Managing maize pollen dispersal and out-crossing

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Managing maize pollen dispersal and out-crossing

by

Juan Pablo Astini

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In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Program of Study Committee:
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To Elena, Roberto, Duky, Santiago and Pilar
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Abstract

Concerns about controlling pollen flow in maize (Zea mays L.) have greatly increased since the introduction of transgenes for insect resistance, herbicide resistance, and production of pharmaceutical and industrial compounds. The primary concern is related to out-crossing with non-transgenic genotypes destined for food uses or organic production. Pollen control is necessary to prevent the introduction of transgenes into sexually compatible crops or wild relatives in locations where these are grown or occur naturally (e.g., Native maize genotypes in Mexico).

The central hypothesis of this dissertation is that out-crossing resulting from pollen flow in maize is a predictable process, which can be simulated and quantified. The thesis is organized into three chapters. Chapter 2 describes a field study to test how a natural vegetative wind barrier might be used to limit pollen dispersal. In 10 independent tests, diminishing wind speed across the maize canopy resulted in a smaller pattern of pollen dispersal in the surrounding field. Chapter 3 presents the first attempt to predict out-crossing in a commercial hybrid seed production field. The analysis demonstrates that out-crossing can be predicted accurately based on inbred flowering dynamics and estimates of pollen dispersal. The field study presented in Chapter 4 tests the potential for predicting the spatial pattern of out-crossing from a transgenic pollen source. The results demonstrate the accuracy of the combined kernel set and pollen dispersal models as well as the benefit of surrounding the transgenic source with a non-transgenic maize crop producing abundant pollen.
Chapter 1

General Introduction

Introduction

Pollen flow in maize (Zea mays L.) has become a widespread concern recently due to the introduction of transgenic technology for generating insect resistance, herbicide resistance, and pharmaceutical and industrial compounds. Control of pollen dispersal may be necessary to prevent dissemination of transgenes into sexually compatible crops or wild relatives in locations where these are grown or occur naturally in the same vicinity as commercial production (Baltazar et al., 2005).

Maize is a monoecious plant, with staminate (male) flowers in an apical inflorescence commonly referred to as the tassel, and pistillate (female) flowers on lateral meristems. These develop into floral racemes commonly referred to as ears. Male and female flowers are physically separated, which ensures a high percentage of pollinations naturally occur between plants (out-crossing). As a result of this uncontrolled pollination, maize is considered an open pollinated crop (Poehlman, 1995).

Pollination in maize can occur only if pollen shed by the anthers on the tassel is captured by the stigmas of the pistillate flowers on the ear. Because the durations of pollen shed and silk receptivity are limited, close synchrony between pollen shed and silk emergence is required for high kernel set in the field and to
ensure minimum out-crossing (Bassetti and Westgate, 1994; Cárcova et al., 2000; Fonseca and Westgate, 2005). Maize pollen is 90 to 125 microns (µm) in diameter, spherical in shape (Jones and Newell, 1948), and weighs approximately 250 ng (Goss, 1968). A maize tassel will shed pollen for 2 to 10 days, depending on genotype and environmental conditions. Daily pollen release depends on moisture and temperature conditions, but it will generally last 4 to 5 hours, starting approximately one hour after sunrise (Flottum et al., 1984, Fonseca and Westgate, 2005). Reported values of 20 to 42 million grains per tassel for old cultivars (Hall et al., 1982; Sadras et al., 1985) contrast with more recent values of 9.6 to 11.3 million grains (Uribelarrea et al., 2002) and 2.2 to 3.3 million grains (Fonseca et al., 2002) observed for modern hybrids. These natural flowering dynamics, general receptivity of female flowers to pollen from any source, and extensive pollen production per plant, contribute to the natural tendency for out-crossing in maize.

The benefit that out-crossing represents for seed production conflicts with the necessity of limiting gene flow and control of genetic purity in harvested seed. It also limits the success of grain producers motivated to achieve market demand for genetically pure products and those not containing transgenes.

Numerous studies have documented the consequences of pollen movement on gene flow in maize. Garcia et al. (1998) reported gene flow from commercial cultivars of maize to landraces and teosinte growing in farming areas of Mexico. They concluded that 185 m of spatial isolation would be necessary to prevent out-crossing associated with pollen dissemination from a transgenic
maize field. These authors argued that the introduction of transgenes would have a negative effect on the genetic diversity of these landraces populations.

Numerous studies have documented the occurrence of out-crossing associated with pollen movement from improved transgenic maize hybrids to non-transgenic genotypes in grain production fields (Luna et al., 2001; Bellon and Risopoulous, 2001; Baltazar et al., 2005; Ma et al., 2004; Lang et al., 2004; Halsey et al., 2005; Jaroz et al., 2005). Results vary in the spatial and temporal isolation required to ensure genetically-pure (transgene-free) seed in a crop planted in proximity to transgenic maize. Without temporal isolation, 200 m were required; with 14 days of temporal isolation between flowering periods, isolation distance could be decreased to 62 m (Halsey et al., 2005). Burris (2001) and Ireland (2006) analyzed the extent of out-crossing reported in over 350 commercial seed production fields. Their analysis indicated, among other things, that the level of out-crossing varied with the size of the seed field, its proximity to an adventitious (external) pollen source, and the level of pollen production within the field.

Seed production companies use several practices to diminish out-crossing in hybrid seed production. These practices include crop rotation, high purity parent seed, mechanical and/or manual detasseling of the female inbred, temporal or physical isolation from maize in nearby fields, and inclusion of border rows of the male parent around the field. Although costly and arduous, these strategies do not always limit out-crossing to desired levels (Ireland et al., 2006). Production of genetically pure seed could be less than desired for several reasons. First, only a fraction of the field population is permitted to shed pollen
(male inbred); seed companies commonly use one male to four female ratios (Ireland et al., 2006). Second, pollen shed density is often less than in a grain field since inbreds typically produce less pollen per plant than do hybrid plants. Third, a major goal in hybrid seed production is to reduce the area dedicated to male rows as much as possible without decreasing the number of kernels harvested per area (Wych, 1988). Finally, pollen shed and silk exsertion on physically separated plants increases the probability of floral asynchrony. Considering these challenges for hybrid seed production, the high potential for out-crossing is not surprising.

Past management approaches to minimize outcrossing have relied primarily on physical and temporal isolation (Luna et al., 2001; Halsey et al., 2005; Ireland et al., 2006). They have not taken full advantage, however, of the predictable nature of maize flowering biology and the physical nature of pollen dispersal in the atmosphere. Lizaso, et al. (2003), for example, developed a flowering model to simulate kernel set based on pollen shed and silking dynamics. This model has recently been incorporated into CERES-maize and dramatically improved the accuracy of its yield predictions (Lizaso et al., 2007). Likewise, Arritt et al., (2007) have developed a lagrangian particle dispersion model to simulate pollen movement from an isolated source field. Combining these models has obvious applicability for simulating out-crossing under commercial field conditions. To that end, we devised several field studies to generate ‘ground truthing’ data for model testing. By necessity, these involve
large scale field trials and experimental procedures designed to modify pollen dispersal.

The objective of the first field study was to determine whether a vegetative wind barrier of sorghum sudangrass surrounding an isolated maize plot would disturb pollen movement sufficiently to limit the extent of pollen dispersal. This experiment was conducted in 2005 and 2006 under standard agronomic practice on a farm in Northwestern Iowa. Both years, one-hectare plots of dwarf maize were planted in the center of two soybean fields of approximately 260 ha in size. In one field, sorghum sudangrass bordered the maize plot as a vegetative wind barrier. The goal was to have a border of sorghum tall enough to decrease wind speed through the maize plot in an attempt to limit pollen dispersal. Pollen production and dispersal were monitored with passive traps distributed inside the maize plot and up to 300 meters in the soybean field along and between cardinal transects (N, NE, E, SE, S, SW, W and NW). Towers also were located in the center, south and north of each maize plot to capture pollen up to 16 m above the ground. The distance reached by pollen grains was greater from the central plot without a wind barrier. In this case, pollen grains were found up to 300 m from the plot. In the field with the sorghum sudangrass border, pollen grains were found up to 160 m away. In both cases, most of the pollen remained within the center plot. These results indicate that using a wind barrier of sorghum sudangrass could be an effective means to limit pollen movement from an isolated plot of maize. This could have important implications for managing maize isolation for genetic purity or pharmaceutical production.
The objective of the second study was to simulate the level of out-crossing resulting from adventitious pollen entering a hybrid seed field having a range of anthesis silking intervals (ASIs) between the male and female inbreds. This experiment was conducted in 2004 in cooperation with Syngenta Seeds Inc. on a commercial seed production field in Southeast Iowa. Female inbred blocks were sown at three planting dates around the sowing date for the male inbred to achieve a range of flowering asynchronies. Sampling stations within the seed field were 100 m and 170 m away from a commercial maize field, which served as a source of adventitious pollen. As expected, the observed level of out-crossing increased and the seed yield decreased with increasing ASI between the male and female inbreds. The levels of out-crossing at 16 locations within the seed field were predicted accurately using the combined kernel set (Lizaso et al., 2003) and pollen dispersal models (Arritt et al., 2007). The results of this research demonstrate for the first time that the observed level of out-crossing in the seed production field can be accurately predicted from the flowering dynamics of the inbred parents and the physical modeling of pollen dispersal.

The objective of the third project was to simulate out-crossing resulting from adventitious transgenic pollen entering an adjacent maize field with pollen densities typical of grain or hybrid seed production. This study was carried out in 2003 and 2004, in Ankeny, Iowa. In 2003, two fields of approximately 36 hectares were planted with non-transgenic white maize. Each white maize field had one central hectare of yellow, RoundUp Ready™, Bt maize planted as an adventitious source of pollen. One field was managed for normal grain production
(not de-tasseled). The second field was managed as a hybrid seed production field (detasseled to a 4:1 female: male row ratio to reduce local pollen density). The field trial with normal grain production was repeated in 2004. Ear samples were harvested following eight cardinal transects (N, NE, E, SE, S, SW, W and NW) up to 250 meters. The extent of out-crossing with distance from the central plot was similar for grain production fields both years. The extent of out-crossing was greater both in distance and amount in the field simulating hybrid seed production. This result was attributable to the lesser pollen density in the detasselled seed production field. Predicted out-crossing values modeled from flowering dynamics of the yellow and white hybrids and pollen dispersal from the yellow, Bt/RR maize plot where highly accurate at distances farther than 35m from the transgenic pollen source. At distance within 35m, imprecise predictions of local pollen density limited the accuracy of predicted out-crossing.

References


Chapter 2

Effectiveness of a Vegetative Wind Break on Maize Pollen Dispersal

*A manuscript to be submitted to Crop Management*

Juan P. Astini, Mark Westgate, Susana Goggi and Raymond Arritt

Abstract

Maize (*Zea mays* L.) pollen is an airborne particle and its dispersal is highly dependent upon wind patterns and turbulence within and above the canopy. The U.S. Animal and Plant Health Inspection Service (APHIS) currently requires transgenic maize crops producing non-approved traits or pharmaceutics to be isolated within a fallow area surrounded by a non-maize crop. This open field design may increase the potential for pollen escape to neighboring fields. Our objective was to determine whether a natural vegetative wind break could be used as a means to limit pollen flow from an isolated maize field established according to APHIS isolation requirements. Two maize plots (approx 1 ha each) were established within 260-ha soybean (*Glycine max* L. Merrill) fields. In one case, a border of sorghum sudangrass (*Sorghum bicolor* L. Moench) was planted around the maize to alter the wind patterns in and around the maize canopy. At anthesis, the sorghum wind break was approximately 1 m taller than the corn and
decreased average wind speed within the maize canopy by 1 m s\(^{-1}\). The wind break significantly altered the pattern of pollen dispersal, compared to the maize plot without the wind break. Without the wind break, pollen grains were detected up to 300 m downwind from the source. With the wind break, the maximum distance reached by pollen was 160 m. In both fields, however, most of the pollen grains were deposited within the maize canopy. These results indicate that a vegetative wind break can reduce pollen dispersal from an isolated stand of maize. Further studies are needed to define the optimum height and density of vegetation for this purpose.

**Introduction**

The development of transgenic crops as production platforms for pharmaceutical and industrial compounds will depend largely on the success of efforts to confine the transgenes and their expressed proteins in field environments. The potential for loss of gene confinement has gained great interest since the introduction of transgenic genotypes in commercial production, particularly for the hybrid seed industry and biotech companies seeking to use maize for production of industrial and pharmaceutical compounds (Rogers, 2005). The rapid expansion of transgenic maize in commercial production has raised concern particularly in areas where maize landraces are grown (Lavigne et al., 2002; Ma et al., 2004; Baltazar et al., 2005; Messean et al., 2006; Weber et al., 2006), and where organic maize production is expanding. Loss of gene confinement is of particular concern during field testing of unapproved transgenic
events (Ma et al., 2004). Temporal and spatial isolation along with detasseling are the primary methods used to control pollen flow from these crops.

The maize plant is monoecious, i.e. the male and female flowers are physically separated. Maize pollen is relatively large (90 to 125 microns in diameter) and heavy ($247 \times 10^{-9}$ g). It quickly reaches a terminal fall velocity, which explains in large part, why maize plants are mostly pollinated by wind and gravity (anemophilous) (Jones and Newell, 1948). As such, a high degree of pollination occurs naturally between plants, and maize is generally considered an open pollinated crop (Poehlman, 1995). A high potential for out-crossing between plants is advantageous for breeding, but conflicts with the need to confine transgenic pollen and limit the potential for gene flow to non-transgenic genotypes. It also limits the ability of grain producers to meet market demand for genetically pure transgene free products.

To some extent, the literature contains contradictory results about the pattern of pollen dispersal to be expected from an isolated maize plot. Abundant evidence indicates that the vast majority of pollen shed from maize plants remains within or in close proximity to the field (Raynor et al., 1972; Emberlin et al., 1999; Goggi et al., 2006; Goggi et al., 2007). This would be expected as the dispersal of maize pollen is affected by its large size and rapid settling rate. But there is general agreement that the typical downwind dispersal pattern of pollen by the airflow in low to moderate wind speeds results in an exponential decrease of pollen deposition with distance from the source (Goggi et al., 2006; Luna et al., 2001; Jarosz et al., 2005). As such, a number of empirical models have been
developed using wind speed and direction to simulate pollen dispersal or out-crossing downwind (Raynor et al., 1972; Halsey et al., 2005; Goggi et al., 2006; Goggi et al., 2007). Since these models are empirically developed and do not incorporate flowering dynamics, however, they can not predict the level of out-crossing expected from pollen dispersal.

Under favorable atmospheric conditions, pollen grains can travel long distances on the airflow. If the pollen is not viable, it is not a problem related to out-crossing (Messeguer et al., 2006). Loss of pollen is not an issue in our experiments, however, because pollen is transported within minutes to 300 m. Jones and Brooks (1950) reported out-crossing levels greater than to 0.5% at 500 m from the pollen source. In chapter 3, we reported out-crossing values in a seed production field of 6.15% at 100 m and 18% at 170 m from an adventitious pollen source.

