

# Transgenic weed beets: possible, probable, avoidable?

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## Summary

1. Weed beets pose a serious problem for sugar beet *Beta vulgaris* crops. Traditionally, the only efficient method of weed control has been manual removal, but the introduction of transgenic herbicide-tolerant sugar beets may provide an alternative solution because non-tolerant weed beets can be destroyed by herbicide. We evaluated the possibility that new, transgenic, weed beets may arise by gene flow between wild and crop plants.

2. In a study area in northern France, weed beets were present in variable densities in sugar beet fields of up to 80 weed beet plants m<sup>-2</sup>. Weed beets arise from a long-lived seed bank, with seeds germinating from depths of 5 cm or less. In addition, diploid F<sub>1</sub> crop–wild hybrids and triploid variety bolters (individuals with a low vernalization requirement) were present in low densities in virtually all sugar beet fields. We found gene flow to be possible between all forms, illustrated by both overlapping flowering periods in the field and successful controlled cross-pollinations.

3. The F<sub>1</sub> crop–wild hybrids result from pollination in the seed-production region by wild plants possessing the dominant bolting allele B for flowering without experiencing a period of cold. In the case of a transgene for herbicide tolerance incorporated into male-sterile seed-bearer plants, such hybrids will contain both the herbicide-tolerance and the bolting allele. Contamination of the fields by transgenic weed beets will be the result unless bolters are removed manually. The same will apply in the case of a cytoplasmically inherited transgene.

4. Incorporation of the transgene into the pollinator plants will prevent the immediate formation of transgenic weed beets. However, in sugar beet fields, variety bolters may successfully cross-pollinate with weed beets in neighbouring fields. The use of diploid pollinator plants instead of tetraploids will considerably enhance gene flow towards wild beets, and is not, therefore, an attractive option.

5. In conclusion, the appearance of transgenic weed beets is possible but can best be retarded if the transgene for herbicide tolerance is incorporated into the tetraploid pollinator breeding line.

*Key-words:* *Beta vulgaris*, biosafety, gene flow, genetically modified organisms, population dynamics, seed bank

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## Introduction

Given the increasing likelihood of genetically modified crops being grown on a commercial scale, there is an urgent need for more knowledge of the possibilities of gene exchange between crops and their wild relatives (Ellstrand & Hoffman 1990; Rogers & Parkes 1995; Hancock, Grumet & Hokanson 1996). The greatest

concern is the escape of transgenes from the crop to the wild and their subsequent introgression into the genome of wild relatives (Raybould & Gray 1993; Dale 1994; Darmency 1994; Gliddon 1994; Kareiva, Morris & Jacobi 1994; Linder & Schmitt 1994; Raybould 1999). A distinction must be made between crops cultivated for their seeds, such as cereals, sunflower *Helianthus annuus* L., rapeseed *Brassica napus* L., etc., and crops cultivated for their vegetative parts that are not supposed to flower in the fields, for example sugar beet *Beta vulgaris* L. and chicory *Cichorium intybus* L. Most seed crops will easily exchange genes in the presence of close wild relatives (Ellstrand, Prentice & Hancock 1999).

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Vegetable crops pose less of a risk, but are cultivated from seed, which has to be produced, thus creating the possibility of gene exchange in seed-production areas (Bartsch *et al.* 1999), albeit on a smaller scale.

In our study species, the sugar beet *Beta vulgaris* ssp. *vulgaris* (biennial, self-incompatible and largely wind pollinated), there is a Mendelian gene of which the dominant B or bolting allele cancels vernalization requirement (Munerati 1931; Boudry *et al.* 1994; Abe, Guan & Shimamoto 1997). Instead of remaining vegetative when sown in late spring, plants possessing this allele are able to produce seeds before the roots are harvested. The B allele is present in wild (ruderal) populations near the main sugar beet seed-production regions in south-west France (Boudry *et al.* 1993) and recurrently contaminates the commercial sugar beet seed at a low frequency, giving a few bolting plants per hectare in the sugar beet fields. These plants can eventually lead to weed beets.

We studied the introgression of wild genes and in particular the B allele into the transgenic crop (creating an agronomic problem), rather than the introgression of transgenes into the wild populations (which may create an ecological problem outside the agro-ecosystem). Nevertheless, the presence of flowering beets in sugar beet fields near the coast may favour gene flow from the crop to the wild beets in their natural habitat, i.e. the sea beets, *Beta vulgaris* ssp. *maritima*. Beets form a crop-wild complex in which genetic information is freely exchangeable between the different parts of the complex (Van Raamsdonk 1995). Several authors have identified the potential for cultivated beet to hybridize with their wild relatives, together with the ability of the crop to produce weedy forms (Keeler 1989; Kapteijns 1993; Bartsch *et al.* 1996a; Van Raamsdonk & Van Der Maesen 1996; Bartsch & Schmidt 1997; Van Raamsdonk & Schouten 1997; Bartsch & Ellstrand 1999).

