SESSION 3

CONSEQUENCES OF GENE FLOW WITHIN SPECIES

Chairman & Session Organiser: Mr J Kilpatrick
ADAS Rosemaund, Preston Wynne

Gene flow in genetically modified herbicide tolerant oilseed rape (*Brassica napus*) in the UK

E C Simpson, C E Norris, J R Law, J E Thomas, J B Sweet
The National Institute of Agricultural Botany, Huntingdon Road, Cambridge, CB3 OLE, UK

ABSTRACT

The environmental and agronomic impact of genetically modified (GM) herbicide tolerant oilseed rape has been studied by NIAB since 1995. This paper reports on results of gene flow frequencies recorded between trial plots at several National List sites and at a large scale release of genetically modified herbicide tolerant rape in the UK. Levels of cross pollination tended to decrease with increasing distance from the pollen source, there was some evidence of varietal differences in receptiveness to foreign pollen. Pollen dispersal was also recorded at distances of up to 400m from a large release of GM herbicide tolerant rape using male sterile 'bait' plants.

INTRODUCTION

In 1995 the Department of the Environment Transport and the Regions commissioned NIAB to monitor some of the first large scale releases of GM herbicide tolerant rape (Sweet *et al.*, 1997, Sweet & Shepperson 1996). The work has recently been extended to include monitoring releases of about 100ha of oilseed rape modified for high lauric acid content. The potential for gene transfer to related *Brassica* species has been examined at a range of sites across the UK in addition to observations made on the incidence and persistence of volunteers and feral populations following GM releases. Further work commissioned by MAFF is investigating agricultural consequences of growing herbicide tolerant oilseed rape and will eventually provide data based on pollen flow between relatively large areas of GM herbicide tolerant and conventional oilseed rape.

Gene flow is an important part of evaluating the risks associated with growing GM herbicide tolerant oilseed rape as the dispersal of transgenes via pollen could potentially contaminate neighbouring crops, feral or volunteer populations or hybridise with related *Brassica* species. Inter and intra-specific transfer of herbicide tolerance transgenes may cause difficulties with weed and volunteer management in agricultural fields.

These studies will provide valuable information required for risk assessment for releases of GM herbicide tolerant oilseed rape and will ultimately contribute to understanding the environmental and economic benefits and costs of the deployment of these crops in farming systems. This paper presents some of the results of initial studies of cross pollination involving herbicide tolerant oilseed rape.

MATERIALS AND METHODS

1. Gene flow In National List Trials - seed and pollen sources

National List (NL) trials of GM herbicide tolerant winter oilseed rape consisted of three replicates containing two GM herbicide tolerant varieties tolerant to the broad spectrum herbicides glufosinate-ammonium and glyphosate. The trials also contained five non-tolerant conventional control varieties (Synergy, Express, Nickel, Falcon and Apex). Each GM trial was isolated from other rape trials by a pollen barrier of a conventional oilseed rape variety which was a minimum of 6m wide. Plots were approximately $40m^2$ and were harvested after swathing by standard small plot combine harvesters. Samples of harvested seed were taken from four NL sites in 1997 by removing the required weight of seed from a bulk of approximately 200g. Samples from non-GM varieties were selected from plots nearest to the GM trial, 50m, and 100m or the furthest point from the GM trial. Seed samples were also tested from the varieties in GM trials to screen for single and double herbicide tolerance.

Screening for herbicide tolerance in National List seed samples

Seed samples collected from NL trials were sown in field plots in a randomised block design replicated three times with negative controls (conventional winter rape variety - Express). Each plot contained an average of 600 plants. In order to allow testing for both glufosinate and glyphosate tolerance the trials were duplicated. Each trial was treated with either 200 g/l glufosinate-ammonium at 3l/ha or glyphosate 360 g/l at 4l/ha when plants were at the 3-5 leaf stage using a tractor mounted sprayer (Sprayranger). The numbers of surviving plants were assessed approximately 7 DAT and 14 DAT for glufosinate and glyphosate treatments respectively. Surviving plants were sprayed on a second occasion with the appropriate herbicide to confirm tolerance.

