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RESEARCH ARTICLE

Spatial sensitivity of maize gene-flow to landscape pattern: a simulation approach

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Abstract Pollen dispersal is a critical process defining connectivity among plant populations. In the context of genetically modified (GM) crops in conventional agricultural systems, strategies based on spatial separation are promoted to reduce functional connectivity between GM and non-GM crop fields. Field experiments as well as simulation studies have stressed the dependence of maize gene flow on distances between source and receptor fields and on their spatial configuration. However, the influence of whole landscape patterns is still poorly understood. Spatially explicit models, such as MAPOD-maize, are thus useful tools to address this question. In this paper we developed a methodological approach to investigate the sensitivity of cross-pollination rates among GM and non-GM maize in a landscape simulated with MAPOD-maize. The influence of landscape pattern on model output was studied at the landscape and field scales, including interactions with other model inputs such as cultivar characteristics and wind conditions. At the landscape scale, maize configuration (proportion of and spatial arrangement in a given field pattern) was shown to be an important factor influencing cross-pollination rate between GM and non-GM maize whereas the effect of the field pattern itself was lower. At the field scale, distance to the nearest GM maize field was confirmed as a predominant factor explaining cross-pollination rate. The metrics describing the pattern of GM maize in the area surrounding selected non-GM maize fields appeared as pertinent complementary variables. In contrast, field geometry and field pattern resulted in little additional information at this scale.

Keywords Cross-pollination · Global sensitivity analysis · Landscape metrics · Multi-scale analysis · Pollen dispersal

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Introduction

Pollen movement is a critical process in the functional connectivity among plant populations in patchy landscapes (Sork et al. 1999). Connectivity here refers to gene flow between two populations. Whereas connectivity plays a fundamental role for the dynamics of natural population it is problematic in agriculture where the objective is to maintain the purity of cultivars. This concern is especially acute in Europe where an EU regulation (EC 1829/2003) stresses the necessity to ensure co-existence between genetically modified (GM) and non-genetically modified (non-GM) supply chains and establishes a 0.9% threshold for maximum admissible level of mixing GM material with conventional material. Maize is a major crop in Europe and is the only GM crop commercially grown there. Accurate prediction of maize gene flow is thus needed to compare coexistence scenarios of GM and non-GM maize under various production systems and landscape features.

Maize pollen is mainly dispersed by wind (Bateman 1947a), and the basic processes leading to connectivity between two fields are well understood. Numerous field experiments have shown that most pollen is transported over short distances (i.e. less than a few hundred meters), although longer dispersal distances (more than 500 m) may occur (Bateman 1947b; Paterniani and Stort 1974; Raynor et al. 1972). Additionally, the spatial arrangement of individuals and the sizes of donor and receptor populations have an influence on the probability of field-to-field cross-pollination (here defined as pollination of non-GM individuals by GM pollen). A meta-analysis on gene flow data for oilseed rape indicated that the width of the recipient field relative to the pollen-source field has a large effect on the proportion of GM pollen received by the recipient field (Damgaard and Kjellsson 2005). Recent simulation studies that used a fat-tailed power-law dispersal kernel between two fields of oilseed rape also stressed the influence of source width and recipient depth on cross-pollination rates (Klein et al. 2006).

Situations are more complex at the landscape scale with a large number of interacting fields and a diversity of spatial arrangements of fields. A detailed understanding of the impact of landscape patterns on cross-pollination among fields is thus required to define management strategies to protect the purity of varieties. However, few datasets are available to address this question yet. Simulation studies with spatially explicit gene flow models such as MAPOD-maize (Angevin et al. 2008; Lavigne et al. 2008) offer an alternative. Those gene flow models account for factors such as crop characteristics, agricultural techniques, climatic conditions and the spatial pattern of crop fields. Global sensitivity methods are necessary to take into account these different input variables and parameters as well as their variability (Saltelli et al. 2000). Applied to spatially explicit models, such methods can describe the sensitivity of model output to variation among landscapes (Crosetto et al. 2000), and determine which spatial variables have the most influence on certain predictions (Jager et al. 2005) or their interaction with other variables or parameters. Colbach et al. (2005) used such methodological framework to address the spatial sensitivity of an oilseed-rape gene flow model to landscape pattern, considering cross-pollination to a central non-GM maize field and using a limited number of field patterns (i.e. the spatial structure of fields). However, the critical point to fully capture the relationship between landscape pattern and cross-pollination is to be able to generate a set of landscapes reflecting the diversity found in agricultural areas where GM maize is or may be cultivated. This landscape diversity arises from field patterns and from the spatial arrangement of GM and non-GM crops within the field pattern.

The aim of this study was to develop a methodology to assess the sensitivity of the gene flow model MAPOD-maize to landscape characteristics. Landscape pattern, here defined as the combination of field pattern and spatial arrangement of GM and non-GM crops in the field pattern, was incorporated into the sensitivity analysis as an explicit input factor. Its influence on model output at the landscape and the field scale was investigated, as were its interactions with other model inputs such as cultivar characteristics and wind conditions. In addition, the relative importance of metrics describing the local environment of individual non-GM maize fields was investigated.