Weed beets have arisen as a consequence of poor agricultural practice (Williamson 1993). Since the mechanization of sugar beet cultivation in the 1950s, farmers have not eliminated bolters from their fields so thoroughly. As a consequence, numerous fields have been invaded by weedy forms of beet carrying the B allele (e.g. in northern France but also in other European countries; Hornsey & Arnold 1979; Longden 1993). Because beet has a long-lived seed bank (Desprez 1980) and can shed thousands of seeds (Bartsch *et al.* 1996a), weeds reappear each spring. They are easily controlled by selective herbicides in crops other than sugar beet, but because they belong to the same species as cropped beet they are insensitive to the selective herbicides used for sugar beet cultures. Traditionally, the only successful remedy has been the systematic manual removal of bolters, which is currently regarded as good agricultural practice for beet growers (Brants & Hermann 1998). Unfortunately, if weed beets are numerous, complete removal by hand is impractical.

Occasionally the beets sown by the farmer also bolt and flower. There are two types of 'sown bolters'. First,

there are the F<sub>1</sub> crop-wild hybrids resulting from sugar beets that were pollinated by wild beets containing the B allele in the seed-production area. These hybrids are probably the original source of the weed beets (Boudry *et al.* 1993; Desplanque *et al.* 1999). The second type of bolter can occur if individuals in the crop, generally selected for a strong bolting resistance, receive sufficient cold for vernalization. The studies by Boudry *et al.* (1993) and Desplanque *et al.* (1999) excluded the possibility that weed beets originated from this type.

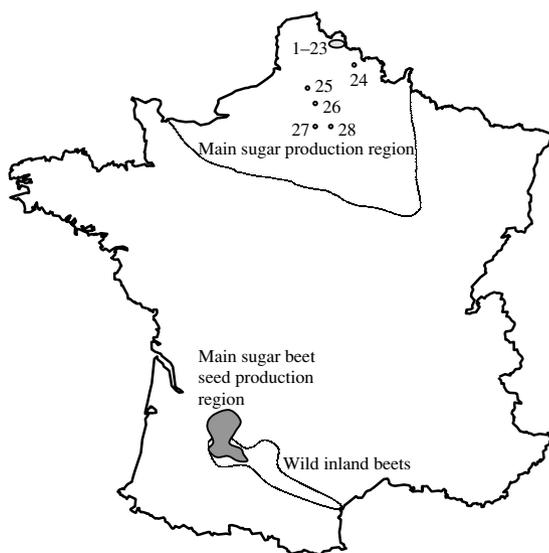
With the advancement of gene technology there is now a new, very attractive, opportunity to tackle the weed beet problem (together with the other weeds; for *Chenopodium album* L. in particular see Freckleton & Watkinson 1998; Watkinson *et al.* 2000); the use of sugar beets possessing a transgene for tolerance to a non-selective herbicide (for a first study on the impact on biodiversity see Elmgaard & Bruus Pedersen 2001). All plants without that tolerance gene, even the very close relatives, could be completely eliminated by the herbicide. This raises the question of the likelihood that the tolerance gene might escape to the weeds, creating a similar weed problem, this time with transgenic weed beets insensitive to the herbicide (for a discussion about 'super-weeds' see Kling 1998). The B allele and the tolerance gene would have to combine in a single individual, being able to survive and reproduce under herbicide treatment. Unsurprisingly, hybrids between wild beets and transgenic sugar beets are easily obtained (Bartsch & Pohl-Orf 1996; Dietz-Pfeilstetter & Kirchner 1998). It should be noted that there is no principal difference between conventional herbicide or transgenic tolerance, so all scenarios considered also apply to conventional tolerance, should it occur.

The most important variables with direct consequences for gene flow are:

1. the parent containing the transgene (the male-sterile seed bearer or the hermaphrodite pollinator);
2. in the case of the male-sterile seed bearer, the genome containing the transgene (the nuclear genome or one of the cytoplasmic genomes);
3. in the case of the pollinator plant, its ploidy level.

Until recently, most European sugar beets were triploid as a result of diploid male-steriles pollinated by tetraploid pollinator plants. The pollen of tetraploids is known to be less competitive compared with the pollen of diploids (Hecker 1988), and triploids may have problems with gamete production (Hecker & McClintock 1988).

In this study we quantified the present weed beet problem in northern France by estimating population dynamic parameters. We examined to what extent gene flow is possible between the different forms of beet in this area. Together with the available information about the situation in the French seed-production region, we wanted to investigate how the three variables listed above play a role in limiting gene flow. The final aim was to find the best scenario for the introduction of herbicide tolerance: the one which minimizes the



**Fig. 1.** The position of the fields sampled (1–23 near the University campus, 24 Bugnicourt, 25 Esquennoy, 26 Gournay sur Aronde, 27 Poulainville, 28 St Just) together with the main sugar production area, the main seed-production area and the wild inland (ruderal) beets in France.

probability or the speed of the appearance of transgenic weed beets.

