Maize Pollen Flow in Contiguous and Non-Contiguous Field Plots

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Abstract

Pollen flow is a major concern faced out in the coexistence of GM and conventional cultivars of maize.

Maize pollen flow research has mostly addressed the flow between contiguous plots. In these cases, it has been shown that, very often, within a few meters from the common edge of the plots, inside the recipient plot, there is a dramatic reduction of the crossing rate by the contiguous donor. This may be accounted for by both the retention of pollen by the canopy of the first rows of the recipient plot and the dilution of the foreign pollen into the pollen of these very same rows. Therefore, for coexistence purposes one should be cautious in applying the isolation distances suggested by data from contiguous plots to spaced plots.

In order to assess the differences between maize pollen flow in contiguous and non-contiguous field plots, two successive studies were drawn. The first, between 2002 and 2003, aimed at evaluating pollen flow between spaced plots with increasing distances between them (from 40 m to 250 m); the donor plot was sown with a *Bt* yellow grain variety carrying the MON810 event, and the recipient plots were sown downwind with conventional white grain varieties; a secondary objective of this study was to evaluate the selective advantage of pollen carrying the MON810 event. This allowed us to confirm the absence of any selective advantage of MON810 bearing pollen and to pursue the research on maize pollen flow with conventional varieties only.

The second study, between 2005 and 2007, aimed at evaluating pollen flow between continuous plots. Donor and recipient plots were sown with yellow and white grain conventional varieties, respectively. Some, but not all of the recipient plots were sown downwind.

In all experiments the choice of varieties and sowing dates aimed at simultaneous flowering of both donor and recipient plots.

Observations of the samples from recipient plots consisted on the distribution and ratio of yellow grains per ear.

Results indicated that the reduction of the crossing rate between donor and downwind recipient plots with distance is much more drastic in contiguous plots than in spaced plots. While in non-contiguous plots at 40 m from the donor plot out-crossing rates were above 20%, in the most unfavourable situation (average wind speed of 10 ms^{-1}) in contiguous plots at 45 m the out-crossing rate was 4%. The differences may however resume to the first rows of the recipient plot. The practical consequences of these results in coexistence are discussed.

Introduction

Pollen flow is a very important cause of transgene dispersal in out crossing crops that are grown for flower, fruit or seed production.

Maize is a wind pollinated out crossing crop, grown either for silage or grain. Therefore, pollen flow is a major issue in maize coexistence (1).

Numerous authors have addressed the subject of maize pollen flow, namely in recent years, due to concerns regarding transgene transmission to non-transgenic maize crops (2, 3, 4). Most reported studies have focused on maize pollen flow between contiguous fields (3, 4) and it has been pointed out (2) that the conclusions driven from these conditions might not apply to non-contiguous fields.

The research aiming to provide information about pollen flow of transgenic cultivars has utilized either both transgenic and non-transgenic cultivars or only non-transgenic cultivars differing by at least a marker gene, usually a grain colour marker. The comparability of results from both types of research materials relies on the assumption that transgene carrying pollen and non-transgenic pollen have the same fitness. But, to our knowledge, this has never been demonstrated, though gametophytic selection has been demonstrated for some traits, namely in maize (5).

Herein experiments aiming to address both the patterns of pollen flow between separated plots and between side-by-side plots are reported and information about the selective advantage of MON810-carrying pollen is provided.

Material and Methods

Two sets of field trials were established between 2002 and 2003 and between 2005 and 2007, respectively. Figure 1 shows the images of the three locations involved: Escaroupim (2002 and 2003), Valada (2006 and 2007) e Taveiro (2005 to 2007). The first two stand in the Tejo valley (Santarém district) and the third in Mondego valley (Coimbra district).