Material and methods

MAPOD-maize model

MAPOD is a spatially explicit model simulating cross-pollination in agricultural landscapes on a daily time-step with a quasi-mechanistic approach (Angevin et al. 2008). During the flowering period pollen is dispersed from each field using an individual Normal Inverse Gaussian dispersal function (Klein et al. 2003). The model uses various types of input data: (1) a landscape pattern delineating the agricultural fields in vector format and transformed into a regular square grid of 'GM maize', 'non-GM maize' or 'non-maize' pixels; (2) daily climate data such as wind data; (3) agricultural practices on each field such as sowing date and density; (4) cultivar characteristics such as the quantity of pollen per plant, the precocity in growing degree days, the heterozygosity of GM cultivars, or the difference in height between male flowers and female flowers of maize. The main output results are the expected proportion of total seeds that are hybrid at harvest (cross-pollination rate) for each non-GM pixel.

Main features considered

A set of contrasting landscapes was generated using real-world field patterns. They differed in field shapes and sizes, GM and non-GM maize areas and crop spatial arrangement. The characteristics of the pollen dispersal curve were studied indirectly through the difference in height between male flowers and female flowers of maize, which influences the distances of GM pollen dispersal and the probability of its reception by non-GM individuals (Klein et al. 2003). Wind speed and direction were also considered. Flowering dynamics were not varied. Consequently, we simulated the maximum risk of cross-pollination, since GM and non-GM cultivars were assumed to flower simultaneously (Romary 2005).

Factorial simulation design

A complete factorial design (Campolongo and Saltelli 2000) was built with the input factors described below, requiring 5760 simulation runs.

Landscape factors

Field pattern (Map) Six 1500 × 1500 m contrasting real-world field patterns located in France were chosen. They differed in the configuration of field size, shape and arrangement (Table 1).

Maize area (Maiz) and GM-maize area (GM) Total maize area was set to either 70% or 20% of the agricultural land, simulating French production areas, where maize is or is not a major crop. GM maize area was set to either 10% or 50% of the total maize area, simulating low or high acceptance of GM maize by farmers.

Maize spatial arrangement (Aggr) In some agricultural areas, maize fields are clustered due to soil constraints, water availability for irrigation or farm organization. Farmers may either collectively decide to isolate GM from non-GM maize fields by allocating them to different areas or to grow GM crops on an individual basis. Consequently, three spatial arrangements of maize fields were considered: (1) random distribution of non-GM and GM maize fields; (2) aggregated distribution of maize with non-GM and GM maize fields in different areas; (3) aggregated distribution of maize with non-GM and GM maize fields mixed within aggregates (Fig. 1).

The four factors *Map*, *Maiz*, *GM* and *Aggr* resulted in 72 (6 × 2 × 2 × 3) combinations. Ten allocations of GM and non-GM maize to fields were randomly generated for each combination under the constraints induced by the factors *Maiz* (±5%), *GM* (±5%), and *Aggr*. One allocation among the ten was selected after visual inspection.

Grid cell size (Step)

Two grid cell sizes were considered: field patterns were divided into either 5 × 5 m or 10 × 10 m regular square grids.

Ratio between GM and non-GM pollen production per unit area (Poll)

The ratio between the quantities of pollen produced by GM and non-GM cultivars per unit area was set to either 0.33 or 3.75. These values represent the large range of possible ratios when considering the range of sowing densities and pollen productivity in France, according to experts consulted at GEVES (Groupe d'Etude et de Contrôle des Variétés et des Semences: French group for the study and inspection of varieties and seeds) and other technical institutes.

Table 1 Main geometrical characteristics and maps of the six field patterns

Field patterns	A1	O1	P1	S1	S4	T1
Number of fields	100	124	175	93	62	180
Mean field area (ha)	2.1	1.7	1.1	2.3	3.5	1.1
Mean field perimeter (m)	610	640	450	660	780	430
Mean field elongation[a]	3.07	6.57	2.47	3.34	2.55	3.95
Field orientation (% fields)						
East–West	27%	56%	30%	35%	32%	38%
North–South	56%	19%	50%	56%	49%	36%
No orientation	17%	25%	20%	9%	19%	26%
Maps[b]						

[a] Elongation equals the ratio between the largest and shortest widths of a field

[b] Grey polygons indicate agricultural fields whereas white polygons indicate non-agricultural areas (roads or built areas)

