

Gene flow in maize fields with different local pollen densities

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Received: 13 October 2006 / Revised: 23 January 2007 / Accepted: 24 January 2007 / Published online: 17 April 2007
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Abstract The development of maize (*Zea mays* L.) varieties as factories of pharmaceutical and industrial compounds has renewed interest in controlling pollen dispersal. The objective of this study was to compare gene flow into maize fields of different local pollen densities under the same environmental conditions. Two fields of approximately 36 ha were planted with a nontransgenic, white hybrid, in Ankeny, Iowa, USA. In the center of both fields, a 1-ha plot of a yellow-seeded stacked RR/Bt transgenic hybrid was planted as a pollen source. Before flowering, the white receiver maize of one field was detasseled in a 4:1 ratio to reduce the local pollen density (RPD). The percentage of outcross in the field with RPD was 42.2%, 6.3%, and 1.3% at 1, 10, and 35 m from the central plot, respectively. The percentage of outcross in the white maize with normal pollen density (NPD) was 30.1%, 2.7%, and 0.4%, respectively, at these distances. At

distances greater than 100 m, the outcross frequency decreased below 0.1 and 0.03% in the field with RPD and NPD, respectively. A statistical model was used to compare pollen dispersal based on observed outcross percentages. The likelihood ratio test confirmed that the models of outcrossing in the two fields were significantly different (P is practically 0). Results indicated that when local pollen is low, the incoming pollen has a competitive advantage and the level of outcross is significantly greater than when the local pollen is abundant.

Keywords Maize · Pollen · Dispersion · Statistical models

Introduction

Stevens et al. (2004) define gene flow in maize (*Zea mays* L.) as the fertilization of a population by pollen from another group of genetically different maize plants. This process has been an intricate part of the evolution of maize improvement. The movement of genes from one population to another has been reported between landraces of maize and teosinte in rain-fed areas of Mexico (Wilkes 1970, 1977; Eubanks 2001; Luna et al. 2001; Doebley 2004; Baltazar et al. 2005), between improved varieties and landraces in grain production fields (Castillo Gonzalez and Goodman 1997; Bellon and Brush 1994; Bellon and Risopoulos 2001), and between grain and seed production fields, or among seed production fields, jeopardizing the genetic purity of the seed produced (Burris 2001). The magnitude of gene flow among plants varies with species, population, genotypes, and environmental conditions (Ellstrand 2003), and can ultimately modify and define the genetic structure of a plant population. The principal means for gene movement are via pollen flow (Arriola and Ellstrand 1996) and, less frequently, by seed dispersal (Dow and Ashley 1998; Ennos 1994; Hardy et al. 2004; Kenta et al. 2004). The interruption of the

This journal paper of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa, Project No. 3638, was supported by Hatch Act, State of Iowa fund and by a USDA-CREES BRA Grant.

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natural flow of genes among plants reduces population variability and causes inbreeding depression and loss of genetic variability in open-pollinated species by promoting self-pollination (Mayr 1970). Therefore, gene flow has both a positive role by enhancing genetic diversity of maize and a negative role by compromising the genetic purity of elite germplasm during seed production for improved varieties.

Earlier maize pollen flow and outcross studies by Jones and Newell (1948), Bateman (1947a, b), Jones and Brooks (1950), Raynor et al. (1972), and Paterniani and Stort (1974) showed that pollen amounts and outcross percentages decline considerably within the first 10 m from the pollen source and that a long-extending tail of pollen dispersal and outcross persist at distances of up to 250 m. The Organization for Economic Co-operation and Development (OECD) minimum requirement for genetic purity of maize hybrid seed is 99%, and the minimum isolation distance required, including border rows, is 200 m (OECD 2005). These standards for seed production have remained unchanged for more than 50 years (Burris 2001).

Recent studies (Garcia et al. 1998; Burris 2001; Jemison and Vayda 2001; Luna et al. 2001; Pleasants et al. 2001; Sears et al. 2001; Henry et al. 2003; Jarosz et al. 2003; Chilcutt and Tabashnik 2004; Ma et al. 2004 and Stevens et al. 2004) found that pollen flow and outcross in maize decrease within the first 10 or 20 m from the pollen source and that small amounts of pollen and outcross are present at distances not greater than 200 m, with the exception of Stevens et al. (2004) who found 0.02% outcross at 300 m.

Most of the studies are characterized by the use of small pollen source fields (50×50 m), small-size receiving plots, and noncontinuous source-receiving field design. Smaller source fields produce smaller pollen clouds. Pollen entering the receiving field must “outcompete” locally shed pollen to achieve an outcross. This competition is controlled by physical factors (e.g., local pollen density and incoming pollen density) and genetic factors (e.g., pollen-silk compatibility and relative rates of pollen tube growth). Current studies fail to explain the role of large pollen clouds, multidirectional wind effects, contiguous fields, and different local pollen density in modifying gene flow and the incidence of outcross.

The development of transgenic maize varieties as factories for pharmaceutical and industrial compounds has generated renewed interest in pollen dispersal and gene flow. It is essential to reevaluate the minimum distance required to isolate conventional hybrids from these transgenic varieties to avoid the adventitious presence of transgenic proteins and other undesired compounds in the food supply (Doering 2004). Source and receiver field size (Burris 2001) had a strong impact on the level of outcross and the distances into adjacent fields that pollen travel. These studies were conducted in seed production fields where

local pollen density was reduced by detasseling of the female plants for hybrid seed production. However, little has been done to explain the differences or similarities in the incidence of outcross when field have different local pollen density. Large-scale studies are needed to assess how local pollen density, continuous source and receiver fields, and multidirectional gene flow affect outcross in order to understand and achieve strict control of pollen flow and successful coexistence of these transgenic crops in the environment. The objectives of the present study were to compare the gene flow and outcross in a field with “reduced” local pollen density (RPB) and a field with “normal” local pollen density (NPD) in maize fields under the same environmental conditions by using statistical models.

