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ISSN 1479-2621

Plant Genetic Resources **4**(2); 96–107
DOI: 10.1079/PGR2005103

Out-crossing between genetically modified herbicide-tolerant and other winter oilseed rape cultivars

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Received 21 February 2005; Accepted 12 September 2005

Abstract

Out-crossing between genetically modified herbicide-tolerant (GMHT) and non-GM rape cultivars was studied using GMHT source field plots of approximately 0.8 ha. Levels of cross-pollination between adjacent fully fertile rape varieties declined rapidly with increasing distance from the interface between plots. A varietal association with low levels of male sterility showed higher levels of out-crossing than other varieties. Out-crossing data were used to compare negative exponential and inverse power-law models for their fit to describe the observed relationship between cross-pollination and distance from source. Results showed that the inverse power-law model provided a better fit of the data.

Keywords: dispersal curves; genetically modified; herbicide tolerance; oilseed rape; out-crossing

Introduction

There has been considerable study of the ability of oilseed rape to disperse pollen and out-cross in anticipation of the deliberate and commercial release of genetically modified (GM) oilseed rape. Studies have shown that although oilseed rape varieties are self-fertile, additional pollination by wind and insects is required to achieve a high seed set. In addition, oilseed rape varieties show a high degree of receptiveness to pollen from other varieties and a tendency to out-cross (Eastham and Sweet, 2002). Concentration of pollen and levels of cross-pollination have been shown to decline rapidly with distance (Scheffler *et al.*, 1993; Colbach *et al.*, 1999). However, studies of movement of oilseed rape pollen have demonstrated dispersal of viable pollen over long distances at low frequencies (Timmons *et al.*, 1995; Thompson *et al.*, 1999; Ramsay *et al.*, 2003).

Both insects and wind are widely recognized as influencing the transport of oilseed rape pollen (Williams, 1987; McCartney and Lacey, 1991) although the relative importance of wind and insect pollination still remains unclear (Ramsay *et al.*, 2003). Differences in experiment design, genotypes and environmental conditions may have contributed to the wide variation in reported rates of gene flow. In field experiments, the relative size of the pollen source and receptor plots is probably one of the main factors causing variation in results. However, common to all studies is an initial rapid decline in cross-pollination frequency with distance from the source plot.

Gliddon (1999) considered that most of the data reported for transgene dispersal experiments are inappropriately presented and are of little use in risk assessment partly because of experimental design (e.g. using small pollen sources and large recipient populations) and most fail to fit a distribution to the data. The dispersal behaviour of small particles such as pollen grains has been studied for many decades and models with various

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degrees of complexity have been developed. Dispersion models have been used to describe the dispersal of fungal spores (McCartney and Bainbridge, 1984; Fitt and McCartney, 1986) pollen (Raynor *et al.*, 1974; McCartney and Lacey, 1991; Lavigne *et al.*, 1998) and seeds (Squire *et al.*, 1997).

Of these models, the simplest are empirical dilution curves which are derived by fitting pre-selected functions of a suitable general form to observed data. Among the empirical dilution curves the two which have received the greatest attention are the negative exponential (e.g. McCartney and Lacey, 1991; Kareiva *et al.*, 1994) and the inverse power-law (e.g. Gregory, 1968; McCartney and Bainbridge, 1984). Simple versions of these models are given as Equations 1 and 2, respectively:

$$y = a\exp(-bx) \tag{1}$$

$$y = ax^{-b} \tag{2}$$

The aim of this study was to measure the dispersal of herbicide tolerance genes from field plot areas of herbicide-tolerant (HT) oilseed rape cultivars to similar size plots of both conventional oilseed rape and the HT oilseed rape. Comparisons were made using two conventional receptors, a varietal association (VA) cultivar which contains approximately 80% male sterile plants together with a standard open-pollinated variety, and the HT varieties. Simpson *et al.* (1999) showed that the out-crossing potential of the varietal associations was higher and that greater isolation from GM crops may be needed to achieve required purity thresholds in the future.

Materials and methods

Trial establishment

In early September 1998, winter oilseed rape cultivars tolerant to the herbicides glufosinate ammonium (LL), glyphosate (RR) and imidazolinone (IMI), and the non-HT varieties were sown in adjacent plots (92 m × 92 m) in two blocks in a 10 ha field at NIAB experimental farm, Cambridge. The conventional plots were equally divided and sown half with the conventional open-pollinated cultivar Apex and half with the varietal association Synergy. The schematic layout of the plots is shown in Fig. 1.

Five varieties acted as potential recipients of HT pollen in the out-crossing experiment; glufosinate-tolerant (LL), glyphosate-tolerant (RR), imidazolinone-tolerant (IMI), and the conventional cultivars Apex and Synergy (VA).

Measurements of crop and factors influencing cross-pollination

Records of the growth and development of the plants and the start and finish of flowering of each plot were taken to estimate synchrony of growth and flowering between varieties. Growth stages of oilseed rape were measured according to Sylvester-Bradley and Makepeace (1984).

Crop density was measured in each plot in November 1998 by counting 101 m crop row-lengths.

The heights of varieties were recorded when the majority of the rape plants had started to flower (growth stage 4.8 on 27 April 1999). Twenty plants from each plot were measured from soil level to the tip of the main raceme.