Seed samples of both GM and conventional varieties from 3 NL GM winter rape trials were also grown in a glasshouse with positive and negative controls. Plants were grown to the two true leaf stage before being treated with either a 1% dilution of 200 g/l glufosinate-ammonium or a 1% dilution of 360 g/l glyphosate using a hand sprayer (Hozelock). The numbers of surviving plants were assessed approximately 7 DAT and 14 DAT after treatment for glufosinate and glyphosate respectively. Surviving plants were treated as appropriate with either glufosinate or glyphosate to detect double tolerance. Leaf tissue samples were taken from plants expressing double tolerance to confirm the presence of multiple transgenes by PCR using specific primers for the PAT and EPSPS GOX genes conferring glufosinate-ammonium and glyphosate tolerance respectively. DNA was isolated from plant tissue using an extraction kit obtained from Qiagen Ltd. UK. PCR reaction conditions were supplied by herbicide manufacturers.

2. Measurement of pollen dispersal from a large area of genetically modified herbicide tolerant winter oilseed rape using male sterile bait plants

Pollen source

The pollen source used for this study was a trial grown at a farm in Cambridgeshire by Plant Genetic Systems (PGS). The trial consisted of an area of approximately 9 ha of winter oilseed rape with about 65% of plants in this area containing the BAR gene conferring tolerance to the herbicide glufosinate ammonium. About 30% of the area contained a mix of 50% male sterile

herbicide tolerant and 50% male fertile herbicide susceptible plants. A pollen barrier of non-GM oilseed rape up to 20m wide was grown around the perimeter of the trial.

Male sterile bait plants

The male sterile portion of the spring oilseed rape composite hybrid variety 'Concept' was grown under glasshouse conditions so that flowering coincided with the onset of flowering of the field crop. Six male sterile plants were positioned (approximately 0.5m apart) in linear plots at a range of distances (100m, 200m, 400m) and directions north, south, east and west from the pollen source. Plants were left in position for the duration of the flowering of the crop and were removed after the main flowering period (approximately 4 weeks). Insect activity, weather conditions and the duration of flowering were observed and recorded on each site visit. Detailed weather data was obtained from the meteorological station at the NIAB farm approximately 25km north of the release site. Seeds were screened under glasshouse conditions for glufosinate tolerance as described previously.

RESULTS

1. Gene flow between GM NL and NL trials

Herbicide tolerance was found in the seed samples of non-GM varieties in 3 of the trials tested, Caxton, Cockle Park and Bridgets. No transgenic plants were found in seed samples from Wye Regional Trial Centre. Mean frequencies of glufosinate and glyphosate tolerance detected at a range of distances from the GM trials are shown in Tables 1-3. Frequencies are expressed as the percentage of herbicide tolerant plants per plot. Overall, frequencies of glyphosate and glufosinate tolerance tended to decrease with increasing distance from the GM trial and varied between varieties with the highest levels detected in the composite hybrid Synergy.
Of 38 samples examined from the Caxton site, 23 had no tolerance to either glufosinate or glyphosate. At both Cockle Park and Bridgets 9 samples had no tolerance to glufosinate or glyphosate out of a total of 18 tested. Varieties being tested in NL trials are coded Var. A-I.

Table 1. Frequency of glufosinate and glyphosate tolerance detected at varying distances from the pollen source at Caxton National List trial site

Variety	Distance from source (m)	% glufosinate tolerant	Variety	Distance from source (m)	% glyphosate tolerant
Synergy	4	2	Synergy	4	0.16
Capitol	8	0.1	Lipton	8	0.16
Apex	14	0.05	Var. A	8	0.05
Synergy	34	0.16	Cobra	8	0.33
Synergy	56	0.05	Var. B	8	0.05
			Synergy	20	0.16
			Apex	34	0.05
			Synergy	34	0.05
			Var. C	50	0.05
			Synergy	54	0.11

Table 2. Frequency of glufosinate and glyphosate tolerance detected at varying distances from the pollen source at Cockle Park National List trial site