## Materials and methods

### STUDY SITE

We studied 23 sugar beet fields within 10 km of Lille University campus (numbers 1–23), and five populations elsewhere in northern France (numbers 24–28; Fig. 1) selected because no weed control was practised. The major seed-production region and the area where wild ruderal beets grow in south-west France are also shown on Fig. 1.

### SEED BANK EVALUATION

The real diaspore in beet is not the seed itself but the seed ball, a non-dehiscent corky structure resulting from the fusion of 1–8 flowers. Each fruit in the non-divisible seed ball contains one seed.

Cylindrical soil samples, 5 cm in diameter, were taken to a depth of 50 cm in six fields (numbers 12–17) in April 2000. The last year in which sugar beet was grown and the qualitative degree of weed contamination (low: less than 100 bolters ha<sup>-1</sup>; high: more than 1000 bolters ha<sup>-1</sup>) was recorded. Density of seed as a function of depth (seed balls per volume) was assessed in 10 soil samples from each of the fields by suspending the soil in water and sifting out all particles larger than 2 mm in diameter. Viability of the beet seed balls detected was estimated by recording the germination rate 3 weeks after sowing in trays with soil at 5 mm depth and at temperature 20 °C.

Seeds obtained previously from field 14 were used to test the influence of soil depth on germination probability. The seeds were buried at various depths in the same type of clay soil as in the sugar beet fields studied. We verified that it was free from beet seeds before use. After 3 weeks at 20 °C the percentage of seed balls from which one or more seedlings had germinated was observed for each depth.

### DETERMINATION OF THE PLOIDY LEVEL

The ploidy level was estimated indirectly by counting the number of chloroplasts in the stoma guard cells (Jahier 1992), a technique frequently used by sugar beet breeders (Brown *et al.* 1991). The mean value of 10 stomata (20 cells) was calculated for each individual. The ranges of values per stoma described for discriminating diploids, triploids and tetraploids were 12–18, 19–25 and 26–28, respectively. There is a small overlap between diploids and triploids (for the values 18 and 19): in the rare cases of ambiguity, more cells were counted.

### MEASUREMENT OF FITNESS

Different numbers of plants were used for fitness measurements: weeds were available in large quantities, whereas F<sub>1</sub> hybrids and variety bolters were rarer and had to be checked for ploidy. The latter, in particular, were not always available for all measurements.

Germination rate was tested in trays with compost in a glasshouse at 20 °C and 16-h day length. After 20 days the seedlings were cut and the containers allowed to dry out. Three weeks later they were moistened again, leading to a second germination period. Total germination was measured for weeds from 11 fields (numbers 13–15 and 21–28) using nine to 15 families (i.e. the seeds harvested on a single plant) per field and 30 seed balls per family. The germination of seeds harvested on F<sub>1</sub> crop–wild hybrids or variety bolters from four fields (numbers 12 and 18–20) was tested using three to eight families per field (the total number of families was 21, of which 16 were harvested on a diploid plant and five on a triploid) and 50 seed balls per family.

Juvenile mortality (before flowering or the formation of adult rosettes) and flowering rate without vernalization were measured in the glasshouse at a constant temperature of 20 °C and a photoperiod of 16 h. There was some doubt whether the triploid variety bolters suffered from aneuploidy in their progeny, possibly with early effects on survival.

Pollen viability was tested using the staining method described by Alexander (1969). For each plant tested, two flowers were used and one anther per flower. The pollen viability was calculated as the proportion of fertile pollen grains, indicated by the cytoplasm staining purple. For each anther more than 300 pollen grains were counted. In total, 222 weed beet offspring and 29 F<sub>1</sub> crop–wild hybrid offspring were tested.

## FLOWERING PHENOLOGY

The bolting and flowering stages of sugar beet plants were qualitatively followed in 10 fields varying in weed beet density. Reproductive plants were counted during one short period (22–25 July 1997) in fields with no weed beets nor any weed control, thus containing only F<sub>1</sub> hybrids and variety bolters that were identified by their ploidy. Four stages were distinguished: plants bolting but not yet flowering; plants that had just started flowering; plants at full flowering; and plants after flowering bearing more or less mature fruits.

## CROSSES

Controlled crosses between plants were performed by isolating couples or groups of (hermaphroditic) plants in pollen-proof cages in the glasshouse. In all cases the plants crossed were dug up from the same field.