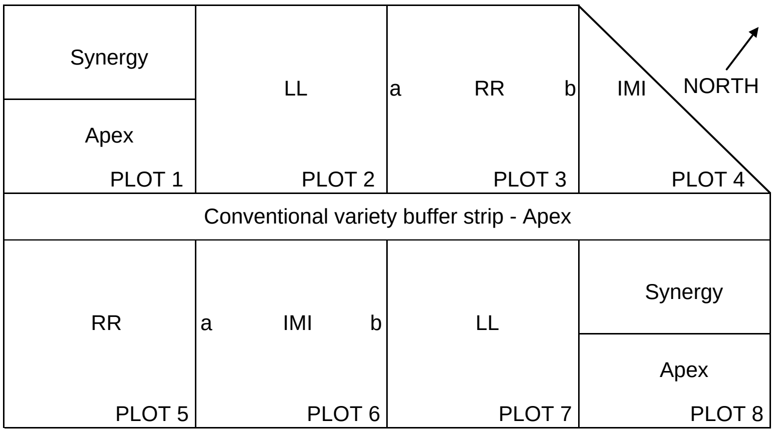

Fig. 1. Plot layout of conventional and herbicide-tolerant winter oilseed rape cultivars in 1998–99 at NIAB, Cambridge, UK. Plot size: 91.5 m × 91.5 m, plot 4 approximately half the area. Conventional variety plots 91.5 m × 45.75 m, a/b: side of plot sampled. Synergy, conventional variety of oilseed rape (varietal association); Apex, conventional variety of oilseed rape; LL, glufosinate-tolerant oilseed rape; RR, glyphosate-tolerant oilseed rape; IMI, imazamox-tolerant oilseed rape.

The incidence of pollinating insects (honey bees/ bumble bees) was recorded during flowering. The number of bees in a 90 m long, 1–2 m wide strip was counted along each edge of each plot. These assessments along the edges of plots were made on three occasions when the oilseed rape was in full flower (6, 12 and 19 May 1999). Observations were made from late morning and into the afternoon, when it was not raining or windy.

Weather conditions were recorded at the NIAB meteorological station on a daily basis from the onset to the end of the main flowering period (approximately 9 April to 27 May 1999).

Seed sampling and testing procedure

Location of sampling points

Three linear transects were established at 20 m intervals across each block and were sampled at 1.5, 6.5, 11.5, 16.5, 21.5, 41.5, 61.5 and 81.5 m from the adjacent oilseed rape variety. Conventional plots had three linear transects established at 10 m intervals across and were sampled at more frequent distances: 1.5, 6.5, 11.5, 16.5, 21.5, 26.5, 31.5, 41.5, 51.5, 61.5, 71.5, 81.5 and 91.5 m.

Seed harvesting

The main raceme was removed from 20 plants within a 1 m^2 quadrat at each sample point. Racemes were collected in large cloth bags and dried on glasshouse benches (18–22°C) for approximately 14 days. Seeds were removed from pods by crushing the racemes in cloth bags and hand sieving (1 mm slot sieve) to remove debris. Seeds were randomly sub-sampled in order to test two replicates of 1000 seeds per sample. Samples of 1000 seeds were prepared using a digital automated seed counter (Pfeuffer, Contador).

Seed testing for herbicide tolerance

The seed samples were sown and grown in seed trays (40 cm × 50 cm, FYBA) containing a multi-purpose peat-based potting compost (Shamrock) under glasshouse conditions (18–22°C; supplementary lighting 400 W HPS, 16 h photoperiod). A herbicide-susceptible control (a conventional non-tolerant winter oilseed rape variety: Falcon) was grown in a similar way as each herbicide tolerance test. Trays were arranged on glasshouse benches using a randomized block design. Seedlings derived from seeds harvested from particular receptor plots were treated with herbicides for which there was tolerance in the adjacent donor plots, at growth stage 1.2 (two leaves). The herbicide treatments were selected based on previous experience with these herbicides and experience from other seedling screening studies at NIAB (Norris & Sweet, 2002; Simpson & Sweet, 2002; Sweet *et al.*, 2004):

- a 1% solution of glufosinate-ammonium (200 g/l);
- a 0.5% solution of glyphosate (360 g/l);
- a 1% solution of imazamox (40 g/l + wetter).

All were applied using a hand sprayer (Hozelock, Polyspray P2).

The numbers of surviving plants were assessed approximately 7 days after treatment for glufosinate, and after 14–21 days for glyphosate and imazamox. Survivors from each replicate of 1000 plants were re-treated with the herbicides at or after growth stage 1.3 (three leaves), and surviving plants counted as in the first treatments. The herbicide treatments were also applied at this time to 12 plants grown from the seed of the herbicide-tolerant varieties initially used to sow the field trials. This was done to confirm that the parent material of the harvested seed showed tolerance to the respective herbicide treatments applied to the test seedlings.

In addition, 10 seedlings expressing tolerance to glufosinate and glyphosate were selected at random from the seed trays and tested by PCR for the presence of the *bar* gene and 5-*enol*pyruvylshikimate-3-phosphate synthase (EPSPS), respectively, to confirm the presence of the herbicide tolerance genes.

Empirical dispersal curves

Out-crossing data were used to compare negative exponential and inverse power-law models for their fit to describe the observed relationship between cross-pollination and distance from source. Estimates of the parameters in Equations 1 and 2 were obtained by ordinary least-squares linear regression after transformation of the original data. The linear forms of Equations 1 and 2 are shown as Equations 3 and 4, respectively:

$$\ln(y) = \ln(a) - bx \tag{3}$$

$$\ln(y) = \ln(a) - b\ln(x) \tag{4}$$

Thus, the parameters of the negative exponential model are obtained as the intercept and gradient of the fit of the logarithm of the number of cross-pollination events against the distance. The corresponding parameters for the power-law are obtained by fitting the logarithm of the number of cross-pollination events against the logarithm of distance. In both cases regression analyses were performed on $\ln(y + 1)$ to avoid missing values when $y = 0$. The transformation affects the estimated constant (a) but not the gradient (b).