Numerous studies have shown that wind breaks can decrease wind speed on the lee side of the barrier, depending on height, width and barrier porosity (Mader et al., 1999; Heiligmann et al., 2006). The downwind area that a vegetative wind break will affect depends on its height, density and porosity. As explained above, pollen dispersal is correlated with wind speed, direction and turbulence. In this project, we are concerned about the impact of a windbreak on the short distance pollen movement where wind speed plays a major role in the pattern of dispersal (Brookes et al., 2004; Halsey et al., 2005; Goggi et al., 2006). To our knowledge, there are no reports demonstrating an effective means of containing maize pollen within of near the source where it was produced.
Therefore, the objective of this project was to investigate the effectiveness of a vegetative wind break surrounding a maize plot for limiting maize pollen dispersal. Sorghum sudangrass was selected as a test windbreak because of the biomass production and potential height difference with the maize canopy. The trial was conducted under field conditions to ensure its relevance for confining pollen dispersal from transgenic maize produced on a commercial scale.

**Material and Methods**

The research was conducted in 2005 and 2006 near Rockwell City, in Northwest Iowa on two field sites located within 2 km of each other. At each site, approximately 1 ha of maize was planted in the center of a 260-ha soybean field. In one case, the maize plot was surrounded by a 7m border of sorghum sudangrass (Fig. 1); in the other field, the maize plot was immediately adjacent to the soybean plants.

Commercial soybean cultivars (MG II) were planted with a grain drill in 24-cm rows (20 May, 2005 and 22 May, 2006) at $350 \times 10^3$ seeds ha$^{-1}$. The canopy reached an average plant height of approximately 0.8 m each year. Uniform closed canopy was achieved in these fields each year, generating a uniform surface for pollen dispersal into the field.

Center maize plots were planted with a dwarf, open pollinated variety (D-G-M PD 2 Dwarf, Nature’s Own Seed Mixtures, Mantano, IL) on 3 June, 2005 and 27 May, 2006. Surrounding maize fields in the area were planted before May 15, ensuring at least 12 days of asynchrony between dwarf maize center plot and
these commercial maize fields. The dwarf variety was selected due to its short stature (average 1.6 m); long pollen shed duration (20 d), and prolific pollen production (approx $7 \times 10^6$ grains per plant). Final plant density in the center plot both years was approximately $6.8 \times 10^4$ plants ha$^{-1}$; distance between rows was 0.76 m. Standard production practices were applied to achieve vigorous plant growth. Variation among plants for plant height, flowering time, and pollen production typical of an open pollinated variety resulted in a long period for pollen shed, which was beneficial to the objectives of this study.

Nutri+Plus BMR sorghum sudangrass (Wolf River Valley Seeds Co., White Lake, WI) was used to create a vegetative wind break around one of the central maize plots each year. This hybrid was selected because of its potential height and biomass production under Iowa weather conditions. The border was seven meters wide seeded at 223 seeds m$^{-2}$ (20.3 kg ha$^{-1}$). The soil was fertilized with 250 kg Urea ha$^{-1}$ to enhance vegetative growth. The sorghum sudangrass hybrid was planted 15 days before the maize plot to establish a maximum height differential during maize pollen shed. The average height difference between the dwarf maize and sorghum windbreak at anthesis was 1 m.

Passive pollen traps were used to monitor pollen deposition within the maize plots and throughout the surrounding soybean fields up to 300 m from the central plot. Nine sampling stations were positioned within the maize plot to document the daily intensity of pollen shed (Fig. 1). Traps were located between rows at ear height (0.9 m). Pollen shed was monitored on 6 days in 2005 and 4 days in 2006. In the surrounding soybean field, 144 pollen trap stations were
positioned along 16 cardinal transects (N, NNE, NE, etc.) at 1, 3, 5, 15, 25, 40, 60, 90, 120, 160, 220 and 300 m from the central plot (Fig. 1). The sampling station platforms positioned the pollen traps at the top of the soybean canopy. Traps were placed in the field between 0630 and 0800 each sampling day. Pollen traps were removed between 1630 and 1830 and covered immediately with a protective cover to avoid collecting extraneous pollen during transport and storage prior to counting.

Pollen traps used for pollen collection were of the type described by Bassetti and Westgate (1994). This method does not affect normal tassel development and provides information relevant to pollen deposition per unit land area. The traps were supported in clear plastic bases (11 by 11 cm) mounted on plastic coated metal stakes. The pollen traps were constructed on a plastic base of white high impact polystyrene sheeting (7 by 9 cm) (Fig. 2A). Two bands of 2 cm wide smooth, black tape (Super 88-3M Scotch Brand, St. Paul, MN) were placed across the white base to produce a high contrast background for imaging (Area = 36 cm²). The black tape was covered with transparent double coated tape (666-3M Scotch Brand). The double sided tape was protected by a white liner, which was removed to expose the sticky surface when the trap was positioned in the sampling station.

An attempt was made to monitor pollen movement above the corn canopy using passive pollen traps mounted on towers located at the South end, North end, and in the middle of the maize plots (Fig. 1). Eight round pollen traps (Fig. 2B) per tower were positioned at 4, 8, 12 and 16 m above the ground. Round
traps were constructed on the same white plastic base used for flat traps, with
two bands of 2 cm wide smooth, black tape generating an area equal to 84 cm².
The black tape as well was covered with transparent double coated tape. Traps were attached to an aluminum cylinder (237 cm³; Fig. 2B).

Pollen collected by the passive traps was analyzed according to Fonseca et al. (2002). Fluorescence images of pollen adhering to the traps were collected with a Nikon Eclipse 200 EPI-Fluorescence microscope equipped with a Nikon Plan Fluor 4X/0.13 NA objective lens (Fryer Company, Huntley, IL). Typically, eight 0.25 cm² images were collected from each trap, and 20 images were collected from the round traps. In both cases, all images collected and saved were from different positions on the trap. The images were saved in tif file format and later analyzed. Traps with less than 30 pollen grains per image were counted manually with an image viewer program (Windows Pictures and Fax Viewer). For traps with pollen density greater than 30 pollen grains per image, Metamorph Imaging System (Universal Imaging Corporation, West Chester, PA) was used for the analysis. Average pollen grain number from the total number of images from each trap was calculated for each sampling station.

Distance between the farthest sampling station from the central plot and the nearest neighboring commercial maize field was greater than 500 m. Nonetheless, neighboring maize fields were carefully examined for evidence of plants shedding pollen. None were found shedding pollen coincident with pollen shed from the dwarf corn in the central plots. Thus, temporal and spatial isolation ensured pollen observed on the pollen traps originated from the central plot of
dwarf maize. In 2005, the field with the sorghum sudangrass wind break had volunteer corn in the surrounding soybean field. These plants were manually removed from the field prior to pollen shed to eliminate them as potential pollen sources.

Weather data were collected throughout the flowering period by mobile weather stations placed in the center plot and within the soybean field near the central plot. A third weather station was located 300 m to the south of the central plot to avoid potential turbulence generated by maize and sorghum. Wind speed and direction data were averaged and stored every 15 minutes throughout the pollen shed period using a Campbell Scientific CR10 data logger.

After analyzing data from 2005, we confirmed that no pollen was detected at sampling stations located far upwind of the central plot. Therefore, in 2006 we limited upwind sampling to those stations located within 10 m of the central plot. All sampling station located down wind and along lateral transects were used to measure pollen movement.

Data were analyzed with GraphPad Prism. Prism fit the pollen dispersal data for both fields with exponential decay equation (Eq. 1) and compared the confident interval of the parameter involved in the equation using an F test at the 0.05 level of significance.

**Results and Discussion**

As expected, the vegetative wind break generated by the sorghum sudangrass reduced wind speed within the central plots. Average wind speed in
the central plot surrounded by sorghum sudangrass was 1 m s$^{-1}$ slower than in the plot without the wind break. Gusts up to 8.1 m s$^{-1}$ were recorded in the plot without sorghum, whereas the maximum wind speed reached was 5.5 m s$^{-1}$ in the plot with sorghum sudangrass. The difference observed in the wind speed between plots translated to an observable difference in pollen dispersal patterns.

Both fields (with/without vegetative wind break) were always sampled on the same dates to ensure a direct comparison in response to pollen shed intensity and local weather conditions. Figure 3 shows that pollen shed for both fields was synchronized and pollen shed amounts were nearly identical. In 2006, the peak of pollen production was less than in 2005, but shedding time was longer. Therefore, total pollen shed was similar for both years.

Sampling both fields on the same day was challenging but essential to compare both cases under the same weather conditions. Wind speed and direction, which are important climatic factors affecting pollen dispersal, varied every day as did the relative humidity. On days with high relative humidity in the morning, the anthers start releasing pollen later than on days with lower relative humidity (Westgate and Arritt, unpublished data). These subtle weather factors, coupled with the variation in pollen shed density, affected the timing and intensity of pollen dispersal. As such, each day of pollen collection was considered unique for analysis purposes.

Figure 4 shows the variation in pollen dispersal from fields with and without a vegetative wind break. As has been observed in other studies, most of the pollen shed in the central plots settled there, and pollen deposition decreased
exponentially with distance from the pollen source (Raynor et al., 1972; Emberlin et al., 1999; Goggi et al., 2006; Goggi et al., 2007). The presence of a windbreak, however, had a significant impact on the observed pattern of pollen dispersal. No pollen grains were found at sampling stations at 220 m from the central plot in fields with the wind break. Pollen grains were observed at 160 m. In the field without the wind break, however, pollen grains were captured up to 300 m from the pollen source. In most cases, more pollen grains were observed outside the central plot in the fields without wind break, compared to those surrounded by sorghum sudangrass.

Comparing sampling dates with different wind speeds demonstrates how important the effect of wind speed and direction were on pollen dispersal. Days with wind coming from a consistent direction had a greater probability of dispersing pollen grains farther from the source plot. Days with variable wind direction generated greater pollen dispersal at shorter distance from the source and less at farther distances. An exponential decay equation (Eq. 1) was fit to the pollen dispersal data from each field. The equation was selected due to its high $R^2$ value and low number of parameters involved.

$$y = a + b/x^{0.5} \quad (Eq. 1)$$

Confidence intervals of equation parameters generated for each field were compared with an F test using Graphpad Prism to establish whether the dispersal pattern with the vegetative wind break was statistically different from
that without the wind break. The results of this analysis, listed in Table 1, indicate that surrounding the maize plot with a vegetative windbreak significantly decreased pollen dispersal on every sampling date.

Figure 5 illustrates the surface patterns of pollen dispersal obtained with and without the sorghum sudangrass windbreak. On this sampling date, winds were steady and predominantly from the North. Wind speed for most of the shedding period (0800 to 1800 h) exceeded 2 m s\(^{-1}\). As expected, pollen dispersal was predominately to the south of the central plot. Yet most of the pollen was deposited within the plot, despite the steady winds during pollen shed. The presence of the windbreak altered the pollen dispersal pattern in the field. Pollen was dispersed over a smaller area, pollen dispersal decreased immediately down wind of the plot, and more pollen was dispersed upwind. Evidently, diminishing the wind speed across the maize plot decreased the potential for pollen dispersal in the immediate vicinity of the central plot. The impact of the windbreak on pollen dispersal patterns for the other nine sampling dates was similar to that illustrated in Figure 5 (see Appendix A). In all cases, the area where pollen grains were captured was larger in the field without the sorghum sudangrass wind break.

The presence of the taller wind break could potentially increase turbulence over the maize plot, generating uplift of pollen. Major quantities of pollen deposition upwind as well as a band of pollen deposition downwind support the possibility of increasing turbulence caused by the taller sorghum. Further study
is needed to explore the potential effects of this added turbulence for long-distance transport of pollen from the central plot.

Since pollen dispersal data have been generally related to wind speed and directions, we examined the 10 days of field data for a common pattern in pollen dispersal related to wind speed and/or direction. As expected, most of the pollen leaving the central plot was deposited in the downwind direction. In general, pollen dispersal downwind was related to the daily average wind speed, but no consistent pattern was apparent. This is probably attributable to the variability in wind speed and direction during the day and among the sampling dates. Pollen dispersal was related, however, to the maximum wind speed during pollen shed (0800 to 1800 h). Sampling dates with high wind speed had more pollen deposition at farther distances from the maize pollen source than did dates with slower wind speed. The presence of a vegetative wind break was effective in generating smaller patterns of pollen dispersion across the entire range of wind conditions encountered in this study.

A few pollen grains were captured by the traps positioned on the towers located in and near the maize plot. Pollen was detected at 12 m at the north and south edges of the plot in the fields with and without the sorghum windbreak both years. Pollen was detected at 8 m in the center of the maize plot, but not at 12 or 16 m. While these results are not quantitative, they do indicate pollen is elevated well above the source level (about 1.5 m), which is prerequisite for dispersal and long-distance transport. Further studies using more sophisticated methods to quantify pollen movement above the canopy are warranted. -
Summary

To our knowledge, this study demonstrates for first time that a vegetative wind break can be an effective means of decreasing pollen flow from an isolated maize canopy. Pollen dispersal pattern was clearly modified with a natural wind break of sorghum sudangrass 1 m taller than the maize plants it surrounded. Wind break height, width and porosity will affect wind speed, which is main factor affecting pollen dispersal. This study specifically shows a vegetative wind break 7 m in width and 2.6 m tall can shorten the maximum pollen dispersal distance from greater than 300 m to less than 220 m. It also decreases the level of pollen deposition throughout the surrounding field. These results necessarily reflect the weather conditions encountered and field dimensions (both of which were typical of corn production in central Iowa). Nonetheless, the results suggest that the addition of a vegetative wind break could decrease the distances required for field isolation of maize containing genes for non-approved transgenic traits. (APHIS requires an isolation distance of 1600 m and a temporal isolation of 28 day at planting date for pharmaceuticals traits). Further study should be directed towards defining the optimum physical characteristics of such wind breaks and exploring the vegetative resources to meet these characteristics.

Acknowledgements

A special recognition to Horan BioProduction, Inc. for providing the opportunity to conduct this study in their fields.
References


Table 1: Equation parameters for data from both years comparing pollen dispersal with and without vegetative wind break. Equation fitted; $y = a + b/x^{0.5}$. The presence of a windbreak resulted in a significantly different pattern on pollen dispersal at all samplings. GraphPad Prism was used for comparing data sets from both fields.

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Figure 1: Field map showing pollen sampling locations. A 1 ha plot of dwarf maize was located in the center of the field as the pollen source. The entire area surrounding the central plot was planted to soybeans. Sampling stations in the soybean field were located at 1, 3, 5, 15, 25, 40, 60, 90, 120, 160, 220 and 300 m from the central plot along cardinal transects. The field shown has a sorghum sudangrass wind break surrounding the dwarf maize plot. Not drawn to scale.
Figure 2: Passive pollen traps placed in the field from 0800 to 1600 h each sampling day. A: Flat pollen trap placed on transparent plastic base located in the central plot and throughout the surrounding soybean field. B: Round pollen traps positioned on towers 4, 8, 12 and 16 m above the ground.
Figure 3: Pollen shed dynamics in A: 2005 B: 2006. Data are the average of nine pollen traps located with the dwarf maize plot.
Pollen (grains cm\(^{-2}\))

Distance from source (m)

Wind speed (m s\(^{-1}\))

- Non-sorghum
- Sorghum
- Regression

A

P = 0.0105

P < 0.0001

P = 0.0002

P < 0.0001

P < 0.0001

P < 0.0001
Figure 4: Pollen dispersal for ten sampling days. A: Distance reached by pollen grains in the field without sorghum sudangrass wind break. B: Distance reached by maize pollen grains in the field with wind break. Wind rose for sampling dates when dispersal was measured from 0800 to 1800 h. Pollen data are pooled for all sampling stations throughout the field. P value from comparison between equations parameters ($y = a + b/x^{0.5}$).
Figure 5: Surface displacement of measured pollen dispersal on one sampling date with uniform wind direction Z values are a log scale expressed as grains cm$^{-2}$. Wind rose data are for 0800 to 1800 h. A: maize plot surrounded by sorghum sudangrass wind break. B: no wind break. More sampling dates in Appendix A.
Chapter 3
Predicting Out-Crossing in Maize Hybrid Seed Production

_A manuscript to be submitted to Field Crop Research_

Juan P. Astini, Agustin Fonseca, Craig Clark, Jon Lizaso, Mark Westgate and Raymond Arritt.