Materials and methods

Experimental fields

Two fields of approximately 36 ha were planted with a nontransgenic, white maize hybrid RX792W in Ankeny, Iowa, USA, in 2003. In the center of both fields, a 1-ha plot of DKC69-71 yellow maize was planted as an adventitious pollen source. The term “adventitious presence” refers to the unintentional mix of one type of seed, grain, or food product with another (Biotechnology Industry Organization 2005), and it will be used interchangeably with the word “outcross” throughout this article. This yellow-seeded hybrid is a stacked transgenic, Roundup Ready (RR)/Bt [Bt-Cry1Ab gene derived from the soil bacterium *Bacillus thuringiensis* (Bt)] hybrid. Planting dates were 20 May 2003 for the white-seeded hybrid and 21 May 2003 for the yellow-seeded, transgenic hybrid. The population for the transgenic hybrid was 84,013 plants/ha and 69,187 plants/ha for the nontransgenic variety. The fields were managed under normal production practices of cultivation and insect and soil fertility management. Soils were silty clay loam to loam, predominantly of the series Nicollet, Webster, Clarion and Harps (USDA-NRCS 2000). Before flowering, the white receiver maize of the RPD field was mechanically detasseled in a 4:1 ratio to reduce the local pollen density. This ratio is typical of a hybrid seed production field. The NPD field was not detasseled. The yellow and white hybrids flowered synchronously and yellow pollen flowed freely into the surrounding white maize field. Sampling in each experimental field was conducted along eight transects (N, NE, E, SE, S, SW, W, and NW) relative to the source plot to cover all possible wind directions. Ear samples were collected at approximately 1, 10, 35, 100, 150, 200, and 250 m away from the source plot (Fig. 1). Sampling locations were recorded using a global positioning system.

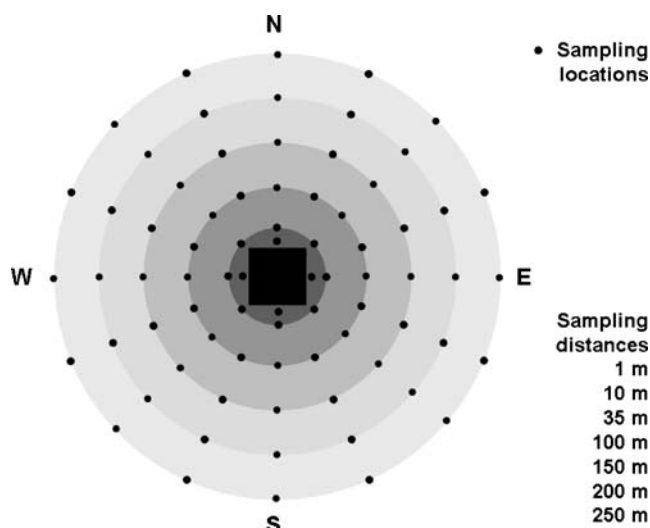


Fig. 1 Sampling locations at increasing distances away from the source plot of maize (*Zea mays*). Samples were collected at 1, 10, 35, 100, 150, 200, and 250 m following the N, E, S, W, NE, SE, SW, and NW cardinal points. Additional samples were taken at 100, 150, 200, and 250 m to minimize separation among sampling locations at the farthest distances from the source plot

At harvest maturity, samples of 25 ears were collected along each transect at 1, 10, and 35 m, and 100 ears were collected at 100, 150, 200, and 250 m, from the yellow pollen source. Sample size was increased for samples taken further from the yellow pollen source to ensure detection of at least 0.005%, if present, according to probability of detection by using Seedcalc6 (Remund et al. 2001). In the RPD field, samples were collected from the two central detasseled rows at each sampling location. In the NPD field, samples were also collected from two rows at each sampling location. Ears were dried to storage moisture (120 mg of water per gram of fresh weight) with forced, 40°C heated air seed driers at the Agronomy Farm, Iowa State University, Ames. Samples were shelled (Custom Seed Equipment (Altoona, Iowa) laboratory-size sheller model LS 91) and color sorted with a 20-channel ScanMaster color sorter model SM-200DE (Satake USA, Stafford, Tex.) at Seed Science Center, Iowa State University. After color sorting, the resulting white and yellow maize subsamples were manually separated until 100% pure subsamples were obtained. Seed samples were weighed and the number of seeds in 454 g was counted using a seed counter (FMC Syntron, model EB00-D). The total number of seeds per sample was calculated from the number of seed per 454 g and the total sample weight. When the weight of the adventitious seeds was less than 454 g, yellow seeds in the sample were counted by hand.

Meteorological data

During pollen shed, a portable weather station placed by the yellow pollen source recorded wind speed and direction. An R.M. Young 3001 Wind Sentry Set with an precision

of $\pm 0.5 \text{ m s}^{-1}$ recorded local wind speed and direction at a height of 3.17 m from the ground. Weather data were averaged and stored every 15 min by using a Campbell Scientific CR10 data logger.