Five varieties acted as potential recipients of GM pollen in the out-crossing experiment: glufosinate-tolerant cv. LL, glyphosate-tolerant cv. RR, imidazolinone-tolerant cv. IMI and conventional cultivars Apex and Synergy (VA). Although all three HT varieties acted as donors of HT pollen, as a result of the trial layout, and because recipient varieties were only tested for the presence of HT traits from their immediate neighbours, only a sub-set of all possible donor–recipient combinations was tested. These combinations are shown in Table 1.

Within each possible donor–recipient combination a separate regression analysis was carried out for each of the three replicate transects, resulting in a set of three estimates of goodness-of-fit for the different dispersal models. A formal comparison of the ability of the negative exponential and inverse power-law models to describe the data was made by carrying out a paired *t*-test on the percentage variance accounted for (%vaf) from the regression analyses.

In order to investigate the influence of the recipient and donor varieties on the cross-pollination behaviour in the trial, variation in the parameters from the negative exponential and inverse power-law models, and the %vaf, were examined using residual estimates by maximum likelihood (REML). For each parameter the data set available for REML analysis consisted of 36 values distributed over the recipient and donor combinations shown in Table 1. For the REML analysis individual replicates were assumed to be a random sample, and formed the residual term of the mixed model. The fixed effects model was specified as the interaction between donor and recipient varieties. Fitting the dispersal curves by regression and the REML analysis of the parameter values was carried out in Genstat 5.4 (for Windows NT).

Table 1. Combinations of recipient and donor variety tested for cross-pollination of herbicide tolerance traits

	Donor		
Recipient	LL	RR	IMI
LL		Yes (3)	Yes (3)
RR	Yes (3)		Yes (6)
IMI	Yes (3)	Yes (6)	
Synergy	Yes (6)	No	No
Apex	Yes (6)	No	No

Values in parentheses are the numbers of replicate transects of each combination examined.

Results

Crop density, crop height, growth stage measurements and pollinating insects

All plots and varieties were within the normal crop densities for oilseed rape restored hybrids, varietal association and normal open-pollinated varieties. A variable seedbed across the field meant that there were some differences in crop establishment (Table 2). Mean crop height differed across the field and between varieties. Plots 1–4 tended to be shorter than plots 5–8 although there was considerable variation across all plots. Growth stages varied between varieties and plots, particularly at the onset of flowering, although this is not clearly evident from the data in Table 2.

The presence of three bee species was identified, *Bombus terrestris*, *Bombus lapidarius* and *Apis mellifera*, and their numbers were recorded. Four bees were recorded on 6 May, 25 on 12 May and 39 on 19 May 1999. Total numbers of bees recorded were extremely low and low numbers of bees were also noted during

Table 2. Mean (and standard error) crop density, height and growth stage range during the main winter oilseed rape flowering period, April to May 1999

				Crop growth stage range during the main flowering period[a]				
Variety	Plot no.	Mean crop density (plants per m^2) ($n = 20$)	Mean crop height (m) ($n = 12$)	6 April	13 April	20 April	27 April	17 May
Apex	1	72.8 (6.793)	1.18 (0.011)	3.5–4.5	3.5–4.5	4.2–4.4	4.8–4.9	5.6–5.8
Synergy	1	67.6 (7.175)	1.06 (0.019)	3.5–4.5	4.0–4.5	4.1–4.7	4.8–5.0	5.7–5.9
LL	2	70.8 (6.365)	1.21 (0.023)	3.5–4.3	3.6–4.0	4.2–4.5	4.8–5.0	5.6–5.9
RR	3	82.0 (7.407)	1.14 (0.015)	3.1–3.5	4.0–4.7	4.7	4.9–5.0	5.7–5.8
IMI	4	98.8 (8.315)	1.13 (0.016)	3.1–4.0	4.0–4.7	4.5–4.8	4.9	5.6–5.9
RR	5	64.4 (5.549)	1.18 (0.016)	3.5–4.3	3.7–4.5	4.5–4.8	4.8–5.0	5.6–5.9
IMI	6	79.6 (4.941)	1.12 (0.017)	3.1–4.5	3.7–4.7	4.7–4.9	4.8–5.0	5.7–5.9
LL	7	82.8 (6.971)	1.25 (0.013)	3.1–4.5	3.7–4.5	4.3–4.7	4.9–5.0	5.6–5.9
Apex	8	86.4 (4.740)	1.25 (0.019)	3.5–4.5	4.1–4.7	4.9–5.0	4.8–5.0	5.8–5.9
Synergy	8	64.0 (7.729)	1.22 (0.012)	3.5–4.5	4.1–4.7	4.7–4.9	4.9	5.8–5.9

[a] Crop growth stages according to Sylvester-Bradley and Makepeace (1984).

general observations of the crop throughout the flowering period. Due to the high frequency of zeros, the data were not statistically analysed. Over all sample days there was a mean of 1.42 bees recorded per 90 m strip (16 × 90 m strips sampled on each day). There was a slight increase in bee activity in the second half of the flowering period, probably due to higher air temperatures. No records of other insects visiting flowers were taken but casual observations made at the same time as the bee records suggested low numbers were present.

The wind velocity and wind directional data showed there was a predominance of approximately south-westerly and north-easterly winds during the main flowering period (9 April to 25 May 1999).