Abstract

In hybrid seed production, controlling pollination of the female inbred is critical to achieve maximum kernel set and high levels of genetic purity. Although kernel set associated with inbred flowering dynamics is fairly predictable, it has not been possible to predict the level of out-crossing resulting from adventitious pollen entering the seed field. Our objective, therefore, was to combine our kernel set model, which calculates kernel numbers formed from inbred flowering dynamics, with a new lagrangian pollen dispersal model, which calculates pollen drift based on local weather conditions and pollen physical properties. Male and female flowering synchrony was varied to provide a wide range for risk of out-crossing. Seed yields varied from 13.4x10^6 to 24.5x10^6 kernels ha^-1 and measured out-crossing varied from 0.5 to 20% as identified using molecular markers. The kernel set model accurately simulated kernel set within the seed field (R^2 = 0.83; RMSE = 0.3 x 10^6), and percent out-crossing (R^2 = 0.78; RMSE
= 0.8) from flowering dynamics and estimates of adventitious pollen density provided by the pollen dispersal model. Combining the kernel set and pollen dispersal models provides a novel quantitative approach for defining optimum management strategies for seed production and genetic purity.

**Introduction**

In maize (*Zea mays* L.) hybrid seed production, controlling pollination of the female inbred is critical to achieve maximum kernel set as well as high levels of genetic purity. The latter has become increasingly important with the introduction of transgenic hybrids, and numerous studies have documented the potential for out-crossing associated with pollen dispersal from transgenic maize (Doebley, 1990; Burris, 2001; Luna et al., 2001; Brunet et al., 2003; Jarosz et al., 2003; Ma et al., 2004; Stevens et al., 2004; Dupont et al., 2005; Halsey et al., 2005).

Maize is normally cross-pollinated and crosses freely with most members of the genus *Zea* (Burris, 2001). Most pollinations result from pollen transported by wind or gravity (anemophilous), but there are reports of pollination carried out by bees and other insects (Emberlin et al., 1999). Maize pollen has particular characteristics that facilitate out-crossing of nearby plants. Maize produces one of the largest pollen grains among the grass family (90 to 125 microns in diameter; Smith, 1990). Maize pollen grains are spherical to ovoid in shape with a slightly protruding aperture (Erdtman, 1952). Pollen volume is approximately $700 \times 10^{-9}$ cm$^3$ with a weight of $250 \times 10^{-9}$ g (Goss, 1968). Even though maize
pollen is disseminated primarily by wind and gravity, the relatively large maize pollen grains normally travel only a short distance compared to pollen from other members of the grass family (Poaceae). Pollen viability also is an important aspect of pollination potential. Reported values vary considerably (1 to 24 h) depending on the genetics and methods used to determine viability (Jones and Newell, 1948; Aylor, 2003; Aylor, 2004; Fonseca and Westgate, 2005). It is safe to assume that pollen shed from the tassel remains viable for several hours under favorable weather conditions (Jones and Newell, 1948). In controlled atmosphere studies, viability is negatively affected by elevated temperatures and reduced relative humidity, although elevated temperature appears to result in a more rapid decline than low relative humidity (Aylor et al., 2003; Fonseca and Westgate, 2003). Pollen exposed to ambient field conditions (20 to 25 °C and 60 to 80% RH) decreased viability to 80% in one hour and to 0% within two hours (Luna et al., 2001). While the distance that pollen can travel is not well defined, the determination of viability is even less well defined. Many investigators have used pollen tube germination in sucrose media, while others have used the development of the extended pollen tube to determine growth into sucrose-agar media (Fonseca et al., 2005). Pollen viability is of great importance for out-crossing values; under conditions where pollen grain viability decrease rapidly, there is less possibility of obtaining out-crossing at greater distances from the pollen source.

Most reported cases of out-crossing involve hybrid seed production where kernel set by the female inbred is limited by pollen production by the male inbred.
Typically, the planting pattern in seed fields has 80% of male sterile or emasculated plants. In some cases as much as 86% of the plants do not produce pollen. Other disadvantages are the quantity of pollen produced per plant; inbreds produce less pollen compared to hybrids. Inbred lines have less vigor than hybrids, generating smaller plants and smaller tassels, which contribute to the limited amount of pollen typically produced. Together, these factors increase the likelihood that out-crossing will occur if an adventitious pollen source is present. Whether or not the level of out-crossing exceeds the 0.5% threshold for transgenes imposed by industry (Burris, 2001; Ireland et al., 2006) depends on the flowering dynamics of the inbred pair and the density of adventitious pollen entering the seed field. Fortunately, both of these components of maize pollination can be accurately simulated (Lizaso et al., 2003; Arritt et al., 2007).

Several plant growth models have been developed to simulate yield formation in maize (Jones and Kiniry, 1986; Keatim et al., 2003; Yang et al., 2004; Lizaso et al., 2007). Of these, only Lizaso et al. (2003) simulates kernel set directly from flowering dynamics, which is essential for addressing the problem of predicting out-crossing. The model of Lizaso et al. (2003) develops temporal profiles of pollen shed and silk exsertion, and then calculates a daily kernel set based on published pollination efficiencies (Bassetti and Westgate, 1994). This model also calculates the number of exposed silks that remain unpollinated and at risk of out-crossing (Fonseca et al., 2004). The proportion of these silks that become out-crossed can be calculated, if the density of adventitious pollen entering the seed field is known.
Numerous models have been developed to simulate downwind patterns of pollen deposition (Fyfe, 2005; Gustafson et al., 2005; Schueler and Heinke al., 2006). These models typically are developed empirically by adjusting equation parameters to match the data observed. Others have used physical principles to move and track pollen movement (Jaroasz et al., 2004; Aylor and Boehm, 2006; Arritt at al., 2007). Arritt et al. (2007) developed a three-dimensional Lagrangian random flight model constructed for numerical simulation of maize pollen dispersal. The model simulates the path of particles, in this case individual pollen grains. The particle motion is determined by the mean flow, wind velocity, and a quasi-random turbulent component. The Lagrangian process is adopted because of its generality and flexibility. It also incorporates the environmental conditions through which the pollen moves on its journey from tassel to silk. As such, the physical effects of wind and turbulence on pollen dispersion are considered together with the biological aspects of pollen release and viability. Predictions of pollen dispersal by the Lagrangian model compare well to field observations and to results generated by a standard Gaussian plume model (Arritt et al., 2007).

Our intention was to predict both kernel production per hectare and percent out-crossing resulting from adventitious pollen entering a hybrid production field. We did so by coupling a kernel set model of Lizaso et al. (2003) with the Lagrangian particle dispersal model of Arritt et al. (2007). The experiment was conducted in a commercial seed field to ensure its relevance to current hybrid seed production practices.
Materials and Methods

The study was conducted in 2004 in cooperation with Syngenta Seeds Inc. on a commercial seed production field in Lone Tree, Iowa. The hybrid seed production field was managed for high yield using the standard production practices of Syngenta Seeds Inc. The inbreds were planted in a 4:1 female: male row ratio at a final population density 5.5 plants per meter of row for the male inbred and 5.8 plants per meter of row for female inbred with distance between rows of 0.76 m. The female inbred was mechanically detasseled before tassels started shedding pollen.

Three floral synchrony treatments were established by varying planting date of the female inbred blocks. A central block was planted as recommended to obtain optimum floral synchrony between male and female inbreds. This block was designated as the ‘Middle’ planting. A second female block was planted six days before the recommended planting data (Early planting), and a third female block was planted six days after the recommended planting date (Late planting). The Early and Late plantings resulted in floral asynchrony of about four days relative to the Middle planting (Fig.1). A commercial grain production field was located to the south of the seed production field, which provided an adventitious pollen source for out-crossing. This field was a normal grain production field with a typical planting density for Iowa of approximately 70,000 pl ha\(^{-1}\) (Duvick, 1997). The peak of pollen production in the commercial maize field coincided with silking of the Early and Middle planting of the female inbred (Fig.1).
Within each female block, six sampling stations were selected three weeks prior to flowering to represent the typical stage of inbred development. Three of the six sampling stations per block were located 100 m north of the commercial maize field. Three additional stations were located at 170 m (Fig. 2). Flowering dynamics were monitored in each sampling station. The sampling area was approximately 125 m$^2$ and at least 25 m from the field border. Pollen density was calculated at each station from the average pollen production per plant and population density recorded at flowering. Ten consecutive plants from each female row (total of 40 ears per station) were selected prior to flowering and their silking date documented. Ears from those plants were individually harvested at about 30% moisture and measured for number of kernels. Silk emergence dynamics were measured on ten plants in one representative sampling station in each treatment. Ears were covered before the appearance of silks, and silks were cut the first, third and seventh day from the appearance of the first silk in the ear. Subsequently, cut silk numbers were manually counted in the laboratory.

Passive pollen traps were used in both fields (seed and grain production) to document daily pollen shed density. Passive pollen traps were placed in the field from 0800 to 1800 h on five days during pollen shed. Traps were located between rows at ear height (90 cm). Pollen counts were determined in the laboratory by fluorescence microscopy according to Fonseca et al. (2002). Pollen production per tassel was measured on twenty plants using clear plastic bags (Pantek, Montesson, France) designed to exclude moisture but allow gas exchange around the tassel. Bags were placed over the tassels before pollen
shed began and until shedding was complete (5 to 7 days). Pollen was washed from the bags in isotonic solution (Isotone II solution, Coulter Corporation, Florida, USA), filtered to remove debris, and counted by particle counter and size analyzer (Beckman Coulter Z2).

Forty ears harvested from each of the eighteen field stations were separated into Early, Middle and Late silking plants relative to the mean for each treatment population. In general, 13 ears were pooled into each group. Kernels from the tip and the base of these ears were sampled separately. Base kernels were collected from the first fifteen kernels from each row in the ear, as those are usually the first ones to exsert their silks (Bassetti and Westgate, 1993a). Tip kernels were sampled from the last ten kernel rows on the ear. Four replications of 96 kernels were individually screened for out-crossing using the Stuber method (Stuber, 1988) by Syngenta Seeds, Inc. Due to the high cost of analysis, only two of the three replicate stations were analyzed for each planting date-distance combination.

Wind measurements (speed, direction) were obtained from the closest Iowa Environmental Mesonet (www.mesonet.agron.iastate.edu) station, located in Washington, Iowa 15 km south of the experimental site.

The Lagrangian particle dispersion model developed by Arritt et al. (2007) used local weather information to simulate daily pollen movement from the adventitious source to the seed production field during the flowering period. Daily adventitious pollen densities at each sampling location were used as inputs to calculate out-crossing by the kernel set model (Lizaso et al., 2003). Measured
flowering dynamics and local pollen shed densities were used as inputs to simulate kernel set at each seed field station. Out-crossing was calculated using flowering dynamic of the seed field plus the value of pollen grain reaching the sampling station generated by the particle dispersal model. An out-crossing percentage was related to the adventitious pollen, the local pollen and the receptive silks at the seed sampling station.

Root Mean square errors (RMSE) and adjusted R square values ($R^2$) were calculated for comparing measured and simulated out-crossing values.

**Results and Discussion**

Measured grain yield for the 18 field sampling stations ranged from 13.5 to 24.6 million kernels ha$^{-1}$ (Fig. 2). Sampling stations in the Late female planting block produced the least yield, due to the delay in silking relative to pollen shed (Fig. 1). Yields were greater in the Early planting block due to closer synchrony between inbreds, but some plants exserted silks up to seven days prior to pollen shed. Rapid loss of silk receptivity in the unpollinated plants likely explains the lower yield compared to the ‘Middle’ planting date (Bassetti and Westgate, 1994). As expected, the ‘Middle’ recommended female planting block achieved the greatest kernel production since the male inbred started shedding pollen at the same time the female inbred exserrated silks (Fig. 1). Edmeades et al. (1993) and Fonseca et al. (2004) observed similar relationships between kernel set and Anthesis-Silking Interval (ASI). These results underscore the critical nature of close floral synchrony between the inbred parents in hybrid seed production.
Measured out-crossing among the field sampling stations ranged from 1.4 to 18.0 % (Fig. 2). The Late planting block had higher levels of out-crossing than the other planting blocks, particularly at sampling stations 170 m from the adventitious pollen source. Premature removal of the male rows from this block likely explains this result. The lack of local pollen permitted more silks to be pollinated by female inbred plants that escaped detasseling (self/sib pollination) and by the adventitious pollen source (out-crossing). Both yield and out-crossing were affected by the imposed floral asynchrony. Because the normal pattern of male inbred pollen shed was disrupted in this block, out-crossing data from these stations were not included in out-crossing predictions.

A negative correlation was observed between yield and percent out-crossing (Fig. 3). This result most likely reflects the impact of variation in local pollen density on competition with the adventitious pollen source. Bassetti and Westgate (1994) showed how daily pollen density effects pollination of exposed silks. At local pollen densities greater than about 100 grain cm$^{-2}$. Most flowers will be fertilized the same day their silks become exposed, increasing yield and decreasing the potential for out-crossing. Thus, even though silking in the Early planting block was more synchronous with the adventitious pollen source (Fig.1), out-crossing was less than in the Late planting block due to the greater amount of local pollen production.

The kernel set model simulated kernel set by fitting mathematical functions to temporal profiles of plant population dynamics to pollen shed and silking exertion measured in the field. Figure 4 is a typical model output showing
the temporal dynamics of silk exsertion, pollen shed, cumulative kernel set, and silks not pollinated. The large number of ‘silks at risk’ in this example provides insight into the high levels of out-crossing observed in the Late planting block. Silks continued to appear after male inbred finished shedding pollen. The only pollen source for these silks was that coming from the adventitious source or the female inbred plants that had escaped detasseling.

