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A model of pollen-mediated gene flow for oilseed rape

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The development of genetically modified (GM) crops has precipitated the need for risk assessment and regulation of pollen-mediated gene flow. In response to this need we present a mathematical model to predict the spatial distribution of outcrossing between progenitor populations of oilseed rape. The model combines the processes of pollen dispersal and pollination, resulting from wind and insect activity. It includes the effects of post-pollination reproductive processes by relating the number of progeny to both pollen deposition and competition at the stigma. Predictions compare well with a range of experimental results for different-sized GM source crops (i.e. 0.0064–0.8 ha) and non-GM target crops with different fertilities (i.e. self-fertile to 80% male-sterile). For these comparisons, we represent the variation caused by wind and insect exposure as a constrained set of random functions and limit the range of insect transport to typical plant-scale distances. In addition, the model is used to examine the relative sensitivity to the factors that determine gene flow. Target-crop fertility and source-crop size are shown to be more important than other factors, including background pollen and the natural range of insect activity. The concept of isolation distance to regulate gene flow is most effective for self-fertile target crops, but is ineffective for male-sterile target crops with low background pollen.

Keywords: wind dispersal; insect dispersal; pollination; outcrossing; risk assessment; genetically modified plants

1. INTRODUCTION

The development of genetically modified (GM) crops has precipitated the need to assess the risks associated with pollen-mediated gene flow and to establish sustainable land-management practices. The current methods of risk assessment assume that growing GM crops at a minimum distance from a non-GM neighbour can adequately regulate this type of gene flow. This 'isolation distance' as it is known varies according to the required threshold for outcrossing between GM and non-GM varieties of the same or related plant species. Other factors affecting isolation distance include the areas of both GM and non-GM crop production, attractiveness to insects, synchronization between flowering of GM and non-GM crops, viability of crosses between GM and non-GM varieties and competition of pollen deposits at the stigmas of non-GM plants (Ingram 2000). Experiments have been performed to study some of these effects based on field trials with maize (Scott 1970; Raynor *et al.* 1972; Klein *et al.* 2003) and oilseed rape (Scheffler *et al.* 1993, 1995; Lavigne *et al.* 1998; Norris & Sweet 2002; Rieger *et al.* 2002; Simpson & Sweet 2004). However, it may be too costly to subject all future new GM plant products to the very extensive range of measurements that are currently used to study pollen-mediated gene flow (Lutman & Sweet 2000).

Modelling systems may therefore be required in the future to reduce the cost of risk assessment and enable the well-structured development of land management and regulatory systems through the improved integration of accumulated field data and theoretical understanding (Colbach *et al.* 2001). The modelling of pollen dispersal from oilseed rape is particularly challenging because both wind and insects have been identified as agents of transport and pollination. A fully validated modelling system for predicting these combined effects is still an elusive goal.

Bees have been identified as one of the most prolific pollinators of oilseed rape (Scheffler *et al.* 1993, 1995). Bumble-bees (*Bombus terrestris*) have been observed to deposit much of the available pollen close to the pollen source (Thomson & Thomson 1989; Cresswell 1994). By contrast, some field studies have linked honeybees (*Apis mellifera* L.) with long-distance transport of pollen (Ramsay *et al.* 1999). In addition to these field observations, a Monte Carlo simulation model and approximate solutions of an advection–diffusion transport equation have been used to predict pollen dispersal resulting from bee movement (Cresswell *et al.* 1995). However, it was not recognized that these models are variants of well-known atmospheric-transport models that are routinely used for predicting the dispersion of airborne particulates (Pasquill & Smith 1983).

Atmospheric-dispersion models of pollen transport (DiGiovanni & Beckett 1990; Tufto *et al.* 1997; Hunt *et al.* 2002; Klein *et al.* 2003) are much more highly developed than the available insect-dispersal models. Furthermore, several models are commercially available and are routinely used for the control and regulation of various particulate emissions to the atmosphere. The current state-of-the-art model, which has the capacity to generate results within *ca.* 50 km of a source for a wide range of environmental conditions (Carruthers *et al.* 1994*a*), has been extensively validated against experimental data

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(Carruthers *et al.* 1994*b*, 1996, 1998) and has been developed to appropriate quality assurance standards.

We develop a deterministic model of pollen-mediated gene flow for oilseed rape. To achieve this we simplify the modelling approach of Hunt *et al.* (2002), based on hourly averages of windborne pollen deposition, and instead use similarity approximations to represent the spatial distribution of atmospheric pollen deposition as a time average for the full flowering period (typically 30 days for oilseed rape). We propose a new pollination model based on the effects that insects have on the transport of pollen across a range of length scales. At local plant-scale distances, pollen transport by insects is modelled by applying a scalar correction to the deposition distribution of windborne pollen, and, at larger distances, it is modelled as a convolution process. We also propose a model for the gross effect of competitive post-pollination reproductive processes to enable the estimation of outcrossing probability based on the predicted distributions of pollen deposition from different progenitor-plant populations. We adapt the model so that it can be used for multiple regression analysis of typical field data (Scheffler *et al.* 1993; Norris & Sweet 2002; Simpson & Sweet 2002). For this application, the predicted outcrossing distribution is based on standardized conditions and the variation caused by the wind and insect exposure, during different field trials, is treated as a constrained set of random functions. We also consider the sensitivity of the deterministic model to key parameters (i.e. source-crop size, background pollen deposition, target-crop fertility and the range of transport distances associated with insect pollination). Finally, we briefly discuss the implications of the results in relation to the use of isolation distance as a method of controlling pollen-mediated gene flow.

