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A pollen-dispersal experiment with transgenic oilseed rape. Estimation of the average pollen dispersal of an individual plant within a field

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Abstract In order to help establish a basis for the assessment of gene flow associated with the large-scale release of transgenic oilseed rape, we previously designed a method which makes it possible to retrieve the average pollen dispersal of a single plant from that of a large source plot. The 'individual' pollen distribution thus obtained is less dependent on the experimental design than pollen distributions usually published and could therefore be used to model the possible escape of a transgene from commercial transgenic crops. In this study we report on a field experiment set up to study the pollen dispersal from an herbicide-resistant transgenic variety of oilseed rape and to test the applicability of the method on the experimental data. Two techniques were used to determine the individual pollen dispersal, and their outcomes are compared. The results suggest that approximately half of the pollen produced by an individual plant fell within 3 m and that the probability of fertilisation afterwards decreased slowly along a negative exponential of the distance. Comparison with the global pollen distribution from the source plot indicates that pollen-dispersal distributions based on dispersal from whole plots instead of individual plants would have underestimated the proportion of pollen that was dispersed over average or long distances.

Key words Risk assessment · Pollen flow · Transgene · Fourier transforms · *Brassica napus*

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Introduction

Advances in biotechnology now make it possible to transfer genes from various classes of organisms into crop plants, thus resulting in the production of genetically modified varieties expressing new traits of agronomic, technological or medical interest. Most of these lines are still at the stage of greenhouse or small-scale experimental field trials, but some of them have already been released on a commercial scale. Because gene technology expands the range of genes available to plant breeders well beyond the gene pools available by sexual crosses, it is usually considered as a technological novelty and, therefore, as requiring specific risk assessment studies. The primary concern in these studies is to limit the uncontrolled escape of the introduced genes from the modified crop.

More specifically, the main agronomic and ecological concerns are (1) that the crop itself becomes a weed in subsequent crops or in "natural" populations outside the fields, (2) that the transgene is transferred to other fields of the same species in which it is undesirable, (3) that the transgene is transferred to a wild relative of the crop, thus creating weeding problems, (4) that the introduced trait exerts an undesirable selective pressure on populations of micro-organisms or insects interacting with the crop and (5) that a dangerous trait (such as antibiotic resistance) is transmitted to micro-organisms.

Of these concerns, two (nos. 2 and 3) will directly depend on the dispersal capacity of pollen grains, which has thus been identified a key process to investigate in studies assessing the ecological risks associated with the release of transgenic crops.

Pollen, whether air- or pollinator-borne, has long been considered to fertilise mainly neighbour plants (Levin and Kerster 1974) and thus to be a limited means of gene dispersal (Ehrlich and Raven 1969). More recent studies, however, focusing on pollen carryover (Waser and Price 1982) or using genetic markers for paternity analyses (Ellstrand et al. 1989; Godt and Hamrick 1993) demonstrated that gene dispersal by pollen might often have been underestimated. Dispersal over distances greater than 100 m have, for example, been reported in a number of species such as maize (Bateman 1947), loblolly pine (Friedman and Adams 1985), *Cucurbita* sp. (Kirkpatrick and Wilson 1988), radish (Klinger et al. 1991), sugar-beet (BRIDGE 1995), milkweeds (Broyles et al. 1994) and potato [Skogsmyr (1994) but see Conner and Dale (1996) for a contradictory analysis of the data]. As part of risk assessment studies, pollen dispersal from crop plants has been more recently specifically investigated. Pollen-dispersal experiments directly performed with transgenic plants were carried out with potato (Tynan et al. 1990; McPartlan and Dae 1994; Skogsmyr 1994), oilseed rape (Scheffler et al. 1993), cotton (Llewellyn and Fitt 1996), tobacco (Paul et al. 1995) and sugar beet (BRIDGE 1995). Furthermore, studies directly addressing the question of risks, although not using transgenic plants, were carried out on oilseed rape (Timmons et al. 1995), raspberry (Luby and McNicol 1995) and *Lolium perenne* (Giddings et al. 1997a, b).

The view that small-scale release experiments are sufficient to predict the fate of a transgene once the transgenic crop is grown on a commercial scale has been, however, questioned (Morris et al. 1994; Stone 1994). Such experiments are, indeed, spatially and temporarily limited and do not allow for the detection of rare, but potentially important, events. This view is further supported by past experiences with disease epidemics, the release of biological control agents and the invasion by plants or animals, which suggest that the scale and frequency of introduction highly determine the ability of a new organism to establish (Tiedje et al. 1989). When the escape of the transgene via the pollen is considered, low-frequency long-distance dispersal might prove particularly difficult to assess but be highly relevant since a small number of migrants per generation is sufficient to modify the genetic structure of a population (Wright 1931). Such dispersal is, in particular, expected to have important consequences when the migrant is at a selective advantage compared to the residents, which would be the case if the transgene coded for pest or stress resistance.

A problem faced by pollen-dispersal experiments is that a large source of pollen is necessary for these rare events to become detectable, and the distribution of pollen observed is therefore not that of a single plant but depends on the shape of the source plot. It is therefore highly dependent on the experimental design. To deal, at least partly, with this problem, we designed a method which makes it possible to retrieve the average pollen distribution of a single plant from that of a large pollen source (Lavigne et al. 1996). The distribution thus obtained could be used in models that simulate the commercial use of transgenic crops in order to expand, both in time and space, the results of small-scale releases and therefore to assess the importance of rare events. It could more generally be used to model pollen-mediated gene dispersal in any situation where different individuals are genetically different. Among fields, it could be interesting to assess the impact of pollen dispersal on the quality of seeds when varieties which differ for oil or meal composition (double-low, high erucic acid, low linolenic acid, pharmaceutical peptides) are produced in the same area. Within a field, this would also, for example, allow simulation of the modification of seed quality resulting from the pollen production of a few undesirable individuals. Such individuals could result from the regrowth of volunteer plants with different genetic background, whether the difference is due to a transgene or not, or from the presence of a few pollen-producing hermaphrodites within a supposedly male-sterile variety. More information on pollen dispersal would also be needed to optimise the alternated design used to produce F_1 hybrid seeds or the percentage of pollen donors included in varietal associations (varieties obtained by mixing seeds of a male-sterile F_1 hybrid with seeds of male-fertile lines) to ensure efficient cross pollination.