The kernel set model was fairly accurate at simulating kernel set for the ‘Middle’ planting block with close synchrony between the male and female inbreds (Fig. 5A). We observed, however, that simulated kernel set was consistently overestimated for the Early female planting and underestimated for the Late female planting block. There were two likely sources of error in these kernel set calculations. First, every harvested kernel was included in the ‘measured’ yield. No attempt was made to differentiate between hybrid, selfed/sib, or out-crossed kernels, whereas the model input for hybrid kernel set assumes only local inbred pollen is available for pollination. When other sources of pollen are present, this assumption inevitably leads to an underestimate of measured kernel set. Mechanical detasseling in the female inbred blocks apparently was not entirely effective, since the laboratory analysis of out-crossing indicated a variable percentage (0.2 - 12.5%) of self/sib-pollinations also occurred within the seed field. The amount of pollen produced by the female inbred initially was not considered in our calculations of kernel set associated with pollen production by the male inbred. Nor was the amount of pollen entering from the adventitious pollen source considered. Since self/sib and out-cross
percentages were known, we could subtract these values from total measured yield. Correcting the measured kernel set had the greatest impact on the Late planting block where high values of out-crossing and self pollination were found due to poor floral synchrony.

Two model assumptions could lead to overestimation of kernel set in the Early planting blocks. First, the model assumes that pollen shed from each plant is normally distributed with a maximum 3 to 4 days after pollen shed begins. The period of pollen shed is often shorter for inbreds (Fonseca et al., 2003), which could have led to an overestimate in pollen shed duration. To test this possibility, we shortened the length of time from beginning to maximum pollen shed within the model. This adjustment, however, did not improve accuracy in the kernel set simulations (data not shown). The second potential source of error was an overestimate for the duration of silk receptivity. The kernel set model assumes silks remain receptive to pollen for 6 days once they are exposed for pollination this. Assumption is based on the documented longevity of silks on hybrid plants (Bassetti and Westgate, 1993). Since inbreds typically progress through flowering stages much more rapidly than do hybrids (Fonseca et al., 2004), we tested the possibility that silk receptivity could be of shorter duration than previously assumed. When the duration of silk receptivity was shortened to 4 days, the accuracy of kernel set simulation was greatly improved ($R^2 = 0.71$ to 0.83; $RMSE = 0.47 \times 10^6$ to $0.3 \times 10^6$), mostly for Early planting block, which had the greatest potential for overestimating kernel set due to extended silk receptivity (Fig. 5B).
Atmospheric vapor pressure and temperature during flowering were favorable for pollen viability generating optimum conditions for obtaining high values of out-crossing in the sampling stations. Wind was predominantly from North during the flowering period, but during the maximum pollen shed days (Fig. 1, 192-195 DOY) there were strong wind gusts from the South and Southwest. This promoted adventitious pollen drift into the seed production field, resulting in high values of out-crossing (as high as 18% at 100 m). Considering wind pattern (Fig. 6) as the only variable affecting out-crossing, however, the values obtained at 170 m would not make sense. In this experiment, the primary factor contributing to the high level of out-crossing observed was variation in local pollen density. In cases where out-crossing values were high, the male: female synchrony was not optimum. Local pollen production was limited while silks were receptive. As such, adventitious pollen arriving to the area had a higher probability of affecting fertilization.

Simulated pollen dispersal from the commercial field indicated seed sampling stations located at 170 m were exposed to less adventitious pollen than those at 100 m (Fig. 7). This simulated result corresponded closely to measured results indicating greater out-crossing at 100 m compared to those observed at 170 m from the source.

Out–crossing values were obtained from 80 ears per sampling station. These ears were separated in three groups associated with Early, Middle and Late silking plants. Base and tip kernels from each ear were sampled resulting in six ‘silking groups’ for which out-crossing was measured (Tip: Early, Middle, Late;
Base: Early, Middle, Late). All data from each group were pooled to obtain an out-crossing value for the entire sampling station. Obtaining out-crossing data from tip and base from each of these three groups provided a wide range of flowering times in which out-crossing was measured. The base kernels from the early silking plants represented the first silks to appear in the plot; kernels from the tip of the Late silking plants represented the latest appearing silks in the plot. Thus, pooling all the data from the six sub-groups at each station provided a fairly robust estimate of total out-crossing percentages to be expected within each sampling station.

The lowest percentage of out-crossing was observed in the Middle planting treatment which is, attributable to the maximum pollen local density during silking. The Late planting treatment had the highest level of out-crossing due primarily to late silk appearance. Out-crossing values were intermediate in the Early planting treatment. Ireland et al. (2006) reported similar levels of out-crossing in commercial seed production fields that were separated by 100 m from the nearest source of adventitious pollen.

Our main objective was to predict out-crossing values observed in the field by coupling the kernel set model (Lizaso et al., 2003) with the particle dispersion model (Arritt et al., 2007). Figure 8 shows that predicted out-crossing values explained about 78% of the variation in measured out-crossing values (within the range of 0 to 6% out-crossing). But predicted values were about 86% of measured values, on average, for all synchrony treatments (Early, Middle, and Late planting). This underestimation might be attributable to several factors. First,
flowering dynamics in the seed production field were measured in only one of the three replicate sampling stations per treatment. Variation in flowering dynamics is commonly observed. Silk exsertion dynamics from one replicate might not perfectly represent the actual dynamics for all sampling reps at each field station. Second, the transport path for pollen drift between the commercial maize production field and the hybrid seed production field was assumed to be uniform. An intervening gravel road may have generated effects on the wind patterns that were not taken into account when simulating pollen dispersal. Third, a ± 0.05% error is typical for the lab technique used for measuring out-crossing (Stuber method). In spite of these potential sources of error, the combined models predicted the range of measured out-crossing values with a RMSE of 0.8 and $R^2$ of 0.77.

**Summary**

This experiment demonstrated for the first time that out-crossing can be accurately predicted by coupling a kernel set model with a particle dispersion model. As such, modeling flowering dynamics and pollen dispersal can be an effective means to assess potential yield and genetic purity of a hybrid seed field well in advance of harvest. Current research is aimed at improving the accuracy of model simulations for a wide range of flowering synchronies.

Our results show that the kernel set model is highly accurate at simulating seed production in fields with close synchrony between male and female inbreds. The accuracy of the combined models would be suitable for predictive purposes;
however, several potential sources of error need to be considered. The overestimate of kernel set associated with silking prior to pollen shed, for example, may be due to a shorter duration of silk receptivity for inbreds than documented for hybrids, as assumed in the model. Additional studies are needed to resolve this issue.

There also were potential sources of error affecting model prediction for out-crossing associated directly with conducting this experiment in a commercial seed production field. These include a non-uniform flow field between the adventitious source and the seed field (in this case a gravel road), border rows at the edge of the seed production field, premature removal of the male inbred, and poor detasseling efficiency. The intervening gravel road may have generated local turbulence that was not considered by the Lagrangian pollen dispersal model. Mechanical removal of the male inbred plants is a common practice in seed production fields, and is often done before they have completed pollen shed. Male removal occurred earlier in the season than expected and precluded a prediction of out-crossing for the Late planted female treatment. Detasseling on the female inbred was not completely effective or uniform, which resulted in greater than expected levels (up to 12.5%) of selfed kernels. There was no direct means to account for the extra pollen generated by the male inbred border rows at the south edge of the field. This source of pollen likely affected measured kernel set and decreased the out-crossing level.
Acknowledgments

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References


**Figure 1:** Flowering dynamics for the male and female inbreds and the adventitious pollen source at the Lone Tree field station. Grey lines represent the cumulative silk emergence (silks female ha$^{-1}$) for the Early planted, Middle, and Late planted sub-plots. Solid black line depicts daily pollen shed intensity from the male inbred (grains cm$^{-2}$ d$^{-1}$). Dashed black line indicates the daily intensity of pollen shed within the adventitious pollen source (grains cm$^{-2}$ d$^{-1}$) approximately 100 m from the nearest seed field sampling site. In each case, the curves represent the average for all sampling stations within planting block. Redrawn from Fonseca et al. (PhD thesis, Iowa State University).
**Figure 2:** Yield (kernels ha\(^{-1} \times 10^6\)) and out-crossing percentages (%) observed within the floral synchrony blocks at 100 m and 170 m from the adventitious pollen source. The female inbred was planted six days prior to the male inbred in the ‘Early’ block, six days after the male inbred in the ‘Late’ block, and on the same day as the male inbred in the Middle planting block (Recommended by Syngenta Seed Inc.). Out-crossing was measured in kernels collected at 2 of the 3 sampling stations at 100m and 170 m within each planting block. *not included in simulation analysis because male inbred was removed prematurely. This figure represents the field experimental design (not drawn to scale)
Figure 3: Relation between yield (kernels ha\(^{-1}\) x 10\(^6\)) and out-crossing percentage. Samples collected at 100 m and 170 m from the adventitious pollen source. Out-crossing on ten ears was measured at each sampling station.
Figure 4: Output of the kernel set model for a sampling station located in the Late planting block. The timing and intensity of pollen shed is calculated from pollen trap data collected within the seed field and adventitious pollen source field. Cumulative silk exsersion dynamics were generated from daily measurements of % silking and silk exsertion kinetics within the planting block. The model calculates daily and cumulative kernel set, as well as remaining unpollinated silks ‘at risk’ of out-crossing.
Figure 5: Relationship between measured and simulated kernel production resulting from contrasting flowering synchronies between male and female inbred. Data represent values measured at 18 field sampling stations within the Early planting block (x), Middle planting block (♦), and Late planting block (◊). Dashed line indicates the 1:1 relation. A: Simulated yield assuming the male inbred was the only pollen source and unpollinated silks remained receptive for six days. B: Simulated yield assuming unpollinated silks remain receptive for only four days. The number of out-crossed and self/sib pollinated kernels was subtracted from measured kernel number.
Figure 6: Wind Rose for Lone Tree, Iowa (186-200 DOY). Weather data were collected on days when both fields were shedding pollen and silks were exposed within the seed production field. Data were collected from 0800 to 1800 hr at 15 min intervals. The concentric circles represent the percentage of the time that the wind came from the indicated direction. Note that the wind direction is presented as blowing from a cardinal direction, not to a cardinal direction.
Figure 7: Simulated pattern of pollen dispersal predicted by Lagrangian-Stochastic analysis for the Lone Tree field site. Pollen dispersal is integrated for all days of pollen shed and scaled to the daily pollen production from the source plot. Data are presented on a Log scale. Horizontal lines in the seed field indicate location of sampling stations 100 m and 170 m north of the adventitious pollen source.
Figure 8: Relationship between simulated and measured out-crossing at 10 sampling stations within the hybrid seed production field. Six stations were 100 m from adventitious pollen source; four sampling station were 170 m from the source. Self/sib pollinated kernels were subtracted from the measured out-crossing values.
Chapter 4
Predicting Out-Crossing in Maize Fields Having Pollen Densities
Typical of Grain and Hybrid Seed Production

*A manuscript to be submitted to Field Crop Research*

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Abstract

Potential use of maize (*Zea mays* L.) for production of pharmaceutical and industrial compounds has increased concern about confinement of transgenic pollen. While numerous studies have documented pollen flow in maize, there has been little success predicting the level of out-crossing to be expected from it. The objective of this project was to predict the measured patterns of out-crossing resulting from the quantitative interactions between local flowering dynamics and the density of adventitious pollen naturally dispersed into the field. One hectare of yellow, RoundUp Ready®, Bt maize was planted in the center of approximately 36 hectares of non-transgenic, white maize. In one case, the entire field was managed for normal grain production; in the second case, the white maize was detasseled to reduce local pollen to levels typical of hybrid seed production. Flowering dynamics were monitored to determine floral synchrony between the
transgenic and non-transgenic hybrids. These data were coupled with local weather conditions to determine the timing and extent of pollen dispersal from the center field. Out-crossing percentages were calculated by coupling the kernel set and pollen dispersal models we have developed. The level of out-crossing predicted by the kernel set model closely followed the field pattern of measured values. It was highly accurate at predicting the distance from the center field where out-crossing exceeded 0.5%. As expected, lower local pollen production resulted in greater observed and predicted out-crossing with increasing distance from the transgenic pollen source. These results indicate that out-crossing in maize can be predicted on a field scale by combining quantitative analysis of flowering dynamics, local pollen production, and adventitious pollen dispersal.

**Introduction**

Gene flow, i.e. the transfer of alleles or foreign genes from one population to another, is a major concern in commercial maize (*Zea mays* L.) production, especially since the introduction of transgenic genotypes. The reproductive biology of maize naturally predisposes it to a high level of out-crossing. The species is naturally cross-pollinated, markedly heterogeneous, and in most cases hybridizes freely (Purseglove, 1972). The maize plant is monoecious and diclinous, with male and female flowers borne separately on the same plant. Other quality favoring out-crossing is that maize often exhibits protandry, which means that pollen is shed before the silks are receptive, but as there is some
overlap, some self-pollination can occur. The tassel usually extends fully before anthesis begins. Opening of the flower begins near the middle of the central spike and passes upwards and downwards, followed by the lateral branches, and ends with the tips and bases of the lower branches (Purseglove, 1972). A maize tassel sheds pollen over several consecutive days with dehiscence occurring in the mornings, although it may be delayed in cold, high RH and cloudy conditions (Herrero et al., 1980; Miller, 1985; Schoper et al., 1987; Fonseca and Westgate, 2005).

Maize pollen is produced in massive quantities. Reported values of 20 to 42.2 million grains for old cultivars (Hall et al., 1982; Sadras et al., 1985) contrasts with more recent values of 9.6 to 11.3 million grains (Uribelarrea et al., 2002) and 2.2 to 3.3 million grains (Fonseca et al., 2002) observed for modern hybrids. Maize pollen is among the largest in the gramineae (grass) family with dimensions of 90 to 125 microns (Erdtman, 1952, Smith, 1990). The pollen grains are more or less spherical and with the aperture slightly protruding (Erdtman, 1952). The grain has a volume of about $700 \times 10^{-9}$ cm$^3$ and a weight of about $250 \times 10^{-9}$ g (Miller 1985; Goss 1968). Published data show that maize pollen can remain viable for several hours under natural conditions. In warm conditions or exceptionally hot weather and low relative humidity (RH), this time could be reduced to less than one hour (Fonseca and Westgate, 2005). In cool and high RH conditions, viability could be extended to several days. Purseglove (1972) mentions maize pollen remains vigorous for close to 24 hours, but loses
viability more rapidly in very hot dry weather. Jones and Newell (1948), Aylor (2003) and, Fonseca and Westgate (2005) reported pollen viability ranging from 1 to 24 hours, depending on vapor pressure deficit. It is essential to consider all these characteristics to predict the level of out-crossing that might result from pollen dispersal.

Numerous field studies have documented the potential for maize pollen dispersal. Most have reported exponentially decreasing gradients of pollen deposition with distance from the source (Jarosz et al., 2003; Stevens et al., 2004; Goggi et al., 2006; Goggi et al., 2007). Others have sought to identify the temporal or spatial isolation needed to limit out-crossing, and reported isolation distances from 50 to 500 m and temporal isolation from 2 to 4 weeks (Doebley, 1990; Burris, 2001; Luna et al., 2001; Brunet et al., 2003; Ma et al., 2004; Dupont et al., 2005; Halsey et al., 2005). Particle dispersion models have been used to simulate downwind rates of pollen deposition and dispersal of pollen in the atmosphere; some models used empirical approaches that basically fit a curve to observed data (Jarosz et al., 2004; Gustafson et al., 2005; Fyfe, 2006; Schueler and Heinke, 2006). Recent mechanistic approaches have obtained accurate result compared to observed patterns of pollen deposition in the field (Aylor and Boehm, 2006; Arritt et al., 2007). In no case, however, has it been possible to predict the level of out-crossing to be expected from the observed patterns of pollen dispersal. The main dilemma is lack of field research linking the predicted pattern of pollen dispersal with flowering dynamics and kernel set.
Lizaso et al. (2003) have successfully simulated maize kernel set in the field by coupling simple measures of flowering dynamics and pollen shed with known pollination efficiencies (Bassetti and Westgate 1994). This kernel set model was used in Chapter 3 to provide accurate predictions of kernel set (RMSE: 0.3, $R^2$: 0.82) in a commercial seed production field. Importantly, the kernel set model also calculates the number of exposed silks left unpollinated each day. These silks are completely ‘at risk’ of being pollinated by an adventitious pollen source. Predicting the amount of out-crossing under different production and environmental circumstances only requires an estimate of the density of adventitious pollen entering the field.