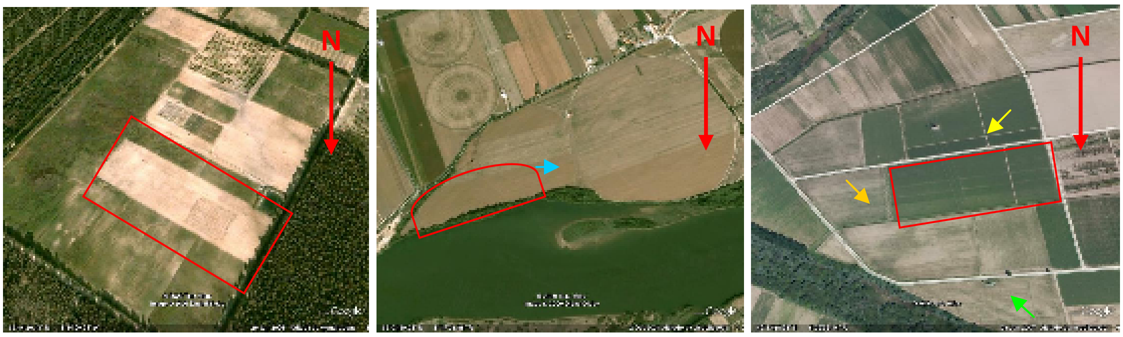

Figure 1. Google Earth images of the locations of the experimental fields, signed with red lines. From left to right: Escaroupim (2002 and 2003), Valada (2006 and 2007) e Taveiro (2005 to 2007). To provide details about surrounding landscape, different scales have been used. Coloured arrows are explained in the text.

The first set of trials aimed at evaluating (i) pollen flow between non-contiguous field plots and (ii) the selective advantage of pollen carrying the MON810 event. The second set of trials aimed at evaluating pollen flow between contiguous field plots.

In each case, varieties within the same group of precocity and sown at the same time were used, in order to maximize the chances of cross-pollination and fertilisation between varieties.

The first set of trials was run for two years, at a single location (Escaroupim). A 100 m x 100 m field was sown with the yellow grain *Bt* cultivar Elgina (FAO class 600), hemizygous for the MON810 event. All around the 1 ha field six (in 2002) and four (in

2003) border rows were sown with a conventional white maize cultivar (Lucila), thus simulating the refuge area that is currently planted in *Bt* maize growing fields. From now on the yellow maize field and its border rows will be referred to as donor plot.

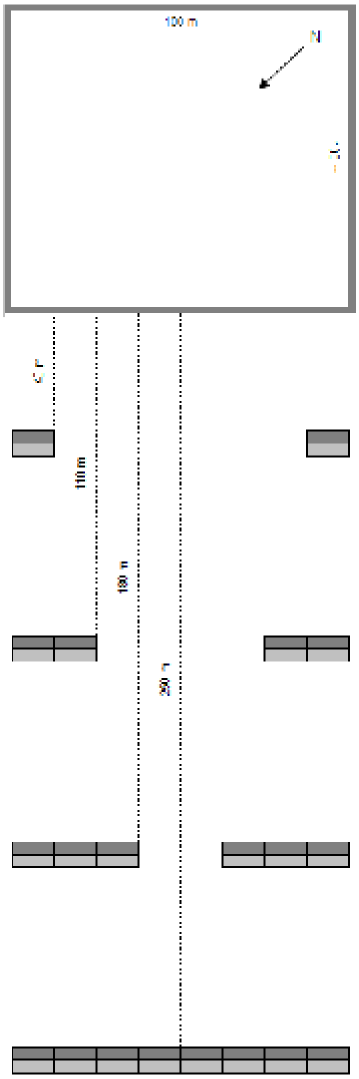

Figure 2. Field layout of the first set of trials: white – cv. Elgina; dark grey – cv. Lucila; light grey – cv. Damiana.

White maize plots were sown at increasing downwind distances (40, 110, 180 and 250 m) from the donor plot, as shown in Figure 2. Each white maize plot consisted of twelve rows of conventional maize cultivars: six rows of cv. Lucila and six rows of cv. Damiana (FAO classes 500-600). From now on these white maize plots will be referred to as recipient plots. The lengths of recipient plots (from 12.5 m to 50.0 m) were chosen in order to evaluate the effect of twelve maize rows barriers on pollen flow between the donor and the final recipient plot.

At maturity, within each 12.5 m length section of recipient plots, a ten ears sample was collected in each odd row. Samples were also collected in all border rows of the donor plot in 2002 and in odd border rows in 2003.

Registration of presence/absence of yellow grains along the ear (base, middle or top of the ear) and counting of yellow and white grains per ear were performed. As flowering along the ear is acropetal, the position of yellow grains along the ears was used to check the flowering overlap of pollen donor and pollen recipient varieties. Chi-square tests were performed for each recipient variety to compare the presence of yellow grains at the base and at the top of the ears; the 1:1 null hypothesis was meant to demonstrate the simultaneous flowering of Elgina and the recipient white variety, while a prevalence of yellow grains at the base or at the top of the ears was meant to indicate, respectively, a later or earlier flowering of the recipient white variety with respect to Elgina.

