Transgenic Research **2**, 356–364 (1993)

Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*)

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Received 25 January 1993; revised 10 March 1993; accepted 17 March 1993

The objective of this study was to evaluate pollen dispersal in *Brassica napus* (oilseed rape). The selectable marker, used to follow pollen movement, was a dominant transgene (*bar*) conferring resistance to the herbicide glufosinate-ammonium. Transgenic and non-transgenic plants of the cultivar Westar were planted in a 1.1 ha field trial, with the transgenic plants in a 9 m diameter circle at the centre, surrounded by non-transgenic plants to a distance of at least 47 m in all directions. A 1 m circle of non-transgenic plants was sown in the centre of the transgenic area to allow estimation of the level of pollen dispersal when plants were in close contact. Honeybee hives were placed at the trial site to optimize the opportunity for cross-pollination. During the flowering period, regular observations were made of the number of plants flowering and the number and type of insects present in 60 1 m^2 areas. These areas were located uniformly around the plot at distances of 1, 3, 6, 12, 24, 36 and 47 m from the edge of the 9 m circle of transgenic plants. Seed samples were harvested from each of the 7 distances so that approximately 20% of the circumference of the plot was sampled at each distance. The centre non-transgenic circle was also sampled. Plants were grown from the seed samples and sprayed with glufosinate to estimate the frequency of pollen dispersal at each distance. In order to screen enough samples to detect low frequency cross-pollination events, seed samples were tested in the greenhouse and on a larger scale in the field. Results were confirmed by testing progeny for glufosinate resistance and by Southern blot analysis. The estimated percentage of pollen dispersal in the non-transgenic centre circle was 4.8%. The frequency was estimated to be 1.5% at a distance of 1 m and 0.4% at 3 m. The frequency decreased sharply to 0.02% at 12 m and was only 0.00033% at 47 m. No obvious directional effects were detected that could be ascribed to wind or insect activity.

Keywords: *Brassica napus*; oilseed rape; transgenic crops; pollen dispersal; insect pollination

Introduction

By the use of molecular techniques it is now possible to produce transgenic plants containing a variety of different genes. Some of the transgenic plants currently available are potential new cultivars and others are being used to conduct studies on plant growth and development. Following initial evaluation of transgenic plants in the laboratory and greenhouse, it generally becomes necessary to monitor their performance under field conditions.

Current regulations require that transgenic plants evaluated under field conditions should be partly contained; this includes isolating them from plants that they might hybridize with. In order to provide sufficient genetic isolation for field experiments, it is essential to have information on the extent of gene dispersal for the species under investigation. This is especially important for the crop species at the forefront of transgenic research, where field release experiments are imminent or already in progress on a small scale. The objective of the study described here was to evaluate pollen dispersal in *Brassica napus* (oilseed rape).

Oilseed rape is self-compatible and can produce a large quantity of seed without cross-pollination (Free and Nuttall, 1968; Williams, 1978); however, cross-pollination is possible if the pollen is transported by wind or insects. At present the relative contribution of wind and insects as pollinators is not clear. Oilseed rape pollen has been detected in the air above rape crops (Langridge and Goodman, 1982; Williams, 1984), and trapped at a

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distance of 40 m from a rape crop (Olsson, 1955). Mesquida and Renard (1982) detected rape pollen 32 m from oilseed rape plots, but noted that the concentration of pollen collected decreased rapidly with distance from the pollen source. McCartney and Lacey (1991) reported similar results and concluded that windborne pollen was unlikely to play a significant role in long distance cross-pollination of oilseed rape.

Insects, especially honeybees (*Apis mellifera* L.) and bumblebees (*Bombus terrestris* L.), collect nectar and pollen from oilseed rape plants, and pollen is transferred as the bees move from plant to plant. As honeybees can travel one to two kilometres from the hive, it is possible that pollen could be transferred far beyond the borders of an experimental plot (Eckert, 1933). However, studies indicate that honeybees tend to visit plants that are located in a small area as near to the hive as possible, and often forage only one plant species per foraging trip, although there are exceptions (Gary, 1975; Martin, 1975; Ribbands, 1953). Bumblebees exhibit similar behaviour, but are more likely to visit more than one plant species during a foraging trip (Alford, 1978).

