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► **To cite this version:**

Etienne Klein, Claire Lavigne, Hervé Picault, Maurice Renard, Pierre-Henri Gouyon. Pollen dispersal of oilseed rape: estimation of the dispersal function and effects of field dimension. *Journal of Applied Ecology*, 2006, 43 (1), pp.141-151. 10.1111/j.1365-2664.2005.01108.x . hal-00174616

HAL Id: hal-00174616

<https://hal.science/hal-00174616v1>

Submitted on 1 Jan 2025

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Pollen dispersal of oilseed rape: estimation of the dispersal function and effects of field dimension

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Summary

1. Debate continues regarding the ecological impacts of genetically modified (GM) crops and their coexistence with non-GM crops in Europe. In this debate, quantitative predictions of gene dispersal by pollen are necessary, and as a result numerous plot-to-plot gene flow experiments have been performed with various crops. However, plot-to-plot cross-pollination rates (CPR) depend on spatial configuration of plots, implying that (i) they are difficult to compare among experiments and (ii) functions directly fitted on CPR data are inappropriate for predictions in other spatial contexts.
2. Modelling pollen dispersal via an individual dispersal function (IDF) circumvents these problems by accounting for spatial designs. We detail for oilseed rape how this approach can be used to both estimate an IDF from field data and predict CPR between two neighbouring fields of various sizes and shapes. Predictions were used to investigate the sensitivity of CPR to the family of IDF, the uncertainty in parameter estimates and the effects of field dimensions and isolation distances.
3. We fitted a range of families of IDF, including several types of tails, on previously published data. The best IDF was a fat-tailed power-law function, meaning frequent long-distance dispersal.
4. The choice of IDF appeared crucial when predicting CPR between fields, occasionally being even more important than the distance between fields. Width of the source field and depth of the recipient field were next in importance. When approximated CPR were calculated without considering field dimensions, using distance between field centres gave better performance than field margins.
5. *Synthesis and applications.* This study demonstrates the value of IDF for quantitative predictions of pollen flow in variable spatial configurations. A spatially explicit model of agro-ecosystems used to define management rules for the commercial release of GM crops in Europe already employs IDF but underestimates long-distance dispersal for oilseed rape. These new parameter estimates will refine the performance of these models. Moreover, the detailed guidelines for estimating an IDF should encourage such statistical analysis of other dispersal data, enabling comparisons of dispersal data obtained for different environments and species and providing new IDF for management models.

Key-words: agricultural landscape, agro-ecosystem, fat-tailed dispersal kernel, gene flow, risk assessment of GM crops, spatially explicit metapopulation

Journal of Applied Ecology (2006) **43**, 141–151

doi: 10.1111/j.1365-2664.2005.01108.x

Introduction

The debate about the commercial release of GM plants in the environment has stimulated research on quantitative modelling of gene flow, particularly by pollen dispersal (Beringer 2000; Gray 2004). Indeed, pollen flow is a main cause of harvest pollution by cross-pollination between genetically modified (GM) and non-GM fields (Rieger *et al.* 2002), introgression of transgenes into wild species through hybridization (Raybould & Gray 1993) and stacking of transgenes following cross-pollination among GM fields (Hall *et al.* 2000) or between fields and feral populations (Desplanque, Hautekeete & Van Dijk 2002). The need for quantitative predictions in different agricultural landscapes has been strengthened recently by European legislation requiring low thresholds of GM presence to avoid GM-labelling of feed and food products. In order to derive landscape management rules to limit cross-pollination, several recent spatially explicit models (Thompson *et al.* 1999; Colbach, Clermont Dauphin & Meynard 2001; Wilkinson *et al.* 2003) predict pollen flow among cultivated, wild and feral plant populations that differ in size, shape, density and isolation distance.

Long-distance pollination has been observed in oilseed rape using male-sterile bait plants (2.5 km in Timmons *et al.* 1996; probably 26 km in Ramsay, Thompson & Squire 2003) and male-fertile plants (400 m in Scheffler, Parkinson & Dale 1995; 800 m in Beckie *et al.* 2003; 3 km in Rieger *et al.* 2002). Pollen is dispersed both by wind and insects (Williams, Martin & White 1986; Mesquida, Renard & Pierre 1988); the main insect pollinators are honeybees and bumblebees (Pierre *et al.* 2003; Cresswell & Osborne 2004). The relative contribution of wind and insects to transport and pollination is as yet unresolved (Cresswell & Osborne 2004; Cresswell *et al.* 2004; Walklate *et al.* 2004). There is some agreement that insects carry pollen over short distances within fields, but questions exist regarding their contribution to pollination among fields or between fields and feral populations. Pollen beetles may also contribute to very long-distance pollen movement (Ramsay, Thompson & Squire 2003). This lack of knowledge about pollen vectors hinders the development of mechanistic models for pollen dispersal at the landscape scale, despite a wealth of published data (for an overview see Eastham & Sweet 2002; for oilseed rape see Beckie *et al.* 2003).

There are two main approaches to studying oilseed rape pollination. The first is mechanistic and includes examples addressing wind dispersal (Aylor, Schultes & Shields 2003; Cresswell *et al.* 2004; Walklate *et al.* 2004), insect-mediated dispersal (Cresswell, Osborne & Bell 2002; Ramsay, Thompson & Squire 2003; Reboud 2003) and their respective contributions (Mesquida, Renard & Pierre 1988). Despite significant advances recently, these mechanistic approaches are far from being integrated into landscape models because of (i) the 'cost' of numerical simulations, (ii) the necessity of many precise data to perform the simulations and (iii) an imperfect under-

standing of the mechanisms. The second approach models pollen dispersal statistically, by fitting dispersal functions to data measured directly at the temporal and spatial scales of interest (Lavigne *et al.* 2004). Two phenomena are still poorly understood in these approaches that render both estimation of dispersal functions and their use for predictions difficult: (i) the impact of sizes and shapes of both source and receptor plots on cross-pollination rates (CPR) and (ii) the amount of long-distance dispersal (Meagher & Vassiliadis 2003).