2. THEORY

(a) *Pollen dispersal and pollination by wind and insects*

There is some experimental evidence to suggest that the stigmas of oilseed rape flowers are partly sheltered from windborne pollen trajectories. For example, the field measurements of outcrossing from caged and naturally exposed oilseed rape plants (Ramsay *et al.* 2003) show that pollination can be dramatically reduced when insects are excluded by cages. Landscape-scale studies of outcrossing (Thompson *et al.* 1999; Squire *et al.* 1999) suggest that the greatest risk of GM pollen transport is from insect pollination closest to the source, and honeybees have been identified as the principal pollinator for oilseed rape at large distances. Observations of honeybee flights of 1–2 km to collect nectar and pollen have been known for some time (Eckert 1933) and recent studies have recorded bees foraging on oilseed rape at 5 km from a hive (Ramsay *et al.* 1999). Other studies have shown that much of the pollen collected by bumble-bees is transferred to other plants within a short distance of the first collection point (Thomson & Thomson 1989; Cresswell 1994). Therefore, it is possible that bees contribute to both long-distance and short-distance transport but not necessarily in equal proportions.

In this study, we neglect the contribution that bees make to direct transport of oilseed rape pollen over distances of greater than 100 m. However, we recognize the contribution that bees (Cresswell *et al.* 2002), and possibly other insects (Kirk 1992), make by transporting pollen direct from local sources and between highly exposed parts of the plant and the more sheltered stigma. The effect of pollen transport by insects, within the limits of local plant-scale resolution (i.e. $-\Delta x/2 < x < \Delta x/2$ and $-\Delta y/2 < y < \Delta y/2$, where x and y are horizontal orthogonal coordinates and Δx and Δy are the corresponding length scales of the plant), is represented by a linear scaling of the hourly averaged windborne pollen deposition at the most exposed part of the target plant, $D'(x,y)$. Therefore, the pollen deposition at the stigmas of target plants is given as $D(x,y) = \gamma D'(x,y)$, where γ is the local exposure coefficient $\gamma \leqslant 1$. The experimental results of Ramsay *et al.* (2003) seem to imply that where insect pollination is excluded, by the use of caged plants, the exposure coefficient is significantly reduced (i.e. $\gamma \cong 0.1$). Thus, if insect transport is limited to plant-scale distances then these results also suggest that the atmospheric deposition of pollen at the stigma is typically one order of magnitude smaller than the deposition at the most highly exposed part of the plant. This, therefore, is consistent with the general observations of very high variability of atmospheric deposition on plant surfaces reported by Chamberlain (1975).

In addition to the local plant-scale transport of pollen by insects, we introduce the effect of large-scale transport as a convolution process for all length-scales greater than the local plant-scale distance. Hence, the pollen deposition at the stigma after insect transport at all length scales is

$$D(x,y) = \gamma \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} D'(x,y) R(x - x_1, y - y_1) \mathrm{d}x_1 \mathrm{d}y_1, \quad (2.1)$$

where $R(x,y)$ represents a suitable convolution function. This process ensures that the effects of insect transport are applied to the deposition distribution $D'(x,y)$ throughout space. The convolution function can be used to conserve pollen mass by using the identity $\kappa = (\Delta x \Delta y)^{-1} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} R(x,y) \mathrm{d}x \mathrm{d}y$, where $\kappa = 1$ for pure redistribution, $\kappa > 1$ for mass gain and $\kappa < 1$ for mass loss. The generalized convolution model (equation (2.1)) therefore represents local plant-scale transport of pollen, assuming pure redistribution, when $R(x,y) = 1$ in the region $-\Delta x/2 < x < \Delta x/2$, $-\Delta y/2 < y < \Delta y/2$ and $R(x,y) = 0$ elsewhere. Estimates of the convolution function for more extensive insect transport may be made by using heuristic methods or specially constructed experiments with different insect groups.