In order to test the selective advantage of MON810 carrying pollen, after grains counting, yellow grains of border rows of the donor plot and of each one of the recipient plots (40 m, 110 m, 180 m and 250 m) were bulked, and a sample of 50 grains was collected from each of the five bulks.

Identification of maize grains carrying the MON810 event was assessed by conventional PCR. A 194 bp fragment of the MON810 construct was amplified using the forward primer HS01 and the reverse primer CR01 (6). With these primers a fragment of the hsp70 intron 1 and a region of the cryIA(b) coding region were covered. Although these sequences are inserted in many other GM maize, the length of the amplicon varies according to the event, being the level of specificity acceptable for the running study. The reactions were performed according to Matsuoka *et al.* (6). The number of grains in each sample carrying the MON810 event was tested for the null hypothesis (absence of selective advantage) of a 1:1 segregation ratio for MON810 presence in the progeny of recipient x donor (white non-transgenic cultivar x yellow hemyzigous transgenic cultivar).

Following the results of the first set of experiments, a second set of experiments was established in two farms, located at Valada and Taveiro. For three years (2005 to 2007) in both locations contiguous plots of conventional yellow (donor) and white (recipient) maize cultivars were sown in order to evaluate pollen flow from donor to recipient plots. Due to the low number of locations, the interest in getting information about downwind recipient plots and the succession of results, more than a single field layout was used in each location along the three years.

At Valada (figure 1, centre) the farm has three systems of circle irrigation, of which two circular and the third half circular, as can be seen in the image. The 2005 experiment was set under the smaller circular system; the recipient plot occupied a quarter of circle (between 3 and 4 ha) in the position indicated by the blue arrow in figure 1; the remaining area under the system was the donor plot at the North-Northwest (NNW) and West-Southwest (WSW) sides of the recipient plot. The 2006 and 2007 experiments were run under the half circular system, which covers a field of 7.5 ha. In 2006 the field was split in two halves: East-Northeast (ENE) used as donor plot and WSW used as recipient plot. In 2007 the field was split in two by a line parallel to the full diameter of the field: the donor plot grew in the area at NNW of the line (partially repeating the 2005 situation), while the recipient plot was sown in the South-Southeast (SSE) side of the line. Therefore, 2006 and 2007 disposals of donor and recipient plots were perpendicular. In the three years the donor and recipient plots were sown, by mid April, with cvs. Pioneer A46 (yellow grain) and PR32B10 (white grain), respectively.

At Taveiro (figure 1, right) the field limited by the red line (4.4 ha), was always used as the recipient plot, being sown with cv. Lucila for the three years (2005 to 2007) of trials. It has always had a border of four rows (3 m wide) of Lucila on the side facing the field indicated with the orange arrow in figure 1. In 2005 and 2006, a single donor plot with 1 ha all along the strip pointed by the yellow arrow in figure 1, was sown with yellow maize cv. PR35P12. Between this strip and the recipient plot there is a field road 2 m wide. The owners of the fields pointed with orange and green arrows also sown yellow maize cultivars, but only in 2007 the owner of the field signed with the orange arrow accepted to participate in the experiment by sowing cv. PR35P12 at the same time of the two other experimental plots. The field with the green arrow stands about 120 m far.

Sampling took place at harvest. In 2005 sampling was done with different grids (from 9 m x 9 m, nearby the donor plots, to 36 m x 18 m in Valada, and 24 m x 12 m in Taveiro). In 2006 and 2007, in both locations, sampling was always performed according to a square grid, with sides 12 m long. The grid sizes were decided taking into account the between rows sowing distance, which was 0.75 m; this choice allowed a combination of counting of rows with direct measuring within rows to perform the sampling. In each sampling point, the ears from two contiguous plants were collected.

The observations made per ear were the same as described above for the first set of trials.

Results and Discussion

Table 1 provides data on wind direction and speed, when available, for both sets of experiments. Valada data were collected on place, 2006 and 2007 Taveiro data were collected in a farm 2 km far from the experimental field (by safety reasons), while the others were provided by the nearest stations of the National Meteorological Institute.

Table 1. Summary of wind data across experimental locations and years: AWSD – Average wind speed (ms^{-1}) and direction; Max – maximum speed and its direction (on an hourly base); [8 am; 4 pm] – period of the day for maize anthesis. In italic: data from the National Meteorological Institute.