The estimation of dispersal functions does not simply require fitting functions to CPR data. Indeed, CPR data obtained from pollen-dispersal experiments vary with the spatial shape of the experimental design and misrepresent the effect of distance alone on gene flow (Lavigne *et al.* 1998; Meagher & Vassiliadis 2003). The impact of experimental design on CPR data is illustrated by the variability of dispersal data when presented only as a function of distance and not as a function of the size and shape of pollen sources (Beckie *et al.* 2003). One way to account for variation in spatial designs of cross-pollination experiments is to retrieve the individual dispersal function (IDF) from observations of CPR at different distances from a pollen source. This IDF, also named dispersal kernel (Clark *et al.* 1999), describes the probability that a pollen grain dispersed by a plant at point $(0, 0)$ pollinates at any point (x, y) (Lavigne *et al.* 1998). It is generally accepted that this function depends less strongly on the spatial design than the function describing the CPR at different distances, and it can thus be used to predict effective dispersal under different spatial designs. Despite the supplementary 'cost' in statistical analysis, this approach has been used several times for pollen (Tufto, Engen & Hindar 1997; Lavigne *et al.* 1998; Klein *et al.* 2003) and seed dispersal data (Clark *et al.* 1999).

Estimating the appropriate shape of the tail of the IDF is critical (Clark, Lewis & Horvath 2001). Most dispersal functions are 'leptokurtic', with dispersal to both short and long distances occurring more frequently than they would under a Gaussian function. However, among leptokurtic functions different types of tails exist, such as thin-tailed functions (quicker decrease than an exponential), exponential-like functions (long-range decrease similar to an exponential) and fat-tailed functions (slower decrease than an exponential). These functions generate different colonization patterns (Clark, Lewis & Horvath 2001), mixing of propagules and gene flow at long distances (Devaux *et al.* 2005).

In this study we (i) illustrate the process of estimating and comparing IDF by reconsidering data from an oilseed rape pollen-dispersal experiment (Lavigne *et al.* 1998); (ii) propose fitted functions for modellers wishing to integrate a dispersal function in a model; (iii) derive CPR predictions for different spatial designs and use these predictions to test the sensitivity of the CPR to shapes and sizes of source and receptor plots and to the choice of the IDF; and (iv) provide a first step in

scaling-up models of dispersal from the plant to the plot scale by comparing different approximations in the description of these source and receptor plots.

Materials and methods

EXPERIMENTAL DESIGN

The experiment was carried out in 1995 in Brittany, France. It consisted of a 90 × 90-m field of conventional winter oilseed rape ('BOO4' line), in the middle of which was sown a 10 × 10-m plot of nearly isogenic winter oilseed rape homozygous for a transgene conferring resistance to oxynil herbicides (obtained by three back-crosses from a Westar × BOO4 cross). Ten seeds of male-sterile (MS) plants (ogu-INRA cytoplasmic male sterility) were sown on each node of a 3 × 3-m grid covering the entire field. At harvest, the seeds produced by these male-sterile plants and by six neighbouring male-fertile (MF) plants (biological receptors) were collected on each of the 841 nodes of the grid.

This experiment took place at a flat site, surrounded by open spaces (maize fields that were still barren when oilseed rape bloomed) without any noticeable obstacle (e.g. forest or hedges). The field was supplied with a bee hive at its northern border. Wind data were recorded at the Institut National de la Recherche Agronomique (INRA) station near the field. Pollen dispersal occurred mainly from week 16 to week 20 (17 April–21 May), with a lower estimated density of flowers in the transgenic plot than in the surrounding conventional field during the two main weeks of female receptivity (weeks 16–18). The GM plants had actually started flowering earlier but their early racemes had been cut to provide better synchrony. Bees were observed throughout the field and we recorded no difference between the plots of susceptible and resistant plants. Wind speed was quite high over the period (maximal daily speed between 4 and 13 ms⁻¹, mean = 7.7 ms⁻¹), mostly southwards and occasionally south-eastwards and eastwards.

Two months later, seeds from each of the biological receptors (841 MF and 841 MS) were weighed and sown on separate plots using a maximum of 50 g seeds plant⁻¹. To estimate the number of seedlings in each plot, we first regressed the number of emergent seedlings on the mass of seeds sown for a subset of 29 plots and then used this regression for all other plots. These seedlings were sprayed twice with the herbicide Oxytril (Bayer Crop Science), at the one- to two-leaf stage and 2 weeks later. Surviving seedlings were counted as resistant.

The proportions of resistant seedlings for each receptor, i.e. CPR, were then obtained as the ratio between the number of resistant seedlings and the estimated number of sprayed seedlings.

MODELS FOR THE IDF

The IDF is a 2-dimensional probability density function $\gamma(x, y)$ describing the probability that a pollen grain

released by a plant located at (0, 0) pollinates a plant located at (x, y). We investigated eight families of IDF, presented in Table 1 (from Tufto, Engen & Hindar 1997; Clark *et al.* 1999; Austerlitz *et al.* 2004).

Thin-tailed functions included the exponential power (with $b > 1$) and Weibull models (with $b > 1$); exponential-like functions included exponential, exponential power (with $b = 1$), Weibull (with $b = 1$) and gamma models; and fat-tailed functions included the exponential power (with $b < 1$) and Weibull models (with $b < 1$) as well as three power-law tails, the geometric, logistic and 2Dt models.

For all models, we ignore the exact behaviour around $d = 0$ because we are mainly interested in the behaviour of the tail, and because a 3 × 3-m grid does not allow proper estimation of the decrease of the function over the first 1.5 m. We use a parameter θ , modelling the proportion of pollen falling within the 3 × 3-m square centred on the source plant. The IDF thus only describes the way the $1 - \theta$ remaining pollen grains are dispersed outside of this square (see Appendix S1).