Variety	Distance from source (m)	% glufosinate tolerant	Variety	Distance from source (m)	% glyphosate tolerant
Var. D	6	0.05	Var. D	6	0.05
Synergy	42	0.05	Var. G	30	0.05
Var. E	50	0.05	Synergy	42	0.33
Var. F	50	0.05	Var. E	50	0.05
			Var. F	50	0.16

Table 3. Frequency of glufosinate and glyphosate tolerance detected at varying distances from the pollen source at Bridgets National List trial site

Variety	Distance from source (m)	% glufosinate tolerant	Variety	Distance from source (m)	% glyphosate tolerant
Var. G	10	0.05	Var. G	10	0.16
Var. H	10	0.05	Var. H	10	0.44
Var. I	10	0.05	Var. I	10	0.05
Synergy	150	0.11	Synergy	20	0.05
			Synergy	150	0.22

Gene flow in GM NL trials

Herbicide tolerance was detected in seed samples from conventional and herbicide tolerant varieties in samples from the three sites tested (Figures 1a-1f). Overall, frequencies of herbicide tolerance decreased with increasing distance from GM plots. Levels of herbicide tolerance observed in the composite hybrid Synergy tended to be higher than in other varieties at the same distance from GM plots. Double tolerance to both glufosinate and glyphosate was detected in seed samples from herbicide tolerant varieties. The presence of the PAT and EPSPS GOX transgenes conferring glufosinate and glyphosate tolerance respectively are currently being confirmed using PCR with specific primers. Glufosinate and glyphosate tolerant varieties are coded GLU1 and GLY1 respectively.

Figures 1a-1f show plot configuration of National List GM winter oilseed rape trials and the levels of glufosinate and glyphosate tolerance detected in seed samples from these plots

Synergy 0	**GLU1**	**GLU1**	**GLY1 0.67**	Express 0	Falcon 1.17
Falcon 0.17	Nickel 9.17	Express 7.5	Falcon 0.33	Synergy 2.17	Nickel 0.33
GLY1 0.17	Express 0.83	Apex 1.0	Nickel 0.5	**GLY1 3.5**	Apex 0
Apex 0	Discard	Synergy 1.17	Discard	**GLU1**	Discard

Figure 1a. % Glufosinate tolerance - Caxton

Synergy 4.33	**GLU1 0.16**	**GLU1 0.83**	**GLY1**	Express 1.17	Falcon 0
Falcon 0.5	Nickel 0.5	Express 0.5	Falcon 7.6	Synergy 9.16	Nickel 0.16
GLY1	Express 0.33	Apex 0	Nickel 0.83	**GLY1**	Apex 0.16
Apex 9.83	Discard	Synergy 1.0	Discard	**GLU1 2.0**	Discard

Figure 1b. % Glyphosate tolerance - Caxton

Falcon 0.17	Synergy1.67	Express 0.33	**GLY1 0.75**	**GLU1**	Nickel 18.33	Apex 0.67
GLU1	Falcon 0.33	Apex 0.5	Synergy 1.5	**GLY1 0.83**	Express1.66	Nickel 0.33
Apex 1.0	Nickel 0.17	**GLY1 0.08**	Falcon 0.33	Express 0.33	**GLU1**	Synergy 21.0

Figure 1c. % Glufosinate tolerance - Cockle Park

Falcon 0.33	Synergy1.83	Express 1.67	**GLY1**	**GLU1 0.58**	Nickel 3.0	Apex 1.5
GLU1 0	Falcon 1.0	Apex 1.83	Synergy 17.5	**GLY1**	Express 2.67	Nickel 0.33
Apex 0	Nickel 0.17	**GLY1**	Falcon 17.7	Express 1.33	**GLU1**	Synergy 1.33

Figure 1d. % Glyphosate tolerance - Cockle Park

Falcon 0	**GLY1 0.17**	Apex 1.17	**GLU1**	Express 10.8	Synergy 4.83	Nickel 1.67
GLY1 0.25	Falcon 0.17	Express 0	Apex 2.5	**GLU1**	Nickel1 4.0	Synergy2.67
Nickel 0.17	Synergy 0.17	**GLY1 1.0**	**GLU1**	Apex 11.5	Falcon 4.17	Express1.83