(b) *Competitive post-pollination reproductive processes*

The competition between different pollen grains on the stigmas of target plants is modelled by considering the deposition of pollen from GM source plants D_g and expressing this as a proportion of the total pollen deposition $D_g + (1 - \eta)D_t + D_b + D_s$, where the product $(1 - \eta)D_t$ is the deposition from extrinsic sources within the target area, η is the proportion of male-sterile plants within the target area, D_b is the deposition from background sources and D_s is the deposition from intrinsic

sources (i.e. from the anthers of the flower in which fertilization takes place). Therefore, for self-fertile target plants the local competition between GM and all other sources of pollen is represented by the ratio

$$P_{\mathrm{sf}} = \frac{D_{\mathrm{g}}}{D_{\mathrm{g}} + (1 - \eta)D_{\mathrm{t}} + D_{\mathrm{b}} + D_{\mathrm{s}}}, \tag{2.2}$$

and for male-sterile target plants (i.e. where $D_{\mathrm{s}} = 0$) this becomes

$$P_{\mathrm{ms}} = \frac{D_{\mathrm{g}}}{D_{\mathrm{g}} + (1 - \eta)D_{\mathrm{t}} + D_{\mathrm{b}}}. \tag{2.3}$$

Very low competition between GM and non-GM pollen thus occurs in target crops containing all male-sterile plants (i.e. $\eta = 1$). Under these conditions equation (2.3) approximates to $P_{\mathrm{ms}} \sim 1$ when the deposition from background pollen is small (i.e. $D_{\mathrm{b}} \ll D_{\mathrm{g}}$).

We postulate that the number of GM progeny N_{g} from the sampled area of target plants is proportional to the integral of the product of pollen deposition rate and the proportion of GM pollen. Therefore, the number of GM progeny from both self-fertile and male-sterile target plants within the sampled area of the target crop is

$$N_{\mathrm{g}} \propto \int_{t}^{t+\Delta t} D_{\mathrm{g}}(\eta P_{\mathrm{ms}} + (1 - \eta)P_{\mathrm{sf}})\mathrm{d}t, \tag{2.4}$$

where Δt is the relevant period of flowering. It has been observed that oilseed rape has high levels of both self-compatibility and cross-compatibility between varieties (Downey & Robbelen 1991). Therefore, we assume that there is no biasing between different cultivars other than that represented by equation (2.4). Similarly, the total number of progeny from both self-fertile and male-sterile plants is

$$N \propto \int_{t}^{t+\Delta t} (D_{\mathrm{g}} + D_{\mathrm{b}} + (1 - \eta)(D_{\mathrm{t}} + D_{\mathrm{s}}))\mathrm{d}t. \tag{2.5}$$

Typically, the outcrossing between different progenitor plant populations is expressed as the probability $F = N_{\mathrm{g}}/N$ given as

$$F = \frac{N_{\mathrm{g}}}{N} = \frac{\displaystyle\int_{t}^{t+\Delta t} D_{\mathrm{g}}(\eta P_{\mathrm{ms}} + (1 - \eta)P_{\mathrm{sf}})\mathrm{d}t}{\displaystyle\int_{t}^{t+\Delta t} (D_{\mathrm{g}} + D_{\mathrm{b}} + (1 - \eta)(D_{\mathrm{t}} + D_{\mathrm{s}}))\mathrm{d}t}. \tag{2.6}$$

(c) *Comparison of model with field experiments*

The general aim of typical field experiments is to harvest sufficient numbers of set seeds from the target crop to estimate the outcrossing probability distribution. To make a similar estimate, based on a deterministic model (equation (2.6)), meteorological data are necessary to determine the time-series of hourly averaged deposition and competition throughout the period of flowering. Assuming that this information is not available, however, we approximate the integrals in equation (2.6) by using large-scale time averages of pollen deposition to make estimates of outcrossing F and compare these with observations, $\hat{F}$, based on the following correlation function for the natural-log transform of outcrossing probability

$$\ln(\hat{F}_j) = \beta_j + \beta_0 \ln\left(F \cong \frac{\bar{D}_{\mathrm{g}}(\eta \bar{P}_{\mathrm{ms}} + (1 - \eta)\bar{P}_{\mathrm{sf}})}{\bar{D}_{\mathrm{g}} + \bar{D}_{\mathrm{b}} + (1 - \eta)(\bar{D}_{\mathrm{t}} + \bar{D}_{\mathrm{s}})} \right). \tag{2.7}$$

Here, the overbar indicates time-averaged properties for the full flowering period, β_0 is introduced to allow for further optimization of the spatial distribution and β_j, for $j \geqslant 1$, is a set of random functions representing the environmental exposure conditions associated with each dataset.

A typical field layout that has been used to study outcrossing between GM and non-GM crops is shown in figure 1. In this example, two rectangular plots of different oilseed rape cultivars, each of length L and width B, are grown side-by-side. The model assumes the following similarity distributions for pollen deposition, expressed as time averages for the full flowering period:

$$\bar{D}_{\mathrm{g}} = \varepsilon_{\mathrm{g}} \sum_{i = x/\Delta x}^{(L + x)/\Delta x} D_i', \tag{2.8}$$

$$\bar{D}_{\mathrm{t}} = \varepsilon_{\mathrm{t}} \sum_{i = 1}^{x/\Delta x} D_i', \tag{2.9}$$

$$\bar{D}_{\mathrm{b}} = \text{constant}, \tag{2.10}$$

$$\bar{D}_{\mathrm{s}} = \text{constant}, \tag{2.11}$$

where ε_{g} and ε_{t} are the exposure coefficients for the combined effects of wind and local plant-scale insect activity for the GM and target crops, respectively, and D_i' is the similarity deposition distribution on the centre-line of the target crop (i.e. $y = 0$) given as

$$D_i' = D^+ \left[\frac{\Delta x}{\Delta x + x} \right]^{\alpha} \mathrm{erf}\left(\frac{Bb\sqrt{2}}{4(\Delta x + x)} \right), \tag{2.12}$$

where x is measured from the centre of an elemental source area of length Δx and width B. Here, we introduce coefficients α and b to represent standardized atmospheric conditions, and D^+ is introduced to maintain dimensional equality.