Modelling the observations with an IDF

Experimental results can be summarized in the set of data:

$$\{x_k, y_k, n_k^{ms}, R_k^{ms}, n_k^{mf}, R_k^{mf}\}_{k=1\dots K}$$

where (x_k, y_k) is the position of the k th node of the grid, n_k^{ms}, n_k^{mf} are the numbers of sprayed seedlings from MS and MF plants at this node, and R_k^{ms}, R_k^{mf} are the observed numbers of resistant seedlings.

The expected values of these observed numbers of resistant seedlings are given by:

$$E[R_k^{ms}] = n_k^{ms} \mu(x_k, y_k) \quad \text{eqn 1}$$

and

$$E[R_k^{mf}] = \begin{cases} n_k^{mf} & \text{if } (x_k, y_k) \in A \\ n_k^{mf} (1 - s) \mu(x_k, y_k) & \text{if } (x_k, y_k) \notin A \end{cases} \quad \text{eqn 2}$$

where s is the selfing rate, and A (respectively B) is the set of only-resistant (respectively susceptible) plants. The function μ provides the proportion of pollen grains containing the transgene (i.e. coming from any resistant plant) in the pollen pool at point (x, y) . It can be calculated from the IDF γ and the experimental design (sets A and B):

$$\mu(x, y) = \frac{\int_{(x', y') \in A} \gamma(x - x', y - y') dx' dy'}{\int_{(x', y') \in A} \gamma(x - x', y - y') dx' dy' + m \int_{(x', y') \in B} \gamma(x - x', y - y') dx' dy'} \quad \text{eqn 3}$$

where m is the relative pollen production of 1 m² of susceptible plants relative to 1 m² of resistant plants.

Table 1. Families investigated for the IDF. For all these families, the parameter a acts as a scale parameter, whereas the b , c and c' are shape parameters that govern the shape of the IDF, particularly of its tail. In these expressions, d stands for $\sqrt{x^2 + y^2}$ and Γ for the gamma function $\left(\Gamma(x) = \int_0^{+\infty} t^{x-1} e^{-t} dt\right)$. To show the effects of the different parameters in each model, we give the formula for the mean distance travelled by a pollen grain $\left(\text{obtained as } \int_0^{+\infty} t\gamma(t)2\pi t dt\right)$. For the first four families, the mean distance decreases with the scale parameter a , whereas it increases with a for the last four families. For the exponential power and Weibull functions, decreasing b leads to heavier tails. For the gamma function, increasing c leads to higher mean distances but the tail remains an exponential-like one. For the last four families, the c and c' parameters are exponents of the power-law tail: decreasing one of them leads to heavier tails, and thus higher mean distances. See Austerlitz *et al.* (2004) for close expressions of these families and descriptions of the higher order moments

Kernel families	Expression	Parameter values	Weight of the tail	Mean distance
Exponential	$\gamma_e(x, y) = \frac{a^2}{2\pi} \exp(-ad)$	$a > 0$	Exponential	$2/a$
Exponential power	$\gamma_{ep}(x, y) = \frac{ba^{2b}}{2\pi\Gamma(2b)} \exp(-ad^b)$	$a > 0, b > 0$	Thin for $b > 1$, fat for $b < 1$, exponential for $b = 1$	$\frac{1}{a^{1/b}} \frac{\Gamma(3/b)}{\Gamma(2/b)}$
Weibull	$\gamma_w(x, y) = \frac{ab}{2\pi} d^{b-2} \exp(-ad^b)$	$a > 0, b > 0$	Thin for $b > 1$, fat for $b < 1$, exponential for $b = 1$	$\frac{1}{a^{1/b}} \frac{\Gamma(1/b)}{b}$
Gamma	$\gamma_\Gamma(x, y) = \frac{a^c}{2\pi\Gamma(a)} d^{c-1} \exp(-ad)$	$a > 0$	Exponential-like	c/a
Geometric	$\gamma_g(x, y) = \frac{(c-2)(c-1)}{2\pi a^2} \left(1 + \frac{d}{a}\right)^{-c}$	$a > 0, c > 2$	Fat-tailed and power law	$\frac{2a}{c-3}$
Logistic	$\gamma_{\log}(x, y) = \frac{c'}{2\pi a^{2/c'} \Gamma\left(\frac{2}{c'}\right) \Gamma\left(1 - \frac{2}{c'}\right)} \left(1 + \frac{d^{c'}}{a}\right)^{-1}$	$a > 0, c' > 2$	Fat-tailed and power law	$a^{1/c'} \frac{\Gamma\left(1 - \frac{3}{c'}\right) \Gamma\left(\frac{3}{c'}\right)}{\Gamma\left(1 - \frac{2}{c'}\right) \Gamma\left(\frac{2}{c'}\right)}$
2Dt	$\gamma_{2Dt}(x, y) = \frac{c-1}{\pi a} \left(1 + \frac{d^2}{a}\right)^{-c}$	$a > 0, c > 1$	Fat-tailed and power law	$\sqrt{a} \frac{\sqrt{\pi}}{2} \frac{\Gamma\left(c - \frac{3}{2}\right)}{\Gamma(c-1)}$
Generalized logistic	$\gamma_{G\log}(x, y) = \frac{c'\Gamma(c)}{2\pi a^{2/c'} \Gamma\left(\frac{2}{c'}\right) \Gamma\left(c - \frac{2}{c'}\right)} \left(1 + \frac{d^{c'}}{a}\right)^{-c}$	$a > 0, cc' > 2$	Fat-tailed and power law	$a^{1/c'} \frac{\Gamma\left(c - \frac{3}{c'}\right) \Gamma\left(\frac{3}{c'}\right)}{\Gamma\left(c - \frac{2}{c'}\right) \Gamma\left(\frac{2}{c'}\right)}$