Flowering synchrony

Silk exertion and pollen shedding were determined for each field during the period 21 July 2003 to 14 August 2003. Approximately 100 plants at 4 locations within the center yellow maize plot, and 26 locations within the surrounding white maize field were monitored for pollen shed and silks emergence. The percentages of plants shedding pollen and silking at these locations were averaged to determine flowering synchrony for each field. Pollen density was determined by placing passive pollen traps within the canopy at the ear level and by collecting pollen every 24 h at approximately 1700 hours for counting. Pollen counts were evaluated in the laboratory by image analysis according to Fonseca et al. (2002) and Bassetti and Westgate (1994).

Screening for the RoundUp Ready® gene

After color-sorting, the remaining seeds mixed in the yellow and white fractions were separated by hand. The yellow seed were screened for the presence of the RR gene by using the preemergence method established by Goggi and Stahr (1997) and AOSA (2003). The yellow seeds were imbibed for 48 h in groups of 200 seeds between paper towels moistened with a 3% a.i. (30 g kg^{-1}) solution of glyphosate (Roundup Ultra). Two hundred seeds from the white seeded, nontransgenic seed in the sample, and 200 seeds from samples of the yellow-seeded, pollen source plot were also tested to assess the segregation and linkage between the yellow and RR gene. The herbicide-imbibed seeds were planted on moistened crepe cellulose paper (Kimpak) on top of plastic trays and germinated at a constant 25°C for 7 days. The number of normal, abnormal, and dead seeds were evaluated following the Association of Official Seed Analysts (AOSA) Rules for Testing Seeds (AOSA 2003). If a seed carried the RR gene, it produced a normal seedling. The percentage of outcross in yellow seeds by using the information from the RoundUp Ready gene was obtained as follows:

Proportion of outcrossed seeds in the sample

$$= [\text{number of normal tolerant seedlings} / \text{total number of seeds}] \times 2$$

where number of normal tolerant seedlings is number of yellow seed that produced a normal seedling after the RR seed preemergence glyphosate imbibition test, and total number of seeds is number of yellow + white seed in the sample. This number is multiplied by 2, because the RR

gene was segregating as a true hemizygote, indicating that only half of the gametes (pollen grains) produced by the source plot carried the RR gene. During plant transformation process, a copy of the gene of interest (e.g., RR gene) is inserted in one member of the chromosome pair. The other member of the chromosome pair does not carry a copy of the inserted gene. Thus, half of the seeds resulting from an outcross with pollen from the center field will carry the RR gene and half will not.

Screening for the Bt-Cry1Ab gene

Normal and abnormal seedlings from the RR preemergence glyphosate-imbibition test also were analyzed for the presence of the Bt-Cry1Ab gene. Control samples collected from the yellow-seeded source plot and from the white-seeded receiver field were also analyzed. In samples collected at 1 and 35 m from the source plot, 360 seeds (180 RR and 180 non-RR seedling) were tested. In samples collected at greater distances from the source plot, all RR-seedlings and 180 non-RR seedlings were tested. If fewer than 180 non-RR yellow seedlings were found in the sample, all seedlings were tested. An enzyme-linked immunosorbent assay (ELISA) Bt kit (Agdia Incorporated, Elkhart, Ind.) was used to detect the Bt protein. The test is a double-antibody sandwich (DAS) ELISA where the protein is captured and detected using a polyclonal antibody. The procedure used is described in detail in the ELISA kit. The protein was extracted by placing a small piece of leaf of the RR-tolerant seedlings or the tip of the coleoptile of the non-RR seedlings into a single well of the 96-well plates and adding PBST protein extraction buffer. The protein was extracted from the tissue using an ultrasonic cleaner (model FS30; Fisher Scientific, Pittsburgh, Pa.) for 30 min. All steps followed were as described in the instructions included with the ELISA kit. The intensity of the color is related with the amount of protein and was quantified by optical density with a plate reader (model ELx800; BIO-TEK Instruments, Winooski, Vt.). Seedlings lacking the Bt-Cry1Ab protein showed a clear or light blue color in the test wells. Data from the plate reader were used to determine the number of seedlings expressing the protein. The average of the data for the four negative control wells multiplied by 2 was used as the base number to determine the wells considered positive and negative for the presence of the protein. Samples in test wells with an optical density reading equal or lower than the average of the negative control wells multiplied by two were considered non-Bt samples. The proportion of Bt-Cry1Ab outcross was obtained as follows:

$$\frac{[\text{number of seedlings with the Bt} - \text{Cry1Ab protein}]}{[\text{total number of seedlings tested}]} \times 2,$$

where number of seedlings with the Bt-Cry1Ab protein is number of wells in the test with optical density readings higher than the average control, and total number of seedlings is total number of seedlings tested. Results are multiplied by 2 because the Bt gene was segregating as a true hemizygote. As with the RR gene, only one member of the chromosome pair has a copy of the Bt gene. Thus, only half of the outcross seeds carry the Bt gene and are identified using the Bt-Cry1Ab protein test.

Data analysis

The statistical analysis to determine whether the RPD field and the NPD field had significantly different distribution of yellow seed outcross at the different distances from the source plot was carried out using the open source statistical software R ("The R Project for Statistical Computing" 2004). Statistical analysis of the outcross percentage in the field with RPD versus the field with NPD involved comparing statistical models of outcrossing profiles. Using the statistical model described in Goggi et al. (2006), the goal was to model the process of pollen dispersal in fields with different local pollen density through the observed outcross data. The rational and development process for the statistical model are explained in subsection [Statistical comparison of outcrossing in the RPD and NPD fields of Results](#). The response variable is the percentage of outcrossing, defined as the percentage of transgenic yellow seeds in a given sample collected at each field location, as a function of distance from the source plot, wind speed, and direction during pollen shed. Parameter estimation was performed using the maximum likelihood estimation technique. Numerical optimizations of the log-likelihood function were performed using a Newton-Raphson algorithm (Press et al. 1986). The theoretical and observed RR and Bt gene frequencies were calculated by χ^2 .