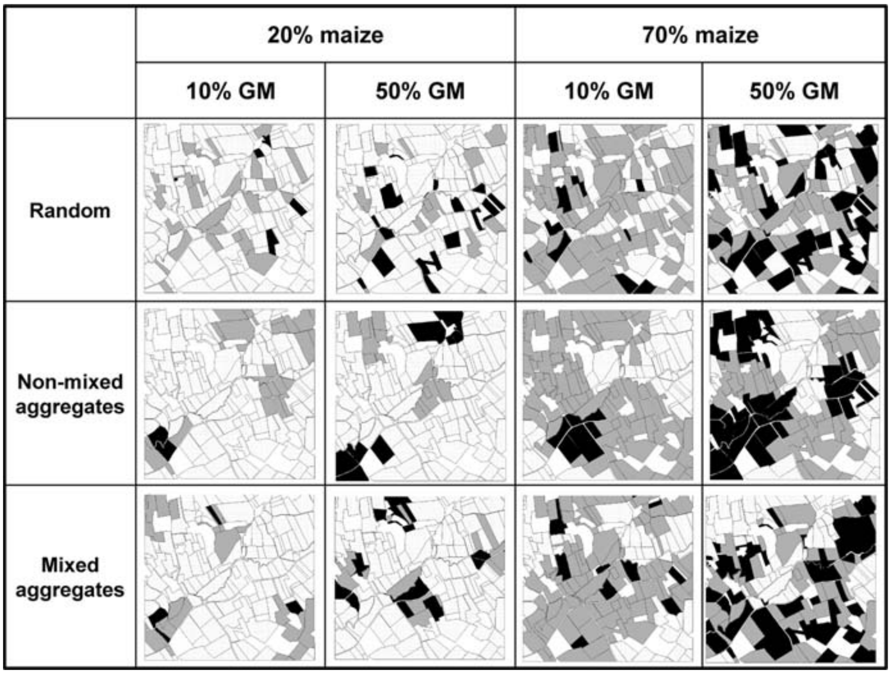

Fig. 1 Maize spatial organization simulated on field map P1. Gray fields indicate non-GM maize, black fields indicate GM maize, and non-shaded fields indicate non-maize land uses

Difference in height between male flowers and female flowers of maize (Heig)

According to measurements of 30 cultivars, the difference in height between male flowers and female flowers of maize varies between 0.83 and 1.52 m. Two levels were considered: 0.8 m and 1.75 m, assumed to be equal for GM and non-GM maize (GEVES, pers. comm.)

Wind direction (Wdir) and speed (Wspe)

Ten different conditions during pollen dispersal were studied, combining wind speed and direction: (1) no wind, implying isotropic dispersion; (2) (3) (4) (5) a 4 ms^{-1} wind uniformly blowing from each on the four cardinal points (North, East, South, West); (6) a 4 ms^{-1} wind blowing from each of the four cardinal points for equal proportions on each day; (7) (8) (9) (10) a 14 ms^{-1} wind uniformly blowing from each of the four cardinal points. The latter correspond to an extreme wind speed.

MAPOD-maize simulations and response variables

MAPOD output was integrated into response variables at two different scales. At the landscape scale, the response variables of interest were the average cross-pollination rate of all non-GM maize cells by GM pollen and the proportion of non-GM maize fields whose average cross-pollination rate by GM pollen was higher than 0.9% (EU threshold) or 0.1% (detection threshold). At the field scale, the response variables of interest were the average GM cross-pollination rates of each non-GM field and a binary response y_x indicating whether average cross-pollination rate y was higher than a targeted threshold x% or not: $y_x = 1$ if $y > x$, $y_x = 0$ if $y \leq x$. We focused on two values of y_x, y_{01} and y_{09}, corresponding to the thresholds 0.1% and 0.9% respectively.

Additionally, geometry and environment of non-GM maize fields were described for each simulation. Area (*Area*), perimeter (*Peri*), main orientation (*Orie*) and elongation (*Elon*) of the non-GM maize fields were calculated. The main orientation was defined as a categorical variable with three modalities: "no preferential orientation"; "North–South"; "East–West". Elongation was defined as the ratio between the largest and shortest widths of a field.

The environment of non-GM maize fields with respect to pollen sources was characterized with two metrics: the surrounding surface area occupied by GM or non-GM maize and the distances to the nearest GM or non-GM maize fields.

The surface areas of GM and non-GM maize within 20, 50 and 100 m-wide buffers around each non-GM maize field, were calculated. The GM and non-GM maize areas were also calculated within 20, 50 and 100 m-wide "orientated buffers" restricted to locations within ±45° of lines extending from each pixel in the field towards the origin of the dominant wind (Fig. S1). Note that in simulations with no dominant wind direction, orientated buffers were the same as non-orientated ones.

Several metrics were then calculated from these measures. Let be $x_1 = 20$, $x_2 = 50$ and $x_3 = 100$, let bx_0 denote the area of the non-GM maize field F, and for i = 1, 2 or 3 let bx_i denote the total area of F plus its x_i-meter-wide buffer. In addition let $bx_i.gm$ and $bx_i.ngm$ denote the corresponding sub-areas occupied by GM and non-GM maize respectively (so that $bx_0.gm = 0$ and $bx_0.ngm = bx_0$), and let obx_i, $obx_i.gm$ and $obx_i.ngm$ denote the same quantities for the orientated buffers. The following quantities were calculated:

(1) buffer area proportion of GM and non-GM maize

$$pbx_i.gm = \frac{bx_i.gm - bx_{i-1}.gm}{bx_i - bx_{i-1}} \quad \text{and}$$
$$pbx_i.ngm = \frac{bx_i.ngm - bx_{i-1}.ngm}{bx_i - bx_{i-1}},$$

(2) GM maize ratio within buffers

$$rx_i.gm = \frac{bx_i.gm}{bx_i.gm + bx_i.ngm},$$

(3) GM maize ratio within orientated buffers

$$orx_i.gm = \frac{obx_i.gm}{obx_i.gm + obx_i.ngm},$$

where $rx_i.gm$ and $orx_i.gm$ are more synthetic measures than $pbx_i.gm$ since they give the proportions of GM area versus total maize area within x_i meters from field F, including F.

Four different euclidean metrics were used to describe the distance to the nearest GM and non-GM maize fields: edge-to-edge distance (*de.gm*), centroid-to-centroid distance (*dc.gm*), edge-to-edge "orientated" distance (*ode.gm*), and centroid-to-centroid "orientated" distance (*odc.gm*). Like for the buffers, orientated distance was defined as the distance to the nearest field in the direction of the dominant wind ±45°. The same variables were calculated for the distance to the nearest non-GM maize field (*de.ngm*, *dc.ngm*, *ode.ngm*, *odc.ngm*).