Equations 1 and 2 assume that the proportion of resistant seeds produced by an MS plant is equal to the proportion of pollen grains containing the transgene in the pollen pool. In contrast, all seeds of an MF plant contain the transgene if the plant is transgenic (plants are homozygous) and only the out-crossed seeds (in proportion $1 - s$) contain the transgene in the proportions given by the pollen pool if the plant is susceptible. Equation 3 expresses the proportion of pollen grains containing the transgene in the pollen pool at point (x, y) , obtained as the ratio of the amount of pollen received from all the transgenic plants to that received from all the plants of the field. This equation relies on the assumptions that (i) all plants of one type produce the same amount of pollen and (ii) all plants disperse their pollen following the same IDF γ . The parameter m takes into account not only a difference in pollen production between transgenic and susceptible plants but also an asynchrony of flowering periods or differential pollination success on an ovule of a susceptible plant.

Besides calculating expectations of R_k^{ms}, R_k^{mf} by equations 1–3, we assume that these observations are independent and follow binomial distributions. This assumption allows a maximum likelihood estimation of parameters s and m , together with the parameters of the IDF: θ and a, b, c or c' depending on the family of functions (Table 1). The method has already been described and applied in Tufto, Engen & Hindar (1997) and Klein *et al.* (2003). We also computed confidence intervals for the estimated parameters from the numerical computation of the variance matrix following the classical methods associated with log-likelihood (Reid 2002).

Predictions of field-to-field CPR in various configurations

Equations 1 and 2 can be used to predict the proportion of seeds in field B that were sired by plants from field A (see Appendix S1). To illustrate the relation between spatial design and CPR, we predicted CPR for fields of

depths and widths $D_A \times W_A$ and $D_B \times W_B$ separated by a distance d .

The distance d varied from 20 m to 230 m in 30-m steps; the width parameters W_A and W_B varied from 60 m to 210 m in 30-m steps; and the depth parameters D_A and D_B varied from 50 m to 200 m in 30-m steps. The largest design thus simulated two fields of area 4.2 ha each, separated by 230 m. To predict CPR, we used a selfing rate of 63% (see the Results) and no difference in individual pollen production between plants of fields A and B ($m = 1$ in equation 3). For each design we computed the CPR with three models (exponential, exponential power and logistic) and three IDF within each model (estimated parameters, minimal and maximal cases based on the bounds of the confidence intervals; Table 3).

The predicted CPR in field B was the mean over set B of the proportions of seeds sired by set A, i.e.:

$$C_B = \frac{\int_{\text{B}} (1-s)\mu(x, y) dx dy}{|\text{B}|} \quad \text{eqn 4}$$

where $|\text{B}| = D_B W_B$ is the area of B.

Analyses of variance were conducted on all or part of the log(CPR) predicted, including effects of the distance d , dimensions (D_A , W_A , D_B , W_B) and, occasionally, model, IDF and model \times IDF. The mean square of each effect was recorded.

The 'exact' prediction was compared with three approximations:

$$C'_B = \frac{\int_{\text{B}} (1-s)\mu'(x, y) dx dy}{|\text{B}|} \quad \text{eqn 5}$$

with

$$\mu'(x, y) = \frac{\int_{(x', y') \in \text{A}} \gamma(x-x', y-y') dx' dy'}{m}$$

$$C''_B = \frac{|\text{A}|(1-s)\gamma(d)}{m} \quad \text{eqn 6}$$

$$C'''_B = \frac{|\text{A}|(1-s)\gamma\left(d + \frac{D_A}{2} + \frac{D_B}{2}\right)}{m} \quad \text{eqn 7}$$

The first approximation (equation 5) is obtained by considering that (i) the amount of external pollen is low and (ii) almost the totality of the resident pollen remains in the field. The denominator of equation 3 is then close to $0 + m \times 1$.

The second and third approximations rely on the supplementary assumption that (iii) γ is sufficiently flat for the integrals to be equal to the value of the IDF at a given distance (chosen as the distance between fields) multiplied by the area covered by the integrals. As between-field distances, equation 6 considers the distance between the closest borders of the two fields and equation 7 that between their central points.

Results

DATA COLLECTED, PARAMETER ESTIMATIONS AND MODELS FITTED

The data showed dispersal events at all distances screened (up to 50 m). The majority were concentrated in the first few metres around the plot of resistant plants. The CPR values observed at different distances (proportions of resistant seedlings among all seeds collected at that distance from the central plot) appeared consistent with those from previous studies, with a rapid decrease over the first metres from the source plot (Fig. 1 and Table 2). The observed pattern of dispersal was globally isotropic (Fig. 1), although a slight anisotropy had been detected in Lavigne *et al.* (1998).

The difference between the proportions of resistant seedlings in the MS progenies and in the MF progenies (Fig. 1) was because of the selfing rate s , which was estimated at 63% regardless of the dispersal model chosen (Table 3). It was estimated that about 52% of the pollen released by one plant remains in a square of 9 m² around this plant (almost independently of the dispersal family, except for the exponential family). This indicated that 52% of the pollen pool originated from the 9 m² surrounding each plant. These two values

Table 2. Number of seeds screened and number of resistant seedlings found for MS and MF plants at different distances from the central plot containing resistant plants. The mean CPR obtained at different distances [CPR(MS) and CPR(MF)] are expressed as a percentage