Out-crossing data from plots of conventional and herbicide-tolerant winter oilseed rape

Out-crossing frequencies are expressed as a percentage of herbicide-tolerant seedlings detected in seed samples from each sample point along each of the three transects (each transect consisted of either 13 or eight sample distances depending on variety), the data presented are a mean of two tests of 1000 seeds per sample distance. The schematic layout of the field plots in the experimental field is shown in Fig. 1. The results of out-crossing frequencies as a function of distance (Figs 2–6) have been separated into three main categories:

(i) *Glufosinate-tolerant oilseed rape as a pollen source*

Out-crossing frequencies were the highest in cv. Synergy in plots growing adjacent to glufosinate-tolerant (LL) oilseed rape (Fig. 2). There was a large difference in the frequencies of out-crossing detected in plots 1 and 8. Samples nearest the interface with the pollinator in plot 8 were approximately twice the level of those in plot 1 (32.0% in plot 8 compared with 15.3% in plot 1). Despite these differences in out-crossing frequencies in the two cv. Synergy plots, there was a consistent pattern of decline of out-crossing with distance from the LL pollen source. The profile of the out-crossing decay curve showed a more gradual decline with increasing distance from the pollen source, compared with the other recipient cultivars. Frequencies of out-crossing at all distances were considerably higher than in all other varieties. The level of out-crossing at the most extreme sample distance (91.5 m) was nearly 50 times higher than the mean out-crossing frequencies from all other plots crossed with either glufosinate- and glyphosate-tolerant rape at 81.5 m.

Frequencies of out-crossing in cv. Apex plot 1 were lower than in cv. Apex plot 8 at the interface with the glufosinate-tolerant pollen source (Fig. 3). This corresponds with the lower frequencies of out-crossing detected in cv. Synergy plot 1. The steep decline in out-crossing level with distance in the plots of cv. Apex was consistent in both plots and differed from the profile of the decline curve of cv. Synergy. The frequencies of out-crossing detected in plot 3a (RR) and plot 6b (IMI) were higher over all distances compared to those detected in cv. Apex (Fig. 4), however the decline rate in out-crossing with distance in the GMHT plots was similar to that of cv. Apex. Similar low frequencies of out-crossing were detected at the most extreme sampling point (81.5 m), GMHT plots ranged from 0.06 to 0.1% and cv. Apex was 0.03%.

(ii) *Glyphosate-tolerant oilseed rape as a pollen source*

Out-crossing frequencies in plots 2, 4 and 6a which were cross-pollinated with glyphosate-tolerant oilseed rape were comparable to levels detected in plots cross-pollinated with glufosinate-tolerant rape (Fig. 5).

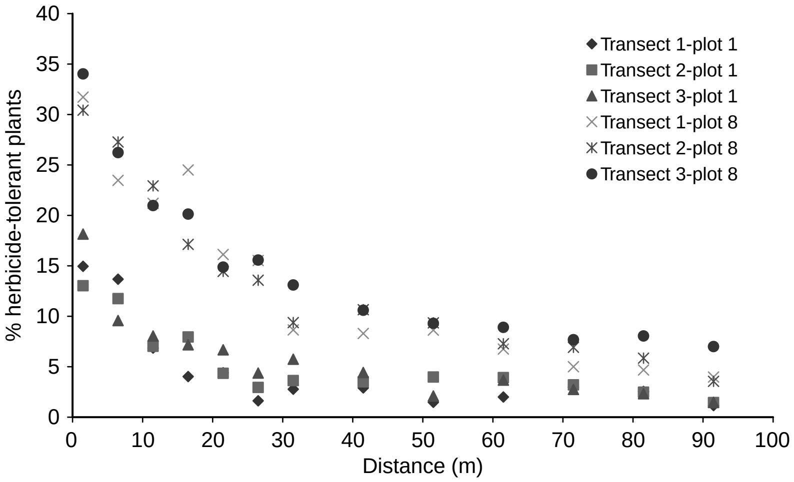

Fig. 2. The percentage of glufosinate-tolerant seeds detected in seed samples from plots of conventional winter oilseed rape (cv. Synergy) growing adjacent to plots of glufosinate-tolerant winter oilseed rape. See Fig. 1 for details of plots.

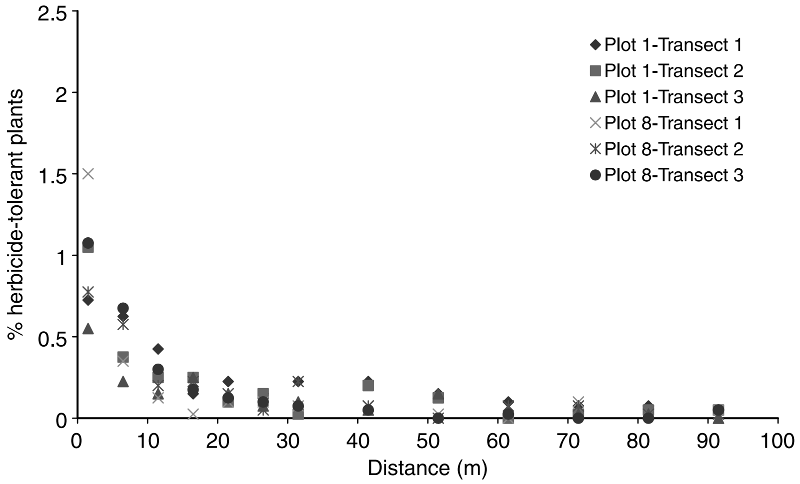

Fig. 3. The percentage of glufosinate-tolerant seeds detected in seed samples from plots of conventional winter oilseed rape (cv. Apex) growing adjacent to plots of glufosinate-tolerant winter oilseed rape. See Fig. 1 for details of plots.