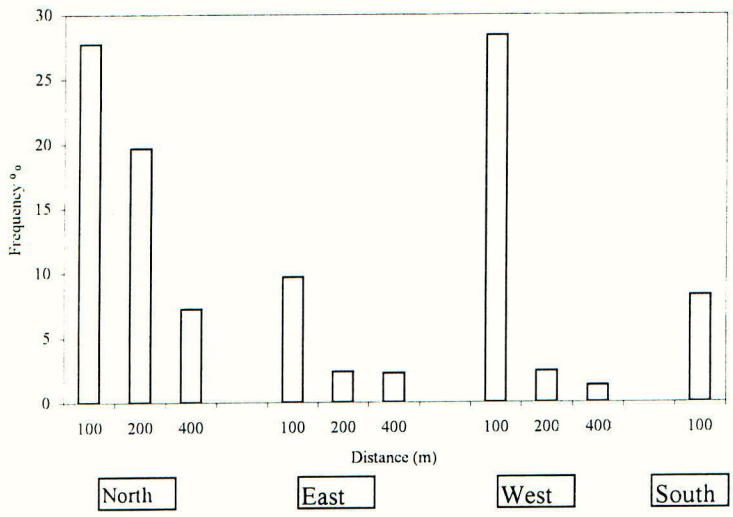
Figure 1e. % Glufosinate tolerance - Bridgets

Falcon 1.17	**GLY1**	Apex 8.33	**GLU1 0.25**	Express 0	Synergy 0.83	Nickel 0.5
GLY1	Falcon 9.67	Express 2.0	Apex 0.5	**GLU1 0.08**	Nickel 0.17	Synergy0.67
Nickel 1.67	Synergy 5.0	**GLY1**	**GLU1 0.57**	Apex 1.83	Falcon 0.67	Express 0.17

Figure 1f. % Glyphosate tolerance - Bridgets

2. Pollen dispersal from herbicide tolerant winter oilseed rape

Male sterile bait plants were pollinated and set seed in all plots. Numbers of herbicide tolerant seed detected decreased rapidly with distance from the pollen source (Figure 2). Plots to the north of the GM pollen source produced the greatest proportion of herbicide tolerant seed. The highest total level of seed set was recorded from plots to the east of the GM trial. The incidence of pollinating insects particularly honey bees (*Apis mellifera*) and bumble bees (*Bombus* sp.) were notably low at flowering. During the flowering period (01.05.98 - 29.05.98) wind direction was predominantly north and north west, mean high and low air temperatures were 18°C and 6°C respectively, mean rainfall was 0.2mm and mean daily sunshine was 5.9hrs.



[1]Total numbers of seed set per plot: north 100m=800, 200m=626, 400m=514; east 100m=1187, 200m=1061, 400m=1174; west 100m=88, 200m=967, 400m=317; south 100m=461

Figure 2. Frequency of herbicide tolerant seed harvested from male sterile bait plants at varying distances and directions from genetically modified herbicide tolerant oilseed rape[1]

DISCUSSION

Gene flow levels, indicated by the frequency of herbicide tolerant plants decreased with increasing distance from the pollen source in both tests with NL trials and a large scale release using male sterile bait plants. Differences in experimental design, particularly pollen source size make direct comparisons with other published data difficult (e.g. Timmons *et al.*, 1995, Scheffler *et al.*, 1993). Some of the fluctuations found in the gene flow levels in this study are likely to be due to different positions of GM plots within NL trials, differences in pollen source size, and various agronomic and climatic factors such as carry over of seed in harvesting machinery and prevailing wind direction. High frequencies of herbicide tolerant plants in seed samples of Synergy demonstrates the 'susceptibility' of composite hybrid material to alien pollen. High cross pollination frequencies have been recorded in composite hybrids in other winter and spring rape trials when growing adjacent to GM plots (E. Simpson, unpublished data). Multiple tolerant hybrids tolerant to both glufosinate and glyphosate were detected at all of the NL GM sites (Figures 1a-1f). The highest levels were observed in adjacent plots, 3.5% and 2% at the Caxton site (Figures 1a & 1b) which correspond approximately to rates of multiple tolerance reported by Messean (1997) of 2% at 1m from a much larger pollen source. Monitoring volunteer incidence at National List sites currently does not indicate that multiple tolerant hybrid plants are more difficult to control than conventional or single tolerant rape varieties. Continued monitoring of these sites will identify any significant future problems with volunteer control.