	MS receptors			MF receptors		
	n	No. R	CPR(MS)	n	No. R	CPR(MF)
Inside R patch	51 403	31 014	60.3			
1.5 m (first crown)	123 108	5 906	4.80	111 661	2088	1.87
4- > 10 m	243 845	1 396	0.57	301 082	421	0.14
10- > 20 m	890 937	662	0.074	920 094	225	0.024
20- > 30 m	1 131 333	259	0.023	1 187 045	81	0.007
30- > 40 m	1 569 338	97	0.0062	1 706 260	102	0.0060
> 40 m (angles)	636 294	15	0.0024	636 945	17	0.0027

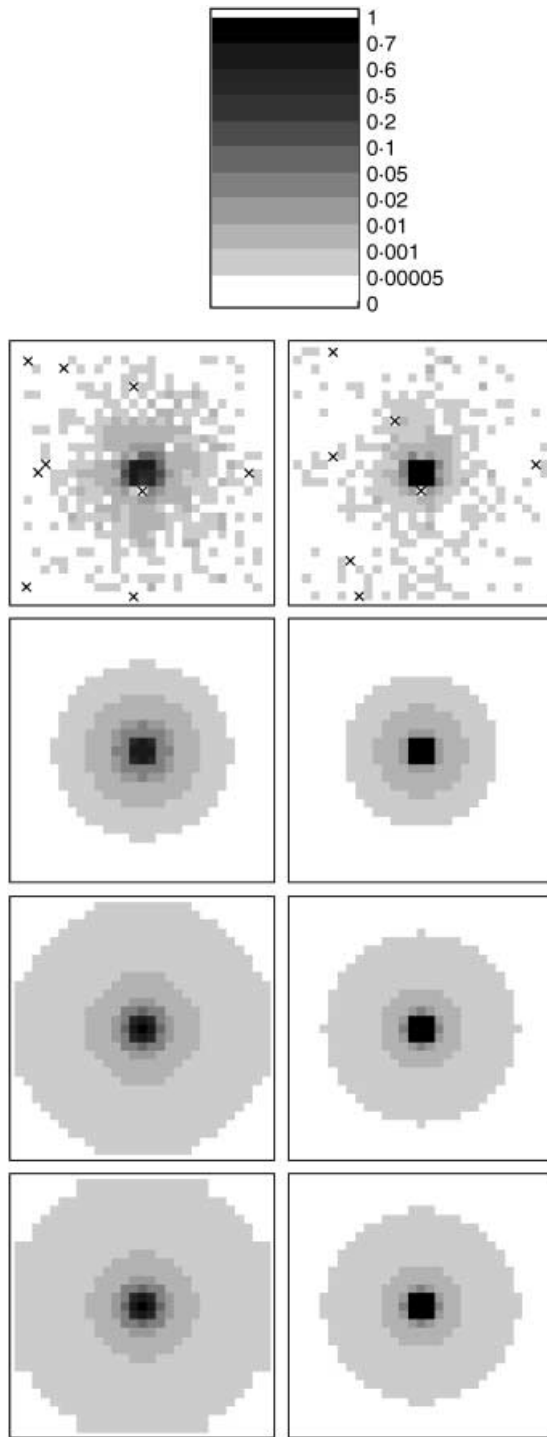


Fig. 1. Observed and predicted proportions of resistant seedlings in progenies as a function of the positions of the sampled plants in the experimental field, for male-sterile plants (left) and male-fertile plants (right). From top to bottom, observations (crosses indicate missing data) and predictions from the exponential, exponential power and logistic models.

explained the low proportions of resistant seedlings in the progenies of susceptible MF plants (no observation above 5%). The expected proportion of resistance in the progeny of a susceptible MF plant growing in the centre of a 9-m² susceptible plot isolated in a resistant field would only be about 15% [i.e. $(1 - 0.63) \times (1 - 0.52)$].

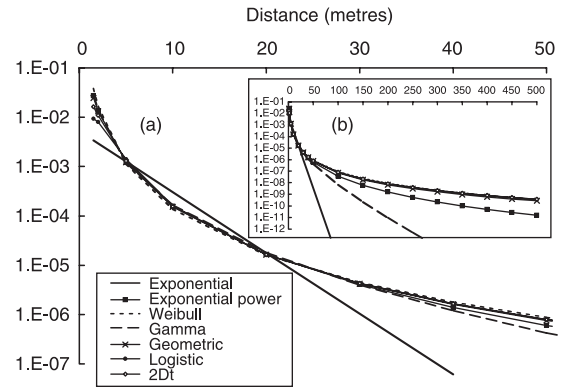


Fig. 2. Log-plots of the estimated IDF for the seven families of curves considered. (a) on a range of distances similar to those observed (0–50 m) and (b) on an extrapolated range of distances (up to 500 m). $1.E - 0x = 10^{-0x}$.

Data collected from MS plants showed a strong asymmetry between the proportions of susceptible seedlings at the inside border of the central resistant patch (around 40%) and the proportion of resistant seedlings just outside its border (around 5%). This asymmetry was not only because of the smaller size of the central patch but also because of the lower pollen production of a GM compared with a non-GM plant. The parameter m was estimated to be around 1.9 (Table 3), which meant that each 1 m² of non-GM plants produced almost twice as many efficient pollen grains as each 1 m² of GM plants. This was probably because of the cutting of early racemes of resistant plants.

Among the families of IDF fitted to the data, the exponential family achieved the poorest fit, with a log-likelihood largely inferior to all the others (Table 3). This was because of an underestimate of the proportions of resistant seedlings both at short and long distances from the central plot (Fig. 1). All the other models succeeded in reaching a similar kind of decrease (Fig. 2), adequately describing the observed pattern of dispersal (Fig. 1) with similar log-likelihood scores (Table 3). However, although the estimated IDF were very similar on the observed range of distances (0–50 m; Fig. 2a), their extrapolation to larger distances (up to 500 m; Fig. 2b) led to differences of several orders of magnitude among models. These differences were mostly dependent on the type of function tail. Note that some models needed an extreme parameterization to a typical geometric decrease that best explained the data (Table 3): a low b estimate for the Weibull model led to a function of form d^{-2} (negligible effect of the exponential term), while an estimate of a close to 0 for the gamma model led to a function of form $d^{-2.51}$ (exponential term almost cancelled).