Statistical analyses

To avoid edge effects only the fields with more than 50% of their area further than 100 m from the map edge were kept in the statistical analyses, since the non-GM maize fields located on the borders of the field map were expected to receive less pollen than more central fields. The sizes of the non-GM maize fields kept in the statistical analyses ranged from 0.25 to 11.70 ha. Grid cell size appeared to have negligible effects on simulated cross-pollination rates. Hence, analyses were restricted to the 2880 simulations performed with the smaller cell size (5 × 5 m).

Landscape scale

The objectives were to assess the global range of cross-pollination rates across simulated situations and to evaluate their sensitivity to the main input factors. Thus, graphical representations and analyses of variance (ANOVA) were performed on three response variables: log-average cross-pollination rate of non-GM maize fields and proportions of non-GM maize fields with a cross-pollination rate higher than either 0.1% or 0.9%. Before log-transformation, the lowest average cross-pollination rates were truncated at the minimum value 4.53×10^{-5}. The ANOVA model combined four fixed synthetic input factors: field pattern (*Map,* 6 modalities), maize pattern (combinations of *Maiz*, *GM*, *Aggr*, 12 modalities), wind condition (combinations of *Wdir*, *Wspe*, 10 modalities), and cultivar traits (combinations of *Poll*, *Heig*, 4 modalities). A sensitivity index of each factorial term was calculated by dividing its sum of squares by the total sum of squares (Monod et al. 2006).

Field scale

The objectives were to assess the sensitivity of the field-average cross-pollination rate to local field characteristics (geometry and maize environment) and its possible dependence on landscape features (*Map*, *Maiz*, *Gm*, *Aggr*), wind (*Wdir*, *Wspe*) and cultivar traits (*Heig*, *Poll*). Thus, we first ranked field characteristics and input factors with respect to their influence on the risk that a non-GM field would exceed the 0.1% or 0.9% thresholds. This was done by random forest analyses on both y_{01} and y_{09}, using the variables for field geometry and environment and the design factors as potential predictors. Random forest is a data mining method based on classification and regression trees (Breiman 2001). It is well suited to the analysis of complex ecological data (De'ath and Fabricius 2000). Random forests were grown on the basis of 1000 bootstrap samples using the R randomForest package (Breiman and Cutler 2003). Interpretation was based on the Gini importance criterion, which measures the decrease in a node's impurity every time the variable is used for splitting (Breiman 2001).

The second step aimed to identify and compare parsimonious logistic regression models that were efficient for predicting the average risk that the cross-pollination calculated by MAPOD would exceed a given threshold. The candidate predictor variables were the field characteristics not discarded in random forest analysis. Model selections were performed separately at the levels of the input factors that had been ranked as highly influential in the random forest procedure. In principle, stepwise selections were performed with the Akaïke information criterion (AIC). In practice, the models minimizing AIC contained highly correlated predictor variables and their parameter estimates were hardly interpretable because of confusion effects. Consequently we further limited the size of the selected models.

In a third step, the classification performances of the logistic models and their prediction accuracy were assessed by Receiver Operating Characteristics (ROC) analyses, and interactions with the field map and input factors were introduced in the models to check how robust they were with respect to landscape features. Recall that the output of the logistic models is an estimated probability P that cross-pollination would exceed the targeted threshold x (0.1% or 0.9%). When associated with a decision threshold (t_d), this output can be used to classify the fields into two classes: if $P > t_d$, the field is classified as "positive"; otherwise, i.e. $P \leq t_d$, the field is classified as "negative". These predicted classes based on the output of the logistic model can then be compared to the true classes as simulated by MAPOD using several criteria. The sensitivity or true positive rate (TP) is defined as the number of fields with $P > t_d$ and $y > x$ divided by the total number of fields with $y > x$, while the specificity or true negative rate (TN) is defined as the number of fields with $P \leq t_d$ and $y \leq x$ divided by the total number of fields with $y \leq x$. The ROC curve is obtained by plotting sensitivity against the false positive rate (1-specificity) when the decision threshold t_d is varied from 0 to 1. The accuracy of the model is measured by the area under the ROC curve (AUC), which is equal to 0.5 for a non-informative model leading to random decisions and to 1.0 for a perfect model (Swets 1988; Makowski 2005).

Results

Landscape scale

The factorial simulation design ensured a large diversity of simulated landscapes with regard to the geometry and environment of non-GM maize fields (Table S1) and resulted in a large range of simulated cross-pollination rates over these landscapes (Fig. S2). The average rate was null in 16 out of the 2880 scenarios due to the specific locations of non-GM and GM maize fields and wind direction in two field maps (maize fields were few, located near the map border, and GM-maize fields were located leeward of the non-GM ones, so that GM pollen was mainly dispersed in the opposite direction). In 150 scenarios, all non-GM maize fields in the map had cross-pollination rates higher than 0.9%. GM-maize proportion was high (50%) in all those cases, and the ratio between GM and non-GM pollen production was also at the higher level (3.75) in 140 of the 150 scenarios. Similarly, all non-GM maize fields had cross-pollination rates higher than 0.1% in 402 scenarios, and GM proportion within maize was high (50%) in most of these scenarios.