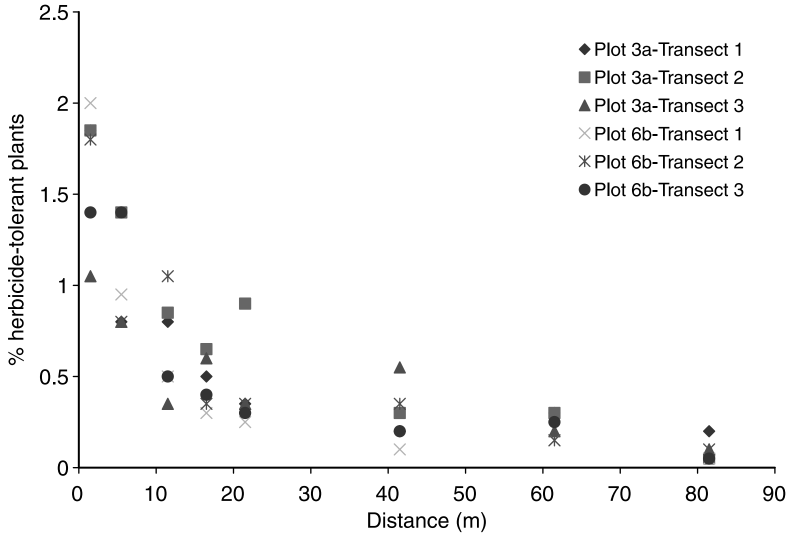

Fig. 4. The percentage of glufosinate-tolerant seeds detected in seed samples from plots of glyphosate-tolerant (cv. RR) and imazamox-tolerant (cv. IMI) winter oilseed rape growing adjacent to plots of glufosinate-tolerant winter oilseed rape. See Fig. 1 for details of plots.

The out-crossing frequencies at the interface between the pollen source and receptor plots were within the same range (1.6–1.7% glyphosate tolerance compared with 1.5–1.7% glufosinate tolerance) and the decline curve with distance was a similar profile to cv. Apex, with a sharp decline in out-crossing in the first 50 m from the pollen source.

(iii) *Imazamox-tolerant oilseed rape as a pollen source*

Out-crossing frequencies detected in plots cross-pollinated with the imazamox-tolerant variety were lower than in crosses with GMHT varieties (Fig. 6). Frequencies of out-crossing detected in plots 3b (RR) and 7 (LL) were considerably lower than all other out-crossing data e.g. 0.1% at 1.5 m (plot 3b) and 0.4% at 1.5 m (plot 7), and were lower than in plot 5 (RR). Although the out-crossing frequencies declined with distance in plots 3b and 7, the extremely low levels detected at all distances meant that the decline profile was different to that of other varieties. Out-crossing data from plot 5 were more comparable with data from other plots crossed with GMHT varieties and followed a similar pattern of decline in out-crossing frequency with distance.

All Falcon seedlings tested showed sensitivity to all the herbicide treatments. Control seedlings of the glufosinate-tolerant variety were not sensitive to the glufosinate treatment applied. Control seedlings of the glyphosate-tolerant variety were not sensitive to the

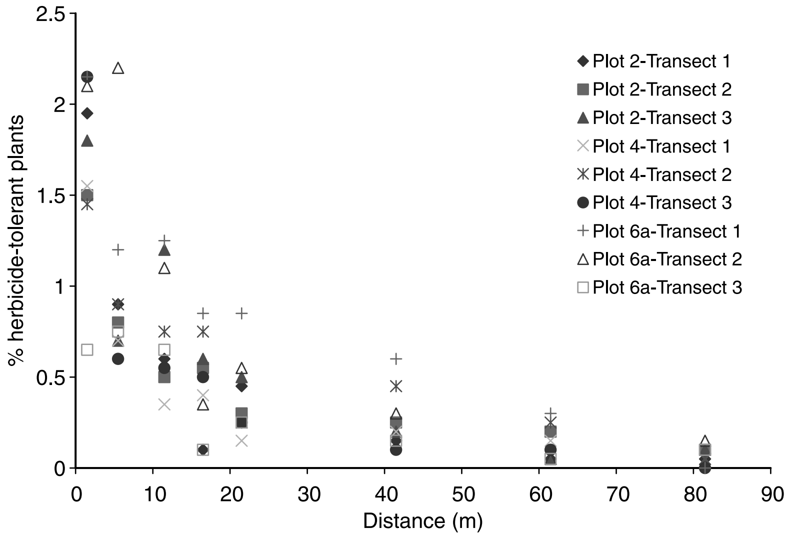

Fig. 5. The percentage of glyphosate-tolerant seeds detected in seed samples from plots of glufosinate-tolerant (cv. LL) and imazamox-tolerant (cv. IMI) winter oilseed rape growing adjacent to plots of glyphosate-tolerant winter oilseed rape. See Fig. 1 for details of plots.