Detection of herbicide tolerance in seed of male sterile oilseed rape plants at distances of up to 400m show that there is potential for oilseed rape pollen to be dispersed by wind and remain

viable over considerable distances. The high levels of herbicide tolerant seed detected in plots to the north and west of the release correspond to prevailing wind conditions. The highest level of total seed set recorded in plots to the east of the pollen source was likely due to dispersal of pollen from a commercial field of conventional winter rape growing near to these plots. Using male sterile plants demonstrates a worst case scenario, as there is reduced competition for foreign pollen, if fully fertile plants had been used, cross pollination levels could have been substantially lower. When seeds (>1000) from feral rape plants flowering at the same time and growing within 120m of the same large scale release of herbicide tolerant rape were tested no herbicide tolerant plants were detected (E. Simpson, unpublished data).

Results from these initial studies of gene flow in oilseed rape show that pollen can be dispersed over considerable distances indicating that gene flow can occur from releases of GM herbicide tolerant oilseed rape. Results of monitoring NL and large scale PGS releases (Norris *et al.*, in press) currently do not indicate that GM herbicide tolerant oilseed rape varieties are any more persistent or invasive than conventional types. Continued monitoring of sites and further gene flow studies using larger areas of GM herbicide tolerant oilseed rape will help to identify any potential ecological and agricultural problems that may occur with the commercial release of these crops.

ACKNOWLEDGEMENTS

The authors would like to thank Ministry of Agriculture, Fisheries and Food and Department of the Environment, Transport and the Regions for financial support, Plant Genetic Systems for their help in carrying out this research, AgrEvo and Monsanto for providing diagnostic primer sequences.

REFERENCES

Messean A (1997) Management of herbicide tolerant crops in Europe. *Proceedings Brighton Crop Protection Conference - Weeds*, **3**, 947-954.

Norris C E; Simpson E C; Sweet J B; Thomas J E (1999) Monitoring weediness and persistence of genetically modified oilseed rape (*Brassica napus*). *BCPC Proceedings Gene Flow and Agriculture Conference, in press.*

Scheffler J A; Parkinson R; Dale P J (1993) Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*). *Transgenic Research*, **2**, 356-364.

Sweet J B & Shepperson R (1996) Monitoring commercial releases of genetically modified oilseed rape. *Proceedings 10th International Weed Biology Conference Dijon*, pp 217-222.

Sweet J B; Shepperson R; Thomas J E; Simpson E C (1997) The impact of releases of genetically modified herbicide tolerant oilseed rape in the UK. *Proceedings Brighton Crop Protection Conference - Weeds*, **4**, 291-302

Timmons A M; O'Brien E T; Charters Y M; Dubbels S J; Wilkinson M J (1995) Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. oleifera. *Euphytica*, **85**, 417-423

Gene flow between sugar beet and weed beet

Y Vigouroux, H Darmency
Laboratoire de Malherbologie, INRA, BV 1540, 21034, Dijon, France.

T Gestat de Garambe, M Richard-Molard
Institut Technique de la Betterave, 45 rue de Naples, 75008, Paris, France

ABSTRACT

Gene flow between transgenic sugar beet and weed beet was investigated at the farm scale. Evidence was given of gene flow between bolters of the crop that occurred at low density in a 1 ha field and weed beet growing spontaneously in an adjacent set-aside field. Up to 0.8 % of the weed progeny was hybrid. A second experiment was specifically designed to check gene flow from a small pollen source of sugar beet to a low density weed beet population. The frequency of hybrids slowed down from 10% three meters away the pollen donor to 1 % at 15 m. The effects of wind, distance, weed aggregation, insects and genotypes on the hybridization rate are discussed.