Results

Table 1 shows precipitation, maximum and minimum temperatures, and growing degree-days (GDD) data from Ankeny (National Climatic Data Center). Figure 2 shows the wind rose for Ankeny from 19 July to 3 August 2003, which coincided with pollen shed. Predominant winds during pollen shed and silk exertion were from the N and the NW. The maximum 15 min average wind speed in 2003 was 4.7 m s^{-1} , recorded on 26 July at 1115 hours, from the south-southwest. Winds during July 2003 were somewhat unusual in that it is more common for winds to be directed from the southerly quadrant (USDA-NRCS 2005).

Synchrony between silking and pollen shed between the yellow and white maize hybrids is shown in Fig. 3 for the

Table 1 Precipitation, maximum and minimum temperatures, and GDD

Parameter	Month						Total
	May	June	July	August	September	October	
Precipitation (mm) ^a	95.3	60.1	89.0	21.8	87.4	20.6	374.2
GDD ₁₀ ^b	171	295	387	399	215	143	
Temp. max. (°C) ^c	20.4	24.8	27.9	28.8	22.1	18.7	
Temp. min. (°C) ^d	8.8	13.6	16.9	16.9	8.6	4.4	

^a *Precip. (mm)* Precipitation in millimeters^b GDD₁₀—growing degree—days in degree Celsius = $[(\text{minimum temperature} + \text{maximum temperature}) \times 2^{-1}] \times -10^{\circ}\text{C}$ accumulated per days
If maximum temperature is $> 30^{\circ}\text{C}$, then maximum temperature = 30°C If minimum temperature is $< 10^{\circ}\text{C}$, then minimum temperature = 10°C ^c *Temp. max. (°C)* Average maximum temperature in $^{\circ}\text{C}$ ^d *Temp. min. (°C)* Average minimum temperature in $^{\circ}\text{C}$

RPD and NPD fields. The RR/Bt yellow maize source plot reached 50% pollen shed by 26 July 2003 and 50% silk exertion by 28 July 2003. The white maize receiver field reached an average of 50% pollen shed by 26 July 2003 and 50% silk exertion by 27 July 2003. The most important synchrony is pollen shed by the yellow maize and silking by the white maize for both RPD and NPD fields. This flowering synchrony was nearly identical for both fields and represents a worst-case scenario for outcrossing resulting

from adventitious presence. Therefore, differences in outcrossing observed in the NPD and RPD fields should reflect differences in local pollen shed density during the period when silks were receptive. Mechanical detasseling reduced the local pollen density in the RPD field to approximately 70% of that of the nondetasseled NPD field (Table 2).

The segregation of the RR and Bt gene was determined in seed samples harvested in the yellow source plot. Both genes segregated with a frequency not significantly different (RR gene, $P=0.28$; Bt gene, $P=0.64$) than the expected 75% RR : 25% non-RR (Chilcutt and Tabashnik 2004). All seeds harvested in the source plot were yellow, indicating there was no segregation for seed color, and thus both alleles were dominant yellow. In addition to testing the yellow seed in the center field, 2,000 white seeds were also tested for the presence of RR and Bt to evaluate the linkage or independent segregation of these genes and seed color. The presence of transgenes in the white seed in the samples tested was 0%. Thus, we applied the expected gene frequencies 1/4 Bt and RR, 1/4 Bt, 1/4 RR, and 1/4 non-Bt or RR to the yellow seed in the samples to determine the number of yellow seeds outcrossed by incoming pollen from the center source plot. We expected hybrid seed purity for the RR gene to be 100% because plants are sprayed with Roundup Ultra during seed production to eliminate plants not carrying the RR gene. Also, due to the simplicity and low cost of the RR biological test, all yellow seeds were tested for the presence of the RR gene. Only 180 seeds from samples harvested closest to the center field (1–35 m from the source plot) were tested for the presence of the Bt gene. For all other sampling locations, all seeds were tested for Bt. Results presented are based on the presence of two genes, yellow seed color and RR, unless otherwise stated. To corroborate the accuracy of this assumption, we also analyzed the data by using total number of yellow seed