Finally, the power-law tails best described the dispersal of oilseed rape pollen and, among them, the logistic model achieved the best fit. This was because of an S-shape at short distances leading to a better description of the observations in the vicinity of the resistant plot (Fig. 2).

Table 3. Maximum likelihood estimation of the parameters for the different models of IDF. $-\text{Ln}$ is the opposite of the log-likelihood: the smaller the $-\text{Ln}$, the better the fit to the data. θ is the proportion of pollen remaining in the 9-m^2 square centred on a plant, and a, b, c, c' are the parameters defined in Table 1. δ is the mean dispersal distance of the estimated dispersal function. δ is not equal to what is expected from the mean distance formula in Table 1 because θ is taken into account. α is the normalizing constant that corrects for the introduction of θ (see Appendix S1); 95% confidence intervals are given for each parameter but because of strong correlations between parameter estimates they should not be read independently

Model	$-\text{Ln}$	θ	a	b	c	c'	δ	m	s	α
Constant + negative exponential (from Lavigne <i>et al.</i> 1998)	19871†	0.56	1.61					(1)	0.589	
Exponential	7236	0.63 [0.58'', 0.67']	0.284 [0.274'', 0.294']	–	–		2.8	1.83 [1.75, 1.91]	0.65 [0.61, 0.69]	0.40
Exponential power	5582	0.52 [0.03'', 1.00']	15.83 [8.94', 36.4'']	0.14 [0.07'', 0.21']	–		3.2	1.90 [1.84, 1.97]	0.63 [0.59, 0.67]	2.24
Weibull	5578	0.50‡	10.97‡	0.086‡	–		4.4	1.91 [1.85, 1.98]	0.63 [0.59, 0.66]	47779
Gamma	5621	0.52 [0.46, 0.58]	0.048 [0.041, 0.055]	–	–1.51 [–1.60, –1.43]		2.9	1.88 [1.82, 1.95]	0.64 [0.61, 0.68]	–0.41
Geometric	5564	0.52 [0.05, 0.98]	1.04 [–0.6, 2.65]	–	3.46 [2.84, 4.08]		4.2	1.90 [1.83, 1.97]	0.63 [0.60, 0.67]	1.02
Logistic	5546	0.55 [0.47'', 0.63']	32.3 [3.8'', 53.2']	–		3.32 [3.12'', 3.52']	4.8	1.90 [1.84, 1.97]	0.63 [0.59, 0.67]	0.53
2Dt	5555	0.54 [0.42, 0.66]	4.06 [1.29, 6.82]	–	1.66 [1.57, 1.76]		4.7	1.91 [1.83, 1.97]	0.63 [0.60, 0.67]	0.66
Generalized Logistic	5546	0.55‡	32.3‡	–	0.999‡	3.26‡	4.8‡	1.91 [1.83, 1.97]	0.63 [0.60, 0.67]	0.53

' and '' in confidence intervals indicate the values used, respectively, as minimal and maximal cases to predict the CPR (see the Material and methods).

†The likelihood of the two sets of observations (MS and MF receptors) has been recalculated with the model fitted in Lavigne *et al.* (1998).

‡For numerical reasons, the computation of the confidence interval failed.

PREDICTIONS OF THE CPR BETWEEN TWO FIELDS

For the best-fitting IDF (logistic model with estimated parameters), the predicted CPR from a field A to a field B ranged from 0.099% to 0.0001% (Fig. 3). The maximal CPR was logically obtained when the largest field A (4.2 ha) was contaminating the smallest field B (0.3 ha) at the smallest distance (20 m) and, inversely, the minimal CPR was obtained for the smallest field A, the largest field B and the largest distance (230 m). As expected, between-field distance had a major effect on CPR: a reduction by almost two orders of magnitude

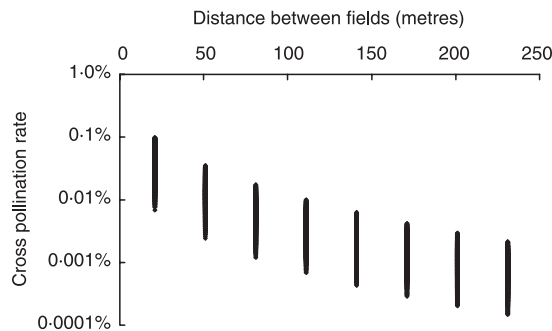


Fig. 3. Log-plot of the cross-pollination rates between two fields as a function of distance between these fields. Each point is the result for one given spatial design (i.e. widths W_A and W_B and depths D_A and D_B).

was obtained when this distance increased from 20 m to 230 m. Furthermore the shape of the design played an important role because, for a given distance, a reduction by one order of magnitude of the CPR was obtained between the 'worst' and the 'best' spatial configurations. The CPR was most sensitive to the distance d [mean squares (MS) = 421.7], the width of field A (MS = 69.7), the depth of field B (MS = 27.0), the depth of field A (MS = 11.3) and the width of field B (MS = 1.2). By considering more precisely the effects of these five parameters on the CPR (Fig. 4), we found that increasing the distance d decreased CPR in relation to the shape of the IDF

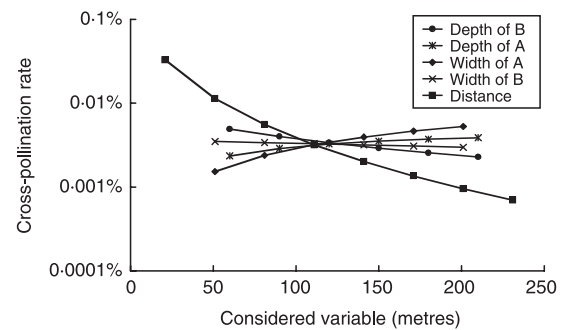


Fig. 4. Effects of the field sizes and distance between fields on the cross-pollination-rate in field B (C_B). For each curve, the corresponding variable d , W_A , W_B , D_A or D_B is plotted on the x-axis. The CPR (mean over all the combinations of the others variables) is plotted on the y-axis.