Location	Escaroupim	Valada			Taveiro		
Year	2003	2005	2006	2007	2005	2006	2007
Flowering	July, 12-21	July, 3-11	July, 1-7	June, 20-30	July, 19-31	July, 10-18	July, 17-30
AWSD	*3.0 WNW*	2.6 W	2.0 W	**9.9** WNW	*2.6 SW*	1,2 W (*2.1 S*)	1,1 SSW (*2.6 WSW*)
AWSD [8 am;4 pm]	*3.3 WNW*	2.9 W	2.4 W	**13.0** NW	*3,5 WSW*	1,3 WNW (*2,9 S*)	1.4 SW (*3,2 W*)
Max [8 am;4 pm]	*5.8 W*	6.1 NW	5.8 NW	**23.1** NW	*6.8 S*	4.0 W (*6.1 SE*)	5.3 W (*6.3 WNW*)

Data in table 1 demonstrate that the layout choices in Escaroupim and Valada went well, as in Escaroupim and for two years (2005 and 2007) in Valada the recipient plots were intended to stand downwind, and in 2006 in Valada it was intended to be the opposite. In Taveiro, however, the first donor plot did not stand from the side of dominant wind direction. But the inclusion of the additional donor plot in 2007 provided the desired source of pollen favoured by wind direction. Therefore, in five out of eight cases shown in figures 4 to 8 – Escaroupim 2002 and 2003, Valada 2005 and 2007 and Taveiro 2007 – pollen flew from donor plots into downwind recipient plots. In one, Valada 2006, pollen flow was against dominant wind direction. In Taveiro 2005 and 2006 the pollen flow under evaluation was more or less perpendicular to the dominant wind direction.

First set of experiments – non contiguous plots

In 2002, the analysis of the distribution of yellow grains along ears (data not presented here) suggested a good overlap of flowering times between donor and recipient plots except for Lucila in the 250 m recipient plot, which proved to be slightly later than Elgina (yellow grains occurred more often at the base than at the top of Lucila ears). In all cases, however, it was in the middle part of the ears that the highest number of yellow grain presences was observed, indicating a large overlapping of flowering periods between Elgina and the white maize recipient cultivars. In 2003 there was again the prevalence of yellow grains in the middle portion of the ears, though in all but three cases (Lucila in border rows of the donor plot and in recipient plots 40 m and 110 m apart from the donor plot), the higher frequencies of yellow grains in the top of the ears indicated a slight flowering delay of Elgina respect both recipient cultivars.

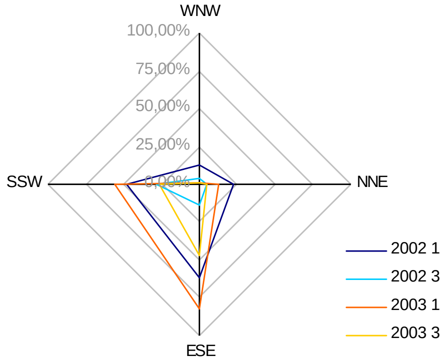

Figure 3. Average percentages of yellow grains in the first and third border rows at each side of Elgina in 2002 and 2003.

Figure 3 shows the average percentages of yellow grains per ear in the first and third border rows along the four edges (WNW, SSW, ESE and NNE) of Elgina, across the two years. It illustrates the relation between the pattern of pollen flow and the prevalence of winds during flowering (West-Northwest) shown in table 1.

2002 data allowed an analysis of row-by-row variation of the ratio of yellow grains in Lucila ears within each side of the donor plot, as shown in figure 4. East-Southeast and South-Southwest sides clearly received more pollen from Elgina then the other two sides, due to wind prevalence, as already seen in figure 3. Where pollen flow was low, a dramatic reduction in cross pollination was immediately observed from the first to the second row (Figure 4, left side); where it was higher, the two first rows had similar ratios and the reduction started only in the third row.