Hühn and Rakow (1979) estimated the frequency of cross-pollination (outcrossing) in winter oilseed rape grown under field conditions in northern Germany. They grew high and low (< 5%) erucic acid cultivars with the distance between plots, of the high and low types, ranging from 2.5 m to 7.5 m. Seeds from the low erucic acid cultivars were analysed, and those with 20% or greater erucic acid content were classified as hybrids between the low and high erucic acid cultivars. They estimated outcrossing to be from 5% to 15%, depending on the cultivar and distance between plots.

In Canada, Rakow and Woods (1987) planted five different low erucic acid genotypes in alternate rows with a high erucic acid genotype. Based on three years data, the average outcrossing rate for adjacent plants of spring oilseed rape was 21.8%. The outcrossing rate recorded for the cultivar Westar was 23.2%. In another study, using petal colour as a marker, outcrossing rates for adjacent plants were estimated to range from 27% to 36% (Olsson, 1952; Persson, 1956).

There are few published reports estimating the frequency of pollen dispersal in oilseed rape over longer distances. Previously, it has been difficult to find genotypes with a stable genetic marker that could be easily detected, as dispersal experiments require the screening of large numbers of plants. One abstract reported using a recessive chlorophyll-deficient mutant as a marker to estimate the frequency of cross pollination in blocks located 47 m, 137 m and 366 m from a field of commercial oilseed rape (Stringham and Downey, 1982). Green progeny from the chlorophyll-deficient plot were assumed to be a product of hybridization. The estimates of outcrossing were 2.1% at 47 m, 1.1% at 137 m and 0.6% at 366 m. At two locations 'where flowering synchrony was not achieved, a background contamination level of 0.2% and 0.5% were recorded'. The study suggested that cross-pollination may have occurred at 47 m and up to 366 m, but the data were inconclusive.

In view of the importance of estimating the distances that will give acceptable levels of genetic isolation for evaluating novel transgenic plants, a study was undertaken using a transgene as the selectable marker. The use of a dominant transgene, conferring herbicide resistance, allowed many thousands of potential hybrid seeds to be screened from a range of distances. The field plot design was based on that developed by M. Renard (INRA, France) in a preliminary experiment. The results of complementary gene dispersal experiments by Plant Genetic Systems in Belgium and INRA in France will be presented in a later publication.

Materials and methods

Plant material

The transgenic and non-transgenic plants used in the study were derived from the spring oilseed rape variety Westar (Klassen *et al.*, 1987). The transgenic plants contained the *bar* (phosphinothricin acetyltransferase) gene (Thompson *et al.*, 1987) which is a dominant gene conferring resistance to the herbicide glufosinate-ammonium (tradenames Basta^R and Challenge^R, Hoechst). The transgenic seed stock was obtained from Plant Genetic Systems (PGS) in Belgium. Greenhouse screening of a sample of the transgenic seed stock supplied showed that, because of the way the seeds had been harvested in the previous year, 65% of the seeds were herbicide-resistant and 35% were not. In a test of progeny of plants from the original sample, 95% of the resistant plants were scored as homozygous for the *bar* gene.

1990 field trial

The field experiment was sown on 10 April, 1990 (Fig. 1). The 1 m diameter circle, in the centre of the plot, was hand sown with non-transgenic seeds and the 9 m diameter circle surrounding it was sown with the transgenic seed stock. Both were sown at a rate of 6 kg ha^{-1}. The purpose of the 1 m circle was to allow estimation of the frequency of cross-pollination with the transgenic plants under conditions where the non-transgenic plants were in close contact with the transgenic plants. Non-transgenic seeds were machine-drilled around the transgenic plants at a rate of 5 kg ha^{-1}, to a distance of at least 48 m from the outside edge of the circle of transgenic plants. The total area of the plot was 1.1 ha.

The seeds emerged evenly and reached the 1–2 leaf stage by the end of April. There was minimal rainfall during the growing season, and the plot was irrigated twice during late May and early June. The plot was sprayed