INTRODUCTION

Risk assessement of transgenic crops on an agricultural scale is currently carried out in France by a group of institutes, seed producers and agrochemical companies. Four different crops are rotating in a farmers' field for several years and are cultivated with usual farmers' practices (see Messean, this volume, for more details). Along with studies on the benefit of growing a given transgenic crop, problems of volunteers in successive crops, pollen flow distances, and gene flow between crops and their wild relatives are monitored and/or investigated. We focus here on the possibility of gene escape from transgenic sugar beet to weed beet in field conditions.

Weed beet are quite common in the sugar beet area in France. They are a real problem in less than 5 % of the fields (Gestat de Garambe et al., 1998). Since weed beet and sugar beet are the same species, no specific herbicide is actually available to solve this problem. Transgenic herbicide-resistant beet have been proposed as a solution for the problem of weed beet infestation (Martin, 1998). However, gene flow between weed beet and sugar beet have been demonstrated to occur in the past: the genetic study of weed beet in the north of France showed they display the cultivated cytoplasm Owen, proving their maternal origin from cultivated beet (Boudry *et al.*, 1993). Although sugar beet is a biennial plant that is not expected to flower the first year, there area few bolting plants occurring per hectare, indicating that pollen escape is possible.

We describe here the pollen flow in a field experiment with transgenic sugar beet and adjacent populations of weed beet in set-aside fields. These results raised questions that were not previously documented in the literature. Especially, pollen flow in beet has been studied in seed production conditions only, not in a root production area (Stewart and Cambell, 1952;

Chamberlain, 1967; Dark, 1971), and the biology of the target weed beet has seldom been considered. Different parameters could interact in cross-pollination as the occurrence of few bolting plants, so that very little transgenic pollen is available, the distance between pollen donor and receiver; and the reproductive behaviour of weed beet and bolting plants (flowering time, incompatibility system, and outcrossing rate). Hence, we designed a specific experiment for studying gene flow using weed beet as pollen receiver in order to assess what gene flow might be when very few pollen donors are present, and how this gene flow is influenced by weed beet as target plants?

MATERIALS AND METHODS

Gene flow between transgenic bolting plants and weed beet in the field

One hectare of sugar beet was sown in 1996 with two transgenic herbicide resistant hybrid lines, one resistant to glyphosate and the other to glufosinate. The plants were heterozygous for the resistance genes. As the main wind direction was South West-North East, ten groups of 20 annual weed beet collected in the Dijon area were planted along the S.W. border of the field. The one ha adjacent field on the N.E. side was left on set aside, and 127 spontaneous weed beet could flower there. Only 94 weed beet were further analyzed for the presence of hybrids because of mortality or no seeds available. In addition, five plots of male sterile beet were planted along the median row of the field, and then five others at 30 m, 90 m and 210 m toward N.E. Every bolting sugar beet (only glufosinate resistant, no glyphosate resistant plants flowered) and all the weed beet were located on a map and their flowering period recorded. Seeds were harvested, weighed and numbered. A sample of 800 seeds from each plant was sown in peat in the greenhouse (22 °C day, 18 °C night). Then 2-4 leaf seedlings were sprayed with 5 l ha^{-1} Liberty (200 g l^{-1} glufosinate ammonium, Agrevo) to check for the presence of resistant plants.

Experimental design to estimate gene flow

Four pollen donor sugar beet were planted out in a small patch (0.2 m^2) in the centre of nine radiating lines. These plants were used as pollen donors and were homozygous for red pigmentation. Red color is inherited as a single dominant allele at one locus (Keller, 1936). One weed beet was planted along the radiating lines at 3, 6, 9, 12 and 15 m. All plants were firstly germinated and grown in the greenhouse before planting. Synchronization of flowering was performed by cutting first flowering ramets. Homogeneous flowering started on July 12 and lasted until the harvest date on August 31. All the beet were harvested, air-dried in bags, and threshed. To prevent contamination during threshing, the more distant plants from the centre of the circle were first processed. Seeds were counted and weighted. A sample of 800 seeds per plant was sown in the greenhouse and the number of red seedlings was recorded.