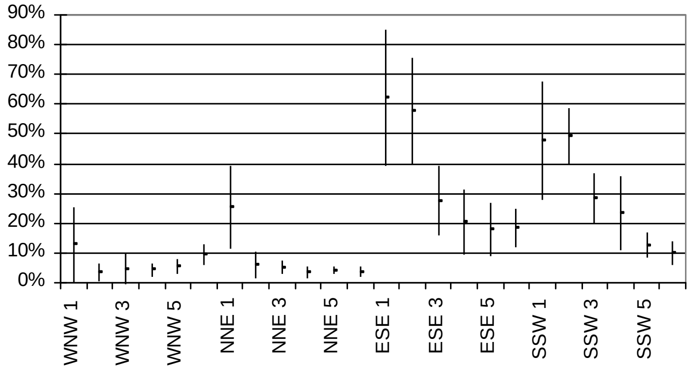

Figure 4. Out-crossing ratios (%) between Elgina and WNW, NNE, ESE and SSW border rows of donor plot in 2002: means and confidence intervals ($\alpha = 0.01$). In each side, border rows go from 1 to six, 0.75 m to 4.5 m far from Elgina, respectively.

The preliminary analysis of ratios of yellow grains per ear in recipient plots indicated that in neither case was there any significant difference between recipient plot sections due to the presence of none, one, two or three maize barriers, which were made by upwind recipient plots. Barriers are apparently only effective in contiguous plots (6).

Significant differences were instead observed between recipient plots equally far from the donor plot. From now on South quadrant and East quadrant will be used to distinguish between them; in figure 2 they correspond, respectively, to the left and right side plots. Therefore, the following analysis was performed keeping apart South and East recipient plots, but pooling all data across any odd row within each recipient plot.

Figure 5 shows the means and confidence intervals ($\alpha = 0.01$) of yellow grains ratios per ear in odd rows of recipient plot.

As expected there was a decrease of out-crossing with increasing distances from donor plot. However, it was much lesser than the decrease reported in literature for similar distances within continuous maize fields (3, 4, 7). At 40 m from the donor plot in the most wind favoured situation (South quadrant), Elgina pollen flow allowed an out-crossing rate in first row plants above 20% in both years. At 110 m this rate was above 6% and reached 12% in 2003, at 180 m it averaged 4% and even at 250 m it was slightly under 1.5%.

The out-crossing rates observed in the first rows of recipient plots were consistently higher than in the rows behind them, except for the East quadrant recipient plots at 250 m, most probably due to the small figures involved (lower than 1%). In all other recipient plots the decrease of out crossing rates across the plot followed a trend similar to that reported above for border rows in donor plot (figure 4).

It has already been mentioned that maize barriers were apparently not effective in reducing pollen flow from the donor plot to the recipient plots placed 110 m or more far from it. When comparing the out-crossing ratios of the final rows of a recipient plot and

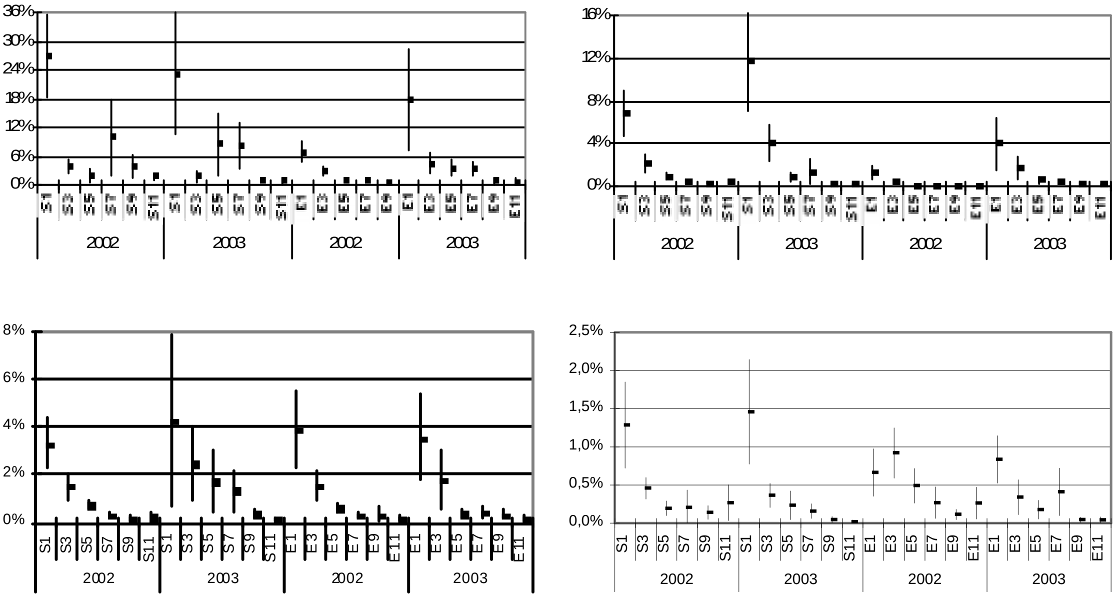

Figure 5. Out-crossing ratios (%) between Elgina and each odd row of recipient plots (means and confidence intervals, $\alpha = 0.01$). From top left to bottom right: results in recipient plots 40, 110, 180 and 250 m far from the donor plot. S and E stand for South and East quadrant, respectively (left and right sides of each graphic).