Almost all variability at the landscape scale was explained by an ANOVA model including all main effects, all two-factor interactions and two three-factor interactions (Table 2; $R^2 = 0.99$, 0.96, and 0.94 for the three response variables). As expected from the variability of the input factors, cultivar traits and maize pattern were the most influential factors. The ranking of factors was similar for all three response variables; however the relative influence of

Table 2 ANOVA type I sums of squares (fixed effects) and sensitivity indices (SI) at the landscape scale, associated with main effects and some interactions of field pattern (*Map*), maize pattern (*Maiz*, *Gm*, *Aggr*), wind (*Wdir*, *Wspe*), and cultivar traits (*Heig*, *Poll*)

Factorial effects	Degrees of freedom	Log average outcrossing rate ($R^2 = 0.99$)		Proportion of non-GM fields >0.009 ($R^2 = 0.94$)		Proportion of non-GM fields >0.001 ($R^2 = 0.96$)	
		Sum of Squares	SI (%)	Sum of Squares	SI (%)	Sum of Squares	SI (%)
Maize pattern	11	7591.7	34.5	313.9	55.6	365.3	62.4
Cultivar	3	6455.5	29.4	100.1	17.7	31.5	5.4
Wind	9	1053.9	4.8	15.1	2.7	25.5	4.4
Map	5	158.9	0.7	2.0	0.4	1.7	0.3
Map × Maize pattern	55	2820.0	12.8	18.4	3.3	29.9	5.1
Maize pattern × Wind	99	309.7	1.4	12.0	2.1	17.4	3.0
Map × Wind	45	261.4	1.2	5.9	1.0	9.6	1.6
Cultivar × Wind	27	35.0	0.2	5.1	0.9	4.2	0.7
Maize pattern × Cultivar	33	23.2	0.1	17.2	3.1	6.1	1.0
Map × Cultivar	15	7.9	0.0	0.3	0.1	0.3	0.1
Map × Maize pattern × Wind	495	2965.1	13.5	36.3	6.4	67.4	11.5
Map × Maize pattern × Cultivar	165	75.3	0.3	4.2	0.7	2.7	0.5

SI equals the sum of squares divided by the total sum of squares

maize pattern was much larger for the proportion of non-GM fields with cross-pollination over 0.9% and even more for those with cross-pollination over 0.1% than for the log average rate. On the contrary, cultivar traits had much less influence on the proportions of non-GM maize fields over a threshold than on the log-average cross-pollination rate. Effects of maize pattern were mainly due to the effects of *Maiz*, *Gm*, and *Aggr*. The influence of field pattern was weak, but field pattern was involved in non-negligible interactions with maize pattern and wind factors. Overall, the sensitivity indices including field pattern summed to over 0.28, 0.12, and 0.19 for log-average cross-pollination rate, proportion of non-GM maize fields with cross-pollination over 0.9% and proportion of non-GM maize fields with cross-pollination over 0.1% respectively.

Field scale

The cross-pollination rate simulated with MAPOD was null in 32% (21081 fields) of the non-GM maize fields. The median cross-pollination rate was 0.003, the mean was 0.047 and the maximum was 0.97. Cross-pollination rate was higher than 0.1% in 57% (38123 fields) of the non-GM maize fields and higher than 0.9% in 40% of them.

Importance ranking of input factors and field characteristics

According to the random forest analyses (Fig. 2), the minimum distance to a GM maize field and the GM maize ratios within buffers were the most important field characteristics to predict the risk of exceeding the 0.9% and 0.1% thresholds of cross-pollination. The orientated versions of these field characteristics had more influence, showing the importance of wind direction. Additionally, edge-to-edge distances were more relevant than centroid-to-centroid distances. A second group consisted of the factors in the factorial design, in particular *Poll,* but also *GM*, *Wspe*, *Wdir* and *Heig*. In contrast, the distances to non-GM maize fields and the variables describing the non-GM environment of the fields and field geometry had low importance. The rank of the top splitting variables was similar for both dependent variables. As expected, *Poll* had a higher Gini importance index for y_{09} than for y_{01}: only 15% of the fields had a cross-pollination rate above 0.9% and, among them, 89% occurred in a scenario with the quantity of pollen produced by GM maize at its highest level. The minimum distance to a GM maize field was more important than orientated ratios for y_{01}, but not for y_{09}.

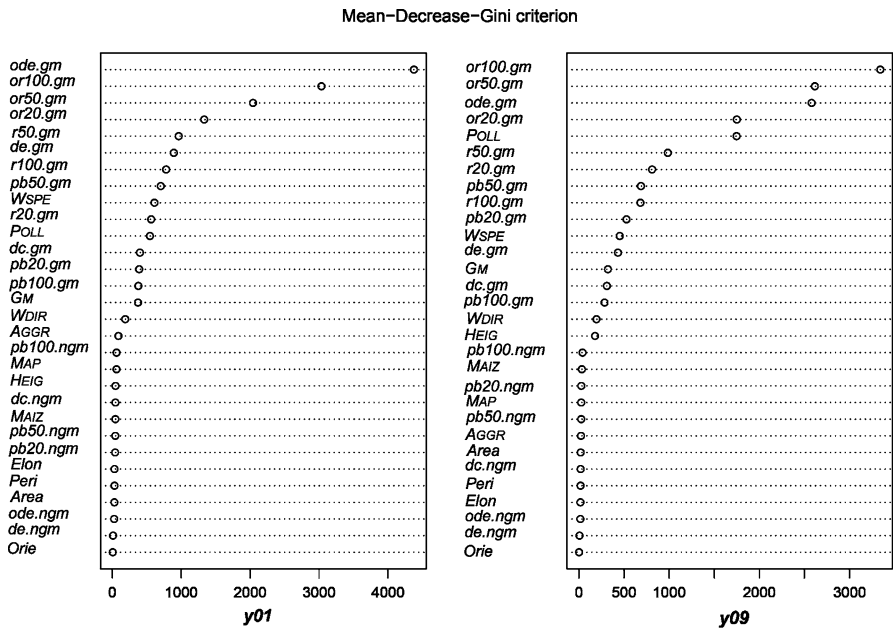

Fig. 2 Random forest importance rankings for the dependent variables y_{09} and y_{01}. Predictor variables are ranked according to the mean decrease in Gini index