Here we present the results from a pollen-dispersal experiment performed with an herbicide-resistant transgenic line of oilseed rape. We measure the pollen dispersal from the whole source plot and thereafter, using these data, determine the pollen distribution of a single average plant of the source plot by two different methods. Comparisons of these distributions with the one that could have been deduced directly from experimental data provide insight on the systematic error introduced in models by using the global pollen distribution instead of the individual one.

Materials and methods

The pollen dispersal experiment

Experimental design

The experiment was performed near Rennes in Brittany (France). The male-fertile (MF) transgenic winter oilseed rape line 'BOO4.*oxy*' homozygous for a gene coding for a nitrilase and conferring resistance to oxynil herbicides was used as the pollen source. This herbicide-resistant line was selected through three backcrosses (B_3F_3 generation) from a cross to a 'Westar' transgenic line (Freyssinet

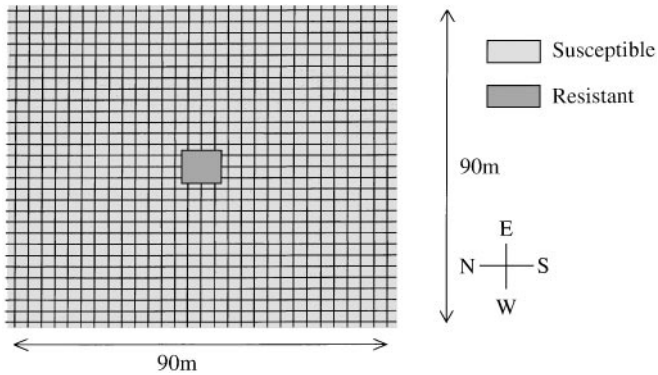

Fig. 1 Schematic representation of the experimental setup. Transgenic herbicide resistant plants were grown in the central area and susceptible plants in the rest of the field. The whole field was covered by a 29×29 matrix of male-sterile recipient individuals

et al. 1995). The plants were sown in October 1994 in a 10-by 10-m square in the middle of a 90-by 90-m field. The area surrounding this central plot was sown with the near-isogenic MF line 'BOO4' susceptible to oxynil herbicides. Distance between rows was 0.5 m. In addition, in order to maximise the detection of pollen and to determine the selfing rate of the male-fertile plants, we sowed ten seeds of the alloplasmic non-transgenic male-sterile (MS) line 'Fu58.BOO4' (*ogu*-INRA cytoplasmic male sterility) on every nod of a 29 by 29 grid (distance between nodes: 3 m) throughout the entire field (Fig. 1). Five weeks after sowing, the transgenic plants of the central plot were treated with the herbicide Buctryl (3 l/ha) to confirm the resistance of the transgenic plants and eliminate potential pollution by susceptible seeds.

Progeny resistance scores

In July 1995, seeds of the male-sterile plants and six fertile plants were harvested separately on each of the 841 nodes of the grid. Out of the 1682 sets of seeds (841 from male-sterile and 841 on male-fertile recipient plots) thus harvested, 14 were unavailable (6 from male-sterile and 8 from male-fertile individuals). Available seeds were sown 2 months later on 1668 1.5-m by 9.6-m plots set at random in a field with a maximum of 50 g seeds per plot to avoid very high densities. A correlation between the mass of seeds sown in a given plot and the number of seedlings in that plot was established from 29 plots (14 from male-fertile plants and 15 from male-sterile ones) in order to estimate the number of seedlings in each plot. All plots were afterwards treated at the one- to two-leaf stage with the herbicide Oxytril (1.5 l/ha), and the number of resistant seedlings per plot was scored. A seedling was considered resistant when it exhibited no sign of chemical burn. A second herbicide treatment was performed 1 month later to confirm the resistance of the plants.

Test of isotropy of pollen dispersal

This was done both for the MF and MS recipient plants. The proportions of resistant progeny were first mapped onto the 29×29 matrix representing the recipient plots of the field. This matrix was afterwards split in four symmetrical quarters which were superimposed by rotation around the plot situated in the centre of the field. In this way, each plot of a given quarter was superimposed with a plot of a second quarter. These two plots were situated at the same distance from the transgenic plants but in a different direction. All pairwise comparisons of these quarters were made with a sign test (Sprent 1989) based on the differences in the proportion of resistant progeny of superimposed plots.

To see if there was a dominant wind direction during the experiment or if insects tended to follow the rows more than they tended to move from row to row while carrying pollen, we split the 29×29 matrix into quarters in two ways: (1) along the diagonals, thus distinguishing the four directions north, east, south and west, and (2) along the lines going through the middle of each side of the field thus distinguishing NW, NE, SE and SW.

Determination of the average individual dispersal function

The principle of the method used and the test of the method on simulated data can be found in Lavigne et al. (1996), and only a short description will be given here.

The total amount of pollen received by a plant can be written as the sum of pollen it receives from every plant in the field. Assuming that all plants have the same pollen distribution and that all pollen grains are equally competitive, the amount of marked pollen from the source plants falling on a given target plant depends on (1) the location and proportion of source plants in every plot of the field, (2) the pollen dispersal distribution, f, from each of the plants and (3) the distance between each source plant and this target plant. More specifically,

$$(g * f)(x, y) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} f(x - x', y - y') g(x', y') dx' \, dy',$$

where $g(x', y')$ is the proportion of marked pollen produced by a plant of coordinates (x', y'), $f(x - x', y - y')$ is the probability that a pollen grain from plant (x', y') falls on a plant of coordinates (x, y) and $(g * f)(x, y)$ is the proportion of resistant pollen falling on plant of coordinates (x, y). Here g only takes the values 0 and 1 since the plants are homozygous and the plots homogeneous.

Here the amount of marked pollen falling from the source plants on the recipient plots was given by the proportion of resistant individuals in the progeny of these recipient plots since the resistance is dominant. The distribution thus obtained is $g*f$, the convolution of f and g defined above. The localisation of all resistant plants on the experimental plot (and therefore g) was also known. Assuming that all plants produced the same amount of pollen, all the necessary information was available to determine the average pollen distribution from each plant (f).

This was done in two ways:

Non-parametric method

A two-dimensional matrix representing individual pollen distribution was obtained directly from the data points by the use of discrete Fourier transforms as described previously (Lavigne et al. 1996). This method uses the fact that the Fourier transform of the convolution of two functions is the product of their Fourier transforms, so that:

$$f = F^{-1}\left(\frac{F(g * f)}{F(g)}\right),$$

where F represents the Fourier transform and F^{-1} the inverse Fourier transform. Fourier transforms were implemented following Press et al. (1992).