the first row of the recipient plot standing behind it in the same quadrant, a rise was noticed, in spite of the 70 m greater distance from the donor plot. These findings might be explained by the fact that pollen flows above the canopy, pushed by the wind, and along its way the quantity of viable pollen decreases as part falls down and other part probably dies. When pollen falls on a flowering maize plot, it will be diluted, by mixing, into the plot own (fresh) pollen. In the plants that stand in the edge of the plot facing the coming wind and the pollen it brings, a part of its own pollen will be pushed away, and foreign pollen will not be strongly diluted, which should explain the higher out-crossing rates of the first rows of recipient plots. Inside de plot, the surrounding plants, particularly those upwind, provide a large amount of pollen to their neighbours and allow a high dilution of foreign pollen and, therefore, a drop of the out-crossing rate.

Second set of experiments – contiguous plots

In all cases but one (Taveiro 2006) the analysis of yellow grains distribution across the years, indicated a strict coincidence of flowering in donor and recipient plots. This was due to the fact that the major source of foreign pollen was the field at the West side of the recipient plot, which had been sown at a time and with a cultivar chosen independently of the experiment.

Figure 6 shows the distribution of samples within the recipient plot of the 2005 experiment at Valada; the sides facing the donor plot are shown in yellow. This type of data presentation puts in evidence the significant drop of the out-crossing rate with increasing distances from the pollen source, but also the sudden occurrence of hot spots of out-crossing in clear contrast with the surrounding area. This is in accordance with what has been reported in literature, and may be due either to air movement or to development anticipation or delay of plants that does not allow them to be preferentially pollinated by their neighbours. Due to this variation, the too large grid used in the larger distances from pollen sources was retained inadequate to provide data for mean estimation. But with the data collected in the 9 m x 9 m grid, a mean of 1.7% was estimated for a strip at 18 m of both pollen sources, 219 m long and 18 m wide.

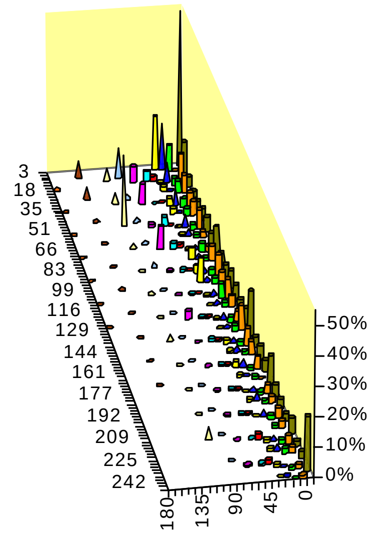

Figure 6. 2005 Valada experiment: out-crossing ratios (%) at increasing distances from the two sides of the donor plot.

The analysis of data of 2006 experiment at Valada provided the means and confidence intervals for out-crossing ratios at increasing distances shown on the top of figure 7. It can be noticed a dramatic drop from 37% to 0.74% in the first 12 m of the recipient plot.

The results of the 2007 experiment at the same location are presented at the bottom of figure 7. Though they follow the usual trend, out-crossing rates are much larger, and at 84 m from the donor plot they still average 1.74%. The out-crossing rate averaged 5.5% in a strip at 24 m of pollen source, 432 m long and 24 m wide. This was more than 3 times higher in a strip more than 2.5 times larger and 6 m farther from donor plot than the 2005 strip facing the same wind direction. The explanation of that high out-crossing rate can be found in the unusual wind speed during 2007 flowering, which was much higher than in