## Results

## WEED BEET DENSITY

Weed beets in sugar beet fields can generally be recognized without ambiguity from their position within the field. The crop sugar beet are sown in rows at regular distances with a final density of about nine plants m<sup>-2</sup>. All beets growing outside the row, bolting or in rosette, were by definition weed beets and were morphologically distinct from the surrounding sugar beet. We found densities varying between none or a few plants per hectare to over 80 plants m<sup>-2</sup>. Usually they were not homogeneously distributed over the field: they often appeared in clusters, probably around the position of their maternal parent in a previous sugar beet culture. At moderate weed beet densities, random samples in two fields (numbers 1 and 2) recorded a mean value of 0.24 weed beets m<sup>-2</sup>, with more intensively infested parts yielding 2.7 plants m<sup>-2</sup>. High weed beet densities showed a more homogeneous distribution, reflecting the presence of seed sources in all parts of the field. We recorded several cases of fields virtually without weed beets adjacent to

highly infested fields, from which we concluded that there was no substantial seed transport between fields.

## THE WEED BEET SEED BANK

No seed balls were detected in two fields that had low infestation of weed beets in the previous sugar beet culture (Table 1). By contrast, in the severely infested fields the density varied between several hundred and several thousand seed balls per m<sup>2</sup>. In all fields the density per volume was almost constant for the first 20 cm, whereas very few seed balls were found at greater depths. Our experiment on the influence of depth on germination showed that germination declined almost linearly from 100% at 0.5 cm to 0% at 5 cm. We calculated that about 12% of all seed balls in the fields were sufficiently near the surface to be able to germinate, and we used this percentage for all further calculations. The potential number of seedlings per m<sup>2</sup> could be estimated after taking into account the mean number of seedlings per seed ball in the various fields. These estimates were very variable among the samples within fields and were in many cases considerably higher than the weed beet densities observed earlier. In field 17 we had the opportunity to verify our estimates, because the field was again used for sugar beet in 2000. The most dense seedling concentrations could easily be spotted by eye, and contained over 2000 plantlets m<sup>-2</sup>. This number was considerably reduced by self-thinning later in the season, coming close to the maximum of 80 adult plants m<sup>-2</sup> observed earlier.

DENSITIES OF F<sub>1</sub> CROP–WILD HYBRIDS AND VARIETY BOLTERS

The variety bolters (i.e. crop plants developing inflorescences) and the F<sub>1</sub> hybrids appeared identical in the field and we were unable to define a diagnostic morphological character (data not shown). Nevertheless, we found a method to distinguish between them using differences in ploidy. The F<sub>1</sub> hybrids are diploid, having resulted from a pollination of the diploid male-sterile seed-bearer plants by diploid wild beets in the

**Table 1.** The seed bank. In order to calculate the potential number of seedlings per m<sup>2</sup> in each of the fields, the number of seed balls found was multiplied by the proportion sufficiently close to the surface (measured once in a separate experiment and its result applied to all fields; see text) and by the number of seedlings per seed ball

Population no.	History of the field		Seed bank data				
	Last sugar beet culture	Last weed beet density	Mean number of seed balls per m <sup>2</sup>	Proportion of seed balls able to germinate with respect to depth	Viability (seedlings per seed ball)	Potential number of seedlings per m <sup>2</sup>	Range among samples
12	1997	Low	0	0.12	–	0	–
13	1996	Low	0	0.12	–	0	–
14	1996	High	732	0.12	0.64	55	0–155
15	1999	High	4028	0.12	1.29	620	0–3005
16	1999	High	2768	0.12	0.68	225	0–660
17	1998	High	1185	0.12	0.60	85	0–559

**Table 2.** Densities of F<sub>1</sub> crop–wild hybrids (diploid) and variety bolters (triploid) in 10 sugar beet fields free from weed beets

Field no.	Area (ha)	Estimated number of sugar beets* ( <i>n</i> )	F <sub>1</sub> hybrids		Variety bolters	
			<i>n</i>	%	<i>n</i>	%
3	1.30	117 000	10	0.09	4	0.03
4	1.43	128 700	20	0.16	4	0.03
5	2.90	261 225	21	0.08	4	0.02
6	4.11	369 720	52	0.14	11	0.03
7	3.98	358 425	10	0.03	3	0.01
8	1.00	90 000	23	0.26	5	0.06
9	1.94	174 420	21	0.12	4	0.02
10	1.49	133 848	33	0.25	5	0.04
11	1.39	124 740	21	0.17	7	0.06
12	4.38	393 750	41	0.10	12	0.03
Mean				0.138		0.032
SD				0.072		0.015

\*According to an average number of 90 000 sugar beets ha<sup>-1</sup>.

seed-production area. The variety bolters in our region, on the other hand, are triploid, resulting from pollination by a tetraploid pollinator breeding line. By counting chloroplasts in the stoma guard cells, we could identify both types of sown bolters. The average densities for 10 fields (numbers 3–12), not substantially invaded by weed beets, were largely under 1% (Table 2), which is the maximum acceptable value imposed by EU legislation. Nevertheless, we observed that no field was completely uncontaminated: both F<sub>1</sub> hybrids and variety bolters were always present. Variety bolters were always less common than F<sub>1</sub> hybrids, but their abundance may vary with spring temperatures and sowing date, whereas F<sub>1</sub> hybrid percentage only depends on the contamination level.