As in Lavigne et al. (1996), the individual distribution obtained presented an oscillation due to the design of the experiment. These oscillations arise from the fact that the Fourier transform of the g function has very small values for periods 2 and 3 due to the fact that it takes a value of 1 on 3×3 square and a value of 0 elsewhere. Indeed, the observations described by $g * f$ are noisy and could be

written as $g * f + \varepsilon$ where ε is a traditional sampling noise. As a consequence, f should be written as:

$$f = F^{-1}\left(\frac{F(g*f)}{F(g)} + \frac{F(\varepsilon)}{F(g)}\right).$$

Since $g * f$ is also expected to have small values at these frequencies, f is dominated by the second term at frequencies where $F(g)$ is small. As a result, the components of the noise that have the same periods have a large magnitude (Rosenfeld and Kak 1982). We again removed the oscillations by averaging each value with that of its neighbours. However, contrarily to what was done previously, we did not simply calculate an even average over the nearest neighbours: the new value was obtained by multiplying the Fourier transform of the individual distribution with two filter functions of the form:

$$filt(x, y) = \left(\frac{\sin(x\Pi/T_1)}{x\Pi/T_1}\right)\left(\frac{\sin(x\Pi/T_2)}{x\Pi/T_2}\right).$$

The two combinations of parameters were $T_1 = 10$ and $T_2 = 10$ or 14.5 in order to remove oscillations of periods equal to two or three. This is equivalent to averaging each value with that of its two nearest neighbours in every direction, giving a larger weight to the closest values.

Parametric method

We calculated the convolution of the g function with four different parametric families of putative f_θ functions and kept parameters for the f_θ function which minimised the distance between the $g*f_\theta$ thus obtained and the data points. The distance chosen was the sum of squares of differences. This analysis was performed using the software MATHEMATICA (Wolfram Research).

The families of f functions tested were (1) negative exponential functions of parameter a, (2) Gaussian functions of mean 0 and standard deviation s, (3) a weighed average of a negative exponential and a function for which pollen simply fell on the plot of origin, and (4) a weighed average of a Gaussian and a function for which pollen simply fell on the plot of origin. For each of these functions, we forced the integral over the (x, y) plane to be equal to one. This is the reason why we did not test the classical inverse power law function which does not have a convergent integral (Peart 1985). For functions 3 and 4 the proportions of the two components of the sum were also optimised. Contrarily to the non-parametric method, this second method can only result in an isotropic distribution of pollen dispersal.

The two methods were compared by calculating the discrepancy (as a sum of squares of the differences) between the experimental data and the two global distributions obtained with the filtered individual dispersal (non-parametric method) and with the best function provided by the parametric method.

Comparison of the global and the individual distributions

The global and the individual pollen dispersal distributions were compared in two ways. First, we computed the proportion of all pollen dispersed that fell within each distance from the source, whether the source was a plot (global distribution) or an individual plant (individual distribution). This enabled us to assess what proportion of the total pollen produced by a source fell at a long distance. Second, by convolving it with g, we calculated the proportions of resistant progeny predicted by the experimental distribution if it had been considered as an individual one and compared these proportions to those obtained by the individual distributions, and to the data.

Results

Test of harvested seeds

On average, the mass of seeds sown per plot to test for the resistance at each distance was smaller for male-sterile (MS) than for male-fertile (MF) plants (25.9 ± 0.48 g and 27.3 ± 0.40 g, respectively, $P < 0.05$). This might suggest that MS plants did not establish as well as MF ones (later emergence due to hand sowing or effects of competition by male-fertile plants), that they were less fertile or that pollen was a limiting factor for male-sterile plants.

There was a very strong positive correlation ($r^2 = 0.957$, $P < 0.001$, $n = 29$) between the mass of seeds sown in a plot and the number of seedlings that emerged. This enabled us to estimate the number of seedlings that emerged from each of the 1668 plots. By counting the seedlings that resisted the two herbicide treatments, we could thus estimate the proportion of resistant seedlings in the progenies of the MS and MF recipient plants.

The ratio of resistant over susceptible MF parental plants before pollen dispersal was 0.0125, and it was 0.0090 in the progeny of the MS plants. This means that, on average, a susceptible plant fathered 1.389 times more progeny than a resistant one on MS plants.

Selfing rate

By comparing the proportion of resistant seedlings in the progenies of male-fertile and male-sterile plants, it was possible to compute the selfing rate of the MF plants. Assuming that differences were only due to partial selfing of the male-fertile plants, the average selfing rate at a given distance could be computed as $1 - (P_{MF}/P_{MS})$ where P_{MF} is the proportion of resistant individuals in the progenies of male-fertile recipient plants and P_{MS} that of the progenies of male-sterile recipient plants. The average selfing rate thus obtained is 0.589 ± 0.065.

The global pollen dispersal

The pollen dispersal from the whole transgenic source plot can be visualised by the proportion of resistant individuals observed in the progenies of MS and MF recipient plants as a function of their situation in the field. As expected, the higher proportions were found in the source plot and in its vicinity, but some resistant individuals were still found in the progenies of plants growing on the edge of the field (Figs. 2 and 3).

The distributions were not isotropic. On male-sterile recipient plots, more resistant progenies were observed in plots situated SW of the source plants than in any other direction. The differences were significant when

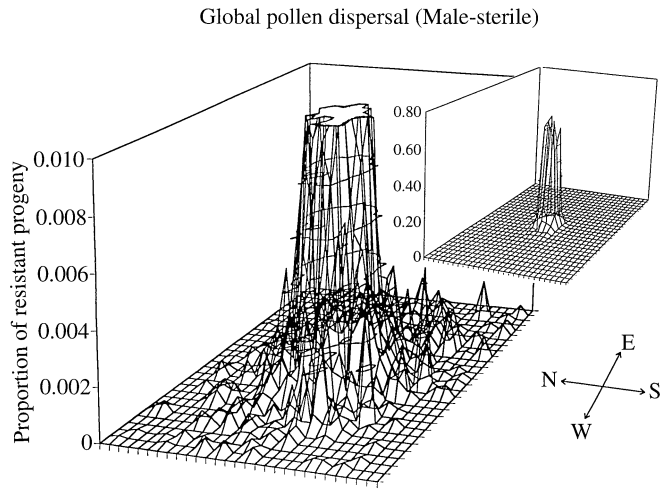

Fig. 2 Proportion of resistant individuals in the progeny of male-sterile recipient plants as a function of their position in the field