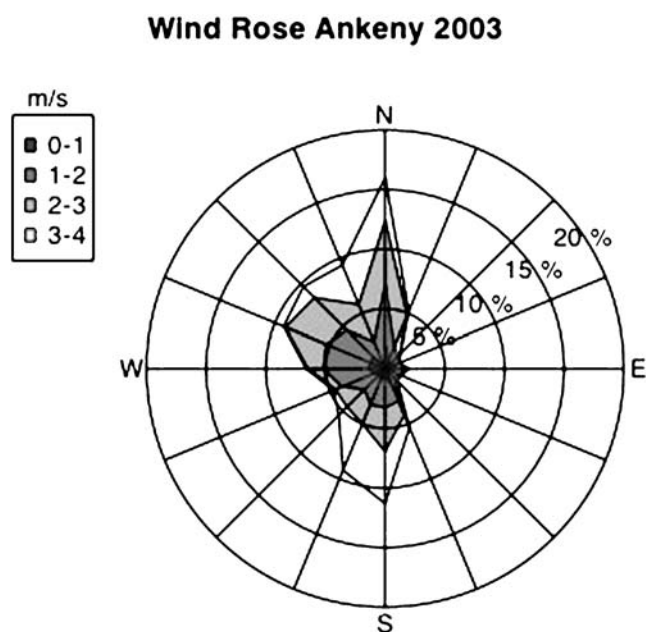
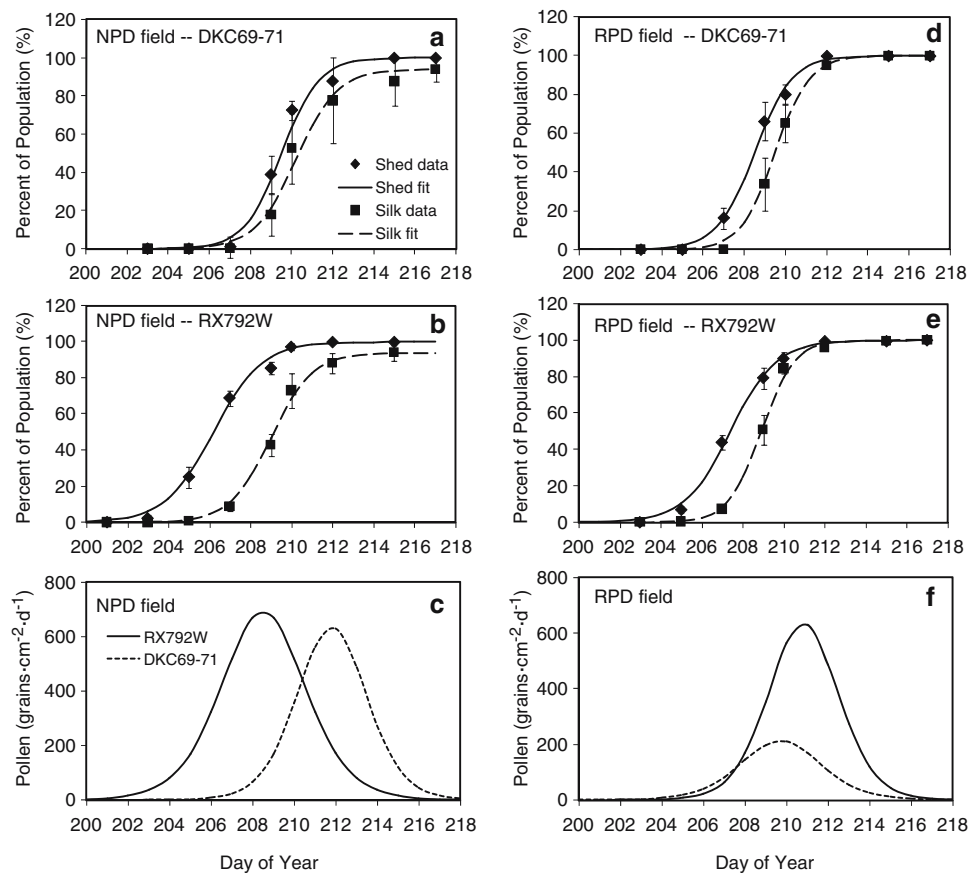


Fig. 2 Wind rose for Ankeny, Iowa from 19 July to 3 August 2003. Wind speed is in meters s^{-1} and concentric circles represent the percentage of the day that the wind came from that direction and that intensity. Data were collected on-site using a R.M. Young 3001-S wind Sentry Set in a portable weather station

Fig. 3 Flowering synchrony – pollen shed versus silk exertion – as a percentage. **a** Pollen shed and silk exertion data for the yellow-seeded DKC69-71 hybrid planted in the NPD field; **b** pollen shed and silk exertion data for the white-seeded RX792W hybrid planted in the NPD field; **c** synchrony and pollen production of both hybrids in the NPD field; **d** pollen shed and silk exertion data for the white-seeded RX792W hybrid planted in the RPD field; **e** synchrony and pollen production of both hybrids in the NPD field; **f** synchrony and pollen production of both hybrids in the RPD field



in the sample, which did not alter the general conclusions drawn from the data.

Mechanical detasseling reduced the local pollen density in the RPD field to approximately 70% of that of the nondetasseled NPD field (Table 2). As expected, the outcross frequency was greatest at sampling locations closest to the source plot in both fields (Table 3). The

largest average percentage of outcross recorded in the RPD field was 64.2% at 1 m within the S transect, whereas the largest percentage of outcross in the NPD field was 47.1% within the SE transect. At greater distances from the source plot (100 m or more), the outcross frequency decreased even further. At 100 m, the largest percentage of outcross for the RPD field was 0.4% in the SE transect, and for the

Table 2 Pollen shed in g cm^{-2} based on pollen trap data collected for several days during pollen shed in two fields in Ankeny, Iowa, in 2003

Date	Pollen production/maize field							
	Pollen source: yellow-seeded hybrid DKC69-71	Receiving white-seeded hybrid RX 792W						
		Transects				Avg. of 4 transects	SE	% of yellow source
		North	East	South	West			
RPD								
29 July	347	73	134	95	106	102	13	29.4
31 July	276	41	135	78	74	82	20	29.7
2 August	138							
NPD								
28 July	716	885		793	697	792	47	110.6
29 July	337	582	472	476	428	490	33	145.3
31 July	246	255	338	196	225	254	31	103.0
2 August	93							

Table 3 Percentage of yellow-RR seed in the samples collected in two white receiver fields with different local pollen density at increasing distances from the yellow-seeded-RR pollen source following eight cardinal directions