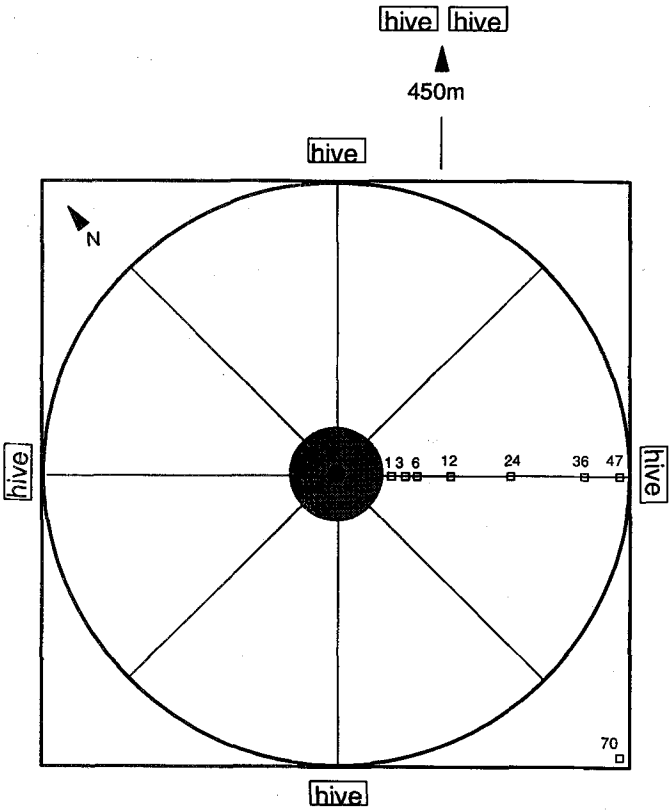

Fig. 1. Field plan for the 1990 oilseed rape (*Brassica napus*) field experiment. The 1 m diameter centre dark circle contained non-transgenic plants. The surrounding 9 m diameter grey circle contained plants from the transgenic seed stock. The remaining area within the square contained non-transgenic plants. The dimensions of the square are 105 m × 105 m. A total of 264 (1 m^2) samples were taken from the eight sections at 1, 3, 6, 12, 24, 36 and 47 m from the edge of the circle containing transgenic plants. Two 1 m^2 samples were also taken from each of the four corners of the plot (70 m). The location of the honeybee hives is shown.

against pollen beetles once before flowering with 1 l ha^{-1} of the insecticide alphamethrin (Fastac^R).

The plot was divided into eight equal sections by making paths 18 cm wide from the centre to the outer edge of the plot (Fig. 1). Along these paths, 1 m^2 areas were marked out at distances of 1 m, 3 m, 6 m, 12 m, 24 m, 36 m, and 47 m from the edge of the circle containing the transgenic plants. Four 1 m^2 areas were also marked out in the transgenic circle. In each of the 1 m^2 areas, the number of established plants was counted. During the flowering period, regular observations were made of the number of plants flowering and the number and type of insects present in 60 of the 1 m^2 areas. Weather conditions were also recorded. Because there was the possibility that no hybrids would be detected, it was important to document that there was an opportunity (via insects and wind) for cross-pollination to occur during flowering.

To ensure that pollinating insects would be present during flowering, one beehive was placed on each of the four sides of the plot and two at a distance of 450 m northeast of the plot. In an effort to determine if the honeybees could be found throughout the plot and not just in one area, approximately 1% of the honeybees in each hive were marked with a coloured dot on the thorax. The marking was done at the beginning of flowering and additional bees were marked during the season to maintain the level of marked honeybees. Different coloured dots were used for each hive, so that the source of any marked bees observed in the plot could be determined.

By 13 July, most of the plants had finished flowering. On 6 August, the centre circle of transgenic plants was hand-harvested. During the same week 272 × 1 m^2 samples were harvested from the non-transgenic area of the plot. Samples were collected so that 20% or more of the circumference of the plot was sampled at each distance from the transgenic circle. Samples were also taken from the four corners of the plot, 70 m from the edge of the transgenic circle. All samples were air-dried and threshed, starting with the 70 m samples and working toward the centre, to prevent contamination of the outer samples by those nearer the centre. To comply with the conditions laid down by the regulatory authorities, all the vegetative matter and seeds not used for further analysis were bagged and burned.

Greenhouse screening

In this study, pollen dispersal was measured as the proportion of the progeny harvested from non-transgenic plants that contained the marker gene, per number of progeny plants tested. Because only the transgenic plants in the centre contained the marker gene, progeny of non-transgenic plants would contain the gene only as a result of hybridization between a non-transgenic seed parent and a transgenic pollen parent.

Owing to space constraints, greenhouse screening of samples from the 1990 field trial was carried out at eight different times beginning in January 1991 and finishing at the end of October. Benches in an unlit greenhouse were filled with John Innes No. 1 soil mixture and divided into equal size sections (approximately 1 m^2) using plastic dividers. Each section was seeded with 1000 seeds from one of the field samples. Each time the samples were screened, at least one 1000 seed sample of non-transgenic Westar and six transgenic Westar plants were included as controls. The transgenic seeds were sown within rubber rings so they could be easily identified. Samples were evaluated for percentage germination.