The exposure coefficients in equations (2.8) and (2.9) can be estimated directly from pollen deposition measurements $D_{\mathrm{g}}^*(t)$ and $D_{\mathrm{t}}^*(t)$ at some reference position $x = x^*, y = 0$ within the target crop, if the exposure coefficient γ in equation (2.1) is known. Thus

$$\varepsilon_{\mathrm{g}} = \left(\gamma/\Delta t \sum_{i = x^*/\Delta x}^{(L + x^*)/\Delta x} D_i' \right) \int_{t}^{t+\Delta t} D_{\mathrm{g}}^*(t)\mathrm{d}t$$

and

$$\varepsilon_{\mathrm{t}} = \left(\gamma/\Delta t \sum_{i = 1}^{x^*/\Delta x} D_i' \right) \int_{t}^{t+\Delta t} D_{\mathrm{t}}^*(t)\mathrm{d}t.$$

Figure 1 presents examples of idealized time-series for hourly averages of deposition $D_{\mathrm{g}}^*(t)$ and $D_{\mathrm{t}}^*(t)$, synthesized from pollen-trap measurements of oilseed rape (McCartney & Lacey 1991) for the full flowering period. However, we will assume that this level of detail is typically unavailable for the purposes of the gene-flow data analysis presented in the next section.

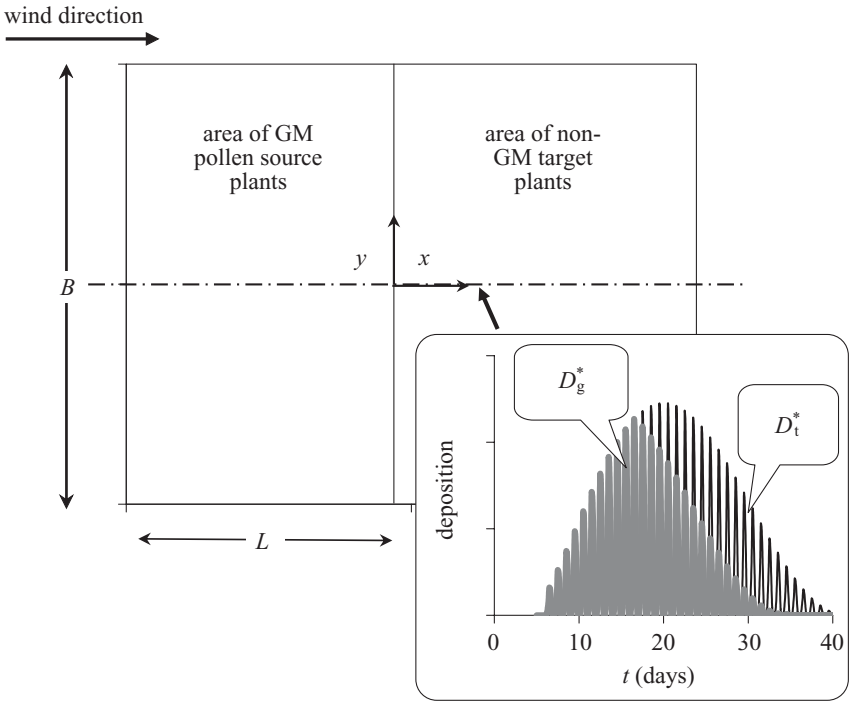

Figure 1. The layout of a typical field experiment to measure gene flow between adjacent areas of GM and non-GM oilseed rape cultivation. The graph shows the hourly averages of pollen deposition (from GM sources $D_g^*(t)$ and non-GM sources $D_t^*(t)$) that would be measured under idealized wind exposure conditions during the flowering period.

3. RESULTS

(a) *Regression analysis of field data*

In this section we compare published measurements of outcrossing probability with model predictions by using the empirical correlation function (equation (2.7)). The measurements of Simpson & Sweet (2002) were used to assess the competition scaling characteristics of the model for adjacent source and target crops of length $L = 92$ m and width $B = 92$ m (figure 2). In the analysis, we used 13 observations of each of two different environmental exposure conditions at each of two target crop-fertility levels (i.e. $\eta = 0$ and $\eta = 0.8$) ($n = 52$ observations in total).

The measurements of Scheffler *et al.* (1993), representing two different environmental exposure conditions for self-fertile targets, were used in conjunction with the previous data to assess the scaling effects of GM source size at similar levels of pollen competition (figure 2). The geometry of the GM source crop for this experiment was a 9 m diameter circle surrounded by a non-GM target crop. However, for the purpose of comparison, the model predictions were based on concentric square plots (i.e. $L = 8$ m and $B = 8$ m for the GM source and $L = 105$ m and $B = 105$ m for the surrounding non-GM target crop). In addition, different wind and insect exposure conditions were represented in the original data by eight transects at 45° spacing about the centre of the plot. However, for the purpose of the analysis presented here, we have recombined the data to give separate environmental exposure conditions for the east and west halves of the target plot.