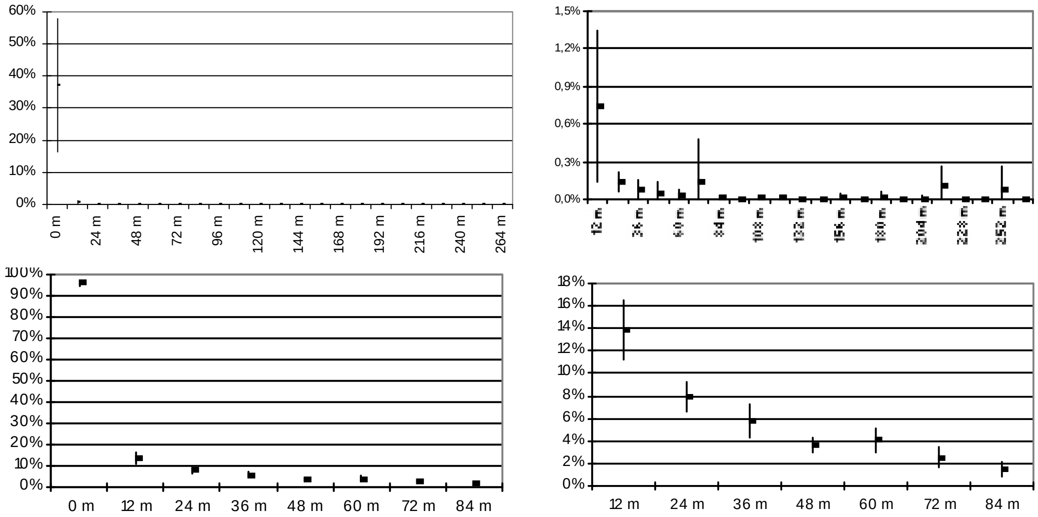

Figure 7. 2006 (top) and 2007 (bottom) Valada experiments: out-crossing ratios (%) at increasing distances from the donor plot (means and confidence intervals, α = 0.01). Graphics on the left include the first row (0 m) of the recipient plot, while those on the right excludes it.

any other situation and well above 2.8 ms^{-1}, the six years (2003 to 2008) local mean.

The differences between the results of 2006 and 2007 experiments were consistent with the differences in their layouts: the donor plots were successively planted at the ENE and NNW side of the recipient plot, and the prevailing wind directions during flowering time were W and NW, respectively. But it must be stressed that the high out-crossing rates in 2007 were due not only to wind direction but also to a high wind speed.

The results of the experiments at Taveiro are presented in figure 8; the surface graphics put in evidence the contribution of the different sources of foreign pollen into the recipient plot.