#### FACTORS INVOLVED IN THE EFFECTIVENESS OF GENE FLOW

##### *Fitness measurements*

Weed beets, F<sub>1</sub> hybrids and variety bolters may each play a role in the formation of transgenic weed beet populations, which must be quantified. The effective seed production of the three types of bolters is difficult to measure in the field, because it depends on the flowering phenology. We were able to harvest some ripe seeds on the variety bolters, which shows that they can produce seeds under natural conditions, although not many. We do not know to what extent the production

of viable seeds is low due to triploidy. For all measured fitness components (seed germination rate, juvenile survival and flowering without vernalization), the offspring of the variety bolters had lower scores than those of the F<sub>1</sub> crop–wild hybrids, which in their turn were lower than the weeds, including pollen viability (Table 3). Except for germination, which is largely a maternal character, the other fitness components also depend on the male parent. The weed seeds came from highly contaminated fields, and probably resulted from pollination by other weeds. The seeds from the two types of sown bolters were collected in fields without weeds, which does not, however, completely exclude pollination by weeds from other fields. Indirect evidence for the latter possibility came from the fact that half the seed from variety bolters bolt without vernalization (Table 3), probably due to pollination by B-carrying pollen.

##### *Flowering phenology and crosses*

The possibility of the three types of beet cross-pollinating in the fields was determined by the start and duration of flowering. Within a field the variety bolters and the F<sub>1</sub> crop–wild hybrids germinated at the same time as the rest of the crop. The variety bolters flowered systematically later than the F<sub>1</sub> hybrids (Table 4),  $\chi^2 = 52.8$ ,  $P < 0.001$ , but there was sufficient overlap to allow crosses between the two categories. About 50% of both categories of sown bolters were flowering when the detailed observations were being made. The weed

**Table 3.** Germination rates of the seeds collected from the three types of bolters together with juvenile survival, flowering without vernalization and pollen viability of the resulting offspring. The standard error of the mean (SE) is given if possible

	Offspring of		
	Weed beets (%)	F <sub>1</sub> hybrids (%)	Variety bolters (%)
Germination rate (SE)	83.6 (1.7)	79.0 (4.5)	18.0 (7.8)
Juvenile survival	99.7	97.8	68.0
Flowering without vernalization (SE)	86.2 (0.9)	69.7 (1.8)	49.7 (5.3)
Pollen viability (SE)	69.1 (5.8)	42.2 (24.3)	(No data)

**Table 4.** Progress of bolting, flowering and fruiting in a series of characteristic sugar beet fields with F<sub>1</sub> hybrids and variety bolters at 22–25 July 1997. Percentage values are given in parentheses

	F <sub>1</sub> hybrids	Variety bolters
Bolting	27 (11)	28 (41)
Beginning of flowering	21 (8)	13 (19)
Full flowering	113 (45)	23 (34)
Fruiting	91 (36)	4 (6)
Total	252 (100)	68 (100)

beets germinated over a longer period. Their time from germination to flowering was variable but was not significantly different from the F<sub>1</sub> hybrids (mean and SD: 92 ± 4 days and 94 ± 6 days, respectively, in an experimental garden). It is therefore not surprising that weed beets were among the first to flower in the fields, and continued to flower throughout the rest of the season. Crosses between all types were therefore possible given the synchronization of flowering. The remaining question was whether such crosses would result in seed set, especially those crosses involving the triploid variety bolters. We tested this experimentally in the glasshouse. Crosses within and between variety bolters and F<sub>1</sub> hybrids were all successful (Table 5): they produced viable seeds giving healthy plants. The (maternal) offspring of the variety bolters appeared to be a mixture of diploids and triploids.

## Discussion

The weed beet densities found in the study area were extremely variable. In a large part of the fields used for sugar beet there was no weed beet problem, but more or less severely infested fields were not difficult to find, for example in field 17 in the year 2000 one part of the field was so saturated by weed beets that no roots could be harvested. Hence there is a real agronomic problem.

The infestation by weed beets starts with the seeds shed by crop–wild hybrid bolters in the sowing line within a field. These seeds stay around the mother plant unless dispersed by mechanical tillage. No zoochory is reported and there is no particular adaptation for attractiveness to animals or dispersion by wind. Our seed bank study makes clear that considerable densities of viable seeds can survive to the next sugar beet culture (usually 3 years after the previous one), for example

field 14 after 4 years. Outside the agro-ecosystem, including recently abandoned fields, we have never found any wild ('ruderal') beets in northern France, although overwintering of beet is sometimes possible (Pohl-Orf *et al.* 1999). Beets are known to be poor competitors in undisturbed sites (Bartsch *et al.* 1996b; Fredshavn & Poulsen 1996) and there is therefore no real risk of invasion of the surrounding vegetation in the root crop region.

