular family.

In general, however, costs associated with transgenic glufosinate resistance were small or undetectable. Presence of the transgene construct did not explain a significant portion of the variability in survival or in the number of seeds per plant in any of the six families, nor did transgenic plants exhibit reduced pollen fertility. Transgenic BC_3 plants produced fewer fruits per plant than nontransgenic plants in two of the six families, but we regard total seed output to be a more relevant component of overall fitness than fruit number. Therefore, we conclude that, under the conditions of this experiment, the costs associated with transgenic herbicide resistance were negligible. Apparent differences among closely related full-sib families were surprising, given the extent of their shared ancestry (Fig. 1), but these differences should be interpreted cautiously because our experimental design confounded the effects of family and growth room. It would be useful to carry out further studies in the field, with more plant families and several levels of competition, to determine whether any costs of transgenic glufosinate resistance can be detected under natural conditions.

A secondary goal of this study was to compare the survival, pollen fertility, and seed production of BC_3 plants with those of pure *B. rapa*. Previous studies have shown that F_1 and BC_1 plants are highly variable in their pollen fertility (U 1935; Jørgensen & Andersen 1994; Jørgensen *et al.* 1996; Hauser *et al.* 1998a,b). In the present experiment, BC_1 plants with > 95% pollen fertility and 20–21 chromosomes were intentionally selected for further crosses in order to utilize the types of individuals that are most likely to succeed in passing crop genes on to successive generations. Thus, as expected, the BC_3 generation had high pollen fertility and high survival. One surprising result was that the seed output of these BC_3 families was similar to or greater than the seed production of pure *B. rapa* grown under the same conditions (Table 2). Earlier generations usually exhibit lower seed production than pure *B. rapa*, perhaps due to meiotic disturbances associated with univalent C chromosomes (U 1935; Jørgensen & Andersen 1994; Brown & Brown 1996; Jørgensen *et al.* 1996; Mikkelsen *et al.* 1996b), but fitness disadvantages of hybrid progeny are expected to diminish with each successive generation of backcrossing (e.g. Chevre *et al.* 1997). In this context, it is interesting that the BC_3 plants in our experiment produced at least as many seeds as pure *B. rapa*, despite the fact that a portion of their genome (about 6%) was derived from *B. napus*. If this pattern also holds for plants in the field, it suggests that after crop alleles have introgressed into *B. rapa* for a few generations, those that are not strongly deleterious and are not linked to other deleterious traits would be able to persist for many subsequent generations.

Few previous studies have tested for costs associated with transgenic herbicide resistance in a rigorous manner, with proper controls for costs that could be due to the transformation process itself (e.g. somaclonal mutations, insertion site effects, effects of marker genes). Thus, it is difficult to interpret results from studies comparing transgenic lines with nontransgenic lines (for example, as in Crawley *et al.* 1993; Hails *et al.* 1997). A better method for identifying costs associated with a transgenic construct is to compare progeny that segregate for the presence or absence of the construct (Bergelson *et al.* 1996). Even with this method, which our study employed, it is not possible to determine whether a reduction in the fitness of transgenic progeny is due to the herbicide-resistance trait itself or to other factors, such as disruption of a coding region of the genome or linkage to other transgenes or deleterious crop genes. Bergelson *et al.* (1996) addressed this problem by comparing four transformed lines of *Arabidopsis thaliana* in which segregating progeny were selfed and then tested in a field plot. Transgenic resistance to the herbicide chlorosulphuron led to a 34% decrease in lifetime seed production, with no differences among the transformed lines. Because the same cost was found in plants from different transformed lines, this penalty is unlikely to be due to position effects.

In our case, we must limit our conclusions to a particular transgenic line of oilseed rape and we acknowledge that further field studies are desirable. Nonetheless, our results support the widespread assumption that once a transgenic trait such as glufosinate resistance has been selected for marketing, it is not likely to entail a strong fitness disadvantage when transferred to weedy *B. rapa* populations. Although our main conclusions are not surprising, it is important to carry out this type of study because the inheritance and expression of transgenes sometimes deviate from what is expected (e.g. non-Mendelian ratios in two of our six BC_3 families, and Bergelson *et al.* 1998). Also, there is some practical urgency for knowing more about this particular weed–crop complex because *B. rapa* is already a serious weed of more than 20 crops in over 50 countries (Holm *et al.* 1997) and glufosinate-resistant oilseed rape is now being grown commercially.

If further field experiments also demonstrate little or no costs associated with transgenic resistance to glufosinate, it is possible that the spread of this transgene to natural populations of *B. rapa* will lead to infestations of this species that are more difficult to control, especially in fields of transgenic oilseed rape where glufosinate applications are used as a primary control method. In the near future, we expect that crops such as oilseed rape will possess additional fitness-related transgenes such as those conferring resistance to lepidopteran pests, fungal diseases, and other types of herbicides such as glyphosate. Further research on ecological implications for weedy relatives should focus on the benefits and well as possible costs associated with multiple fitness-related traits.

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The authors are investigating gene flow, introgression, and the ecological consequences of escaped transgenes in several crop–weed systems in Europe and the USA. This research was carried out while Allison Snow was on a sabbatical leave from Ohio State University, where she is an associate professor. Rikke Bagger Jørgensen is a senior scientist and Bente Andersen is a staff researcher at Risø National Laboratory.