The multiple linear regression tool available in Microsoft Excel 2002 SP-2 was used to estimate fitted parameters β_0 and β_{1-6} in equation (2.7) for the combined data representing a total of six different environmental exposure conditions (table 1). The large-time-scale averaged deposition distributions $\bar{D}_g$ and $\bar{D}_t$ were estimated from equations (2.8) and (2.9), respectively. For these we used the similarity distribution function D_i' given by equation (2.12) and the standardized set of model parameters (table 1). These distributions represent pollen release and deposition under neutral to slightly unstable atmospheric conditions for oilseed rape (McCartney & Lacey 1991) and for local plant-scale insect activity.

An optimum value of the deposition from intrinsic sources of pollen $D_s = 8D^+$ was determined by iterating the regression analysis with successive estimates to satisfy the constraint for the range of re-estimated exposure coefficients (i.e. $\varepsilon_{g_j} \cong \exp(\beta_j/2\beta_0) \leqslant \varepsilon_t$). Table 1 gives a summary of these estimates, based on the 95% confidence limits for β_j. These represent the combined effects of wind and insect exposure for each dataset. However, without hourly averages of relevant atmospheric conditions (i.e. wind direction, wind speed and atmospheric stability) it is not possible to separate these estimates into their component parts for wind exposure and insect exposure.

The exposure coefficients associated with the experiments of Simpson & Sweet (2002) for 80% male-sterile target plants are significantly different from each other and this may reflect, at least in part, the enhanced sensitivity to background pollen deposition at low competition. Furthermore, there are significant differences in the estimated exposure coefficients (table 1) for experiments with self-fertile targets, and this may be indicative of the low levels of bee activity, reported by Simpson & Sweet (2002). In the analysis, we have assumed that the largest value for the exposure coefficient is $\varepsilon_g = \varepsilon_t$. Therefore, when the source and target crops are grown side-by-side, this implies that the general direction of pollen transport is perpendicular to the boundary between the plots

Table 1. Summary of the estimates of fitted model parameters in equation (2.7) and derived exposure coefficients.
(These results are based on ANOVA output from the regression analysis tool in Microsoft Excel. The following deterministic model parameter values were used as the basis for this analysis: $\alpha = 1.0$, $b = 10$, $D^{+} = 1$, $D_{\mathrm{b}} = 0$, $\Delta x = 1$ m and $R(x,0) = 1$ in the region $-\Delta x/2 < x < \Delta x/2$ and $R(x,0) = 0$ elsewhere. It was assumed that the exposure coefficients were $\varepsilon_{\mathrm{g}_j} = \varepsilon_{\mathrm{t}} = 1$, and the estimate of $D_{\mathrm{s}} = 8D^{+}$ was obtained by iterative adjustments to D_{s} until the fitted model parameters for β_j obeyed the constraint for re-estimating the exposure coefficient (i.e. $\varepsilon_{\mathrm{g}_j} \cong \exp(\beta_j/2\beta_0) \leqslant \varepsilon_{\mathrm{t}}$). The adjusted coefficient of variation for this analysis is $r^2 = 0.94$.)

dataset description			β_j [a]		$\varepsilon_{\mathrm{g}_j}$ [b]	
j	plot id	observations	mean	s.e.	upper	lower
0	all	90	1.12	0.048	n.a.	n.a.
self-fertile targets (Simpson & Sweet 2002); model parameters $L = 92$ m, $B = 92$ m, $\eta = 0$						
1	1	13	−1.71	0.26	0.59	0.37
2	8	13	−2.26	0.26	0.46	0.29
80% male-sterile targets (Simpson & Sweet 2002); model parameters $L = 92$ m, $B = 92$ m, $\eta = 0.8$						
3	1	13	−1.36	0.17	0.64	0.47
4	8	13	−0.33	0.17	1.00	0.74
self-fertile targets (Scheffler *et al.* 1993); model parameters $L = 8$ m, $B = 8$ m, $\eta = 0$						
5	east half	20	−0.87	0.29	0.88	0.53
6	west half	18	−0.84	0.28	0.88	0.54

[a] These values are based on the outcrossing probability, expressed as a fraction. However, the model predictions plotted in figure 2 are expressed as percentages.
[b] These values are based on the 95% confidence limits for β_j.