RESULTS

Transgenic field

They were 58 sugar beet plants bolting in the field, but only 29 were resistant. Hybrids between transgenic sugar beet and male sterile plants were found at a rate of 0.76% (Table 1). Most of the hybrids, 34 out of 36, were found on plants grown within the sugar beet field. The two other hybrids were found at 30 meters and 210 meters from the sugar beet field. As transgenic bolters were diploid and heterozygous, and only half of bolting plants carried the resistance gene, the percentage of pollen that carried the transgene should be 25%. We can deduce that bolters furnished 3% of the pollen cloud that fertilized the male sterile plants.

The progeny of the weed beet planted in the S.W. border contained 0.0065 % of resistant plants (Table 1). That of plants growing naturally in the set aside contained 0.2 %. Using the same correction as above to account for heterozygous resistant and susceptible bolters, that makes 0.026 and 0.8 % hybrids, respectively. The lower frequency of hybrids observed for S.W. planted weeds could be explained by the experimental design. Twenty weed beet were planted together in small plots so that mating inside group within plot was probably enhanced. Moreover, the prevailing wind was coming from S.W. resulting in transport of pollen by air toward the set aside field on the N.E. rather than toward the S.W. border.

Table 1. Hybridization between transgenic bolting sugar beet and other beet as indicated by herbicide resistance

Kind of plant	Number of plants	Number of seedlings	Number of resistant	Number of plants that produced at least one hybrid
Weeds planted in S.W. border	186	30850	2	2
Weeds in N.E. set aside	94	10394	21	4
Male sterile	119	4762	36	19

The 21 resistant hybrids observed in the progeny of the spontaneous weeds in the set-aside were produced by four plants only (Table 1). This could not be expected in the case of random spread of the transgenic pollen, but the fact that three quarters of the hybrids have not been detected is perhaps misleading. From a biological point of view, no data on seed production, weight of seeds and seed viability could indicate that these four plants behaved differently than others (Table 2).

Moreover, this does not seem to be a matter of distance of pollination. These four plants were not closer to bolters, on average, nor closer to the nearest bolters than the other plants which did not produced hybrids (Table 3). However, the number of other weed beet growing in the immediate vicinity of these four plants, within a circle of three meter radius, was slightly lower than for the rest of the weed beet.

Table 2. Comparison of plants that produced hybrids to all other weed beet in the set aside

Type of plant	Number of seeds	Thousand seed weight (g)	Number of seedlings	Number of hybrids	% of hybrids
Plants that produced hybrids (min - max)	658 (330 - 1060)	13,2 (10,8 - 19,1)	148 (52 - 200)	5 (1 - 10)	3,3 (1,8 - 5,8)
All other plants (se)	665 (54)	11,0 (0,36)	110 (11)	0	0

Table 3. Localization of weed beet that produced hybrids

Type of plant	Number of weed plants within a 3 m radius	Average distance from all transgenic bolters (m)	Distance from the neare transgenic bolter (m)
Plants that produced hybrids (se) (min - max)	1,25 (0,25) (1 - 2)	87 (6,0) (77 - 96)	59 (6,6) (47 - 73)
All other plants (se) (min - max)	1,73 (0,16) (0 - 7)	94 (1,7) (61 - 139)	63 (1,4) (34 - 90)

Experimental design

The number of seedlings germinated from samples of 800 seeds was 571 (se 49), on average, and at least 176 seedlings. Only five plants presented no red pigmented hybrids, at 6, 9 and 15 m. Pollen flow from red beet to weed resulted in 10.7 % hybrids found at 3 metres (Table 4). At a distance of 9, 12 and 15 m, the rate of hybrids decreased to values around 1 %. Quite homogeneous percentages of hybrids were observed among plants at a same distance, except for the six meters distance where values ranged from 0% to 16.4%. There was no evidence of marked effect of wind direction.

Table 4. Average rate of hybridization between red beet as pollen donor and weed beet as pollen receiver (standard error in brackets).