We examined bolters in the sowing line in 10 fields and noted that each field was contaminated by crop–wild hybrids (Table 1), although below the acceptable maximum value of 100 ha<sup>-1</sup>. This makes clear that the arrival of hybrids in the fields is a recurring phenomenon, which is in agreement with a study based on molecular polymorphism (Desplanque *et al.* 1999). In that study the large diversity of the weed beets compared with their short history could be explained by recurrent crop–wild hybridizations during seed production in spite of the precautions taken for more than 20 years in the seed-production region.

## EVIDENCE FOR GENE FLOW IN THE CROP–WILD COMPLEX

Gene exchange between wild plants and the crop is possible in both the seed-production area and in the sugar beet fields, and is visualized in Fig. 2. The normal seed-production process and transport to the north is indicated by 1; all other figures indicate unwanted gene flow possibilities.

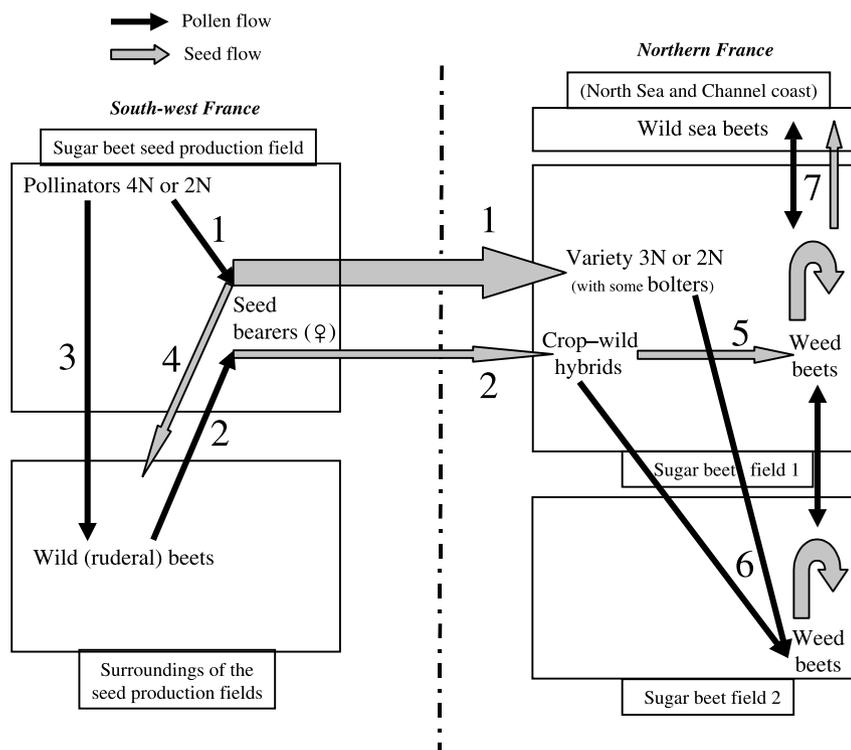
### Gene flow in the seed-production area

Gene flow from wild plants to crop plants cannot be measured in the sugar beet seed-production area, but is manifested by the presence of diploid bolters in a triploid crop as well as by the weeds that are genetically intermediate between the ordinarily used sugar beet varieties and the wild (ruderal) plants in the area surrounding the seed-production fields (Desplanque *et al.* 1999). In Fig. 2 this crucial form of gene flow is indicated by 2. Gene flow from crop to wild in these areas may happen in two ways: by seeds lost during the harvest (arrow 4 in Fig. 2) or through pollination of wild plants by cultivated pollen (arrow 3).

Modern sugar beet varieties (since the 1960s) have a characteristic mitochondrial genotype, Svulg,

**Table 5.** Crosses in the glasshouse using various combinations of variety bolters (triploid) and F<sub>1</sub> hybrids (diploid). Female parent and pollinator plants are in all cases from the same field

Female parent:	Variety bolter	Variety bolter	F <sub>1</sub> hybrid
Male parents:	Variety bolters	F <sub>1</sub> hybrids	F <sub>1</sub> hybrids
Number of female parents analysed	4	2	9
Germination rate of the seeds produced ± SE	36 ± 11	37 ± 4	74 ± 13
Total number of offspring tested	18	9	45
Number of triploid offspring	6	2	0
Survivors to adult stage	17	7	45



**Fig. 2.** A schematic presentation of the possibilities of gene flow by seeds and pollen in the sowing seed-production area (left) and in the sugar-production area (right). The seed bearers are male-sterile, the pollinator plants are hermaphrodite; all other plants can be both. The pollinator plants can be tetraploid (4N) or diploid (2N), leading to triploid (3N) or diploid (2N) varieties, respectively; all other plants are usually diploid.

associated with male sterility (Senda, Onodera & Mikami 1998), which is only maternally transmitted. This genotype has never been found in Mediterranean coastal populations nor in the Atlantic and western Channel populations (Cuguen *et al.* 1994). Desplanque (1999) found the Svulg cytoplasm in five out of 86 plants, from five different populations out of 23, within or near the area where seed production is allowed. This argues seed flow (arrow 4 in Fig. 2): the five plants are probably maternal descendants from seeds lost during the harvest.