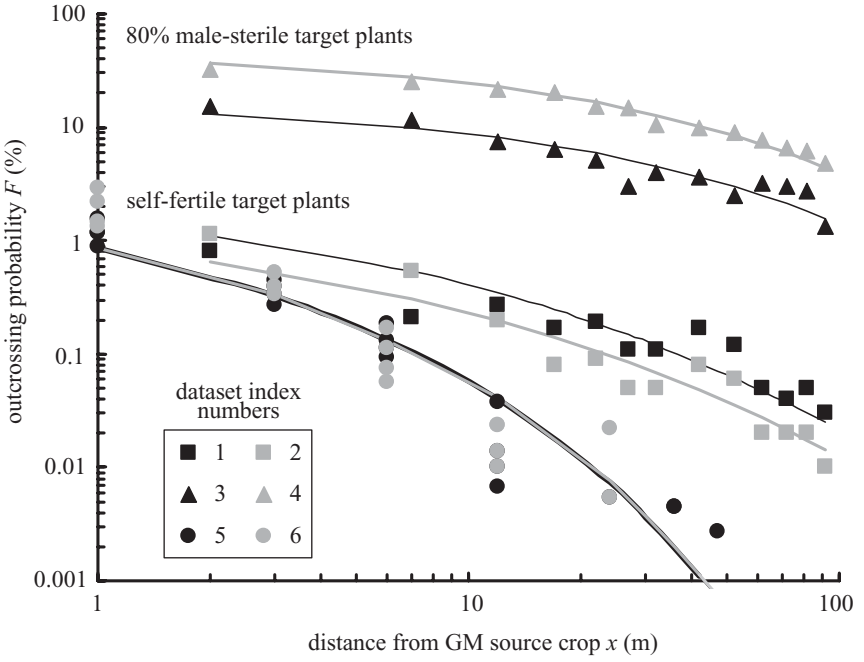

Figure 2. A comparison between measurements and predictions of outcrossing probability for different non-GM target crops after exposure to pollen from different-sized GM source crops. Triangles represent experimental data for a 0.8 ha GM source crop and an adjacent target crop containing 80% male-sterile non-GM plants (Simpson & Sweet 2002). Squares represent experimental data for a 0.8 ha GM source crop and an adjacent target crop containing non-GM self-fertile plants (Simpson & Sweet 2002). Circles represent experimental data for a 0.0064 ha GM source crop surrounded by a target crop containing non-GM self-fertile plants (Scheffler *et al.* 1993). Lines represent model predictions for the fitted parameters defined in table 1.

throughout the period of flowering. In practice, this scenario is unlikely for oilseed rape because of the high variability of wind direction and insect flight direction during the long flowering period.

The mean estimate of the common fitted parameter $\beta_0 = 1.12 \pm 0.096$ (± 95% confidence interval) is slightly greater than the value of unity that might be expected if all the modelling assumptions and the deterministic model parameters were fully optimized before the data-fitting process. Therefore, the key components of the model should be investigated further to obtain independent verification of the assumptions. In particular, the standard value for the wind dispersion parameter $\alpha = 1$ for atmospheric boundary layers may not be appropriate. Larger values of α are consistent with the effects of greater atmospheric instability and particle inertia (Hunt *et al.* 2002).