At the first true leaf stage, the plants were sprayed twice with 4 l ha^{-1} of a commercial preparation of glufosinate. There was a 2 to 3 day interval between sprayings. One week after the second glufosinate

application, the survivors were counted and moved to a high level containment greenhouse where they were allowed to flower and set seed. Because of space constraints, only half the number of survivors (minimum 10 plants) from the 1 m distance samples were saved for seed production.

Field screening

The number of seeds that could be screened in the greenhouse was too small to allow detection of cross-pollination events at the low frequencies predicted for the samples at the outer distances. Therefore, an additional field screening of samples was planted in early April of 1991 (Table 1). There were 449 machine-drilled plots planted in five tiers, with two plots of all samples from distances of 12 m to 70 m included to increase the number of seeds screened at those distances. Transgenic and non-transgenic Westar plots were included as controls. Five thousand seeds were sown per 1.5 m × 20 m plot. In order to detect uneven spray application in the trial, the area between the tiers was seeded with non-transgenic Westar and spiked with 24 labelled transgenic plants, which were transplanted into the trial prior to spraying. Final plant populations were estimated for each plot by counting the number of plants in four 0.5 m row lengths selected at random.

The field was irrigated weekly until most of the plants had reached the 3 to 4 leaf stage. The plots were then sprayed twice with 4 l ha^{-1} glufosinate, with nine days between applications. With the exception of the 1 m distance plots, all surviving plants from each plot were moved to a high-level containment greenhouse and allowed to set seed. Because of limited space in the greenhouse, only ten plants from each 1 m distance plot were saved. To comply with the Regulatory Authorities' conditions, all transgenic plants and survivors, not saved for seed production in 1991, were pulled up and burned as soon as the spray test was completed.

Progeny testing of survivors from greenhouse and field screening

In order to estimate the number of putative cross-pollin-

Table 1. The distribution of plots in the 1991 field spray test, with the number of seeds sown and the estimated percentage of established plants.

Distance from transgenic circle in 1990 (m)	*1m² samples harvested in 1990*	*Plots in 1991 field spray test*	*Seeds sown*[a]	*Plant establishment %*
1	24	8	40000	32
3	24	16	80000	37
6	24	16	80000	37
12	32	64	320000	34
24	32	64	320000	37
36	48	96	480000	37
47	80	159	784190	38
70	8	10	39200	33
Total	272	433	2143390	-
Transgenic controls	-	2	10000	32
Non-transgenic controls	-	14	70000	24
Total	-	449	2223390	-

[a]All seed used in the 1991 field spray test, except the transgenic and non-transgenic controls, came from the 1990 field trial. The transgenic controls were greenhouse-grown seed homozygous for the *bar* gene. The non-transgenic control was commercial Westar seed. A few samples from 47 m and 70 m had insufficient seed to plant 5,000 seed in each plot. In these, the seed samples were evenly divided between the two plots, except in one case where a 47 m sample with insufficient seed was sown in a single plot.

ation events (between transgenic and non-transgenic plants) that escaped herbicide treatment rather than being genetically resistant, a sample of progeny seed from the greenhouse and field screenings was evaluated in a spray test. Twenty-six seeds, from each putative hybrid plant tested, were sown in plastic trays and sprayed as described for the greenhouse screening. Four non-transgenic Westar seeds were sown in each tray as a negative control. Thirty-seven samples of progeny seed, from the transgenic plants used in the greenhouse or field screening tests, were included as a positive control to confirm that plants containing the *bar* gene could survive spray treatment at the rate applied.

The numbers of germinated seeds and resistant plants were recorded, and those segregating within the limits of 3:1 as determined by a χ^2 test, were scored as hybrids. Any samples that were on the borderline were retested using a second sample of 26 seeds. Tissue samples from a subset of putative hybrids and their progeny were checked using Southern blot analysis (Sambrook *et al.*, 1989) to confirm presence or absence of the *bar* gene. The proportion of plants surviving the initial screening and having resistant progeny was calculated and used to adjust the initial estimates of pollen dispersal frequency.