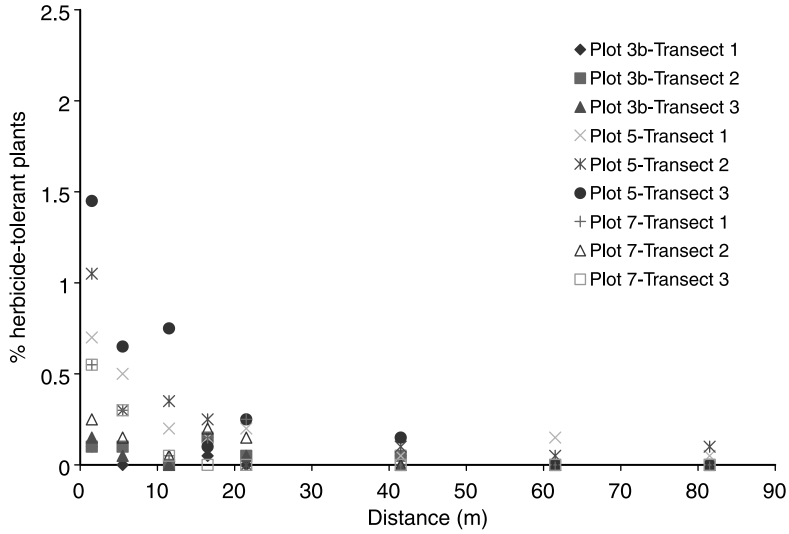

Fig. 6. The percentage of imazamox-tolerant seeds detected in seed samples from plots of glyphosate-tolerant (cv. RR) and glufosinate-tolerant (cv. LL) winter oilseed rape growing adjacent to plots of imazamox-tolerant winter oilseed rape. See Fig. 1 for details of plots.

glyphosate treatment applied. Control seedlings of the imazimox-tolerant variety were not sensitive to the imazimox treatment applied.

All glufosinate-tolerant seedlings and all glyphosate-tolerant seedlings tested by PCR contained their respective herbicide tolerance genes.

Empirical dispersal curves

The observed cross-pollination data could be fitted to the negative exponential and inverse power-law models with varying degrees of success. Examples of the fitted models and observed data (for cultivars Apex and Synergy) are shown in Figs 7 and 8. In each case the fitted model used the mean of the parameter estimates (given in Table 3). The percentage variance accounted for (%vaf) data for the fitted models is given in Table 4.

The paired *t*-test on the %vaf data suggested that the inverse power-law model gave a better description of observed out-crossing than the negative exponential model ($P < 0.001$). The %vaf for the inverse power-law model was greater than that for the negative exponential model in 27 of the 36 transects examined.

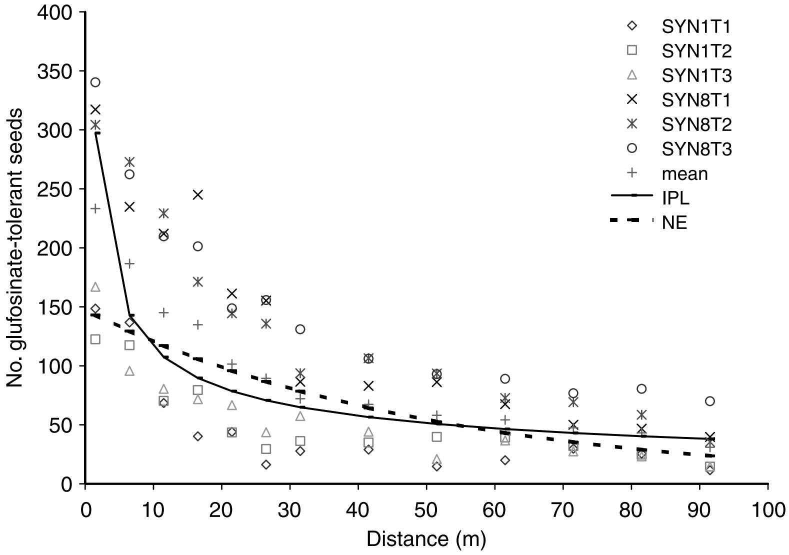

Fig. 7. Fitted and observed out-crossing data where cv. Synergy is the recipient crop and cv. LL (glufosinate-tolerant oilseed rape) is the donor crop. SYN1T1 … SYN8T3, Synergy plot 1, transect 1 … Synergy plot 8, transect 3; IPL, inverse power law; NE, negative exponential.

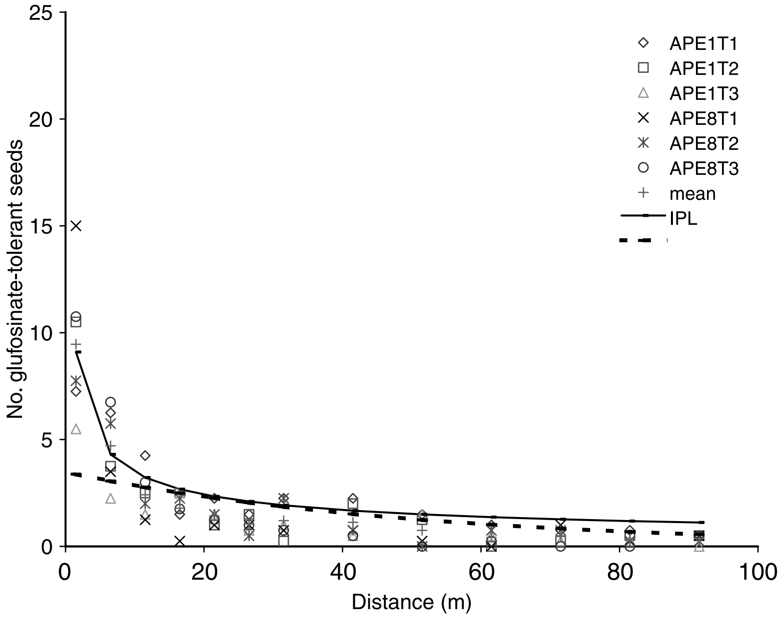

Fig. 8. Fitted and observed out-crossing data where cv. Apex is the recipient crop and cv. LL (glufosinate-tolerant oilseed rape) is the donor crop. APE1T1 … APE8T3, Apex plot 1, transect 1 … Apex plot 8, transect 3; IPL, inverse power law; NE, negative exponential.