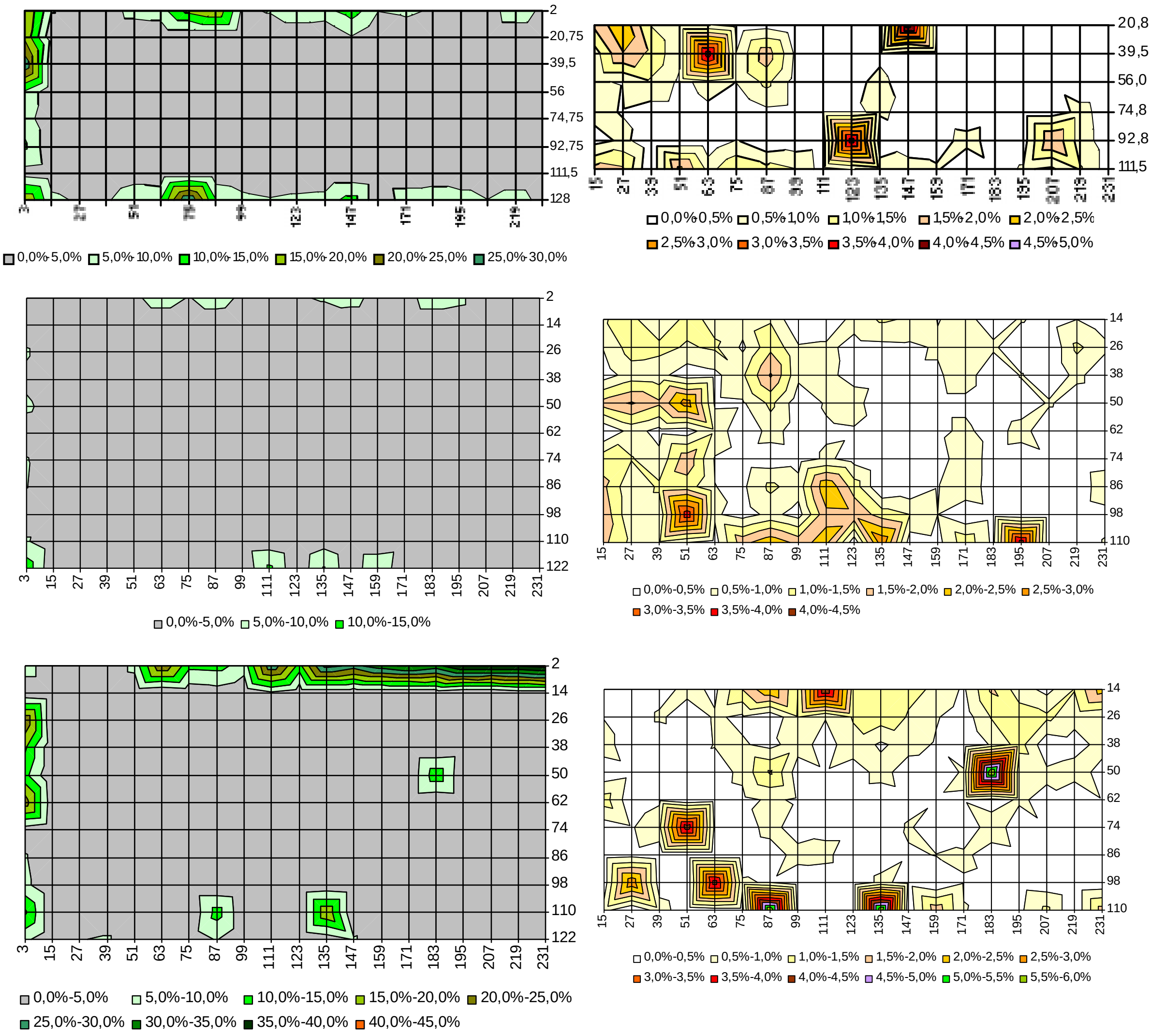

Figure 8. Out-crossing ratios (%) of Taveiro recipient plot. From the top to the bottom: 2005, 2006 and 2007. Left side includes all sampling grid; right side excludes the top, left and bottom first line of the grid. Figures indicate de distances (m) from the donor plots.

Though with different magnitudes, across the three years pollen from both near (top and left side of the images) and eventually 120 m far (bottom of the images) sources flew into the recipient plot, as shown by the higher out-crossing ratios in all but one of its sides. In spite some hot spots, the out-crossing rates for the areas of the recipient plot shown in the right side of figure 8 were 0.6% ±0.8%, 0.7% ±0.7% and 0.9% ±2.0%, respectively. It should be noticed that the three darker hot spots in 2007 ranged between 12% and 19%, the one well inside the field being 13%. The high standard deviations are nothing but another way to state the heterogeneous distribution of out-crossing rates within recipient plots as shown in figure 8, as well as in figures 6 and 7.

The different sources of wind data in Taveiro, and the differences that can be stated between them in 2006 and 2007, do not allow much comparisons between 2005 and the other years. Nevertheless, in spite of the wind direction, the low speeds appear to have kept out-crossing rates inside the recipient plots under 1%.

Conclusions

For the first set of experiments only 2003 wind data are available; when compared with the average of six years (2003 to 2008) for the month of July, which is 2.8 ms^{-1} WNW, it can be expected that the results presented above provide an average pattern of downwind pollen flow between non-contiguous plots. It is, therefore, a different pattern from that observed in contiguous plots, but consistent with other results in literature (8). While in non-contiguous plots at 40 m from the donor plot out-crossing rates were above 20%, in contiguous plots in the most dramatic situation (Valada 2007) at 45 m (assuming a 3 m wide border strip in the donor plot) the out-crossing rate was 4% (5 to 6 times smaller). The out-crossing by foreign pollen in a plot, while depending on the distance of the pollen source, wind direction and wind speed, is also greatly dependent on foreign pollen dilution by the plot own pollen, as has been consistently demonstrated by others (9).

In spite the significant out-crossing rates in the edge of distant recipient plots, the reduction in the following rows may allow a low out-crossing average in the whole plot (7). However, recommendations of isolation distances for maize coexistence should discriminate between spaced and non spaced fields, namely by the introduction of border rows in recipient plots that are separated from donor plots either by fallow or low canopy crops; under these circumstances, such border rows might be more effective in pollen flow containment than the border rows in the donor plot, whose plantation appears to be a good coexistence measure only in contiguous fields (1).

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