Arritt et al. (2007) developed a three-dimensional Lagrangian random flight model for numerical simulations of maize pollen dispersion. The model simulates the path of particles, which are individual pollen grains in this case. The particle motion is determined by the mean flow and turbulence. The Lagrangian process has been adopted because of its generality and flexibility. It also incorporates the environmental conditions to which the pollen grains are exposed during its flight from tassel to silk. Therefore, the physical effects of wind and turbulence on pollen dispersion can be coupled with the biological aspects of pollen release and viability. Predictions of pollen dispersal by the Lagrangian model compare well both to observations and to results from a standard Gaussian plume model (Arritt et al., 2007).

The objective of this project was to predict kernel set and out-crossing on a field scale using established models of kernel set (Lizaso et al., 2003) and
pollen dispersal (Arritt et al., 2007) for maize. Because out-crossing depends on the timely interaction of flowering phenology, local pollen density, and weather conditions during flowering, we conducted these studies over two years, and at pollen shed densities typical of commercial grain production and hybrid seed production fields.

**Materials and Methods**

The study was conducted in the Iowa State University research farm in Ankeny, Iowa in 2003 and 2004. One hectare of DKC69-71 yellow, Roundup Ready® (RR), Bt (carrying the Bt-Cry1Ab gene) maize was planted in the center of approximately 36 ha of non-transgenic, RX792W white maize. The transgenic yellow maize in the center plots provided the source of adventitious pollen. The transgenic maize was seeded at $85 \times 10^3$ plants ha$^{-1}$ and the non-transgenic maize seeded at $70 \times 10^3$ plants ha$^{-1}$ both years, which is typical for Iowa maize production (Duvick, 1997). The soil was predominantly Nicollet, Webster, Clarion and Harps (USDA–NRCS, 2000) characterized as silty clay loam to loam. Both hybrids were managed under normal production practices for cultivation, planting, insect control, and soil fertility. Center plots were planted 21 May in 2003, and 4 May in 2004. The surrounding white maize was planted the next day in both years. The center plot planted with the yellow transgenic hybrid (DKC69-71) was sprayed with glyphosate after emergence to remove non-resistant plants.
Two fields were planted with white non-transgenic maize in 2003. One field was treated as a normal grain production field; the other field was mechanically detasseled to a 4:1 female: male row ratio to simulate pollen density in a hybrid seed production field. The detasseled field had a local pollen density approximately 80% less than its non-detasseled counterpart. The field trial simulating normal grain production field was repeated in 2004.

Flowering dynamics were monitored at 25 seed sampling locations in the non-transgenic maize and at 4 locations in the transgenic maize plot (Fig. 2). Approximately 100 plants were monitored at each location for silk emergence and pollen shed. Percent silking was estimated by counting plants with at least one silk exposed. Similarly, percent pollen shed was estimated by counting plants that had begun to shed pollen. Passive pollen traps described by Fonseca et al. (2002) also were used to quantify daily pollen production. Pollen production per tassel was estimated on 10 plants per hybrid by collecting pollen in clear plastic bags (Pantek, Montesson, France) that were placed over the tassels prior to the initiation of pollen shed. Pollen was removed from the bags in isotonic solution (Isotone II solution, Coulter Corporation, Florida, USA) and counted using a particle size analyzer (Beckman Coulter Z2). Passive pollen traps were placed in the field every other day before plants started shedding pollen (0800 h) and were collected after plant finished shedding pollen (1800 h). Pollen deposited on the traps was analyzed in the laboratory by fluorescence microscopy as described by Fonseca et al. (2002).
Temperature, relative humidity, rainfall, wind speed and wind direction were monitored using weather stations throughout the flowering period. Data were collected by portable weather stations placed at the edge of the transgenic maize plot. Wind speed and direction data were averaged and stored every 15 minutes by using a Campbell Scientific CR10 data logger.

Seed sampling stations were located at 1, 10, 35, 100, 150, 200 and 250 m from the central plot following the N, NE, E, SE, S, SW, W and NW transects (Fig. 1). At harvest maturity, ear samples were collected at each location. At 1, 10 and 35 m, 25 ears were collected at each sample station. At 100, 150, 200 and 250 m, 100 ears were collected per seed sampling station, and there were two seed sampling stations at each distance. Harvested ears number was increased with distance from the transgenic yellow pollen source to ensure detection accuracy of at least 0.005% out-crossing at each location (Remund et al., 2001). The location of the seed sampling stations was verified using a global positioning system (GPS).

Ear samples were shelled in a seed sheller LS91 (Custom Seed Equipment, Altoona, IA), and color sorted in a 20 channel ESM ScanMaster, model SM-200 DE (SATAKE, Stafford, TX) to separate yellow seed out-crossing in each sample. To ensure that the yellow maize in the center plot was not segregating for color, seed samples were also collected within the source field and examined with the color sorter. No color variation was found, thus both alleles were dominant yellow.
Yellow kernels from the center field, as well as all yellow seeds detected at the surrounding field seed sampling stations, were checked for the presence of the RR and Bt traits. The methodology described by Goggi and Stahr (1997) was used for RR detection. The seeds were imbibed for 48 h between paper towels moistened with a 3% a.i. solution of glyphosate (Roundup® Ultra). Seeds from the center plot segregated with a frequency of 70% RR: 30% non-RR in 2003 and 73% RR: 27% non-RR in 2004. These values were not significantly different (P = 0.63) from the expected ratio of 75% RR: 25% non-RR (Chilcutt and Tabashnik, 2004). An enzyme-linked immuno sorbent assay (ELISA) Bt kit (Agdia Inc., Elkhart, IN) was used to detect the presence of the Bt protein. The test is a double antibody sandwich (DAS)-ELISA, which detects the protein using a polyclonal antibody. Test protocols followed manufacturer’s recommendations.

The segregation frequency was 77% Bt:23% non-Bt in 2003, and 88% Bt:12% non-Bt in 2004. This segregation frequency also was not significantly different (p= 0.64) from the theoretical 75% Bt and 25% non-Bt. Final out-crossing numbers were obtained by dividing the percentage of yellow, RR and Bt kernel over the total number of kernels in each seed sample harvested at each location.

Out-crossing at each seed sampling station was simulated by coupling our kernel set (Lizaso et al., 2003) and pollen dispersal (Arritt et al., 2007) models. The daily density of pollen (grains cm\(^{-2}\)) reaching each seed sampling station were calculated from rates of pollen shed in the central plot and local weather data using the pollen dispersal model created by Arritt et al. (2007). Daily
adventitious pollen grains arriving to the seed sampling station was added to the daily local pollen density (white maize pollen) and total pollen value was used to calculate daily kernel set. Total out-crossing was an addition of daily out-crossing values obtained.

Root Mean square errors (RMSE) were calculated for comparing measured and predicted out-crossing values. ArcMap (ESRI) was used for geo-statistical analysis and plotting the surface map of outcrossing values. Data in between seed sampling stations were calculated using a combination of local and global interpolation in geo-statistical analyst.

**Results and Discussion**

A combination of genetic traits was used to ensure the patterns of out-crossing observed in the 36 ha fields resulted solely from pollen dispersal from the central plots planted to yellow/Bt/RR maize. Surrounding maize fields were tested for the presence of Bt and RR transgenic events, and no fields in the area contained the same combination of transgenic characteristic as DKC69-71 (yellow kernels, Bt, RR) sown in the center of the test fields.

Shedding period from both pollen sources and silking time for white hybrid was well synchronized (Fig. 2). In both years, pollen was being shed in the central plot at the time silks were being exerted by the surrounding white maize plants. In the simulated seed production field in 2003, peak pollen production by the white hybrid was about 200 grains cm\(^{-2}\), compared to about 700 grains cm\(^{-2}\) in the non-detasseled grain field (Fig. 2A).
Measured out-crossing values decreased exponentially with increasing distance from the adventitious pollen source (Fig. 3). Less than 0.5% out-crossing was detected in the grain field at distances greater than 35 m; a nearly identical pattern was observed both years. Halsey et al. (2005) found similar out-crossing values at similar distances from a pollen source. Greater values of out-crossing were obtained at all seed sampling stations in the simulated hybrid seed production field, which had lower local pollen density. At least 100 m of isolation was required to achieve less than 0.5% out-crossing. These data confirm that 200 m of isolation hybrid maize production field are required for minimum isolation for obtaining out-crossing values lower than 0.5%. These results indicate that local pollen density has a large impact on the isolation distance required to meet the European threshold for transgene presence (0.9%). Approximately 100 m of isolation would be required if the transgene containing plot were surrounded by commercial maize production; isolation would need to increase to about 200 m if local pollen density were typical of hybrid seed production.

Temperatures and RH were average for Iowa during pollen shed providing high quality conditions for pollen viability. Rain fall patterns and wind speed were typical for the area both years (Fig. 4). In 2003, the distance between the two test fields was about 800 m, i.e. close enough so that wind direction and speed were essentially identical during pollen shed (Fig. 4). Also, there was close synchrony between silking in the surrounding white maize and pollen shed in the transgenic pollen source. These conditions provided a unique opportunity to use the same
weather data for predicting the pattern of pollen dispersal from the two central plots. The difference between fields relative to out-crossing and pollen dispersal was the amount of pollen released from center fields each day and the amount of local pollen released in the surrounding white corn field. Figure 2 A-B shows the difference in timing for pollen shed from the adventitious pollen source. The shedding time generated different pollen dispersal patterns for both field. Pollen quantities liberated each day were different for each field, as were wind speed and direction.

Pollen dispersal patterns followed the primary wind patterns (Fig. 5). Pollen deposition declined exponentially with distance. The pollen flow was closely related to wind speed and direction. Pollen mainly flowed down wind from the transgenic maize source.

Out-crossing patterns are presented as surface graphs to aid in visualizing the relationships between pollen dispersal and out-crossing throughout the fields. This also provides an immediate comparison between measured and predicted patterns of out-crossing.

The out-crossing area is larger in the field treated as hybrid seed production than in the field treated as grain production (Fig. 6). Since floral synchrony between adventitious pollen shed and silking by the white hybrid was nearly identical in the two fields, the lesser local pollen density due to detasselling evidently provided a greater competitive advantage to the adventitious pollen. The proportion of adventitious pollen to local pollen was approximately four times greater in the hybrid seed production field than in the
grain production field. The predicted patterns of out-crossing were similar to measured patterns of out-crossing in all three fields (Fig. 6).

A correlation analysis was performed to assess the accuracy of the coupled kernel set/pollen dispersal model predictions of out-crossing in the three fields studied in 2003 and 2004. In general, there was close agreement between predicted and measured values (Fig. 7). Based on root mean square error (RMSE) comparisons, however, model predictions were more accurate at distances farther from the transgenic pollen source. Predicted levels of out-crossing RMSE values decreased dramatically at distances farther than 35 m from the source in the grain production fields and farther than 100 m from the source in the seed production field. At closer distances, the model was less accurate. This is most likely attributable to incorrect calculation of local pollen density and white-corn plant density adjacent to the central RR plot. There was an access alley 2.5 m wide with no plants surrounding the yellow transgenic center plot. This open space permitted free movement of transgenic pollen and decreased the effective white pollen density. This led to much higher percentage of measured out-crossing at the adjacent edge of the white corn field than simulated by the out-crossing model. The model’s failure to predict out-crossing at shorter distances also could be attributable to incorrect values for flowering dynamics and lower than estimated plant densities. Goggi et al (2007) reached a similar conclusion in their statistical analysis of out-crossing patterns in these fields. Nonetheless, the combined models would be useful in predicting isolation distances required to meet the European community (0.9%) and US industry
(0.5%) thresholds for transgene presence in seed shipments (dotted lines in Fig. 7).

Residuals (Measured % - Predicted %) were calculated to document the discrepancy between predicted and measured out-crossing values. Residuals were large (ranged from 55 to -18%) at the interface between the yellow and white maize (Fig. 8). This error is likely attributable to the absence of plants in the alley which was not calculated in the kernel set model. Residuals were similar for the two fields in 2003; lower values were observed in 2004. We hypothesize that lower wind speeds in 2004 contributed to the apparent decrease in simulation error.

**Summary**

To our knowledge, this study demonstrates for the first time that out-crossing due to pollen drift in maize can be simulated accurately by combining a quantitative analysis of flowering dynamics, local pollen production, and adventitious pollen dispersal. The model accurately simulated out-crossing in grain and hybrid seed production on a field scale and results from the model follow the out-crossing patterns observed in the field data. These results also confirm the accuracy of the Lagrangian particle dispersion model for estimating pollen dispersal. The importance of documenting flowering dynamics must be emphasized for obtaining these results. Another advantage of the combined kernel set model is that it provides a predictive basis for harvest management based on real-time weather conditions and crop development.
Out-crossing predictions were progressively less accurate at distances closer than 35 m from the pollen source. The main source of error is most likely an inaccurate estimate of the local pollen density. The field access alley between the central plot and the surrounding field was the source of this variation because the kernel set model and pollen dispersal models do not account for the dramatic change in plant population on local pollen density between the central and surrounding plant stands.

Nonetheless, these results show that reducing the out-crossing levels to less than 0.5% is possible and predictable. Surrounding the central adventitious pollen source with a 35 m maize barrier of corn capable of high pollen production was sufficient to reduce the out-crossing levels below 0.5% both years. Moreover, the combined kernel set/pollen dispersal model was highly accurate at predicting out-crossing at distances farther than 50 m from the pollen source. In most cases, this is less than the most common isolation distance (100 m) employed by the hybrid seed industry (Ireland et al., 2006), indicating that the model is applicable for testing such situations.

These isolation distances depended on the amount of pollen produced by both fields. If the plants in the adventitious pollen source had produced more pollen per unit area, these distances might have been greater. Likewise, if less pollen is produced in the intervening space, the adventitious pollen will have less competition, and more out-crossing would be expected at the same distances. If the intervening isolation terrain has no maize plants (as current APHIS permitting regulations require), isolation distances might need to be considerably greater.
This conclusion is supported by the results of our windbreak studies described in Chapter 2.

Obviously, weather patterns during pollen shed have a major impact on the effectiveness of distance as an isolation mechanism. Weather conditions for the two years of this study were typical for central Iowa. More extreme weather conditions would likely lead to different patterns of out-crossing and require greater isolation distances to achieve the same low level of out-crossing.