Pollen flow from crop (all forms of *Beta vulgaris* ssp. *vulgaris*: sugar beet, fodder beet, red table beet and Swiss chard) to wild (*Beta vulgaris* ssp. *maritima*) in this area will result in cultivated characters in the progeny harvested on wild plants in the neighbourhood of the seed-production fields, and/or in triploid progeny in the case of tetraploid pollinator plants. Desplanque (1999) found evidence that 1–2% of all plants had progeny with a cultivated-like morphology, but mostly (in 10 populations out of 64) the plants concerned were pale green with broad leaves and very thick veins but no special thick root. This suggests a hybrid with Swiss chard rather than with sugar beet. Swiss chard is frequently grown in kitchen gardens and sometimes bolts and flowers before being removed. In two populations progeny were found possessing the characteristics of red table beet and in two other populations they possessed sugar beet characteristics. Desplanque *et al.* (1996) did not find any triploids after examination of

81 seeds harvested on different wild populations in the seed-production area. The evidence for pollen flow (arrow 3 in Fig. 2) is therefore weak, but cannot be excluded.

#### Gene flow in the sugarbeet fields

Notwithstanding the biennial character of the sugar beet crop, sexual reproduction can obviously happen in the sugar beet fields. Pollen and seeds can be produced by beets both inside and outside the sowing line (variety bolters and  $F_1$  hybrids vs. weed beets, respectively). The seed-bearer plants used to produce the hybrid variety are fully male-sterile. We showed, however, that this is not so for variety bolters or  $F_1$  hybrids: at least some of them can release pollen, as can be concluded from the cross results (Table 5). This can be explained by the restoration of male fertility by nuclear restorer alleles transmitted by the pollinator line or the wild (ruderal) beets, respectively. Male sterility is sometimes described in risk assessment as a way to reduce gene flow (Raybould 1999) but this is not a valid argument for safety in transgenic beets.

In the first stage of contamination of a field by weed beets (i.e. a clean field with only a few bolters per hectare), pollination could be limiting due to self-incompatibility and wind pollination. However, it is notable that we observed intense activity by Diptera (mainly syrphids) with 10–30 individuals per flowering plant, thereby confirming older reports (Archimovitch 1949; Free *et al.* 1975). A partial insect pollination

could therefore exist (Darmency *et al.* 1998) and should be studied more carefully.

The offspring of the F<sub>1</sub> hybrids will evolve to weed beets (arrow 5 in Fig. 2). Other gene flow possibilities are the cross-pollination of weed beets in neighbouring fields and the variety bolters or the F<sub>1</sub> hybrids (arrows 6) and the gene flow by pollen or seed from weed beets to neighbouring wild coastal populations (arrows 7).

#### THE RISK OF THE SPREAD OF A TRANSGENE FOR HERBICIDE TOLERANCE

The weed beet problem as it exists in several parts of Europe may be combated successfully by the introduction of sugar beets carrying a transgene for non-selective herbicide tolerance. This would allow destruction of all the weeds including those belonging to the same species (Dhalluin *et al.* 1992). However, our results suggest that such a transgene could combine with the B allele for flowering without vernalization, thus leading to transgenic weed beets, which would mean that the success was only temporary. We discuss the following scenarios.

1. The transgene is incorporated in the nuclear genome of the (diploid) male-sterile seed bearers.
2. The transgene is incorporated in the nuclear genome of tetraploid pollinators.
3. As 2 but with diploid pollinators.
4. The transgene is incorporated in one of the cytoplasmic genomes of the seed bearers.

##### *Scenario 1: transgenic male-sterile seed bearers*

The advantage of having the transgene in the male-sterile seed bearers is that it will never be transferred by pollen to the wild populations in the seed-production region because of the absence of transgenic pollen in the seed-production process. The only direct way of transgene escape is by seed loss during or after the harvest (arrow 4 in Fig. 2). However, with the same precautions as currently used, there will be a low level of contamination of the seed by wild pollen carrying the B allele. The resulting F<sub>1</sub> bolters in the sugar beet fields have to be systematically eliminated, otherwise transgenic weed beets will be the immediate result (arrow 5). Their removal must take place before flowering in order to prevent the arrival of the transgene in neighbouring fields with weed beets in a non-transgenic crop (arrow 6). The total eradication of bolters by hand includes the removal of the variety bolters, which, although they do not contain the B allele, may also transmit the transgene to weeds in other fields (arrow 6).

##### *Scenario 2: transgenic tetraploid pollinator plants*

Using this option, each contamination of the seed by the B-carrying wild pollen will lead to non-tolerant hybrids that are destroyed by the herbicide after germination. There is therefore no direct risk of the installation of new transgenic weed beet populations,

at least when the herbicide treatment covers 100% of the field. There is the possibility, however, that the wild populations in the seed-production region would receive the transgene (arrow 3 in Fig. 2), thus generating plants that combine B and the transgene. Their pollen would be capable of contaminating the seed and thus producing herbicide-resistant hybrid bolters in the sugar beet fields (arrow 2).