Distance (m)	Transect							
	NW	N	NE	E	SE	S	SW	W
Outcrossing (%)								
RPD^a								
1	24.4	26.9	26.1	41.8	58.8	64.2	55.7	26.2
10	1.1	4.3	1.4	5.7	10.5	12.1	8.5	1.0
35	0.1	0.4	0.8	0.9	3.3	2.0	0.9	0.02
100	0.03	0.05	0.07	0.07	0.4	0.2	0.04	0.02
150	0.02	0.06	0.02	0.03	0.09	0.1	0.01	0.02
200	0.03	–	0.01	0.01	0.06	–	0.02	0.02
250	–	–	0.01	0.01	–	–	–	–
NPD^a								
1	20.7	26.1	20.9	25.7	47.1	43.5	45.1	9.8
10	3.0	1.8	2.4	3.1	3.9	3.1	2.9	0.2
35	0.2	0.2	0.2	0.9	0.9	0.4	0.6	0.0
100	0.005	0.05	0.05	0.01	0.05	0.06	0.04	0.005
150	0.001	0.02	0.03	0.002	0.03	0.03	0.005	0.0
200	0.005	0.005	–	0.006	0.01	0.01	0.011	0.0
250	0.000	–	–	–	0.005	–	0.005	0.005

^a Field RPD is the detasseled field or low pollen density field; field NPD is the nondetasseled or high pollen-density field.

– No data collected

NPD field was 0.06% in the S transect. At 250 m from the source, the percentage of outcross decreased to 0.01% in the RPD field and 0.005% in the NPD field, but it never reached zero at the distances evaluated in this experiment. Because 60,000 kernels were sampled, the lower limit of outcrossing we could detect with 95% confidence was 0.005% (Remund et al. 2001). The results in Table 3 are the average outcrossing at each sampling distance and direction away from the nearest edge of the central source field; the raw data and not the averages presented here were used in modeling.

Wind speed and direction had a strong influence on the observed pattern of outcrossing at different distances and directions from the central pollen source. In 2003, the predominant wind direction during pollination was from N to NW (Fig. 2). This direction is unusual because, historically, the predominant summer wind directions in Iowa are from SE to SW (USDA-NRCS 2005). There was an exponential decline in the percentage of outcross with distance from the pollen source. At 35 m from the source plot, the percentage of outcross was less than 1% in both fields and all directions, with the exception of the S and SE transects in the RPD field. When local pollen density was lower, as in the RPD field, the percentage of outcross at 35 m downwind was approximately 2% (S) and 3% (SE) (data not shown). Thus, the impact of reduced pollen density on potential outcrossing significantly increased at

all distances from the pollen source. To facilitate visual interpretation of the data, we present the results as contour plots of logarithmic values of the observed outcross in Fig. 4a,b. Logarithmic values are used to enable visualization of both, the near-source and distant values. The percentage of outcross downwind (in the direction of the predominant winds during pollination) was greater than upwind (in the direction from which the predominant wind was blowing during pollination) in both fields. This increase

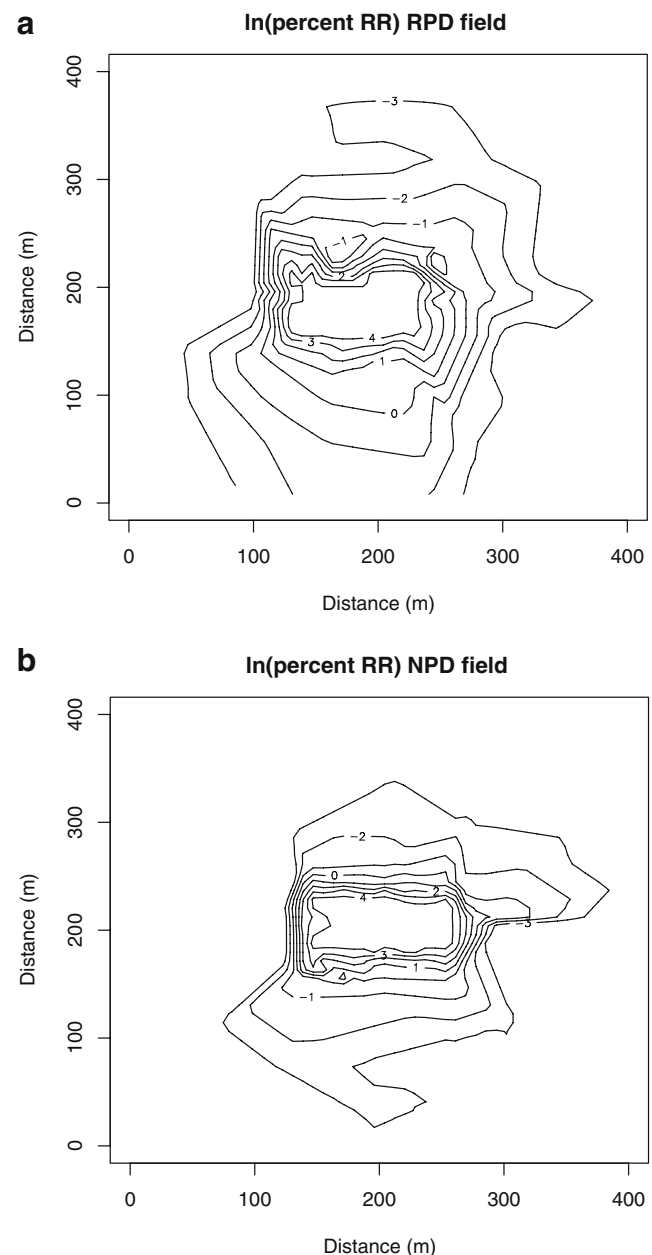


Fig. 4 Distribution of percentage of RR yellow seed outcrossing in two fields in Ankeny, Iowa, based on a natural logarithmic scale; **a** in a detasseled field RPD (reduced local pollen density); **b** in a nondetasseled field NPD (normal local pollen density). Distance on the x and y axes are in meters. Contour plots are based on the natural logarithm of the raw data