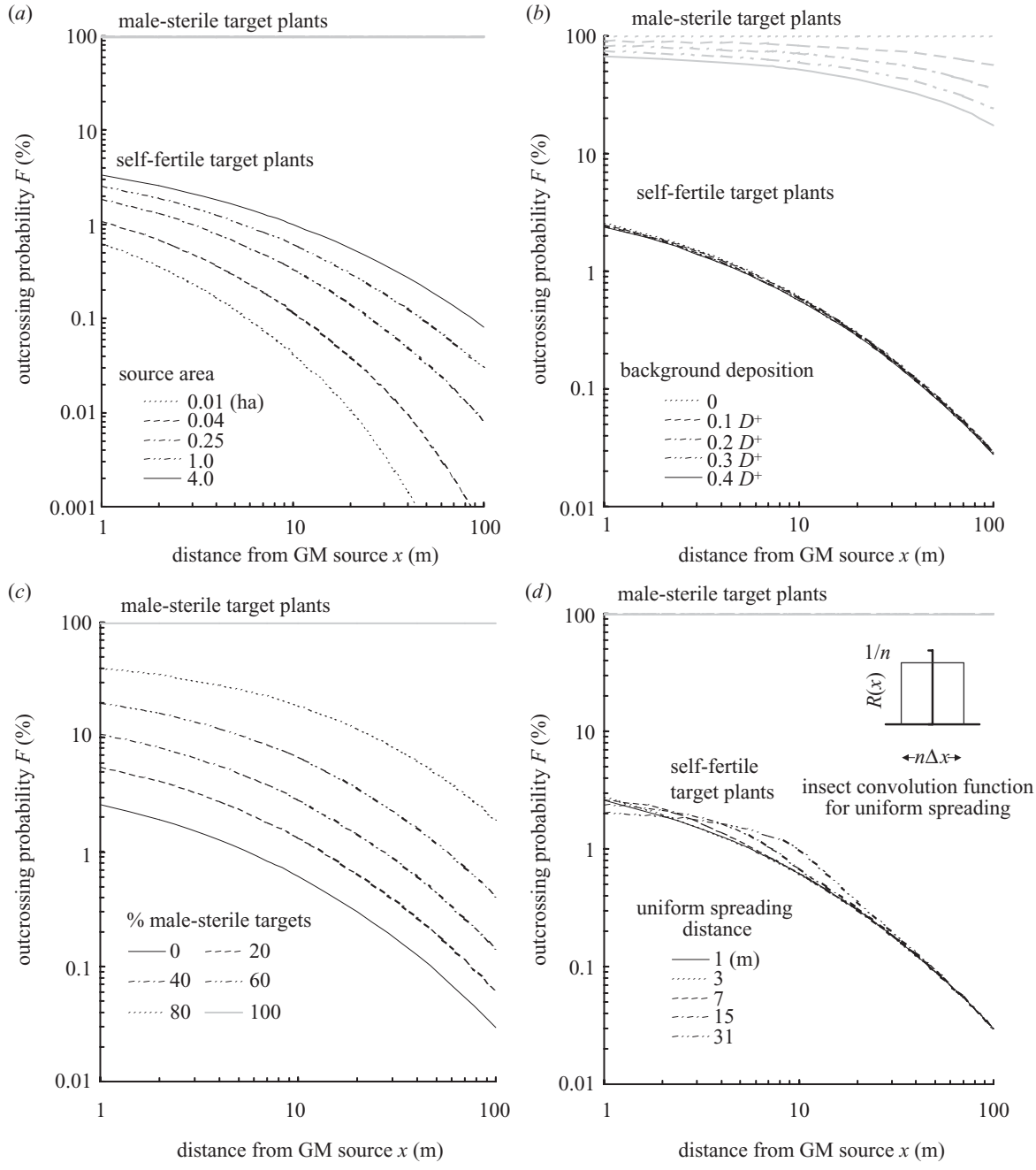

Figure 3. Spatial distributions of outcrossing probability predicted by the model of pollen-mediated gene flow for oilseed rape. Output from the model compares the scaling effects of (*a*) GM source size, (*b*) background deposition, (*c*) target crop fertility and (*d*) pollen transport distance associated with insect activity. The following default values were used for this comparison: 1.0 ha square GM source (i.e. $L = 100$ m and $B = 100$ m), zero background pollen deposition, two extremes of competition represented by self-fertile targets (high competition) and male-sterile targets (low competition), local plant-scale insect activity $\Delta x = 1$ m and exposure coefficients $\varepsilon_g = 0.5$ and $\varepsilon_t = 1$. (*a*) The scaling effects of GM source-crop area. (*b*) The scaling effects of background non-GM pollen deposition. (*c*) The scaling effects of target-crop fertility. (*d*) The scaling effects of insect activity modelled as a one-dimensional top-hat function with different widths (i.e. $n\Delta x = 1$, 3, 7, 15 and 31 m) to represent uniform spreading.

In addition, the spreading effect of insects at scales beyond the local plant scale could change the estimate of β_0. Some large-scale spreading effects of insects are investigated further in § 3b. However, a fully systematic investigation of these effects is beyond the limits we have set for the present study.

(**b**) *Deterministic characteristics of the model*

In this section we use the gene-flow model, represented by equation (2.7), to examine the sensitivity of the outcrossing frequency to the following parameters: area of the GM source crop L^2 (i.e. for square plots), background pollen deposition D_b, target-crop fertility η and insect transport distances represented by the convolution function $R(x)$. Figure 3 compares the predictions of outcrossing probability for the extremes of high and low pollen competition, represented by self-fertile and male-sterile target plants, respectively. For these predictions, we have assumed the same standard atmospheric conditions as in table 1 but we have adjusted the exposure coefficient to

give $\varepsilon_g = 0.5$. The default values for the dimensions of the source crop were $L = B = 100$ m.

The effect of changing the area of the GM source in the range $L^2 = 0.01$–4 ha with no background pollen deposition is plotted in figure 3*a*. This shows that gene flow to self-fertile target plants increases as the GM source area increases but decreases with increasing separation distance x. For male-sterile target plants, gene flow can be more than two orders of magnitude greater than the equivalent for self-fertile target plants and is insensitive to GM crop size and distance.

The effects of changing the background pollen deposition from the default value of $D_b = 0.0$ are plotted in figure 3*b*. This shows that the gene flow to self-fertile target plants is insensitive to large changes in background pollen deposition. However, male-sterile target plants are more sensitive, with gene flow increasing as background deposition decreases.

The gene-flow characteristics for intermediate values of target-crop fertility, represented by the range $0 \leqslant \eta \leqslant 1$ in incremental steps of $\Delta\eta = 0.2$, are plotted in figure 3*c*. These results show that gene flow is very sensitive to crop fertility throughout the range $0 \leqslant \eta \leqslant 1$. In addition, the shape of the distribution changes rapidly with changes in target-crop fertility at low competition (i.e. in the range $0.8 \leqslant \eta \leqslant 1.0$). For this reason, the current model is particularly useful for interpreting field experiments performed under low-competition conditions, when it may be necessary to extrapolate the results to the higher pollen-competition conditions associated with intensive oilseed rape production.