[at least far from (0, 0)]. Increasing the width of A (W_A) increased CPR almost linearly (at least for large distances between A and B; results not shown). This was because increasing W_A increased the number of donor plants at similar distances from B. Increasing the depth of B (D_B) resulted in a reduction of CPR because fewer and fewer contaminated recipient plants were added as D_B increased, i.e. there was a dilution effect. Similarly, increasing the depth of A (D_A) increased CPR less than linearly, because increasing D_A resulted in adding donor plants at increasing distances from B. No effect of D_A would have meant that only the border of the donor field contributed significantly to the CPR in the recipient field. Finally, increasing the width of B (W_B) only slightly decreased CPR. The recipient plants added to increase W_B were at similar distances from A as recipient plants already present when B was not wider than A, and at slightly larger distances otherwise. This latter effect was larger for short distances, d (results not shown), because new plants were added at relatively larger distances in that case.

The relative influence of these four parameters was model dependent. The thinner the tail, the greater the contribution of depth of B and the lesser the contribution of the depth of A [$MS(D_B)/MS(W_A) = 0.38, 0.62$ and 1.56 , and $MS(D_A)/MS(W_A) = 0.16, 0.08$ and 0.008 , for logistic, exponential power and exponential IDF, respectively]. Thinner tails were steeper, resulting in a larger dilution effect when increasing D_B and a larger role of the donor border.

The IDF was the main factor underlying the predicted CPR. Just considering the two fat-tailed models with three IDF each (estimated, minimal and maximal cases; Table 3), the model, IDF and their interaction had effects similar to that of distance and more important than that of spatial design [$MS(d) = 3913$; $MS(\text{model}) = 3119$; $MS(\text{IDF}) = 8080$; $MS(\text{model} \times \text{IDF}) = 1205$; $MS(W_A) = 404$]. Finally, when only the three IDF from the logistic model were considered, the effect of the IDF was in-between that of distance and that of the width of the donor field [$MS(d) = 1269$; $MS(\text{IDF}) = 529$; $MS(W_A) = 209$].

The three approximations proposed to simplify the predictions of CPR led to contrasting results. The first approximation, C'_B (equation 5), always slightly underestimated the true CPR (Fig. 5a) independently of the spatial design. In contrast, the two linear approximations of the effect of field size (C''_B and C'''_B) led to large biases in the approximated CPR (Fig. 5b) and were particularly poor at smaller distances d (results not shown). The border-to-border approximation C''_B performed particularly badly, always leading to an overestimation of the CPR, often by one order of magnitude and sometimes by two. The middle-to-middle approximation C'''_B performed better, leading to underestimations that almost never reached one order of magnitude.

Discussion

IDF can be estimated from data and used for predictions, as shown for oilseed rape pollen using data from Lavigne

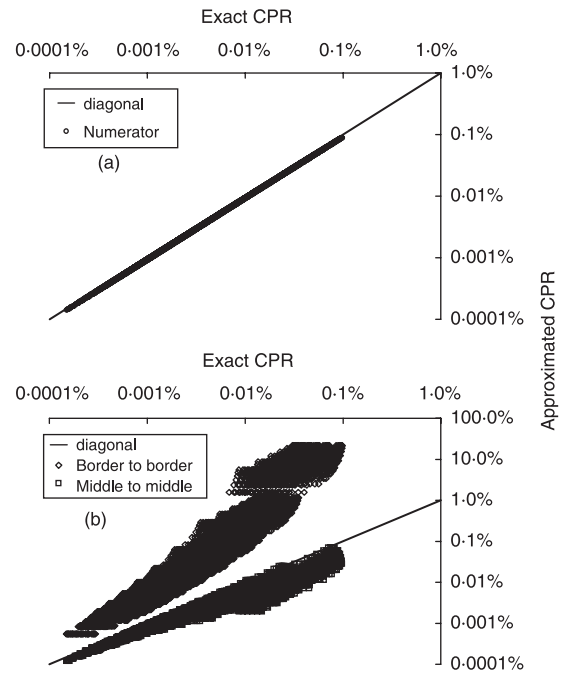


Fig. 5. Comparisons of exact (on x -axis, and thus diagonal) and approximated (on y -axis) CPR. (a) Approximation given by equation 5, considering the denominator of equation 3 is equal to m . (b) Approximations that assume linear effects of the field sizes and use border-to-border distance (diamonds, above the diagonal) and middle-to-middle distance (squares, below the diagonal) (equations 6 and 7).

et al. (1998). Lavigne *et al.* (1998) mainly presented a non-parametric approach leading to a 2-dimensional estimation of the IDF that proved difficult to use to predict gene flow in various spatial configurations. A first parametric estimation had also been attained (recalled here in Table 3) but was much improved in the present study by (i) taking into account the difference in pollen production between resistant and susceptible plants, (ii) simultaneously using the two data sets from MS and MF plants, (iii) exploring a wide range of dispersal models and (iv) using a maximum likelihood estimator rather than a least-square estimator. These improvements led to satisfactory fits and to more convincing IDF.