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References


Figure 1: Field map showing approximate locations of seed sampling stations along the North transect. Seed sampling stations were located along each of 16 transects at 1, 10, 35, 100, 150, 200, 250 m from the central plot of yellow transgenic maize. Not drawn to scale.
Figure 2: Flowering synchrony between central transgenic maize plots (adventitious pollen) and surrounding non-transgenic maize field (local pollen and silks). Data are averaged for four locations within the adventitious pollen source and 26 locations within the surrounding field. A: 2003 seed production field (4female: 1male row ratio) B: 2003 grain production field. C: 2004 grain production field. Redrawn from Goggi et al. (2006).
Figure 3: Measured out-crossing with distance from the adventitious pollen source for three fields measured in 2003 and 2004. Data are the mean ± Standard Deviation (SD) for all seed sampling stations at the indicated distance from the source plot. (♦) Hybrid seed field, 2003; (△) Grain field, 2003; (○) Grain field, 2004. Dotted lines indicate the 0.9% and 0.5% out-crossing thresholds.
Figure 4: Wind rose data 2003(A) and 2004(B) for Ankeny, Iowa. Wind speed is in m s\(^{-1}\). Wind roses were generated with data collected on days when both fields were shedding pollen, from 204 to 215 DOY in 2003, and 195 to 207 DOY in 2004. Data were collected from 0800 to 1800 h at 15 minutes intervals. The concentric circles represent the percentage of the time the wind came from the direction. Note that the wind direction is presented as blowing from a cardinal direction, not to a cardinal direction. Redrawn from Goggi et al. (2006).
Figure 5: Simulated pattern of pollen dispersal from the adventitious sources A: seed production field 2003 B: Grain production field 2003 C: Grain production field 2004. Pollen deposition values are per cm$^2$ expressed on a Log scale.

Figure 7: Correlation between simulated and measured out-crossing values. A: Seed production field 2003 B: Grain production field 2003. C: Grain production
field 2004. RMSE: root mean square error. The dashed line represents the 1:1 relation.

Figure 8: Out-crossing residuals (Measured % - Simulated %) with distance from the pollen source. Root mean square error is parsed to illustrate increased precision of simulation with distance from the pollen source.
Chapter 5

General Conclusion

Conclusion

Since the introduction of genetically modified (GM) hybrid maize for commercial production, much effort has been directed towards controlling pollination to ensure high levels of genetic purity. Controlling pollen dispersion is an important consideration in seed production, particularly for managing transgenic genotypes, due to the potential pollen flow into landraces, wild relatives of maize, and non GM commercial hybrids.

Managing for high levels of genetic purity requires greater knowledge of the factors that affect pollen production and dispersion coupled with simple methods to quantify these processes.

This project creates a basis for achieving levels of genetic purity. It employs enhanced understanding of pollen dispersion dynamics, pollination processes related to weather conditions, and a quantitative estimation of out-crossing based on flowering dynamics.

A method to control pollen flow, by reducing wind speed in the maize field, is studied. This research demonstrates the capacity of a natural wind barrier on decreasing wind speed, therefore, decreasing pollen dispersion and out-crossing.
A mathematical model developed to simulate potential kernel set (Lizaso et al. 2003) was coupled with a Lagrangian pollen dispersion model (Arritt et al., 2003) to simulate out-crossing levels from simple maize flowering dynamic and local weather conditions. We evaluated the out-crossing simulation model under different scenarios, with different anthesis silking intervals (ASI) between male and female inbreds, and diverse production strategies.
Appendix A

Pollen Dispersal Pattern With and Without Sorghum Sudangrass Wind Barrier

Appendix A: Measured pattern of pollen dispersal. “Z” values are log scaled and expressed as maize pollen grains cm$^2$. Wind rose generated with data obtained from 0800 to 1800 h. Top: Pollen dispersal pattern for the field without sorghum sudangrass wind barrier. Bottom: Pollen dispersal pattern for field with sorghum sudangrass barrier. Six days in 2005 and four days in 2006 were sampled.
Appendix B

Coupling Time to Silking With Plant Growth Rate in Maize

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Abstract

In maize (Zea mays L.), progress towards pistillate flower maturity (silking) is highly dependent upon the environmental conditions around flowering. Under conditions that inhibit plant growth, female flower development is delayed relative to that of the male flowers resulting in an increase in the anthesis-silking interval (ASI). Although variation in ASI has been extensively documented, its relationship to plant growth is not well understood. Therefore, we developed a conceptual basis and experimental approach for quantifying and analyzing the process of female flowering in maize in response to variation in plant growth rate during the flowering period.

Time to silking depends on biomass accumulation at the ear level, as silking for each plant is a developmental stage dependent upon ear expansion growth. Because plants within a maize canopy differ in their growth rate around flowering, plants with rapid growth rate reach silking earlier than the ones
growing at lower rates. This is a consequence of differential accumulation of ear biomass around anthesis. As such, quantifying canopy plant-to-plant variability in ear growth around anthesis is a critical component for resolving time to silking for the population of plants. Moreover, plant biomass partitioning to the developing ear (ear growth rate / total plant growth rate around flowering) differs depending on the plant growth rate, and among genotypes. In order to resume this complexity, we developed a simple plant biomass growth framework to quantify time to silking for maize plant populations that takes into account plant-to-plant growth variability and partitioning of biomass to the developing ear around flowering.

**Introduction**

As in most field crops, variation in maize yield is related more to the number of harvested kernels than to variations in the individual kernel weight (Early et al., 1966; Borrás et al., 2004). As such, the period of development when kernel number is defined has been referred as the “yield critical period”. In maize, this period is centered around flowering. Numerous studies have shown that maize kernel number (and yield) is a function of crop growth rate during this time (Moss and Stinson, 1961; Early et al., 1966; Tollenaar et al., 1992; Andrade et al., 1999). Environmental conditions that alter plant growth during this period also affect the temporal separation of male (anthesis) and female (silking) floral maturity (referred to as the anthesis-silking-interval, ASI) mostly due to changes in time to female flowering. Reduced plant growth reduces ear biomass
accumulation, and delays in silking have always been interpreted as a consequence of reductions in biomass allocated to the developing ear at reduced plant growth. However, a direct relationship between ear biomass accumulation and time to silking has yet to be established.

A negative relationship between final grain yield and ASI has been described in numerous studies (Moss and Stinson 1961; Woolley et al., 1962; Edmeades and Daynard, 1979a; Hall et al., 1982). This relation has attracted considerable attention from maize breeding programs (Fischer et al., 1989; Bolaños and Edmeades, 1996; Bruce et al., 2002; Bänziger et al., 2004; Campos et al., 2004), as ASI can be easily measured in large populations. We currently understand that kernel number is a function of the rate of biomass accumulation at the ear level around flowering (Andrade et al., 1999; Vega et al., 2001; Echarte et al., 2004). If kernel number and time to silking are both related to biomass accumulation at the ear level, the connection between yield and ASI will be defined.

The objective of the present article is to establish the conceptual connection between plant growth around flowering and time to silking, and connect ear biomass accumulation and time to silking. We discuss the importance of capturing plant-to-plant variability in plant growth rate around flowering to understand time to silking at the canopy level, and how plant biomass partitioning among competing sinks around this period affects time to silking. We latter integrate these ideas in a framework that help understand how do maize canopies reach silking.
Phenology at the plant and population levels

Maize is a monoecious plant, with staminate (male) flowers borne on an apical inflorescence (commonly referred to as a tassel) and pistillate (female) flowers produced on one or more lateral branches, which develop into grain bearing rachises (commonly referred to as ears). At the individual plant level, anthesis (i.e. functional maturity) for the male flowers is defined by the beginning of pollen shed from the tassel. A plant has reached ‘anthesis’ when at least one anther has dehisced and is liberating pollen. Anthesis for the female flowers is defined by the appearance of the first pollen receptive stigmas (commonly referred to as silks) from within the surrounding husks on the primary ear. Anthesis for the female flowers is commonly referred to as silking. For both flower types, these descriptors are qualitative traits that define a change of state. At any point in time, a plant either has reached the flowering stage (anthesis or silking), or it has not.

When these flowering processes are considered at the population level, anthesis and silking stages are achieved when a pre-determined proportion of plants in the population (typically 50%) reach the stage. This simplification reflects the fact that not all plants in a population achieve anthesis or silking at the same time. Rather, flowering throughout the population is a continuous (but finite) process. Thus, floral anthesis for the population is a quantitative phenomenon; for individual plants, it is a qualitative one.

Using a mechanistic framework to analyze biological phenomenon involving a qualitative process at the individual level and a quantitative process at
the population level has met with considerable success. An example is the prediction of seed lot performance across contrasting environments from quantitative information of germination at the population level and a qualitative assessment of individual seed germination (Ni and Bradford, 1992; Bradford, 2002). In the case of silking dynamics for a maize canopy, a clear understanding of the flowering process at the individual plant level is critical for resolving environmental effects on phenomenology at the population level, and for resolving the connection between plant growth around flowering and time to silking. The need to understand the silking process at the plant level is particularly evident when plant-to-plant variability within the population is large as is often the case in maize crops, especially under stressful growing conditions.

**Biomass Partitioning during Flowering**

Biomass partitioning to the developing female reproductive structures in maize varies with plant growth rate. Edmeades and Daynard (1979b) showed that biomass partitioning to tassels was greater than to ears when plant growth is reduced at high population density. Likewise, Schussler and Westgate (1991) showed that stem development was greatly favored over ear development when plant growth was slowed by water stress. Figure 1, redrawn from Andrade et al. (1999), shows how partitioning to ear growth varies over a wide range of individual plant growth rates during the 30 day period bracketing flowering. Detailed comparisons of ear and plant growth indicate that ears grow more rapidly as plant growth rate increases, but ear growth rate clearly is not a linear
function of plant growth rate (Fig. 1). It is particularly noteworthy, that positive ear growth was recorded only when aerial biomass increased at a rate greater than \(~1 \text{ g pl}^{-1} \text{ d}^{-1}\). At the very low plant growth rates, biomass partitioning to the ear was quite low, about 1:18 (g g\(^{-1}\)). Above this threshold, biomass partitioning to the ear greatly increased, and achieved a partitioning ratio near 1:3 (g g\(^{-1}\)). Over the entire range of plant growth rates, the average partitioning to the ear was about 1:6 (g g\(^{-1}\)) (Fig. 1). These data indicated that the proportion of the biomass allocated to the ear varies dramatically with plant growth rate, with a non-linear response and a minimum threshold for ear growth.

It is important to emphasize that these biological relationships between ear and plant growth were exposed by growing plants at a range of population densities much broader than used in commercial production. Also, examining the response of individual plants within each population, by the means of allometric measurements (Vega et al., 2000; Vega et al., 2001), rather than using population averages (Tollenaar et al., 1992) enabled the authors to quantify the response to a much greater range of plant growth rates. Recent studies using this approach have identified significant genotypic differences in the minimum threshold for ear growth at low plant growth rates, and in the maximum ear growth rates at very high rates of plant growth (Echarte et al., 2004; Echarte and Tollenaar, 2006; Pagano and Maddonni, 2007).

At the population level, altering plant growth rate has dramatically different effects on the male and female flower phenology. Yao et al. (1991), for example, subjected one population to various levels of defoliation to decrease light
interception and crop growth around flowering. Figure 2 shows that the appearance of the male inflorescence was not affected by the decrease in resource capture, while the silking pattern of the population was closely coupled to it. The less resource captured by the crop, the greater the delay in silking. Under the most extreme resource limited treatment, fewer than half of the plants reached the silking stage, even though all the plants reached anthesis (Fig. 2). A similar differential response for anthesis and silking has been reported in studies examining maize response to drought (Hall et al., 1982) or shading (Moss and Stinson, 1961). Numerous studies have associated the progress toward silking indirectly with biomass allocation to the ear (Moss and Stinson, 1961; Buren et al., 1974; Jacobs and Pearson, 1991; Yao et al., 1991) or with ear expansion growth (Westgate and Boyer, 1986; Cárcova et al., 2003). In each case, a delay in silking or failure to reach silking was interpreted as a consequence of decreased biomass allocated to the ear at reduced plant growth, as described in Fig. 1. But in no case was a direct relationship between time to silking and biomass partitioning to the ear firmly established.

**Impact of Plant-to-Plant Variability on Time to Silking**

It is important to recognize that the defoliation treatments from Yao et al. (1991; Fig. 2) affected the time to silking of individual plants within each population differently. Under severe defoliation (slowest plant growth) a small proportion of plants reached silking. Under rapid growth all plants reached silking, but some plants reached the stage earlier than others. Therefore, the
impact of plant-to-plant variability on time to silking at the population level must be considered. Under typical field conditions, each plant perceives intense competition from its neighbors (Donald, 1968) so plants within the canopy grow at differential rates. We studied if variability in time to silking was related to the plant-to-plant variability in growth that is commonly observed within canopies.

We integrated biomass and flowering data from two experiments to establish mechanistic relationships between plant growth, ear growth and time to silking.

*Experiment 1: Time to Silking at the Population Level*

In the first experiment, we examined how maize canopies growing at different rates reach silking. To do this, we monitored the time to anthesis and silking of two commercial hybrids sown at 1, 8 and 18 pl m\(^{-2}\). The experiment was conducted in Ames, Iowa, during the 2001 growing season. Individual plant and ear growth rates were measured according to Andrade et al. (1999) and Vega et al. (2001) over a period of 21 days around flowering. Samples for pre-flowering biomass were collected 6 to 9 days before 50% silking and post-flowering samples were taken 12 to 14 days after plots reached 50% silking in each genotype x density combination.

Population density had a dramatic impact on the dynamics and extent of silking of both hybrids. There was little impact on time to anthesis (Fig. 3). All plants grown at low population density of 1 pl m\(^{-2}\) reached silking rapidly, once silking for the population began. All plants grown at the commercial population
density of 8 pl m\(^{-2}\) also reached silking, albeit more slowly. Silking progressed very slowly at 18 pl m\(^{-2}\), and only about 60% of these plants reached the silking stage. The time interval between initial silk appearance and 50% silking for the population increased with population density (2, 3 and 7 days for the 1, 8 and 18 pl m\(^{-2}\), respectively). This result agrees with early studies showing the interval between 25% and 75% silking increased with plant population density (Buren et al., 1974), as well as with any other stress condition around flowering, like drought, shading or defoliation. Time to 50% anthesis was delayed only 2 days as population density increased from 1 to 18 pl m\(^{-2}\), and all plants reached anthesis even at the highest stand density (Fig. 3).

The mean plant growth rate for Holdens LH198xLH185 at the commercial population density of 8 pl m\(^{-2}\) was 3.5 g pt\(^{-1}\) d\(^{-1}\) (Fig. 4A). The coefficient of variation (CV) for growth rate at this population density was about 27%. At the low population density of 1 pl m\(^{-2}\), at which plants are essentially isolated, the mean plant growth rates for LH198xLH185 increased to 10.5 g pt\(^{-1}\) d\(^{-1}\). The CV for the growth rate of these plants was about 18%. At 18 pl m\(^{-2}\), the mean plant growth rate was 1.6 g pt\(^{-1}\) d\(^{-1}\) for LH198xLH185, and the CV was 44%. Similar results were obtained with Dekalb DK611 (Fig. 4A), although growth rates and plant-to-plant variability were slightly higher than LH198xLH185. Thus, as plant-to-plant competition for resources increased with increasing population density, mean plant growth rate decreased and the relative variability between plants within the population increased. These results indicate that relative variability in growth and time to silking within the population increase in more stressful
environments (e.g., 18 pl m$^{-2}$). This is in general agreement with results reported by Edmeades and Daynard (1979a) and Vega and Sadras (2003).