When compared with scenario 1, it is clear that such a circuitous contamination of the seed, if any, will take considerable time. In the first place, the diploid pollen of the tetraploid pollinator plants appears to have a low chance of pollinating wild plants. The diploid pollen of a tetraploid beet is less readily released by the anthers (Scott & Longden 1970), and Hecker (1988) showed that when a diploid beet has an equal chance of being pollinated by haploid and diploid pollen, 89% of the effective pollinations are made by haploid pollen. Consistently, we found no triploids in the seeds sampled on wild plants in the seed-production area (Desplanque *et al.* 1996), in spite of the presence of high quantities of diploid pollen for several decades. Once such triploid hybrids are formed, their fitness may be low due to their triploidy, as we observed for the variety bolters. Hecker & McClintock (1988) for example, mentioned a weak germination ability (3%) for the pollen from triploid beets compared with pollen from diploid or tetraploid beets (about 40%). Seeds of the triploid varieties are sometimes described as non-viable (Longden 1993) but this is in contradiction to our findings (Tables 3 and 5).

Seeds may escape to the wild, also leading to triploids in the next generation, but, although still carrying the Svulg mitotype, they may evolve during later generations into diploid wild plants indistinguishable from normal individuals. On the other hand, introgressed ruderal plants exhibiting a strong domestication syndrome (i.e. large rosette and biennial character) will mainly escape from our investigation, as they are easy to spot by the seed producers who continuously try to destroy all ruderal beets they can find. An unwanted consequence of this behaviour could be the selection of the B allele, as annuality (together with a short life cycle) allows the ruderal plants to produce offspring before destruction.

##### *Scenario 3: transgenic diploid pollinator plants*

The crucial difference with the previous scenario is the easy passage of the transgene to the wild beets in the seed-production area. The massive amount of pollen, together with the absence of the ploidy handicap, which would also enhance the pollination of weeds in neighbouring fields by variety bolters in the crop, makes this an unattractive option.

##### *Scenario 4: cytoplasmic herbicide tolerance*

The incorporation of the transgene in one of the cytoplasmic genomes (e.g. in the chloroplast; Daniell *et al.*

1998) excludes all forms of exchange of the transgene by pollen, because these organelles are strictly maternally inherited in beet (Corriveau & Coleman 1988). This scenario closes a potential escape route for transgenes into the environment (Gray & Raybould 1998; Daniell 1999). The possibility of contamination by the wild B-carrying pollen, however, is not influenced. As a consequence, weed beet infestations will occur in each case of inattention to bolters, as in scenario 1. The passage to other fields or to wild sea beets at the Channel and North Sea coasts via pollen, on the other hand, is excluded, although still possible through seed transport.

#### CONCLUSION

Two mutually exclusive scenarios (1 and 2) both have their advantages and disadvantages, which means that a preference is not easy to justify. In the first scenario (incorporation of the transgene in the male-sterile seed bearers) there is little risk of the surrounding wild populations receiving the transgene during seed production. Transgenic weed beet populations, however, could very probably arise in a part of the sugar beet fields. It is unrealistic to think that all farmers will remove all the bolting beets from their fields. Finally, the transgene may arrive in natural sea beet populations in those areas where sugar beet is cultivated near the coast (Fig. 1).

In the second scenario (the incorporation of the transgene into the pollinator plants), the disadvantages are different. Large quantities of transgenic pollen will be generated in an area where wild plants of the same species are growing. We know that the diploid pollen of the actual tetraploid pollinator plants will probably have a low chance of pollinating the wild diploid plants, but the increasing tendency to use diploid sugar beet varieties suggests that scenario 3 (transgenic diploid pollinator plants), which certainly is the worst of all four, will be more likely. A second problem, for the seed producers, is that all pollinations by other varieties without tolerance for the same herbicide will result in plants dying after herbicide treatment of the sugar beet fields, whereas at present such unwanted pollinations are hardly visible and do not have real economic consequences.

Scenario 4 (a cytoplasmic inheritance of the transgene), has few advantages when compared with scenario 1 and suffers from the same drawback, the probable installation of transgenic weed beet populations in some of the fields.

The use of herbicide-tolerant transgenic sugar beet varieties will only be economically successful if transgenic weed beet populations do not arise within a few decades, which otherwise would bring us rapidly back to the current situation in which no herbicide is available for weed beet control. We have shown that the eventual formation of transgenic weed beets is not only possible, but even probable, whatever the scenario

adopted. However, the speed with which this happens, and the scale whereon, are both of decisive economic importance. We recommend the incorporation of the transgene into the tetraploid pollinator line (scenario 2) as being the best strategy in this respect.

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