Fat-tailed and especially power-law functions achieved the better fits, with a slight advantage for the logistic model. This result emphasizes the relevance of considering long-distance dispersal. In particular, the classical exponential decreasing function (Timmons *et al.* 1996; Staniland *et al.* 2000) clearly appeared inadequate for describing the pattern observed in our experiment. The exponential power model also led to an even fatter tail (b smaller) than those estimated by Gliddon (1999), probably because we disconnected the behaviour of the function at 0 and at long distances. Finally, the best fit was obtained with a power-law decrease (with exponent -3.32 ± 0.20). This power law is consistent with other recent models for the dispersal of oilseed rape pollen. Wilkinson *et al.* (2003) used a power law with exponent -0.75 and Walklate *et al.*

(2004) present a mechanistic model leading to a power-law decay with exponent ~ -4 .

The parametric estimation of an IDF makes it possible to predict gene flow in spatial configurations including larger distances than those used for the estimation. However, this benefit must be used with caution because, as shown, extrapolations to larger distances may be very sensitive to the dispersal function, and particularly to characteristics of its tail. The major importance of the model effect in the sensitivity analysis also stressed the necessity to explore several types of models. Furthermore, predictions were also largely sensitive to uncertainty in parameter estimates. IDF thus need be estimated with care, and the effects of environmental conditions (e.g. wind regime, insect activity, local topography and obstacles) on the shape of IDF need further investigation. More generally, extrapolations need to be validated on a broader range of distances by collecting data over larger scales (Beckie *et al.* 2003; Ramsay, Thompson & Squire 2003; Devaux *et al.* 2005).

As a first attempt, we compared the CPR predicted from the logistic model fitted here with experimental results from the literature. Our predictions roughly agree with experimental CPR (in parentheses) for adjacent plots in Scheffler, Parkinson & Dale (1993): we predicted CPR of 2.0% (1.6%), 0.47% (0.4%), 0.16% (0.11%), 0.10% (0.016%), 0.02% (0.0041%), 0.006% (0.0011%), 0.001% (0.00034%) and 0.0004% (0.0%). Our predictions are also close to those of Walklate *et al.* (2004), who fit their model to these same data. However, we underestimated, sometimes greatly, the CPR between distant fields. For example, the predicted maximal CPR for a 1×1 -km field to a 0.5×0.5 -km field was $2 \cdot 10^{-4}$ %, $5 \cdot 10^{-5}$ % and $8 \cdot 10^{-6}$ % for isolation distances of 200 m, 1000 m and 3000 m, respectively, while Rieger *et al.* (2002) observed on average $9 \cdot 10^{-3}$ %, independently of the distance. Not considering the effect of gaps between two plots (Reboud 2003) may explain these underestimations, especially as oilseed rape is partly insect pollinated. This confirms that the functions fitted here are more adequate for predictions in the range where they were fitted (no gaps, several dozens of metres) than for large-scale extrapolations. It thus appears inappropriate to use these predictions directly for determining isolation distances (which would probably be underestimated) or for providing management rules for GM crops. Furthermore, risk assessment and management should be considered at the landscape scale (e.g. GeneSys; Colbach, Clermont Dauphin & Meynard 2001).

The models presented here are based on a dispersal kernel approach (Clark *et al.* 1999). They thus rely on the fundamental assumptions that all MF plants disperse their pollen following the same IDF, and that female flowers simply sample pollen grains in the resulting pollen pool without affecting the dispersal pattern. Although this approach is not specific to wind-borne pollen, these assumptions are probably less well founded for insect pollination. For example, plants at the field border may disperse their pollen further than plants inside the field

because their pollinators may more often fly between fields. This would lead to higher CPR than predicted with no effect of gaps (Reboud 2003). Furthermore, even within species, plants differ in their attractiveness depending on their genotype, physiological status and level of isolation. New modelling approaches may be necessary for large-scale pollen dispersal of oilseed rape in an agricultural landscape.

Although extrapolations must be considered cautiously, some general conclusions on the impact of spatial design emerge from the predictions. Our results indicate that a spatial approach appears necessary when modelling between-field gene flow. Representing fields as point sources, possibly weighted by their area, is insufficient. Although the distance between fields appeared as the main parameter determining the CPR, the shape of the design also had a large impact, modifying the CPR by one order of magnitude. For fat-tailed IDF, width of the source plot and depth of the receptor plot influenced most the CPR, followed by the depth of the source and, lastly, the width of the receptor. Mechanisms underlying these differences in sensitivity are rather simple. Increasing the depth of the receptor plot dilutes the pollen flow from the source plot, while increasing its width only has a dilution effect if the new plants are set at larger distances than plants already present. Similarly, increasing the width of the source increases the number of plants with high contributions to the CPR, while increasing its depth increases the number of distant and thus little contaminating plants.

Predicting CPR with IDF is the approach currently used in two models, GeneSys on oilseed rape (Colbach, Clermont Dauphin & Meynard 2001) and Mapod on maize (Angevin *et al.* 2003), for coexistence studies at the European level. One IDF is used in each of these models, each fitted on a single data set. Although their predictions are being validated on numerous data sets, the lack of other available IDF makes it impossible to test the sensitivity of their predictions and conclusions on management strategies to the uncertainty on pollen dispersal. The approach presented here could thus be applied to other data sets to provide such IDF. Running these models has proved that the exact calculation of CPR described here is very time consuming when dealing with large areas. We thus investigated the validity of classical approximations that could enhance modelling efficiency. The approximations tested here were not very satisfactory for solving this practical drawback. The first approximation (equation 5) gave good predictions but required the time-consuming calculation of a convolution product. The second (equation 6), considering only the distance between field borders, greatly overestimated the CPR and was obviously not adequate. The third (equation 7) performed better but always underestimated the CPR because plot areas do not have linear effects on CPR. For an efficient integration of the IDF approach in landscape models, further work is still needed to find an approximation balancing accuracy of the CPR prediction and ease of computation.

Acknowledgements

We are grateful to P. Hulme, J. Cresswell, J. Pierre, J. Shykoﬀ and C. Devaux and referees for improving this manuscript. This work was partially supported by the programme 'AIP OGM et environnement' funded by INRA.

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