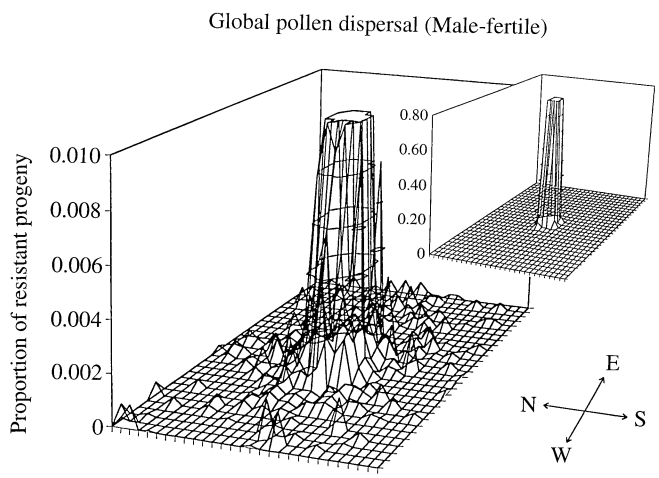

Fig. 3 Proportion of resistant individuals in the progeny of male-fertile recipient plants as a function of their position in the field

comparing SW to NW and NE ($P = 0.034$ and $P = 0.007$, respectively) but not when comparing SW to SE (P = 0.13). No other differences were significant. When N, E, W and S were compared, significantly fewer resistant progenies were observed north and east of the source plants than west of the source plants ($P = 0.002$ and $P = 0.026$, respectively) and significantly more south than north ($P = 0.01$). Other comparisons were not significant. This suggests that pollen was preferentially dispersed toward the southwest. The same analysis on progenies of MF plants gave somewhat different results. When NE, NW, SE and SW directions were compared, no significant differences were observed. When N, S, W and E were compared we observed no significant differences between opposite directions (i.e. north against south or east against west) but significantly more pollen towards west than north or south ($P = 0.022$ and $P = 0.029$, respectively) and marginally more towards east than north or south ($P = 0.08$ and P = 0.14, respectively). This suggests that west was again a preferential direction for pollen dispersal, but that contrarily to what was observed on the progenies of MS plants, more pollen dispersal occurred along the rows than between rows.

Although the distributions were not isotropic, we averaged the proportions of resistant progenies at each distance to have an overview of the decrease in these proportions with distance. For the MS and MF recipient plants, the decrease was well-described by equations

$$P = 68.76\, d^{-1.281}\, e^{-0.0736d}$$

and

$$P = 32.95 d^{-1.993}\, e^{-0.01133d},$$

respectively (d is the distance to the source plot, P is the proportion of resistance expressed in per 1000, sum of squares of differences of 2.27 and 2.66, respectively).

By summing the proportion of resistant individuals within each distance from the source plants, and normalising them, we obtained the proportion of all resistant pollen that fell within each distance from the centre of the grid (Fig. 4). This assumes no differences in pollen production or competitivity.

Individual pollen dispersal

Non parametric method: deconvolution of the data points

Only data from the male-sterile recipient plants were analysed. The average pollen distribution from 1 plant of the field f is given in Fig. 5. It represents the probability that a pollen grain from a plant situated on the centre of the grid fell on each node of the grid. Because the individual distribution is strongly peaked, and filtering was equivalent to averaging each value with its neighbours, filtering reduced the skew of the individual distribution (maximum value around 0.6 on the central point before filtering, and 0.1 after filtering). In order to lose a minimum of information we also did not attempt to remove the parasitic oscillations on two of the sides of the grid by more filtering.

To avoid taking the bias due to these oscillations into consideration we, however, excluded the 2 most exterior rows on each of the four sides when testing for the isotropy of the distribution. The individual distribution was not isotropic. More pollen was being dispersed towards the west and southwest, which is consistent with data from the global distribution (NW > NE, $P = 0.016$; SW > SE, $P = 0.045$; SW > NE, $P = 0.025$; other differences not significant).

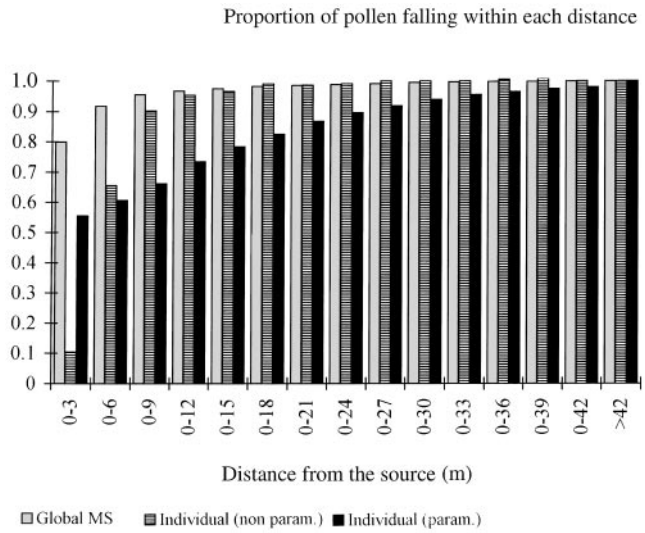

Fig. 4 Proportion of total pollen emitted by the source that fell within each distance from the source. The three distributions represented are the distribution estimated by the proportion of resistance in the progeny of male-sterile recipient plants ('Global MS'), and the best individual distribution obtained by each of two deconvolution methods

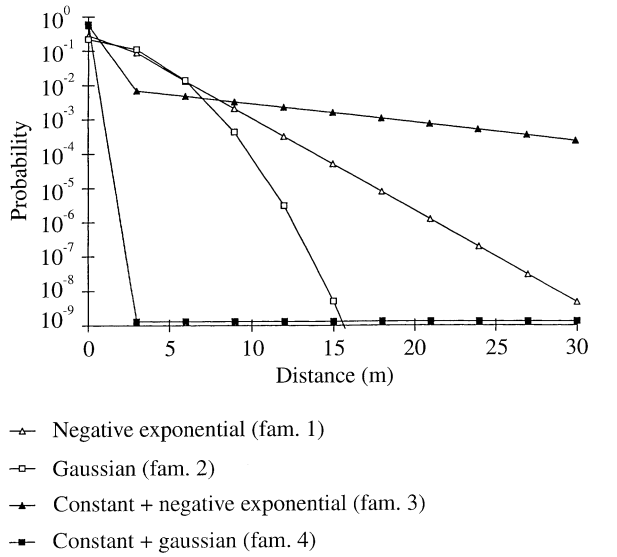

Fig. 6 The individual distribution that best fitted the data for each of the four families of the functions tested. A one-dimensional representation is given since the distributions are isotropic (see text for details). Among the four, the function of family 3 gave the best fit