Results

The densities of plants that became established in the plot were estimated to be 57 per m^2 for the circle containing the transgenic plants and 38 per m^2 for the large non-transgenic area. Of the transgenic seed sample, 65% contained the *bar* gene. The presence of plants without the *bar* gene would reduce the number of rape pollen grains carrying the marker gene; however, the 67% higher plant density in the transgenic circle would help compensate for this.

Flowering was synchronous between the transgenic and non-transgenic plants during the flowering period which lasted approximately 25 days. Throughout the flowering period, the number of bumblebees, honeybees and flies was monitored regularly. Although the number of insects foraging at any one time was affected by the weather, the overall trend showed that the number of insects was positively associated with the number of plants in flower (Fig. 2). There was a small number of flies in the plot, but they were not specifically working the flowers.

A total of 25 marked honeybees from the hives on the four edges of the plot, were sighted in the centre transgenic circle and throughout the rest of the plot; indicating that the short travelling distance from these hives allowed them to forage freely over the whole hectare plot. Twelve marked bees from the two 450 m hives were also sighted. These were observed only on the side of the plot closest to their hives.

Germination percentages for the samples sown in the greenhouse screening test ranged from 95% to 99%, supporting the premise that there was no difference in plant establishment between hybrids containing the marker gene and those without the gene.

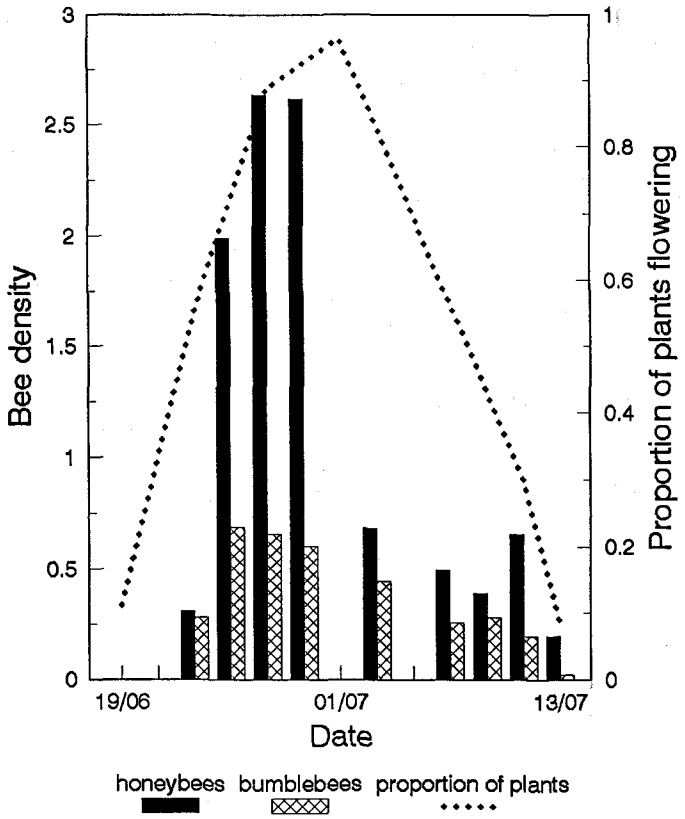

Fig. 2. The mean density per m^2 of bees observed in 60 1 m^2 sample areas, on nine days throughout the flowering period. The line graph indicates the proportion of plants flowering in the same 60 sampling areas.

The progeny tests of plants from both the greenhouse and the field, indicated that there were plants which survived the initial herbicide application that were not hybrids. The results of the progeny screening were then confirmed by analysing a sample of plants using Southern blot analysis, with the *bar* gene as the probe. Based on the results of the progeny screening, the pollen dispersal frequencies were adjusted, with the frequency estimate for each distance being multiplied by the proportion of spray test survivors with resistant progeny (Tables 2 and 3).

The survival of non-hybrid plants may have been the result of incomplete herbicide coverage or low photosynthetic activity owing to low light intensity and low temperatures. As expected, the greenhouse screening gave fewer escapes, which were distributed throughout all the samples tested. There were survivors in the non-transgenic control plots of the field screening, but all scored as susceptible in the progeny test.