Interactions between the main input factors and field characteristics

Above, GM orientated factors and the distance to the nearest GM maize field were identified as the most important variables to predict cross-pollination. Their interactions with *Poll* and *Wspe* were investigated graphically through conditioning plots (Venables and Ripley 2002). The relationship between *or*100.*gm* and the cross-pollination rate was approximately linear, with a slope highly dependent on the levels of *Poll* and *Wspe* (Fig. 3). For a given ratio of GM maize inside the 100 m-wide orientated buffer, the cross-pollination rate was higher and much more variable when the ratio between GM and non-GM pollen production was high, and it increased with wind speed. Similar effects were observed for the ratios in the 20 and 50 m-wide buffers. Similar but smaller effects were also observed for interactions with *Heig*.

The relationship between *or*100.*gm* and the cross-pollination rate was much less dependent on the levels of *Map* and *Wdir* (Fig. S3). However the slope was systematically larger with unidirectional wind than with wind blowing uniformly from all four cardinal points. In addition, the slopes for an O1 field pattern were greater between north or south winds and east or west winds. Note that O1 is made of long narrow fields, elongated in the east-west direction (Table 1).

Logistic regression models to predict the risk of exceeding cross-pollination thresholds

Logistic regression was applied to the binary variables y_{01} and y_{09} as dependent variables and field characteristics as predictors. Based on the random-forest results, the field characteristics *pbxi.ngm* were discarded, and the orientated (*orxi.ngm*) rather than the non-orientated GM maize ratios within buffers were used. To get a better view of model behaviour, analyses were performed separately for different wind speeds (0, 4 or 14 ms^{-1}) and two levels pollen ratio. Thus, twelve stepwise regressions (2 dependent variables × 3 wind speeds × 2 pollen ratios) were performed, starting from the null model with no intercept and limited to three predictors for simplicity and parsimony (Table 3). In all twelve cases, edge-to-edge orientated distance (*ode.gm*) and one of the (*orxi.ngm*) characteristics were the first two selected predictors. Next came either a geometry field descriptor (*Area* or *Peri*) or another distance ratio. The best models according to AIC included some additional predictors, but most of the deviance reduction was obtained by the first three predictors. The deviance reduction was much larger at null wind