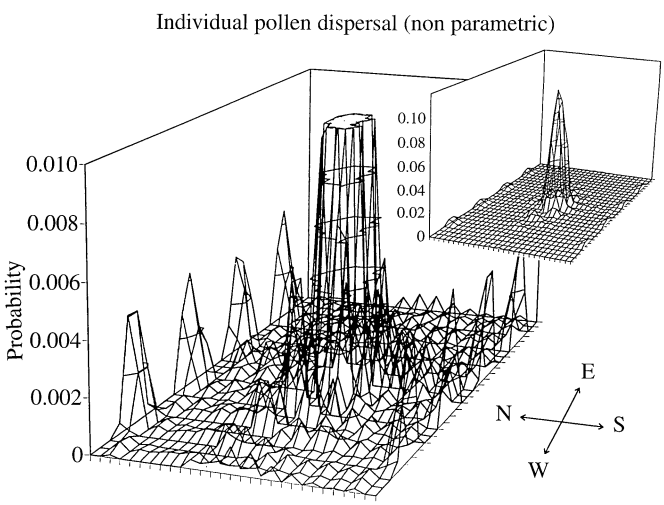

Fig. 5 Individual pollen distribution obtained by method 1 after removing the oscillations of period two and three. Each value is the probability of receiving a pollen grain released by a central plant

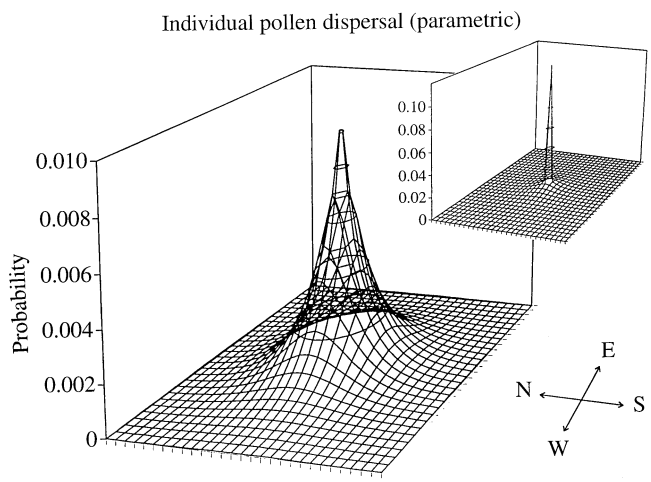

Fig. 7 Individual pollen distribution obtained by method 2. Each value is the probability of receiving a pollen grain released by a central plant

The resulting proportion of pollen falling within each distance from the source is presented in Fig. 4.

Parametric method:
test of putative individual functions

For each of the four putative f family functions, we obtained parameter values that minimised the distance (measured as the sum of squares of the differences, SS) between $g*f$ and the data points (Fig. 6). Larger sum of squares of differences were expected for family 1 than for 3 and for 2 than for 4 because they only included one parameter. The best fit to the data was a function (Fig. 7) of family 3. For this function, a proportion 0.5465 of the pollen falls on the central plot and, the rest of the pollen (i.e. a proportion of 0.4535) falls along a negative exponential of equation:

$$f(d) = \frac{(0.375)^2}{2\Pi} e^{-0.375d},$$

where d is the distance between the pollen donor and the pollen recipient (the unit for d is the distance between two adjacent nodes of the grid, i.e. 3 m) (SS = 0.0836). Worse fits were obtained for the negative exponential (family 1) and the Gaussian (family 2), which both resulted in a very marked peak on the central recipient plot for the $g * f$ function.

Comparisons of individual distributions and that directly derived from the data

Figure 4 presents the estimated proportion of pollen that fell within each distance of the source, whether the source was the central plot or an individual plant. In the pollen distribution that could have been deduced directly by considering the source plot as a punctual source, 80% of the pollen fell within the source and 12% within 3 m. When parametric and non-parametric individual distributions are considered, there is a discrepancy between them for the amount of pollen falling within 3 m due to the filtering of the peak of the distribution obtained for the non-parametric method. They are, however, consistent when considering larger distances: for both of them, about 60% of the pollen emitted by an individual plant fell within 6 m, which means that 40% of the pollen emitted travelled more than 6 m, a much larger proportion than that observed for the distribution directly deduced from the experimental data.

The difference between the global distribution resulting from the convolution of the individual distribution obtained by the non-parametric method and the data points is not null since this individual distribution was filtered. The sum of squares of differences is 0.987. It is therefore larger than that calculated for the convolution of the individual distribution obtained by the parametric method (SS = 0.0836). The proportions of resistance obtained by the two methods and from the experimental data are presented on Fig. 8. Whereas the parametric distribution best fitted short-distance data, it resulted in excessive rates of resistance at long distance. Conversely, the non-parametric method resulted in a better estimate of long-distance resistance rates. To assess the consequences of considering directly the experimental distribution with ('Global MS' on Fig. 4) as an individual distribution, we considered the proportions of resistant progeny predicted by this distribution after convolution with g. The discrepancy with the data was intermediate between that obtained with the two individual distributions (SS = 0.774). The shape of the proportion of resistance with distance is clearly different from the data since short-distance proportions are greatly overestimated, although long-distance proportions are well-estimated.