The consequences of pollen transport by insects with different spreading distances are plotted in figure 3*d*. This shows the predictions of gene flow when the effect of insect transport is represented as a uniform spreading of pollen in direction x over distances defined by $n\Delta x =$ 1, 3, 7, 15 and 31 m. We have ignored insect transport effects in the y direction because the gradient of mean pollen deposition is negligible on the centre-line. The results show that gene flow is insensitive to the uniform spreading effects of insects when male-sterile targets are used and when self-fertile targets are used at spreading distances below 7 m. Self-fertile targets do show some sensitivity to the effect of insects but only at spreading distances that are much greater than 7 m. Perhaps future observation of large-scale insect activity would therefore be better served by the use of self-fertile targets, if it is practical to increase the level of sampling to compensate for the reduced gene flow.

(c) *Simplifications of the model*

The model can be simplified by considering the asymptotic characteristics at the extremes of high and low competition if large-scale insect transport of pollen is ignored. High competition in this context means that there is a large enough number of self-fertile target plants (i.e. $\eta \rightarrow 0$) to ensure that the deposition of GM pollen is small compared with the deposition of non-GM pollen from intrinsic and extrinsic sources. For this condition, the outcrossing probability, given by equation (2.7), can be approximated as $F_{sf} \sim (\bar{D}_g/\bar{D}_s)^{2\beta_0}$. Furthermore, the GM pollen deposition distribution, given by equation (2.8), approximates to $\bar{D}_g \sim \varepsilon_g D^+(L/x + L)^{1+\alpha}$ when $x > L$. Therefore, the asymptotic distribution for outcrossing approximates to $F_{sf} \sim (\varepsilon_g D^+/\bar{D}_s)^{2\beta_0}(L/x + L)^{2\beta_0(1+\alpha)}$ when $x > L$. The measurements presented by Scheffler *et al.* (1993) for $x > 8$ m show good agreement with this asymptotic characteristic.

By contrast, very-low-competition asymptotic characteristics occur when there are low levels of background pollen deposition (i.e. $D_b \ll D_g$) and the target crop contains a high proportion of male-sterile plants (i.e. $\eta \rightarrow 1$) or when male-sterile target plants are used in isolation. For these conditions, the outcrossing probability, given by equation (2.7), approximates to $F_{ms} \sim 1$. Some of the experimental conditions reported by Ramsay *et al.* (1999) and Ramsay *et al.* (2003) may represent this low-competition condition where male-sterile target plants have been used in isolation and very high levels of gene flow have been observed at 5 km from the nearest GM source crop.

4. DISCUSSION

Current risk assessment practice is commonly based on the assumption that pollen-mediated gene flow can be adequately regulated by growing GM crops at a minimum isolation distance from non-GM neighbours. Furthermore, it is possible to estimate these isolation distances, for a range of different scenarios, by using the gene-flow model we have developed. These are given by using equation (2.7) to determine the distance at which the outcrossing probability distribution reaches a suitable threshold. Worst-case estimates of isolation distance are obtained by assuming the similarity pollen deposition distributions based on equations (2.8)–(2.12) and exposure coefficients of $\varepsilon_g = 1$, $\varepsilon_t = 1$.

In general, the isolation distance is well defined when the outcrossing probability distribution has a high gradient (e.g. target crops containing a high proportion of self-fertile target plants) and where the isolation distance is typically greater than the size of the GM source crop. For low pollen-competition conditions (i.e. target crops containing a high proportion of male-sterile target plants) the model shows that it is not practical to regulate gene flow by using rules based on isolation distance.

The model shows that the spatial distribution of the outcrossing probability is insensitive to large-distance transport of pollen by insects or wind under conditions of very low competition, which may be obtained with the use of male-sterile target plants and very low levels of background pollen. However, male-sterile target plants have been used to investigate cross-pollination at large distances from a GM pollen source to enhance the sensitivity of experimental monitoring of cross-pollination. Therefore, caution is necessary when interpreting these observations. The model we have developed could be used to improve the interpretation of these experiments. We have shown that the use of targets containing self-fertile plants would be better suited to observing the spatial effect of insects, if it is practical to increase the level of sampling to compensate for the reduced outcrossing.

Some previous studies have questioned the significance of atmospheric wind dispersion as a basis for predicting

the spatial distribution of pollen-mediated gene flow for oilseed rape and instead have favoured a linkage to direct observations of insect activity (Scheffler *et al.* 1993, 1995; Cresswell 1994; Ramsay *et al.* 1999). However, by appropriate scaling of atmospheric wind dispersal characteristics, we have shown that a good correlation between gene-flow predictions and field measurements can be obtained by modelling only short-distance insect transport. In this study, we have shown that the significant effect of insects can be limited to a range of typically below 1 m. We have also shown that the gene-flow characteristics appear to be insensitive to uniform spreading of pollen by insects at distances below 7 m. These findings are consistent with the results of a recent study by Cresswell *et al.* (2002) showing that bumble-bees produce a strong exponential decay of outcrossing with distance between source and target flowers in a sequence of up to 20 short flights. Therefore, as a first-order approximation, it seems reasonable to neglect the large-distance transport effects of insects for estimating the outcrossing characteristics of oilseed rape.

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