Distance (m)	3	6	9	12	15
Hybrid %	10.7 (2.2)	7.1 (3.4)	1.0 (0.3)	0.8 (0.2)	1.3 (0.3)

DISCUSSION

The obvious result is that hybridisation between bolting transgenic sugar beet and weed beet occurred in this study under field conditions and should occur when transgenic material is used in farmers' fields. No genetic barrier separates weed from cultivated beet, as already reported in other studies (Boudry *et al.* , 1993; Bartsch and Pohl-Orf, 1996). The rate of hybrids found in the progeny of weed beet in the set aside was around 0.8 %. That means that the pollen cloud that fertilized the weed beet contained, on average, more than 99 % of pollen produced by the 127 weed beet, and that the 58 crop bolters contributed for 0.8 % only. This is not surprising as the smallest distance between a weed beet and its closest bolter ranged from 34 to 90 m, a distance at which gene flow is expected to be equal to, or lower than, 1 % according to the result of our specific pollen flow experiment. However, hybrid production was not at random. Only four plants out of 94 produced the 21 hybrids. These plants were not placed in particular conditions and had no peculiar reproductive output, so that random pollination by the wind is questionable. Even if we imagine that the rate of 1 % hybridization, that was observed at 9, 12 and 15 m in the second experiment, keeps stable at farther distances, it cannot account for the higher rate of hybrids produced by these four plants (2 to 6 %). This level in our experiment of gene flow could only be observed for weeds at a distance close to 6 m, while plant mapping showed that these four plants were 47 to 73 m from the closest bolter.

To explain why all the hybrids were restricted to four families only, two hypothesis could be proposed. The one is that a complex relationship between self-fertility and incompatibility among weed and cultivated beet leads to this pattern of gene flow. But little is known about the reproductive behavior of weed beet, which is currently approached by the study of half-sib progeny. The alternate hypothesis is that insects could have been pollen carriers, which could explain the high level of hybrids for a few weed beet that should have been visited by a single insect bearing pollen from a transgenic bolter, while other weeds have not been visited under similar conditions. Work on pollination of sugar beet carried out in seed production fields where high density of flowering plants occurs has concluded that wind is the main vector of pollen. A few studies also showed that some insects could carry beet pollen and contribute to pollination (Free *et al.* 1975). Perhaps, the effect of insects is more important in root production fields where there is a low density of pollen donors. In order to gain improved understanding on transgene flow in production fields of transgenic sugar beet, therefore, more studies on the biology of the pollen recipient plants, the weed beet, are needed.

ACKNOWLEDGEMENTS

We wish to thank the Burgundy Region Council (France) for a grant to Y. V.

REFERENCES

Bartsch D; Pohl-Orf M (1996). Ecological aspect of transgenic sugar beet : transfert and expression of herbicide resistance in hybrids with wild beet. *Euphytica* **91**, 55-58

Boudry P; Mörchen M; Saumitou-Laprade P; Vernet P; Van Dijk H (1993). The origin and evolution of weed beet : consequences fore the breeding and release of herbicide resistant transgenic sugar beet. *Theoritical and applied genetics* **87**, 471-478.

Chamberlain AC (1967). Cross pollinisation between fields of sugar beet. Quarterly Journal of the Royal Meteorology Society **93**, 509-515.

Dark SOS (1971). Experiments on the cross-pollination of sugar beet in the field. Journal of National Institut of Agricultural Botany **12**, 242-266.

Free JB; Williams IH; Longden PC; Johnson MG (1975). Insect pollination of sugar-beet (Beta vulgaris) seed crop. Annals of Applied Biology **81**, 127-134.

Gestat de Garambe T; C Muchembled; Richard-Mollard M (1998). Utilisation de betteraves tolérantes à un herbicide non sélectifs-conséquences sur les pratiques agricoles. In: *Seventeenth COLUMA conference*, pp951-958.

Keller W (1936). Inheritance of some major color types in beet. Journal of Agricultural Research 52, 27-38.

Martin J L (1998). Betteraves Roundup Ready™ : intêrets et utilisations. In: *Seventeenth COLUMA conference*, pp975-982.

Stewart D; Campbell SC (1952). The dispersion of pollen in sugar beet seed plots. Proceeding of the America Society of Sugar Beet Technologists **7**, 459-469.