Discussion

Efficient pollen dispersal, i.e. pollen dispersal that can potentially lead to fertilisations, is a major issue when considering the escape of a transgene from genetically modified plants, in particular for crops that can hybridise with wild relatives growing around the fields. Most studies dealing with pollen dispersal of crop plants had, until recently, been more focused on determining isolation distances for seed production and contamination of seed crops than on the contamination of wild species by pollen from crops. However, since the transgene might be at a selective advantage when outside the field, even very low levels of long-distance pollen dispersal have become a subject of attention. In confirmation with the leptokurtic, or negative exponential distribution of pollen that had been reported by earlier

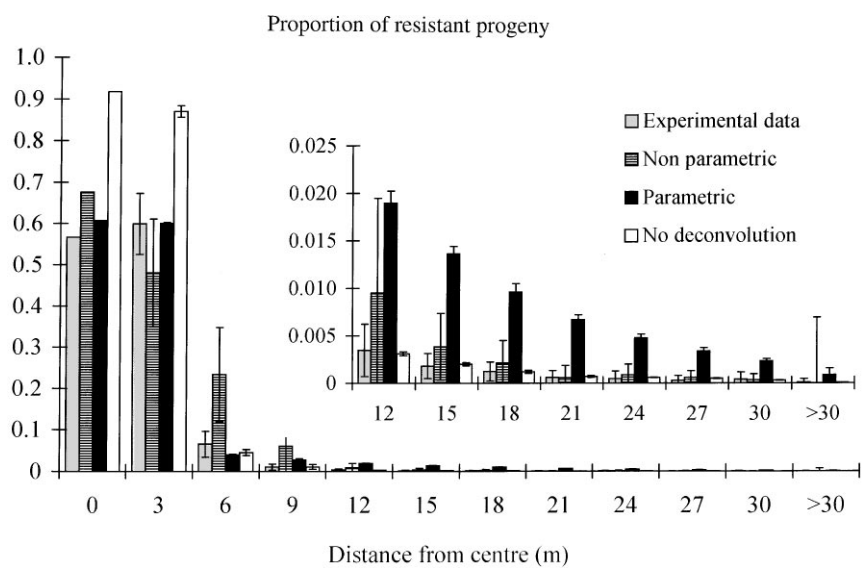

Fig. 8 Proportion of resistant progeny as a function of distance from the source. The four distributions are for the experimental data on male-sterile recipient plants, the distributions obtained after convolution of the individual dispersals obtained by the two deconvolution methods (non-parametric and parametric, respectively) and after convolution of the dispersal distribution directly obtained from the data (no-deconvolution)

authors (Levin and Kerster 1974; Tonsor 1985), most experiments with transgenic crops report that most of the pollen fertilises plants growing within a few metres around the source plot and that some rare long-distance dispersal results in the fertilisation of plants growing away from the source (Tynan et al. 1990; Umbeck et al. 1991; Scheffler et al. 1993; McPartlan and Dale 1994; Timmons et al. 1995; Conner and Dale 1996; Llewellyn and Fitt 1996).

These experiments, in addition to providing quantitative data about pollen dispersal, generate an interesting discussion about the design of pollen-dispersal experiments. One main conclusion arising from this discussion is that two types of experimental designs can be considered: *continuous* designs, such as the one described here, where the recipient plants are evenly spaced around the source plot, and *discontinuous* designs, where recipient plants form patches situated at various distances from the source. When pollen is at least partly carried by insects, the second kind of design seems to favour long-distance dispersal [e.g. Skogsmyr (1994), but see Conner and Dale (1996) for potato, and Manasse (1992) and Morris et al. (1994) for oilseed rape] because pollinators fly over non-recipient plants. The second conclusion is that it appears to be very difficult to compare quantitatively different experiments even when the same crop is used (see, for example, the discussion of Scheffler (1993) for oilseed rape).

The experiment described in this study (of the continuous type) first aimed at estimating pollen dispersal from transgenic oilseed rape. It was furthermore specifically designed to compare a parametric and a non-parametric method which allows retrieval of the average pollen dispersal of a single plant growing within a large source plot. Because recipient patches were situated 3 meters from each other, no precision on the shape of the distribution within 3 m was achievable. Another field study of pollen dispersal of transgenic oilseed rape was performed previously by Scheffler et al. (1993). Their source plot was a ring 9 m wide that surrounded a circle of central recipient plants 1 m in diameter. This source plot was set in the centre of a 1-ha field grown with non-transgenic male-fertile recipient plants. The authors report that the frequency of recipient plants fertilised by pollen originating from the source decreased sharply with distance from the source plot. In the experiment presented here, we obtained similar results: the frequency of resistant individuals in the progeny of MF plants decreased sharply with distance around the source plot, but some resistant progeny were still observed at the edge of the field. More resistant progeny were observed on the west/south west part of the field, which is consistent with the fact that the winds were mainly from the north, north east and north west from March 15th to May 15th on the experimental unit of Le Rheu in 1995.

The use of both MS and MF recipient plants in this experiment provided additional information. First, because they did not self, MS recipient plants better reflected the composition of the pollen cloud and therefore gave better insight into gene dispersal than did the surrounding MF recipient plants that had much lower frequencies of resistant individuals in their progeny. Using MS recipient plants also provided us with values for the proportion of resistant individuals in the progenies that did not depend on the selfing rate of the recipient plants. These values can therefore be used for comparisons across experiments. The MF plants around the source plot were, however, necessary because had only MS plants been used as recipients the plants growing near the source would have been saturated with transgenic pollen and would have given no information on the shape of the pollen distribution. Only farther from the source, when pollen would have become limiting, would seed set have decreased and, thus, given information on the shape of the distribution. A platikurtic distribution would then have been observed, even if the pollen distribution had been leptokurtic [see Tonsor (1985) for such an experimental design].

The comparison of the progenies of MS and MF recipient plants also enabled us to derive the average selfing rate of the MF plants. The value we obtained (58.9%) is very consistent with values reported in the literature, which vary between 45% and 95% under field conditions (Olsson 1960; Rakow and Woods 1987).

All pollen-dispersal experiments are faced with the difficulty that, in order to track rare events of long-distance dispersal, they need to use a large number of plants as source plants. As a result, the function describing the proportion of transgenic individuals in the progeny of recipient plants grown at different distances from the field depends on the shape and the size of the source plot. When different experiments are compared, it is therefore difficult to disentangle differences in pollen distributions that are due to the size or shape of the source plot with actual differences in the pollen dispersal of each plant.

However, such a comparison becomes possible when the individual dispersal retrieved by the deconvolution method is considered. Indeed, the pollen distribution obtained after the deconvolution no longer represents a sum of pollen distributions from a number of source plants but rather the average distribution of 1 plant. As a result, if differences are observed among experiments for this new distribution, they will reflect actual biological differences in the pollen dispersal of each plant. This would allow testing for the effect of environmental conditions (climatic conditions, plant density etc.) on pollen dispersal.