Ear growth increased curvilinearly with plant growth rate above a minimum threshold growth rate (Fig. 4B). The threshold for LH198xLH185 was approximately 0.9 g pl$^{-1}$ d$^{-1}$ and 1.3 g pl$^{-1}$ d$^{-1}$ for DK611. All plants grown at 1 or 8 pl m$^{-2}$ had plant growth rates during flowering above the minimum threshold for ear growth, and all plants grown at these densities reached silking (Fig. 3B). At a population density of 18 pl m$^{-2}$, however, 35 to 40% of the plants had growth rates below this threshold. A similar percentage of the population failed to reach silking in these two hybrids (Fig. 3B). Thus, this analyze indicated that (i) ear growth increases with plant growth rate above a minimum threshold, (ii) silking date is reached earlier and faster at higher plant growth rates, and (iii) relative variability in plant growth rates increases with population density even though absolute variability decreases. We formalized these relationships to create the conceptual framework linking silking dynamics to plant growth rate.

Experiment 2: Time to Silking at the Individual Plant Level

In the second experiment, we examined how altering plant growth around flowering affected the time to reach silking and the interval between anthesis and silking for individual plants within the population. The experiment was conducted in Ames, Iowa, during the 2005 growing season. A maize inbred (MBS Genetics, L.L.C.) was planted at 10 pl m$^{-2}$ and exposed to one of three conditions to alter plant growth rate around flowering. One group of plants was partially defoliated
before anthesis (~75% of the green leaf area removed) to reduce plant growth rate. A second group was thinned to 5 pl m$^{-2}$ at the same time to increase individual plant growth rate. A third set was left at 10 pl m$^{-2}$ to serve as control. Plant growth rate was measured according to Andrade et al. (1999) and Vega et al. (2001), over a period of 20 days around flowering. The pre-flowering biomass sample was taken 8 days before 50% anthesis, and post-flowering samples collected 14 days after plots reached 50% anthesis in all treatment combinations.

Average plant growth rates during flowering were 0.87, 2.63, and 3.70 g pl$^{-1}$ d$^{-1}$ for the defoliated, control, and thinned treatments, respectively (Fig. 5A). The CVs were 36, 26 and 20 % for the defoliated, control and thinned treatments, respectively. These results were in general agreement with observations from our first experiment in that relative variation within the population increased under more stressful conditions (Fig. 4). Time to silking was monitored for each individual plant in the population and plotted against their individual growth rates during flowering. Days to silking were much greater for the slowest growing plants in each population, and increased exponentially at very low plant growth rates exhibited by a number of the defoliated plants (Fig. 5B). But all plants reached anthesis within a few days, regardless of treatment or plant growth rate (Fig. 5C). Delay in time to silking for plants with slower growth rates increased ASI for these plants, which was most dramatic at very low plant growth rates exhibited by defoliated plants (Fig. 5D).
Combining Plant and Population Flowering Processes

Two important insights from these experiments enabled us to couple the response of population silking dynamics to plant growth rate and ear growth. Because silking can be analyzed as a qualitative trait (i.e., either a plant has reached silking, or it has not) and ears are growing continuously when they exert their first silks, there should be a quantifiable value for ear biomass at which silking occurs. Figure 6 illustrates this point for ears collected from the defoliated, control, and thinned plant populations in experiment 2. The left side of Fig. 6 shows that at any time point around anthesis there is a wide variability in accumulated ear biomass at each individual plant. This is related to the variability among plants in their growth. The right side of Fig. 6 shows the ear biomass at which plants silked. The observed ear biomass at silking was remarkably similar across these three treatments. Regardless of the treatment intended to alter plant growth rate, no ears with less than 0.5 g of biomass reached silking. All ears, however, had exerted silks by the time they accumulated 1.0 g of biomass. There was a tendency for plants in the defoliated treatment to exert silks from smaller ears than those in the thinned treatment (Fig. 6). This likely was due to more vigorous growth of the surrounding husks at high plant growth rates in the thinned treatment, which would delay silk exertion slightly. Nonetheless, it is clear from this analysis that each plant in the population must attain a minimum ear biomass (about 0.7 g ear⁻¹) to reach the silking stage. To our knowledge, Fig. 6 shows the only reported values of individual ear biomass at silking. Edmeades et al. (1993) showed that ear biomass increased rapidly
prior to anthesis, but failed to indicate at what point in development silks emerged. Taking their pattern of ear biomass accumulation and reported ASI into account, we estimate that ear biomass at silking was about 1 g ear\(^{-1}\) for the genotypes in their study, which is close to our measured values. Otegui (1997) reported higher values, around 4 g ear\(^{-1}\), probably because average ear biomass was recorded only after 50% of the plants reached silking.

The second insight connecting time to silking with plant growth rate during flowering is that the rate of ear growth can be defined if plant growth rate is known. As illustrated in Figs. 1 and 4, a more rapid rate of plant growth corresponds to a higher rate of ear growth, and therefore, a shorter time required to accumulate the minimum biomass necessary to reach the silking stage. This is evident at both the individual plant and population levels. All plants grown at 1 pl m\(^{-2}\) had faster rates of ear growth than those grown at 18 pl m\(^{-2}\) (Fig. 4) and had correspondingly shorter times to silking for the population (Fig. 3). Plants grown at 8 pl m\(^{-2}\) were intermediate for both ear growth and time to silking.

It was also evident from observing the silking behavior of individual plants that variability in plant growth rate intrinsic to each population defined the range of ear growth, and therefore, the silking dynamics for the plant population. The curvilinear relationship between plant growth rate and ear biomass at the end of the flowering period results in a skewed distribution of ear growth between (Fig. 7A) and within populations (Fig. 7B) as plant growth rate increases. To assign an appropriate ear growth to each plant, we parsed the population into fractions from low to high rates of plant growth. In doing so, it became quite evident that a
small increment in plant growth rate for a slow growing population (or among slow growing individuals in the population) should lead to a large change in time to silking. A similar increment in plant growth rate for a rapidly growing population (or individuals) would result in a much smaller change in time to silking.

Our previous analysis of silking behavior on an individual plant basis revealed that plants with similar growth rates reached silking at about the same time regardless of the specific treatment (Fig. 5B). For example, the first plants to silk at 18 pl m\(^{-2}\) had the same growth rate and silking date as did the last plants to silk at 8 pl m\(^{-2}\). Thus, results from both experiments confirmed that, within a genotype, plants having the same plant growth rate will reach silking at around the same time, independently of the mean growth rate of the population.

Variability in time to silking for the population was greater when mean plant growth for the population was slow (Figs. 4 and 5). This phenomenon was also evident in other studies in which plant growth during flowering was purposefully altered (Buren et al., 1974; Edmeades and Daynard, 1979a; Yao et al., 1991; Uribelarrea et al., 2002). Our population based analysis provides an explanation for the greater plant-to-plant variability in time to silking in populations with slower mean plant growth rates. At low plant growth rates, more plants in the population are closer to the minimum threshold for ear growth (Fig. 4). Low ear growth increases the time required for ears to accumulate the necessary biomass to reach silking. Also, at plant growth rates from 1 to 3 g pl\(^{-1}\) d\(^{-1}\), a small change in plant growth rate translates into a large change in ear
growth (Figs. 1, 4 and 7). As such, plant-to-plant variability at slow growth rates has a greater impact on time to silking because a larger proportion of plants are growing at or near the threshold for ear growth. This analysis provides a plausible explanation for the commonly observed relationship between delayed silking, increased ASI, and plant barrenness in stressful environments (e.g., Woolley et al., 1962).

In summary, the mean plant growth rate and variability in plant growth are fundamentals when understanding time to silking for a population of maize plants. The non-linear nature of biomass partitioning to the female reproductive structures as well as the lack of partitioning below a minimum growth rate result in large differences in ear growth as plant growth varies. This partitioning determines which fraction of the population will achieve the minimum biomass to reach the silking stage, and if so, how quickly the stage is attained. Our experimental results directly relating plant growth rate and ear growth combined with those relating time to silking with a minimum ear biomass, provide the mathematical basis for simulating the observed silking dynamics of a maize population.

A Framework for Population Time to Silking based on Biomass Allocation to the Ear

Creating a Framework

The foregoing analysis indicated silking dynamics of a population of plants was directly linked to biomass partitioning to the ear and the distribution of
partitioning rates within the population. Therefore, we generated a conceptual framework to estimate ear growth based on the plant growth rate, taking into account the plant-to-plant variability intrinsic to the population.

The first step towards predicting the silking dynamics of the population is to describe the mean plant growth rate for the population and the distribution of growth rates within it (see Fig. 4). These variables are specific to the genotype and its response to the environment (Glenn and Daynard, 1974; Vega and Sadras, 2003). The second step is to parse the distribution of plant growth rates into fractions. Figure 8A shows a typical distribution of plant growth rates for each fraction of the population (PGR_{g}). Ear biomass 15 days after anthesis varies for each fraction (EB15DAA_g) according to genotypic coefficients that define partitioning of plant biomass accumulation into ear growth (Fig. 8B) according to Vega et al. (2001):

\[
\begin{align*}
    \text{EB15DAA}_g &= 0; & \text{for } \text{PGR}_g &\leq \text{PGR}_b \\
    \text{EB15DAA}_g &= \frac{\text{IS} \ (\text{PGR}_g - \text{PGR}_b)}{1 + C \ (\text{PGR}_g - \text{PGR}_b)}; & \text{for } \text{PGR}_g &> \text{PGR}_b
\end{align*}
\]

Where IS is the initial slope relating ear biomass (EB) to plant growth rate (PGR_g), PGR_b is the minimum threshold for ear growth, and C is the attenuation factor for the hyperbolic function. PGR_b, IS, and C are genotypic coefficients applied to all plants in the population. Applying these coefficients uniformly to all plants is supported by several studies showing a consistent relationship between
ear growth rate and plant growth rate around flowering across environments (Andrade et al., 1999; Vega et al., 2001; Echarte et al., 2004).

Once EB15DAA$^g$ is determined for each fraction of plants, it is used to calculate the developmental pattern of ear biomass accumulation for the corresponding plants. There is little information on the pattern of ear growth prior to and during silking. Often, reported rates of ear growth around flowering have been estimated by 2-point biomass analysis of a 30 day period bracketing anthesis or silking (Figs. 1 and 4). In general, ear biomass is assumed to be zero in the pre-flowering sample, and the second one is collected 14 to 20 days later (Figs. 1 and 4; Andrade et al., 1999; Vega et al., 2001; Echarte et al., 2004). This approach obviously assumes biomass partitioning to the ear is constant during this interval. This is unlikely, however, since our data show that biomass accumulation in ears is increasing exponentially when plants are silking (Fig. 6). Others have reported similar findings (Edmeades et al., 1993; Schussler and Westgate, 1991; Birch et al., 1999).

Since the response of ear growth around flowering to variation in plant growth rate was fairly consistent across a range of environments (Fig. 4), we normalized the pattern of ear biomass accumulation to a single exponential growth curve (Fig. 8C). The model was based on ear growth observed in the control treatment from Experiment 2 (10 pl m$^{-2}$, Fig. 6). This approach is in accordance with previous observations showing a single curve for ear elongation across genotypes and environments (Otegui and Bonhomme, 1998). The growth curve was normalized to a maximum value of 1.0 at 15 days after anthesis and
progress of ear development monitored relative to anthesis, as this date was far less sensitive to variation in plant growth rate (Figs. 2, 3 and 5). Daily accumulation of ear biomass was calculated for each fraction of the population according to:

\[ EB_g \text{ day}_n = \exp(-2.66 + 0.177 \times \text{ day}_n) \times EB_{15\text{DAA}g}, \]

For clarity, we present the patterns of ear development only for fractions of the population having the slowest 10%, the mean, and the fastest 10% plant growth rates (Fig. 8D). Silking will be reached when a minimum ear biomass associated with the initial exsertion of silks from the husks is attained. We defined this value as the threshold ear biomass at silking \( EB_t \) and assumed it is a genotype coefficient equal for all plants in the population. We set \( EB_t \) as 1.0 g ear\(^{-1}\), as silking was always reached with ear biomass higher than this value (Fig. 6). As such:

if \( EB_g \text{ day}_n < EB_t \) the fraction of plants g has not reached silking, and

if \( EB_g \text{ day}_n \geq EB_t \) the fraction of plant g has reached silking.

Figure 8D shows that silk exsertion is achieved about 1 day earlier than the mean by the fastest growing plants, and about 3 days later than the mean by the slowest growing plants. As such, the population would have completed silking over a period of about 4 days. The relative timing of these events is
determined directly from the relationship between PRG\textsubscript{g} and EB15DAA\textsubscript{g}, and between EB\textsubscript{g} accumulation and silk exsertion.

\textit{Sensitivity Analysis}

The following examples illustrate how the population based approach can be used to identify underlying physiological bases for genotype or environmental variation in time to silking. We tested if changes in traits known to vary due to the genotype or the environment would result in already described population silking patterns for these scenarios. The literature on silking dynamics is fragmented and incomplete, so these analyses are somewhat speculative. And, the outcomes of the model might not perfectly reflect the exact situation. But they demonstrate the importance of quantifying intrinsic plant-to-plant variability within the population and genotype-specific partitioning of biomass to the ear as determinant of time to silking for the population.

The first case illustrates how differences in plant-to-plant variability affect silking dynamics of the population, which is an important characteristic known to affect yield (Maddonni and Otegui, 2004; Martin et al., 2005; Pagano and Maddonni, 2007). The second depicts how genotypic differences in PGR\textsubscript{b} impact the silking pattern under contrasting growth conditions. And the third illustrates how already described physiological changes in plant growth and partitioning around flowering by traditional breeding can explain the observed changes in silking dynamics of modern vs. old commercial genotypes. In each case, the
analysis assumes a population with normally distributed plant growth rates (Vega and Sadras, 2003) divided into twenty fractions.

The impact of variation in plant growth rate on the silking pattern for the population was tested by comparing three populations having the same mean plant growth rate (PGR$_{50}$) with CVs of 15, 30, or 60% of this mean value. This is graphically shown in Fig. 9A, where the PGR$_{50}$ was set to be 3 g pt$^{-1}$ d$^{-1}$. The relation between plant growth rate around flowering and ear biomass 15 days after anthesis was the same for all plants in the three populations (Fig. 9A). Greater variability among plants in their growth rates lead to a longer time interval between initial and 100% silking for the population (Fig. 9B). It is interesting to note that the progress of silking for the population was not normally distributed around the 50% silking date, even though this date was similar for all three populations and plant growth rates were normally distributed in all three cases. This result is in accordance with experimental observations showing an extended delay in silking for the late silking plants (Edmeades and Daynard, 1979a), and is expected from the curvilinear relationship between PGR$_g$ and EB15DAA$_g$ (Fig. 7). Plants growing at rates closer to the minimum for ear growth (PGR$_b$) take longer to reach silking. This analysis also confirms earlier observations of Uribelarrea et al. (2002) calculating ASI as the time interval between population 50% anthesis and 50% silking is not equivalent to measuring plant-by-plant individual ASI and calculating the mean for the population.