in the percentage of outcross was more pronounced, however, when the local pollen density was low (i.e., in the RPD field). Outcross levels in the RPD field were generally higher, especially downwind. Outcross at 200 m in the RPD field was as high as 0.12% at the SE and 0.20% at the S, whereas in the field NPD outcross was 0.01% at both the SE and S (data not shown). Lower local pollen density resulted in a significantly higher level of outcross in the RPD compared with the NPD at all distances away from the source plot, as determined by statistical comparison. Adventitious pollen density was similar in both RPD and NPD fields. Local pollen density, however, was lower in the RPD (70% of the NPD field), which resulted in a significantly higher outcross level at all distances and all directions from the pollen source plot.

Statistical comparison of outcrossing in the RPD and NPD fields

Our goal was to model the process of pollen dispersal through the information gathered on observed outcrossing percentages. Several statistical models based on the distance from the source plot, recorded wind direction and speed were considered. The statistical model that best fitted these data was used to compare outcrossing in the RPD and NPD fields. The exponential nature of this relationship agrees with previous studies (Jones and Brooks 1950; Aylor et al. 2003; Ma et al. 2004).

Initial data analysis indicated that the estimated percentage of yellow outcrossing counts $\hat{Y}_i$ were distributed as Poisson random variables (Neter et al. 1996) with mean μ_i :

$$Y \sim \text{Poisson}(\mu_i)$$

After reparametrizing the mean μ_i of the Poisson process as a function of the percentage of outcrossing λ_i (i.e. $\mu_i = \lambda_i t_i$) model diagnostics showed that the model that best fits the data was Goggi et al. (2006) given by,

$$\lambda_i = \exp(\beta_0 + \beta_1 d_i) + \beta_2 W_i$$

In this equation has an exponential component where d_i is the distance of a given location i from the source plot. The model also includes a linear component where W_i is a composite covariate, based on the hourly wind speed and direction during the peak shed period (28 July to 1 August) and recorded distance from the source plot:

$$W_i = \sum_{h=1}^H \frac{Z_{ih}}{d_i} I(i \in T(\text{direction}_h))$$

where Z_{ih} denoted the hourly wind speed at location i , for hour h , with H being the total number of hours for which the wind speed and direction were recorded during the pollination period. I is the indicator function. In other

Table 4 Parameter estimates of the model fitted to the data collected at all sampling locations and their corresponding standard errors

	Parameters ^a		
	β_0	β_1	β_2
Field RPD			
Estimate	−0.73	−0.25	0.004
SE	(0.006)	(0.003)	(0.0008)
Field NPD			
Estimate	−0.76	−0.54	0.003
SE	(0.010)	(0.010)	(0.000007)

^a β_0 is a scaling constant; β_1 is a parameter that takes into account the distance from the source plot; β_2 is a parameter that takes into account the wind speed, direction, and distance.

words, I controls which locations are included in the covariate W_i for a given wind speed and direction. Its value is 1 if a location lies in the tolerance region $T(\text{direction}_h)$, and 0 if the location is not in the tolerance region. $T(\text{direction}_h)$ denoted the tolerance region for wind direction recorded during the h -th hour. The tolerance region for the wind was constructed based on standard dispersal statistics for moderately unstable stratification (USEPA 2005). The basic idea behind the tolerance region was to include the downwind component in the statistical model: if a location is downwind from the source plot for a given hour it will receive more pollen than a location that is not downwind.

The model was fitted to data from both the RPD field and the NPD field. The estimated values of β_0 , β_1 , and β_2 are presented in Table 4. A graph of the resulting fitted values for each field superimposed on a scatterplot of the observed proportion of RR yellow seeds by distance from

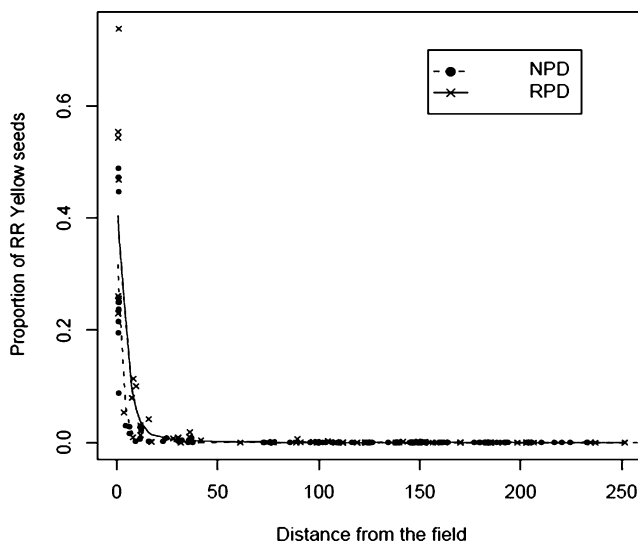


Fig. 5 Graphic representation of the model-fitted values of percent outcrossing for the field RPD and field NPD superimposed on a scatterplot of the proportion of observed adventitious RR yellow seeds by distance from the field

the field is presented in Fig. 5. Based on a likelihood ratio test, the models of outcrossing in the two fields were significantly different (observed P is 10^{-15} , i.e. practically 0). Results indicated that when local pollen is low, the incoming pollen has a competitive advantage and the level of outcross is significantly greater than when the local pollen is abundant. High production of local pollen (228.4×10^9 grains ha^{-1}) and close synchrony between pollen shed and silk exertion decreases the risk of outcross from incoming adventitious pollen.