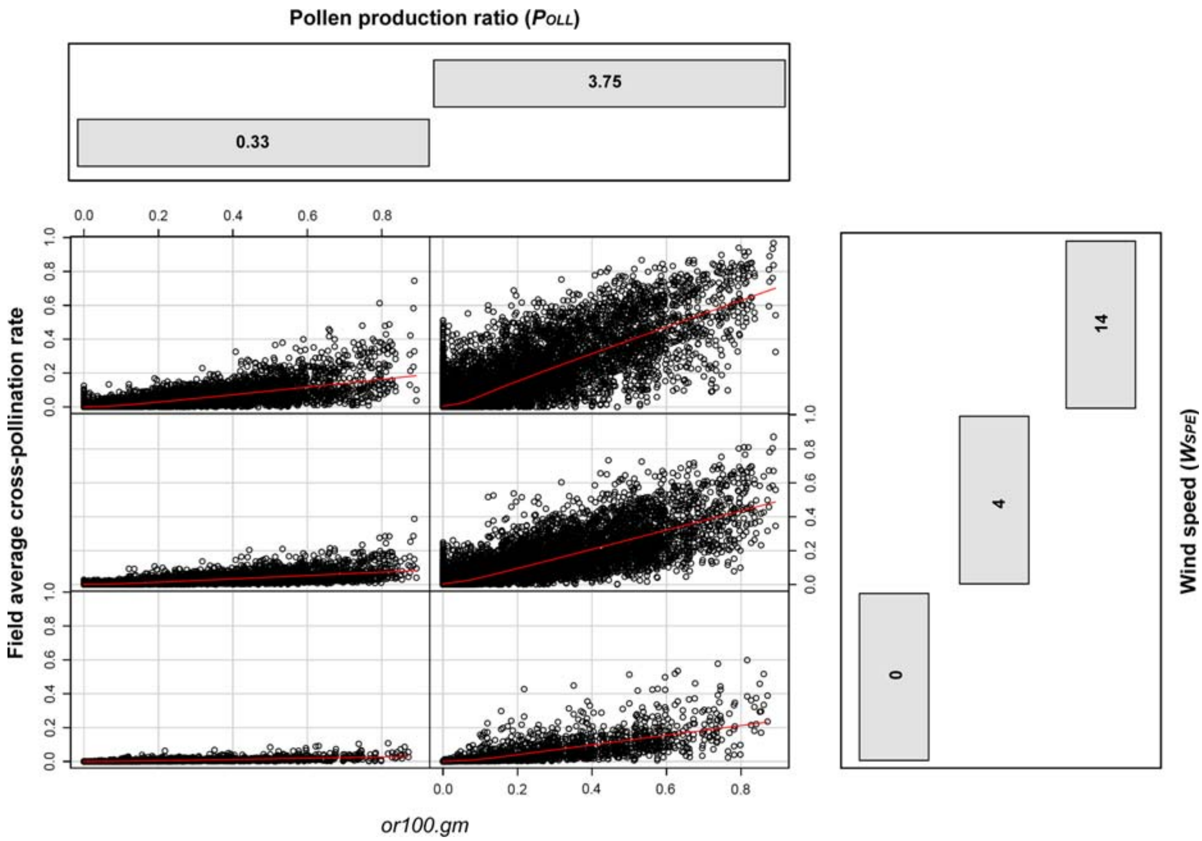

Fig. 3 Cross-pollination rate *versus* 100 m-wide orientated GM maize ratio as a function of pollen production ratio *Poll* (0.33 or 3.75) and wind speed *Wspe* (0, 4 or 14 ms^{-1})

speeds (more than 85%) than at the non-null wind speeds (less than 70%) (Fig. 4). As a consequence, the ability to predict the risk for a field to exceed a threshold was higher when wind speed was null. As distance to the closest GM maize field increased, the risk of exceeding a threshold decreased more slowly as wind speed or GM pollen ratio increased.

Interactions between local field characteristics and global landscape features

Models including interactions between local predictors and the landscape pattern input factors were fitted to evaluate whether global landscape features still had influence after accounting for the local field environment in the previously selected logistic models. *Gm* was the input factor showing the largest interactions with the local field characteristics (results not shown). However, ROC analyses showed that including these interactions helps to predict the risk of exceeding cross-pollination thresholds only marginally (Fig. S4).

Discussion

Methodology

Sensitivity analyses are essential complementary tools to extract information from models such as MAPOD. In this study, we investigated the influence of landscape pattern and field environment on cross-pollination in co-existing GM and non-GM maize crops. Formalized sensitivity analyses procedures are not commonly used to test for model sensitivity to landscape pattern (Delgado and Sendra 2004). However, similar approaches are used in spatial uncertainty analyses in order to assess the range of

Table 3 Results at the field scale. Estimated parameter values for logistic regressions using y_{01} and y_{09} as dependent variables as a function of pollen ratio (*Poll*) and wind speed (*Wspe*). Models are restricted to the best three predictors among the field characteristics

Pollen ratio	Dependent variable	Wind speed 0 ms^{-1} (3248 fields, null deviance = 4502.7)			Wind speed 4 ms^{-1} (16240 fields, null deviance = 22513.4)			Wind speed 14 ms^{-1} (12992 fields, null deviance = 18010.7)		
		Factor	Estimate	*z*-value	Factor	Estimate	*z*-value	Factor	Estimate	*z*-value
0.33	y_{01}	*ode.gm*	0.092	11.3	*ode.gm*	0.0046	53.6	*ode.gm*	0.0048	37.5
		or20.gm	−35.4	−5.2	*or20.gm*	−76.7	−11.1	*or100.gm*	−11.3	−26.5
		or50.gm	−17.1	−7.0	*or100.gm*	−12.5	−23.9	*Peri*	−0.00060	−11.4
		Residual deviance		646.3	Residual deviance		10966.8	Residual deviance		10431.7
	y_{09}	*ode.gm*	0.41	13.3	*ode.gm*	0.017	25.7	*ode.gm*	0.0069	32.1
		or20.gm	−28.5	−16.5	*or20.gm*	−32.0	−33.0	*or100.gm*	−9.31	−33.8
		Area	0.00011	2.0	*Peri*	0.0010	18.5	*Peri*	0.00084	14.7
		Residual deviance		744.8	Residual deviance		7096.7	Residual deviance		7921.1
3.75	y_{01}	*ode.gm*	0.019	17.8	*ode.gm*	0.0046	38.5	*ode.gm*	0.0047	37.5
		or20.gm	−365.0	−2.1	*or100.gm*	−30.5	−18.6	*or100.gm*	−16.4	−21.0
		or100.gm	−49.8	−12.8	*Peri*	−0.0017	−29.5	*Peri*	−0.0013	−22.3
		Residual deviance		586.4	Residual deviance		11754.9	Residual deviance		10190.4
	y_{09}	*ode.gm*	0.044	8.5	*ode.gm*	0.0038	50.4	*ode.gm*	0.0046	37.0
		or50.gm	−45.8	14.4	*or20.gm*	−85.6	−10.2	*or100.gm*	−11.6	−26.0
		Peri	0.0017	7.0	*or100.gm*	−14.7	−24.4	*Peri*	−0.00067	−12.8
		Residual deviance		599.5	Residual deviance		11662.4	Residual deviance		10611.2