Based on the greenhouse screening, the percentage of pollen dispersal in the 1 m centre non-transgenic circle

Table 2. Greenhouse screening results from seeds harvested from the 1990 experiment, showing the number of seeds sown from the seven distances, the number of plants surviving initial spraying with the herbicide glufosinate, the proportion of those survivors confirmed to have herbicide-resistant progeny and the estimated pollen dispersal frequency

Distances from the central plot of transgenic plants (m)	*Seeds sown*	*Plants surviving spray treatment*	*Survivors' progeny tested*	*Proportion of survivors with resistant progeny*	*Estimate of resistant seeds per million seeds*	*Estimated pollen dispersal frequency (%)*
1	16000	256	54	0.87	13920	1.4
3	16000	66	34	0.85	3506	0.35
6	16000	7	4	0.75	328	0.033
12	16000	4	4	1.00	250	0.025
24	24000	0	0	–	0	0.0
36	32000	1	1	1.00	31	0.0031
47	48000	0	0	–	0	0.0
70	12000	0	0	–	0	0.0
Centre non-transgenic[a]	7000	353	22	0.95	47907	4.8
Transgenic control[b]	104	100	5	1.00	–	–
Non-transgenic control[c]	15000	0	0	–	–	–

[a]Seeds from non-transgenic plants located in the inner 1 m diameter circle of the 1990 field trial.
[b]Seeds used as transgenic controls were all from glasshouse-grown plants homozygous for the *bar* gene, except for the first set where stocks segregating for the transgene were used. The four susceptible transgenic controls were from the first set of samples screened.
[c]Certified seed of Westar.

was 4.8%. The frequency of pollen dispersal for the 1 m distance samples was estimated to be 1.4% and only 0.4% for the 3 m distance samples. There was a sharp decrease in frequency of cross-pollination events between 3 m and 6 m, and again between 12 m and 24 m. In the greenhouse screening, only one hybrid was detected (at 36 m) in the samples from 24 m to 47 m (Table 2).

The field screening allowed larger numbers of seed to be screened (Table 1), and a small number of cross-pollination events was detected in samples from 24 m to 47 m (Table 3). The estimated frequencies for samples taken from 1 m to 12 m were similar to those from the greenhouse screening.

The data from the greenhouse (data not shown) and field screenings were separated by compass direction and distance, in order to evaluate the relative effect of bees and wind on the frequency of pollen dispersal (Table 4). Weather records showed that the prevailing wind was towards the east for 16 and to the south for 9 of the 29 days that the plot was in flower. The wind was to the north (two days), west and southeast on the remaining four days. The wind speed ranged from 3.6 kph to 29.5 kph and averaged 14.8 kph. Based on the prevailing wind data, the east, northeast and southeast samples should have the highest frequency of cross-pollination events. In this study, the east and southeast samples had the lowest recorded pollen dispersal frequency.

Discussion

The essential feature of the experiment was that there was a central source of transgenic plants producing pollen containing the *bar* gene and that the gene's movement could be tracked outwards by the mass screening of seedlings.

The transgenic glufosinate-resistant plants proved to be ideal material to study the extent of pollen movement in oilseed rape. Resistance was conferred by a single dominant gene that was easily detected in the F_1 progeny. Both the greenhouse and field methods of screening for hybrid

Table 3. Field screening results from seeds harvested from the 1990 experiment, showing the estimated number of plants established from the seven distances, the number of plants surviving initial field spraying with the herbicide glufosinate, the proportion of those survivors confirmed to have herbicide-resistant progeny and the estimated pollen dispersal frequency

Distance from the central plot of transgenic plants (m)	*Estimate of plants established in the field*	*Plants surviving spray treatment*	*Survivors' progeny tested*	*Proportion of survivors with resistant progeny*	*Estimate of resistant seeds per million seeds*	*Estimated pollen dispersal frequency (%)*
1	12650	284	56	0.71	15940	1.6
3	29811	165	104	0.73	4040	0.4
6	29206	50	44	0.68	1164	0.11
12	110169	38	34	0.47	162	0.016
24	117240	6	5	0.80	41	0.0041
36	179788	2	2	1.00	11	0.0011
47	295176	1	1	1.00	3	0.00034
70	12884	0	0	0	0	0.0
Transgenic controls	3209	–	15	1.00	–	–
Non-transgenic controls	17020	3	3	0	0	0.0

Table 4. Number of plants surviving spray treatment in the field screening test, partitioned by distance and compass direction of seed samples screened from the 1990 field trial