Other methods are being used in field studies investigating the effect of environmental conditions on pollen-mediated gene dispersal. They include marking the pollen with microtags (Nilsson et al. 1992), radioactive tracers (Schlising and Turpin 1971; Massaux et al.

1976) or polymorphism (Thomson and Thomson 1989). More indirect measurements of pollen movement have also been inferred from pollinator flight distances (Cresswel 1997) and the movement of fluorescent dyes (Linhart et al. 1987; Campbell and Waser 1989; Cresswell et al. 1995). Results from genetic markers, however, tend to show that such methods underestimate the actual movement of genes (Chapbell 1991; Godt and Hamrick 1993). The deconvolution method also relies on the use of genetic markers, but contrarily to methods such as paternity analyses, which rely on the existence of individuals all differing for polymorphic genetic markers, it is based on the existence of numerous individuals that all share a similar marker. This makes it particularly suited for the study of pollen dispersal by crop plants for which it is easy to find genetically similar individuals.

In the present study, our first goal was to compare two different methods (the parametric and the non-parametric one) based on the deconvolution principle to retrieve the average pollen distribution of a single source plant from that of a whole plot. We then wanted to assess whether these distributions would be different from the distribution of the whole plot when the latter was considered as a point source. An earlier study on dispersal data generated by computer (Lavigne et al. 1996) had validated the non-parametric method and shown that considering the source plot to be a point resulted in an underestimation of long-distance dispersal.

The main problem with the first method used, i.e. the non-parametric method, is that the regular distribution of the recipient and source plants on a grid creates an oscillation which results in large oscillations for the individual pollen distribution, particularly at long distances from the source. To remove these oscillations of period two and three, we had to average each value with its closest neighbours. Instead of doing a plain average among neighbour values as before (Lavigne et al. 1996), we chose here to filter the Fourier transforms with a function that was equivalent to a weighted average, thereby giving more weight to closer values (Rosenfeld and Kak 1982). This appeared to us as the function which would least modify the global shape of the distribution. This filtering still strongly diminished the peak value of the proportion of pollen that fell within 3 m and increased that which fell between 3 m and 9 m. The resulting individual distribution had a lower skew than if values had not been averaged, which made it more similar to the distribution directly deduced by considering the plot as a point source. Consequently, it did not correctly reflect short-distance dispersal. This method had the advantage, however, that it correctly took into account the lack of isotropy of the pollen dispersal. The parametric method was more satisfactory concerning the skew of the distribution and the fit to experimental data (measured as a sum of squares of differences) but had the drawback that it was necessary to choose (1) a priori functions to describe the individual pollen distribution and (2) a criterion to quantify the fit to the data. As in this study, it would, however, be possible to test different families of putative individual functions and, if necessary, to introduce some asymmetry into the pollen distribution. Other criteria than the sum of squares of differences could also be tested.

Although the two methods gave somewhat different results, a similar picture emerged when the individual distributions were compared with the distribution directly retrieved from the experimental data. As had been observed on simulated data (Lavigne et al. 1996), in both cases the proportion of pollen that fell long distances was greater for individual pollen dispersal than for that deduced from the dispersal of the whole plot, in particular for the individual distribution obtained by the parametric method. In contrast to the study on simulated data, however, pollen production, time of release or competitive abilities of the transgenic and non-transgenic individuals were probably different since the total number of resistant progeny was 1.389 times smaller than expected. This difference is responsible for the fact that, although considering the source plot as a point source was probably similar to largely overestimating the proportion of pollen that fell short distances, and therefore underestimating long-distance dispersal, the fit to the proportion of resistant progeny was rather satisfactory at distances which exceeded 6 m. Indeed, less pollen was, in total, dispersed in the experiment than what was assumed in the convolution of any distribution. Consequently, overestimating short-distance proportions of resistant progeny did not result in an underestimation of long-distance proportions (when considering the distribution directly derived from the data). Similarly, a good estimation of the short-distance proportions of resistant progeny would necessarily result in an overestimation of these proportions at long distance. These results thus suggest that the average plant in this experiment had a pollen distribution with a larger proportion of short-distance dispersal than that which is found with the parametric method, but a much smaller one than that which is found when the source plot is considered as a point source. To assess more precisely the shape of this distribution, we need, more information on the quality and quantity of pollen produced by all plants.

Until more information is obtained on the pollen dispersal of individual plants growing within a field and, in particular, differences in pollen production and competitive ability can be better estimated and taken into consideration, our results suggest that pollen dispersal from an individual plant might be viewed as resulting from a combination of two phenomena. A proportion of the pollen would fall around the source plant at a rate decreasing very rapidly with distance as a result of insect movements among neighbour plants and weak winds. The rest would be distributed almost

uniformly over the field as a result of long-distance insect flights or of strong winds and associated turbulence. In the present study, individual dispersal was best-described by a large quantity (around 50%) of pollen falling within 3 m and the remaining 50% falling at larger distances with a probability that decreased very slowly with distance (Fig. 7). This type of pollen distribution was different from the pollen distribution directly deduced from dispersal of the source plot, since the latter underestimated the proportions of long-distance dispersal. Such individual distributions could be used for a number of simulations of pollen dispersal, such as, for example, models trying to predict the effect of different continuous containment designs on the dispersal of a transgene from a field of oilseed rape, the consequences of the regrowth of volunteer plants with different seed quality in a later crop, the efficiency of pollination in mixtures containing male-sterile individuals or, on a longer term, the ecological consequences of the release of transgenic crops.

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References

Bateman AJ (1947) Contamination in seed crops. II. Wind pollination. Heredity 1:235–246

BRIDGE (1995) Safety assessment of the deliberate release of two model transgenic crop plants, oilseed rape and sugar beet. In: Vassarotti A, Kalbe-Bournonville L, Baselga de Elonz J, de Taxis du Poët Ph (eds) Final Report. European Commission, Luxembourg, 469–474

Broyles SB, Schnabel A, Wyatt R (1994) Evidence for long-distance dispersal in milkweeds (*Asclepia exalta*). Evolution 48: 1032–1040

Campbell DR (1991) Comparing pollen dispersal and gene flow in a natural population. Evolution 45:1965–1968

Campbell DR, Waser NM (1989) Variation in pollen flow within and among populations of *Ipomopsis aggregata*. Evolution 43:1444–1455

Conner AJ, Dale PJ (1996) Reconsideration of pollen dispersal data from field trials of transgenic potatoes. Theor Appl Genet 92:505–508