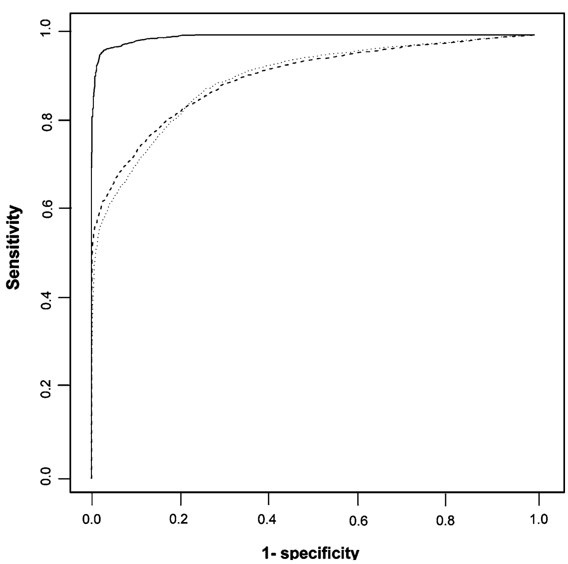

Fig. 4 Receiver-operating characteristics (ROC) curves for the logistic model including *ore.gm+or100.gm+peri* fitted on the dependent variable y_{01}, when GM pollen ratio equals 3.75. Null wind speed (AUC = 0.995) (black line); wind speed 4 ms^{-1} (AUC = 0.902) (broken line); wind speed 14 ms^{-1} (AUC = 0.899) (dotted line)

model predictions generated from spatial uncertainty in landscape data. The methodology described in this paper offers a way to derive operational rules of landscape management from simulations with spatially explicit ecological models. However, sensitivity analyses describe the behaviour of a model and not the behaviour of the real system under study. Hence, caution must be exerted when interpreting results.

The use of global sensitivity analysis methods was justified by the complexity of the MAPOD model and by the numerous potential interactions of input data with landscape-pattern and field-environment variables. Exploring the effect of landscape organization on cross-pollination rate within the known range of variability of other input variables and parameters is indeed relevant since the effects of landscape characteristics were shown to depend on wind conditions and cultivar traits.

With respect to the global-sensitivity principle, this study is innovative in two important aspects for models with landscape features as input data. First, analysis was based on realistic landscapes designed to represent the diversity of landscape patterns where

GM maize is cultivated in France and other European regions. We used real-world field patterns with contrasting field size, shape, and allocations of GM and non-GM maize. Second, several metrics were compared that had been identified in previous two-field studies (Klein et al. 2006; Kuparinen et al. 2007).

The sensitivity analyses were based on the combined use of classical statistical methods (factorial design and ANOVA) dedicated to analysis of variability and of data-mining methods (random forest, ROC curve analysis). The analyses were associated with a complete factorial simulation design, which was simple to implement, facilitated the interpretation at the landscape scale, and made it possible to perform analyses at the field scale as a function of the most influential factors at the landscape scale.

Sensitivity of cross-pollination to landscape pattern and field environment

Contrasting maize patterns and cultivar traits were used in the simulations and identified as the most influential factors at the landscape scale. Maize pattern had a particularly strong influence on the number of fields that were cross-pollinated by GM pollen and on the average risk for a field to exceed the 0.1% or 0.9% thresholds of cross-pollination. This result confirmed the results of other simulations on maize (Messéan et al. 2006) and on oilseed rape (Ceddia et al. 2007). Beyond these major effects, additional factors such as wind speed and direction or overall field pattern were shown to have a large impact on cross-pollination at the landscape scale, and these impacts depended on the maize and GM maize proportions at the landscape scale as well as on their arrangement.

Among the metrics describing landscape patterns in the local environment of non-GM maize fields, distance to the nearest GM maize field was confirmed as a major variable to predict cross-pollination rate. The ratio of GM maize versus total maize within a given distance from the target field appeared as a pertinent complementary variable. It can be considered as a synthetic measure of the field environment and the field geometry, as it includes field area in its denominator and, indirectly, the field perimeter (with GM maize area) in the buffer surrounding the target field. The protective effect of a non-GM maize environment around these fields appeared to be small compared with the effect of the other factors.

In our simulations, the geometry of the non-GM maize fields was shown to play a weak role in predicting the risk of cross-pollination, first, indirectly through the GM-maize ratio variables, second, directly through the perimeter variable. However, effects of elongation, size and perimeter of the non-GM crop fields were weaker than those in other simulation studies (Klein et al. 2006; Kuparinen et al. 2007). This is at least partly due to the focus of other studies on a single emitting GM crop fields and single recipient non-GM crop fields. We assume therefore that, when upscaling from two fields to a landscape, the effect of field geometry becomes less crucial because of the spatial interactions among fields and because of other factors varying at the landscape scale. Field geometry, for example, had only a small effect as compared to that of the cropping system in a simulation model for oilseed rape (Colbach et al. 2005).

For given crop-cultivar properties, local field characteristics provided efficient predictors of the risk for a non-GM maize field to exceed a cross-pollination threshold when wind speed was null, but they were less efficient when non-null wind speed was introduced. Our results indicate that the extent to which a neighbouring area influences cross-pollination rate depends greatly on wind conditions. This suggests that the definition of separation distances between GM and non-GM crop fields should be adapted to the local climatic context. The effect of wind conditions on field-to-field cross-pollination also has been stressed by Hoyle and Cresswell (2007). However, further work is needed to evaluate these dynamics; in particular, the influence of the dispersal kernel used in MAPOD-maize must be evaluated more closely by specific sensitivity analyses.

Field pattern and characteristics of the landscape were not substantially informative for local cross-pollination rate. On the other hand, our results show that considering only a non-GM crop field's topological and geometrical relationships with its nearest GM crop field is not sufficient to explain cross-pollination observed at the field scale. Our study thus suggests that considering an intermediate scale, one between the local and the landscape scale, will be most relevant when establishing management rules for controlling cross-pollination from GM crops.

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