For the inverse power-law model, %vaf ranged from 17.9 to 95.8, with a mean of 76.0, while for the negative exponential model the range was 0.3 to 92.6 with a mean of 63.8, while the standard errors for the %vaf data were generally higher for the negative exponential model than the inverse power-law model.

The pattern of parameter estimates across recipient and donor combinations was similar for both models. The estimated constants were higher for cv. Synergy as a recipient than for any other variety in both cases, with the imidazolinone-tolerant variety (IMI) showing lower values than the others. This pattern

Table 3. Parameter estimates[a] (and standard error) for linear versions of two empirical dispersal functions fitted to observed out-crossing data for herbicide tolerance traits in several combinations of recipient and donor varieties of oilseed rape

	Inverse power-law model			Negative exponential model		
	Donor			Donor		
Recipient	LL	RR	IMI	LL	RR	IMI
Parameter: constant (*a*)						
LL		3.3 (0.13)	1.9 (0.18)		2.4 (0.10)	1.1 (0.12)
RR	3.1 (0.25)		1.7 (0.45)	2.4 (0.18)		1.1 (0.14)
IMI	3.3 (0.05)	3.3 (0.19)		2.3 (0.04)	2.4 (0.16)	
Synergy	5.9 (0.06)			5.0 (0.02)		
Apex	2.5 (0.09)			1.5 (0.07)		
Parameter: gradient (*b*)						
LL		−0.60 (0.056)	−0.45 (0.056)		−0.03 (0.002)	−0.02 (0.002)
RR1	−0.46 (0.059)		−0.34 (0.097)	−0.02 (0.003)		−0.01 (0.004)
IMI	−0.59 (0.036)	−0.59 (0.041)		−0.02 (0.001)	−0.03 (0.002)	
Synergy	−0.50 (0.036)			−0.02 (0.002)		
Apex	−0.51 (0.029)			−0.02 (0.001)		

[a] The values shown are the transformed values obtained by fitting Equations 3 and 4 with $\ln(y+1)$ as the dependent variate. Estimated values for the fitted negative exponential and inverse power-law models can be obtained by substituting the values for *a* and *b*, above, into $y = [\exp(a) - 1] \times [\exp(-bx)]$ and $y = [\exp(a) - 1] \times [\exp(-b\ln(x))]$, respectively.

Table 4. Percentage variance accounted for (and standard error) for linear regression models fitted to observed out-crossing data for herbicide tolerance traits in several combinations of recipient and donor varieties of oilseed rape

	Inverse power-law model			Negative exponential model		
	Donor			Donor		
Recipient	LL	RR	IMI	LL	RR	IMI
LL		87.0 (4.22)	70.90 (4.57)		73.7 (6.11)	50.6 (10.60)
RR	79.2 (7.36)		49.8 (12.35)	73.4 (9.92)		36.5 (12.35)
IMI	88.7 (3.74)	78.0 (4.87)		71.3 (2.27)	72.9 (4.91)	
Synergy	82.5 (3.48)			80.5 (8.94)		
Apex	82.6 (2.38)			58.1 (6.50)		

was repeated to some extent for the gradient parameter in the case of the inverse power-law model, but not for the negative exponential model.

The REML analysis suggested that the effects of both recipient ($\chi^2 = 112.2$ (4 df), $P < 0.001$) and donor ($\chi^2 = 77.9$ (2 df), $P < 0.001$) on the estimated constant (*a*) of the inverse power-law model were significant. However, in the case of the gradient parameter (*b*), only the effect of donor was significant ($\chi^2 = 13.0$ (2 df), $P < 0.01$). For the negative exponential model, the effects of both donor recipient ($\chi^2 = 200.4$ (4 df), $P < 0.001$) and donor ($\chi^2 = 100.8$ (2 df), $P < 0.001$) on the estimated constant (*a*) of the inverse power-law model were significant. In case of the gradient parameter, again, only the effect of donor ($\chi^2 = 19.2$ (2 df), $P < 0.001$) was significant. For both models, the imidazolinone-tolerant variety (IMI) as a donor gave rise to relatively flat dispersal gradients, while the glyphosate-tolerant variety (RR) as a donor gave rise to relatively steep dispersal gradients.

Discussion

Out-crossing levels obtained from all varieties in the experiment followed the same rapid decline in cross-pollination frequency with distance. The differences between the two conventional varieties (cultivars Apex and Synergy) in plots 1 and 8 are due to the high proportion of male sterile plants in the varietal association cultivar Synergy and thus the reduced competition from self-pollen. A higher 'susceptibility' of a varietal association to foreign pollen was also reported by Simpson *et al.* (1999). The large differences in out-crossing values from the same varieties in this experiment (plots 1 and 8) can be partially explained by stunting of growth and poor crop vigour in plots 1 and 2 during the winter and early spring. Differences in uniformity of flowering within plots may have also contributed to fluctuations in data more than initiation of flowering.

The hemizygous male and homozygous female parents of the hybrid glufosinate-tolerant variety (LL) contain the *bar* gene at two loci so that the hybrid produces glufosinate-tolerant and non-tolerant pollen in a 5:3 ratio. This means that using herbicide tolerance in seedlings to measure cross-pollination from glufosinate-tolerant varieties will only detect five-eighths of the actual level (G. Ramsay, SCRI, personal communication, 2000; B. Uijtewaal, Bayer Crop Science, personal communication, 2000). By contrast, the glyphosate-tolerant cultivar is homozygous so that all pollen will carry the herbicide tolerance trait. This should theoretically have resulted in lower out-crossing frequencies from the glufosinate-tolerant plots in this experiment. However, the levels of glufosinate and glyphosate tolerance in out-crossed seed from adjacent plots in this study are approximately equivalent, so that other factors such as flowering time, weather conditions and pollen from other plots may have compensated for this predicted theoretical effect.