Cresswell JE (1997) Spatial heterogeneity, pollinator behaviour and pollinator mediated gene flow: bumble-bee movements in variously aggregated rows of oil-seed rape. Oikos 78:546–556

Cresswell JE, Bassom AP, Bell SA, Collins SJ, Kelly TB (1995) Predicted pollen dispersal by honey-bees and three species of bumble-bees foraging on oil-seed rape: a comparison of three models. Func Ecol 9:829–841

Ehrlich PR, Raven PH (1969) Differentiation of populations. Science 165:1228–1232

Ellstrand NC, Devlin B, Marshall DL (1989) Gene flow by pollen into small populations: data from experimental and natural stands of wild radish. Proc Natl Acad Sci USA 86:9044–9047

Freyssinet M, Créance P, Renard M, McVetty P, Derose R, Freyssinet G (1995) Development of transgenic oilseed rape resistant to oxynil herbicides. In: Murphy D (ed) Proc 9th Int Rapeseed Cong., Cambridge, 974–976

Friedman ST, Adams WT (1985) Estimation of gene flow in two seed orchards of loblolly pine (*Pinus taeda* L.). Theor Appl Genet 69:609–615

Giddings GD, Sackville Hamilton NR, Hayward MD (1997a) The release of genetically modified grasses. Part 1: pollen dispersal to traps in *Lolium perenne*. Theor Appl Genet 94:1000–1006

Giddings GD, Sackville Hamilton NR, Hayward MD (1997b) The release of genetically modified grasses. Part 2: the influence of wind direction on pollen dispersal. Theor Appl Genet 94: 1007–1014

Godt MJW, Hamrick JL (1993) Patterns and levels of pollen-mediated gene flow in *Lathyrus latifolius*. Evolution 7:98–110

Kirkpatrick KJ, Wilson DH (1988) Interspecific gene flow in *Cucurbita*: *C. texana* vs *C. Pepo*. Am J Bot 75:519–527

Klinger T, Elam DR, Ellstrand NC (1991) Radish as a model system for the study of engineered gene escape rates via crop-weed mating. Conserv Biol 5:531–535

Lavigne C, Godelle B, Reboud X, Gouyon PH (1996) A method to determine the mean pollen dispersal of individual plants growing within large pollen source. Theor Appl Genet 93: 1319–1326

Levin DA, Kerster HW (1974) Gene flow in seed plants. Evol Biol 7:139–220

Linhart YB, Busby WH, Beach JH, Feinsinger P (1987) Forager behavior, pollen dispersal, and inbreeding in two species of hummingbird-pollinated plants. Evolution 41:679–682

Llewellyn D, Fitt G (1996) Pollen dispersal from two fields trials of transgenic cotton in the Namoi valley, Australia. Mol Breed 2:157–166

Luby JJ, McNicol RJ (1995) Gene flow from cultivated to wild raspberries in Scotland: developing a basis for risk assessment for testing and deployment of transgenic cultivars. Theor Appl Genet 90:1133–1137

Manasse RS (1992) Ecological risks of transgenic plants: effects of spatial dispersion on gene flow. Ecol Appl 2:431–438

Massaux F, Tchiendji C, Misse C, Decasy B (1976) Etude du transport du pollen de cacaoyer par marquage au 32P. Cafe Cacao The 20:163–171

McPartlan HC, Dale PJ (1994) An assessment of gene transfer by pollen from field-grown transgenic potatoes to non-transgenic potatoes and related species. Transgenic Res 3:216–225

Morris WF, Kareiva PM, Raymer PL (1994) Do barren zones and pollen traps reduce gene escape from transgenic crops? Ecol Appl 4:157–165

Nilsson LA, Rabakonandrianina E, Pettersson B (1992) Exact tracking of pollen transfer and mating in plants. Nature 360:666–668

Olsson G (1960) Self-incompatibility and outcrossing in rape and white mustard. Hereditas 46:241–252

Paul EM, Capiau K, Jacobs M, Dunwell JM (1995) A study of gene dispersal via pollen in *Nicotiana tabacum* using introduced genetic markers. J Appl Ecol 32:875–882

Peart DR (1985) The quantitative representation of seed and pollen dispersal. Ecology 66:1081–1083

Press WH, Teukolsky SA, Vetterling WT, Flannery BP (1992) Numerical recipes in C. Cambridge University Press, Cambridge

Rakow G, Woods DL (1987) Outcrossing in rape and mustard under Saskatchewan prairie conditions. Can J Plant Sci 67:147–151

Rosenfeld A, Kak AC (1982) Digital picture processing. Academic Press, London

Scheffler JA, Parkinson R, Dale PJ (1993) Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*). Transgenic Res 2:356–364

Schlising RA, Turpin RA (1971) Hummingbird dispersal of *Delphinium cardinale* pollen treated with radioactive iodine. Am J Bot 58:401–406

Skogsmyr I (1994) Gene dispersal from transgenic potatoes to conspecifics: a field trial. Theor Appl Genet 88 : 770–774

Sprent P (1989) Applied nonparametric statistical methods. Chapman and Hall, London

Stone R (1994) Large plots are next test for transgenic crop safety. Science 266 : 1472–1473

Thomson JD, Thomson BA (1989) Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. Evolution 43 : 657–661

Tiedje JM, Colwell RK, Grossman YL, Hodson RE, Lenski RE, Mack RN, Regal PJ (1989) The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70 : 298–315

Timmons AM, O'Brien ET, Charters YM, Dubbels SJ, Wilkinson MJ (1995) Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. *oleifera*. Euphytica 85 : 417–425

Tonsor ST (1985) Leptokurtic pollen-flow, non leptokurtic gene-flow in a wind-pollinated herb, *Plantago lanceolata* L. Oecologia 67 : 442–446

Tynan JL, Williams MK, Conner AJ (1990) Low frequency of pollen dispersal from a field trial of transgenic potatoes. J Genet Breed 44 : 303–306

Umbeck PF, Barton KA, Nordheim EV, McCarty JC, Parrot WL, Jenkins JN (1991) Degree of pollen dispersal from a field test of genetically engineered cotton. J Econ Entomol 84 : 1943–1950

Waser NM, Price MV (1982) A comparison of pollen and fluorescent dye carry-over by natural pollinators of *Ipomopsis aggregata* (*Polemoniaceae*). Ecology 63 : 1168–1172

Wright S (1931) Evolution in mendelian populations. Genetics 16 : 97–159