	Distance from the edge of the transgenic circle (m)								*Estimate of plants sprayed*	*1 m^2 samples from the 1990 field trial tested*
Direction	*1*	*3*	*6*	*12*	*24*	*36*	*47*	*Total*		
North	26	20	7	4	1	0	0	58	94873	53
Northeast[a]	35	23	10	2	0	1	1	72	91153	53
East	26	17	6	3	0	1	0	53	97755	53
Southeast	20	14	5	11	0	0	0	50	97426	53
South	33	17	9	3	1	0	0	63	99429	53
Southwest	49	20	3	4	0	0	0	76	100637	53
West	30	27	6	7	4	0	0	74	93570	52
Northwest	65	27	4	4	0	0	0	100	99197	53
Total	284	165	50	38	6	2	1	546	774040	423

[a]The near beehives were located on the edge of the northeast, northwest, southeast and southwest sections. The distant hives were 450 m to the northeast of the plot.

progeny proved effective. In the greenhouse, there were fewer escapes (susceptible plants surviving glufosinate treatment) than in the field. However, in the field, a larger number of seeds could be screened, making it easier to detect rare cross-pollination events at the furthest distances.

An important factor influencing the frequency of escapes in the greenhouse test was the quality and quantity

of light received by the plants after spraying. Low temperatures increased the length of time between spraying and the appearance of definite symptoms, but did not affect the expression of symptoms. The frequency of escapes in the greenhouse was decreased to less than one percent when optimum lighting conditions were used. Another factor that may have influenced the number of survivors in a sample was shielding of sensitive plants by resistant plants during spray application.

For all pollen dispersal estimates made, the frequency of escapes was determined and the pollen dispersal frequency adjusted accordingly. After this adjustment, there was good agreement in frequency estimates between the glasshouse and field, with detectable pollen movement decreasing dramatically over the first 12 m and becoming negligible at 47 m.

When compared with the gene dispersal results from a comparable experiment in Belgium (P. Rüdelsheim, personal communication), the trend was similar, but the frequency of cross-pollination for the centre 1 m diameter non-transgenic circle was approximately twice that found in this study. Results from the French experiment estimated frequency of cross-pollination in the 1 m circle to be 6.6%. Genotypic differences were not the cause of the variation, as both the transgenic and non-transgenic seed used in all the experiments came from the same source. Environmental variation is a likely cause of the differences observed, and confirms the importance of testing gene dispersal in more than one environment. While a small number of hybrids was detected at 36 m and 47 m in the UK study, none were observed beyond 32 m in the Belgian or French screening tests.

In the UK field trial, the prevailing wind was predominantly towards the east, but no obvious directional effects were detected from examining the location of confirmed cross-pollination events. Similarly, no directional effects were observed in the Belgian or French trials.

This study showed that under the conditions described, there was the possibility of pollen travel for distances up to 47 m, but that at 47 m the frequency was very low. No pollen dispersal was observed in the corners of the non-transgenic area at a distance of 70 m from the nearest transgenic plants, but the sample size at these positions was small, with less than 25000 seeds screened, compared with 340000 at 47 m.

The dynamics of pollen movement may depend on the size and orientation of the plots used, as well as the climatic conditions and insect movements (Bateman, 1947; Crane and Mather, 1943). Because of this, there is now a need to measure the extent of pollen dispersal, in typical research and breeding plots, for distances beyond 50 m. This is necessary to determine if the 200 m and 400 m isolation distances commonly used at present provide an adequate genetic barrier for the evaluation of transgenic plants.

Any test to measure pollen dispersal frequencies at distances beyond 50 m would involve screening millions of seeds, and testing could not easily be done in a greenhouse. A field experiment is currently being conducted in which progeny seed of all the non-transgenic receptor plants is screened *in situ*. This method allows the maximum number of seeds to be rapidly screened with a minimum of handling.

Acknowledgements

We acknowledge the European Community Biotechnology Action Programme for sponsoring our collaboration on gene dispersal from transgenic oilseed rape plants, with Plant Genetic Systems, Gent, Belgium and INRA, Rennes, France. We thank PGS for providing the transgenic seeds, Peter Beckley for marking the bees and maintaining the hives, and Jamie Claxton for technical assistance. This research was supported by the UK PROSAMO Programme, a consortium of the Department of Trade and Industry, the Agricultural and Food Research Council, and industrial members. The sponsoring companies were: Advanced Technologies (Cambridge) Ltd, Agricultural Genetics Company, CIBA-Geigy plc, Du Pont (UK) Ltd, Hoechst AG, ICI plc, Monsanto Europe, Plant Genetic Systems, Shell Research Ltd and Unilever (UK) Ltd. The research was conducted under MAFF Licence No. PHF 1185A/46(100).

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