Overall, the frequency of out-crossing from the imazamox-tolerant oilseed rape cultivar was the lowest (Fig. 6). There are a combination of factors that may have caused this. (i) Imazamox tolerance is conferred by two semi-dominant genes, and it was possible that the lower frequencies of imazamox tolerance were due to difficulty in distinguishing between susceptible hybrid seedlings and heterozygotes with low levels of tolerance, which may not have survived the glasshouse screening test. Control plants that were known hybrids between the imidazolinone-tolerant variety and the recipient varieties would have been able to confirm this but were not available at the time of this study. (ii) Waterlogged soil during the winter reduced the vigour and flower production of one imazamox-tolerant plot. (iii) Imazamox-tolerant plot 4 (adjacent to plot 3) was approximately half the area of all other plots and it is considered that relative pollen source size is a major influence on the level of out-crossing (Timmons *et al.*, 1995). (iv) The cultivar flowered earlier so that the period of overlap with other cultivars for pollen exchange to occur was less than for other cultivars.

It is widely recognized that both insects and wind influence both the transport of pollen and pollination of recipient flowers in oilseed rape. Oilseed rape pollen has frequently been detected above and downwind of different-sized source crops during flowering (McCartney and Lacey, 1991). Some studies have indicated that bees are an effective vector for long-distance dispersal (Scheffler *et al.*, 1995; Ramsay *et al.*, 2003) and short-distance dispersal (Cresswell *et al.*, 1995; Bilsborrow *et al.*, 1998). It is possible that there were significant flights of bees on days when the crop was not observed, but the extremely low numbers of all bee species recorded suggested that wind or other insect species may have been important in the short-range transport of rape pollen.

There does not appear to be a detectable relationship between the summarized wind data and the out-crossing levels detected. According to the summarized wind speed and directional data, the wind direction was mainly along the length of the experimental field approximately equally in both directions (Fig. 1), which makes correlation of the out-crossing data with prevailing wind direction difficult, particularly without quantitative measurements of airborne pollen densities. Wind velocity and directional data recorded on one occasion each day is of limited use when attempting to explain precise directional differences in out-crossing levels. It is difficult to conclude the relative importance of wind and insects as vectors for the dispersal of pollen and subsequent fertilization in this study.

Kareiva *et al.* (1994) stressed the importance of the form of the dispersal curve in risk assessment for GM crops. They examined best-fitting versions of the exponential and Weibull functions for data from GM cotton over a similar range of distances as used in this study. Examination of the data presented in Fig. 3 shows that both the exponential and Weibull functions underestimated observed data at the tail of the data. Thus, in this study, the observed relationship between cross-pollination and distance from sources of GMHT pollen was better described by an inverse power-law function than an exponential function. One of the main consequences of this difference is that for a given source of GM pollen, dispersal behaviour which is described by a power-law is more likely to lead to cross-pollination at large distances than dispersal behaviour which is described by an exponential decay. Pollen dispersal described by the inverse power-law function also showed higher estimates at short distances compared with the negative exponential function. However, the power-law model does not fit the data perfectly, and a function with a steeper initial decline and a slower decay in the long tail would be a better fit to most data sets. Models to provide this better fit are being investigated in the current SIGMEA project (www.dyndns.sigmea).

In addition, the shape of the slope varies across some of the data sets. In particular, the plot 1 cv. Synergy data looks superficially like a negative exponential curve. This change in shape across data sets is typical for gene flow data being studied in the SIGMEA project, and indicates that there are likely to be several processes at work (e.g. insect and wind pollination) that will probably not fit one mathematical function.

Where dispersal of pollen follows an inverse power law there are implications for the management of GM crops of oilseed rape. Most importantly, establishing effective isolation distances for GM and non-GM crops when grown at large scale would become more problematic because of the long probability tail of the dispersal curve, particularly where varietal associations or partially restored hybrids are being grown. Thus 'safe' isolation distances may need to be considerably greater than suggested by dispersal levels indicated by the negative exponential function. When considering the development of sampling methods for regulatory control of GMHT traits, a concentration on dispersal distances from known sources of pollen is unlikely to be effective. Rather, methods focused on detecting whether contamination has, in fact, occurred in known 'target' populations and crops may be more appropriate.

The results presented here are from a large number of comparisons made in one season in the same location and some of the results may be overestimates of gene flow levels due to the multiple sources of GMHT pollen in the design of the experiment. However, because of the heritability of herbicide tolerance in the glufosinate-tolerant restored hybrid and the imazamox-tolerant variety, cross-pollination from these sources will not have resulted in the expression of herbicide tolerance in all subsequent seedlings, and thus there will be an underestimate of out-crossing frequency. Data from studies as these need to be corrected by these heritability factors in order to calculate total out-crossing frequencies.

The data show that isolation distances between fields required to achieve any specified threshold levels for non-GM purity would need to be much greater for varietal associations than for open-pollinated fully fertile varieties. Further data are required on the isolation of varietal associations and partially restored hybrids and on the impact of multiple sources of pollen from GMHT oilseed rape in agricultural landscapes.

Acknowledgements

The authors would like to thank Bayer Crop Science, Monsanto, Cyanamid (now BASF) and Pioneer Hi-Bred UK for supplying seeds, Juliet Osborne for bee expertise, DEFRA for funding the research and Prof. George Marshall for critical scrutiny of this report.

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