Even though the proposed model captured the main behavior of the process, analysis of the residual plots and model diagnostics suggested it did not fit very well for the data collected at the locations farthest from the source plot. Based on these analyses and the differences in pollen dispersal patterns near the source plot and at greater distances from it, a second data analysis for the locations situated farther than 70 m from the field was performed. Model comparison suggested again that the model for λ_i described earlier fits these data best. Values of estimated β_0 , β_1 , and β_2 are presented in Table 5. Figure 6 displays the fitted values based on the alternative model for each of the two fields, superimposed on the scatterplot of the observed number of RR yellow seeds at large distances from the source plot. Likelihood ratio tests reinforced the conclusion that there were significant differences in model parameters between the two fields (observed P is 10^{-15} , i.e. practically 0). One interesting observation related to Fig. 6 was that the number of RR yellow seeds from the source plot in the RPD field exhibited more variable behavior than did the ones for the NPD field, with about 10% of the locations having extremely high values. If the local pollen density was high, the percentage of adventitious RR yellow grain decreased rapidly and remained low as the yellow pollen moved away from the source plot. If the local pollen density was low (70% of the NPD field), the percentage of adventitious RR

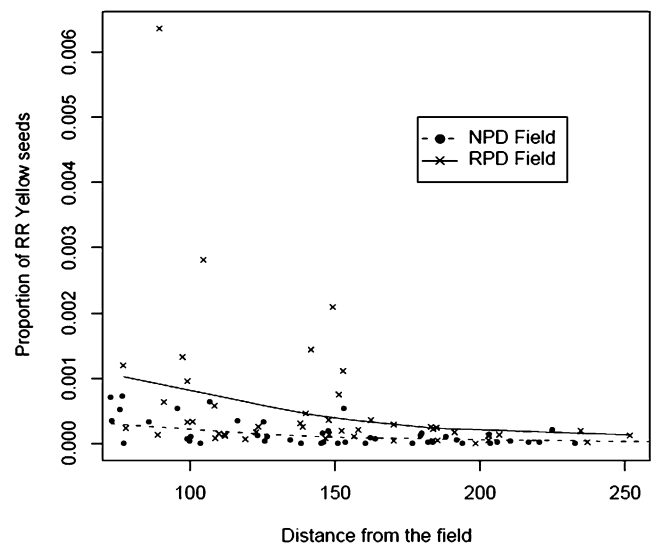


Fig. 6 Graphic representation of the model-fitted values of percentage of outcrossing for the field RPD and field NPDs at locations farther than 70 m from the source plot, and the superimposed scatterplot of the proportion of observed adventitious RR yellow seeds

yellow seed presence was significantly higher as pollen moved away from the source plot and levels fluctuated widely.

Discussion

Several studies have used seed color to trace outcrossing in maize (Paterniani and Stort 1974; Garcia et al. 1998; Jemison and Vayda 2001; Stevens et al. 2004). The yellow or white color in the maize endosperm is owed to a single gene ($y1$), with yellow being dominant over white (Weber 1994). We expected all progeny of the yellow pollen source to bear a yellow seed. However, external contamination from surrounding fields also can produce yellow seed. In our study, two additional genes, the RoundUp Ready (RR) gene and the Bt-Cry1Ab gene, were used to identify the seed resulting from an outcross with pollen from our source plot. This allowed us to monitor the adventitious seed using these three genes.

The results of our study are in agreement with those of Ma et al. (2004), Stevens et al. (2004), and Luna et al. (2001), where outcrossing frequencies were as low as 0.01% at 200 m from the pollen source. Our results, however, show the large impact of local pollen density on reducing the potential for outcrossing at these distances from an adventitious pollen source. Under the same environmental conditions, wind speed and direction, frequency of outcrossing was significantly higher in the reduced pollen density field.

Maize gene flow away from a central pollen source plot into surrounding maize differs with local pollen density. If the local pollen density is low, as in a typical seed production field, the chances for outcrossing are signifi-

Table 5 Parameter estimates of the model fitted to the data only from sampling locations farther than 70 m from the source plot and their corresponding standard errors

	Parameter ^a		
	β_0	β_1	β_2
Field RPD			
Estimate	-5.67	-0.02	0.002
SE	(0.177)	(0.001)	(0.0002)
Field NPD			
Estimate	-5.90	-0.03	0.0009
SE	(0.467)	(0.005)	(0.00009)

^a β_0 is a scaling constant; β_1 is a parameter that takes into account the distance from the source plot; β_2 is a parameter that takes into account the wind speed, direction, and distance.

cantly greater, and the chances of an outcross event are higher at greater distances from the pollen source. These results suggest that surrounding a transgenic maize field with a dense crop of the same height could minimize the chances of outcross into neighboring fields located beyond 200 m, while minimizing the incidence of outcross in the immediate field. Further research is needed to test this hypothesis.

Acknowledgements This research was funded by United States Department of Agriculture CSREES Biotechnology Risk Assessment Research Grants Program proposal 2003-04365, and by the Integrated Studies in Agroecosystems initiative sponsored under an endowment to the Department of Agronomy, Iowa State University.

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