The second example illustrates how our population based approach can be used to analyze differences in genotype response to environmental conditions
that inhibit plant growth during flowering. Genotypic differences in silking patterns and yield performance under source limited conditions around flowering have been well documented (Moss and Stinson, 1961; Woolley et al., 1962; Buren et al., 1974; Soriano and Ginzo, 1975; Bruce et al., 2002). The physiological mechanism(s) underlying these differences in stress tolerance, however, remain obscure. An important implication of our framework is that an earlier time to silking can be achieved by a higher \( \text{PGR}_{50} \) or a lower \( \text{PGR}_b \). At present, genotypic differences in rapid silking under stress conditions seem to be more related to differences in biomass partitioning than to plant biomass production around flowering. In one of the earliest studies on the problem, Moss and Stinson (1961) concluded that the genotypic differences they observed in time to silking and stress tolerance were not related to the current rate of photosynthesis or plant growth. Improved drought tolerance and a more synchronous ASI resulted from increased partitioning of biomass to the developing ears, rather than greater plant growth during flowering, in different maize tropical populations (Edmeades et al., 1993; Edmeades et al., 1999; Monneveux et al., 2005).

In this example, we compared two genotypes that varied in the minimum plant growth for positive ear growth (\( \text{PGR}_b \): 0.5 g pl\(^{-1}\) d\(^{-1}\) for genotype A and 1.5 g pl\(^{-1}\) d\(^{-1}\) for genotype B). Population time to silking was simulated for two environments. One that supported rapid plant growth during flowering (\( \text{PGR}_{50} \): 6 g pl\(^{-1}\) d\(^{-1}\)) and a second that limited plant growth during this period (\( \text{PGR}_{50} \): 2 g pl\(^{-1}\) d\(^{-1}\)). For simplicity, EB15DAA\(_g\) responded similarly to increases in \( \text{PGR}_g \) and plant-to-plant variability was set to 30% for all four populations (although these
variables can be adjusted readily for each population). Figure 9C shows in a graphical fashion the genotypic differences in the relation between plant growth rate and ear biomass accumulation, and the plant growth rates from each population in each environment. Under conditions favoring rapid plant growth, the two genotypes were virtually indistinguishable in terms of their silking patterns (Fig. 9D, closed symbols). Both populations started and completed silking about the same time. In the environment that limited PGR$_{50}$, however, genotype B was much more sensitive to the reduction in plant growth around flowering. The higher minimum plant growth for ear biomass accumulation (PGR$_b$) in genotype B delayed silking and prevented a larger percentage of plants from silking. The differential response of these two genotypes to a similar reduction in plant growth resulted directly from the natural variation in plant growth rates within the population, and the inherent genetic variation in partitioning to ears.

Selection for high yield has decreased the ASI by reducing time to silking (Duvick, 1997; Duvick et al., 2004) especially under stressful conditions (Luque et al., 1998; Sangoi et al., 2002). Results from studies suggested that increased yield of modern maize hybrids is associated with decreased plant-to-plant variability (Tollenaar and Wu, 1999), decreased PGR$_b$ and increased EGR$_g$ at high PGR$_g$ (Tollenaar et al., 1992; Echarte et al., 2004; Luque et al., 2006). We tested whether such physiological changes could explain earlier and faster silking dynamics typically exhibited by modern hybrids. Evidence that increased biomass or mean plant growth rate around flowering has contributed to
differences between old and new genotypes is inconsistent (Tollenaar, 1991; Tollenaar et al., 1992; Luque et al., 2006). So this sensitivity analysis does not include changes in this aspect. We compared the silking dynamics of an ‘old’ hybrid with those of a ‘modern’ one in two environments supporting significantly different plant growth rates. The ‘old’ hybrid population was assigned to have a coefficient of variation (CV) for plant growth of 35%, a PGR_{b} = 1.5 g pl^{-1} d^{-1} and an attenuation factor C=0.2 relating EB15DAA_{g} and PGR_{g} (Fig. 8B). The ‘modern’ hybrid was assigned a CV= 25%, PGR_{b} = 0.5 g pl d^{-1}, and C= 0.1. Results in Fig. 9F showed the modern genotype to have a faster and earlier pattern of silking under both growing environments. The delay in time to silking in the environment with the lower PGR_{50} was considerably longer for the ‘old’ hybrid. These differences are in accordance with previous observations comparing changes in phenology of modern and old genotypes (Duvick, 1997; Luque et al., 1998; Sangoi et al., 2002; Duvick et al., 2004).

Discussion

Plant Growth and Time to Silking

Growth involves irreversible changes in size, whereas development often involves more subtle qualitative changes (e.g., apex shifting from vegetative to reproductive). For some processes, however, growth and development are closely linked. This is the case with time to silking in maize. Early work by Moss and Stinson (1961), Woolley et al. (1962), Early et al. (1966) and Edmeades and Daynard (1978a) firmly established that time to silking was affected by
environmental conditions that alter plant growth around flowering. The simple biomass framework we developed couples the developmental event of silking to plant growth, which can be evaluated at the population level.

At present, absolute silking dates are linked to anthesis date. Fortunately, current crop growth models predict tassel initiation and anthesis fairly accurately, when genotypic and environmental effects are primarily a function of temperature and photoperiod (Warrington and Kanemasu, 1983; Jones and Kiniry, 1986; Birch et al., 1998). Our framework for time to silking could readily be incorporated as a module into such models.

To capture the intrinsic variability in plant growth and time to silking, we borrowed the notion of combining qualitative and quantitative elements in the same model from work on seed germination by Bradford (2002). This helped integrate the qualitative change of stage at the plant level when silking is reached, to the quantitative process that is observed at the population level. Most important, it helped us integrate growth and development at the individual plant and population level. It would be interesting to test if these concepts help understand other developmental process known to be affected by growth, as time to maturity in cotton (Bange and Milroy, 2004).

Additional Knowledge to Further Understand Time to Silking

The foregoing analyses imply a direct relationship between ear growth and time to silking. Although it would seem intuitive that time to silking would be a function of biomass allocation to the ear, published examples illustrating this
relationship were lacking. We observed a minimum biomass of about 0.7 g ear\(^{-1}\) was necessary before the first silks were exerted from the husks (Fig. 6). Defining this minimum value was critical for linking plant growth rate with silking date. Environmental effects on minimum ear biomass to reach silking stage are basically unknown, but there was little difference among the three growing conditions we explored, which suggests variation could be small. Nonetheless, additional study is needed to determining the extent of genotypic and environmental variation in this parameter for a wider range of growing conditions.

It was necessary to normalize ear development to the date of anthesis because the point in time at which ears start accumulating significant biomass is not well defined. Otegui and Bonhomme (1998) reported that rapid ear elongation started at 227 degree days before silking. Although this time-point likely coincides with the initiation of rapid ear biomass accumulation, reference to tassel initiation or anthesis would provide a far more robust baseline for treatment comparisons. In contrast to silking date, developmental benchmarks for the tassel are not affected dramatically by environmental conditions affecting plant growth. The early initiation of rapid growth in the reproductive structures is an important trait for improving grain yield in other crops. In wheat, for example, greater partitioning of biomass to spikes in modern vs. old genotypes around flowering has been attributed to initiating spike growth earlier, when there is less biomass in the stems (Slafer and Andrade, 1993). Partitioning of phloem delivered nutrients between competing sinks is governed by their relative ability to unload major osmotic species from the importing phloem sieve (Patrick, 1997).
As such, an earlier transition to rapid spike growth lead to a greater competitive ability for attracting photo-assimilates later in development, when the number of fertile florets is being determined (Miralles et al., 1998). Although maize breeding has increased biomass partitioning to the ear around flowering (Tollenaar et al., 1992; Edmeades et al., 1993; Echarte et al., 2004; Monneveux et al., 2005; Luque et al., 2006), this shift in partitioning has not been directly coupled to an earlier initiation of rapid ear growth. Determining the point at which ears initiate rapid growth would improve our capacity to predict time to silking and to evaluate ear and tassel development as independent, but temporally associated processes.

Concluding Remarks

A framework based on the physiological processes regulating time to silking at the population level provides a rational basis for resolving genotype differences, environmental effects, and genotype x environment interactions at the higher levels of organization (plant and crop levels). It also provides a rational basis for future studies aimed at identifying the genetic factors regulating gene-to-phenotype relations (Yin et al., 2004; Hammer et al., 2006) particularly as they relate to ear development and silking behavior.

The key issues emerging from this study are:

1. Understanding maize flowering dynamics as a quantitative trait at the population level but as a qualitative trait at the plant level is essential. In a field crop each plant suffers from intense competition from its neighbors,
and individual plants are sensing, exploring and reacting to their environment independently. We showed the value of using a population based approach by taking into account the intrinsic plant-to-plant variability in maize populations to understand time to silking in maize crops.

2. Time to silking needs to be understood as an ear expansion growth process. As such, any results directly relating plant growth rate and ear growth rate, and those relating time to silking with a minimum ear biomass become imperative.

3. It is important to note the value that allometric models offer in estimating individual plant growth rate (Vega et al., 2000; Vega et al., 2001), as it gives the possibility of a new method of analysis with a population-based approach. As population-based models are required to understand and interpret data like time to silking, and most importantly, to design experiments not confounded by averaging, the use of methodologies like this becomes crucial.

4. For the first time a framework to quantify time to silking of a maize population is presented that can be used to study environmental and genotypic differences affecting plant growth. The key parameters that need to be quantified as genotypic coefficients in relation to silking behavior are: (i) the relationship of ear growth to plant growth rate around flowering, (ii) the pattern of ear biomass accumulation during early growing stages, and (iii) the amount of accumulated biomass an ear needs to reach to attain the
silking stage. We believe current information regarding early ear biomass growth is an important gap at present.

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Figure 1: Relationship between ear growth rate and plant growth rate around flowering for individual maize plants. The variability in plant growth rate was achieved with population densities raging from 2.2 to 16.9 pl m$^{-2}$. Adapted from Andrade et al. (1999). Dotted lines show similar biomass partitioning to the ear during the flowering period (1:3, 1:6, and 1:18 g g$^{-1}$ ratios). Rhombus indicates plants with a single ear, and squares indicate the sum of the apical and sub-apical ear growth from prolific plants.
Figure 2: Effect of reducing the leaf area index of a maize population around the flowering period from 2.3 (black circles, control treatment) to 0.6 and 0.3 (grey and white circles, respectively) on the tasseling and silking dynamics of the population by defoliation. Adapted from Yao et al. (1991).
Figure 3: Anthesis (A) and silking (B) dynamics of two commercial genotypes (Holdens LH198xLH185 and Dekalb DK611) grown at three contrasting population densities (1, 8 and 18 plants m$^{-2}$; black, grey and white circles, respectively).
Figure 4: A: Frequency distribution of plant growth rate for two commercial genotypes (Holdens LH198xLH185 and Dekalb DK611) grown at three contrasting population densities (1, 8 and 18 plants m$^{-2}$; black, grey and white circles, respectively). Lines show the normal distribution of plants for each genotype x population density combination. For each genotype and population density combination the mean plant growth rate and the coefficient of variation (CV) of this mean plant growth rate are shown. A minimum number of 72, 153 and 235 plants (1, 8 and 18 plants m$^{-2}$, respectively) were measured for each genotype. B: Relation between ear biomass at the end of the flowering period (12 to 14 days after 50% silking) and plant growth rate for all the individual plants measured. Symbols as in A. Arrows indicate the adjusted plant growth rate threshold from where increases in plant growth gave positive ear growth rates. Plant growth rate and ear growth were measured following the procedures from Andrade et al. (1999).
Figure 5: Distribution of individual plant growth rate (A) and its relationship to time from planting to silking (B), time from planting to anthesis (C) and anthesis-silking interval (D) for plants in the three treatments of Experiment 2. A single maize inbred line was planted at 10 plants m⁻² and defoliated (to reduce plant growth; ~75% of green leaf area reduced; white circles) or thinned (to increase the plant growth of the remaining plants; 50% of the plants were uniformly thinned; black circles) around 8 days before anthesis. Mean individual plant growth rates around flowering were 0.87, 2.73 and 3.61 g pl⁻¹ d⁻¹ of the defoliated, control and thinning treatments respectively. The CVs of the individual plant growth rates within each population were 36, 26 and 20 % from the defoliation, control and thinning treatments respectively. A total number of 60 plants per treatment were measured (20 consecutive plants in the row in each of three replicates). Negative power functions in B and D were fitted to the 180 data points ($r^2 = 0.37$ and 0.52 in B and D, respectively), curve parameters where not different between the three populations (p<0.05) so a single curve is shown. Model fitted in B: time to silking = 70.60 + 3.77 * PGR ^(-0.699). Model fitted in D: anthesis-silking interval = 0.449 + 2.74 * PGR ^(-0.908).
Figure 6: Left: Ear biomass accumulation as a function of time after anthesis from Experiment 2. A total number of 123 plants per treatment were sampled (41 plants in each one of three replicates) around the flowering period, and individual ear data are shown. A single maize inbred line was planted at 10 pl m$^{-2}$ and defoliated (to reduce plant growth; $\sim$75% of green leaf area reduced) or thinned (to increase plant growth of the remaining plants; 50% of the plants were uniformly thinned) around 8 days before anthesis. Right: Silking as a function of ear biomass accumulation in individual ears. Silking was monitored for each individual ear at the moment of sampling. The dotted line shows the accumulated ear biomass necessary to reach silking. The experiment, same as Fig. 5, was conducted in Ames, Iowa, during the 2005 growing season.
Figure 7: Schematic diagram showing mean plant growth rate related to ear biomass at the end of the flowering period for three populations of plants differing in mean plant growth around flowering (Fig. 6 left), and the same for a single population in which the different fractions of the population are divided (Fig. 6 right). Because of the relation between plant growth rate and ear growth rate around flowering (see Fig. 1), changes in plant growth rate are not linearly transferred to changes in ear growth rate around flowering. Numbers in the X and Y axis are arbitrary.
Figure 8: Schematic diagram for the proposed mechanistic theoretical framework of genotypic and environmental effects on time to silking. Environmental conditions affect plant growth rate (PGR$_g$) and its variability (CV) (A). This PGR$_g$ determines ear growth and its biomass 15 days after anthesis each fraction of the population of plants achieves (EB$_{15DA}$). The EB$_{15DA}$ is determined by the hyperbolic function with parameters (base plant growth rate, PGR$_b$; initial slope, IS; curve attenuation, C) that are genotype dependent (B). Figure 8B shows the adjusted model to the data from Holdens LH185xLH198 (Fig. 4B). The ear growth rate is further divided during the flowering period in an exponential curve (C) and daily ear biomass accumulation calculated (D). Silking is reached at the time-point accumulated ear biomass is higher than a particular biomass threshold, here called ear biomass threshold (EB$_t$). This EB$_t$ is also a specific genotypic coefficient. In Fig. 8D accumulated ear biomass is shown for the fraction of the population that shows the mean plant growth rate (PGR$_{50}$, solid line), and for the 10% fastest and slowest growth fractions of the population (PGR$_{10}$ and PGR$_{90}$, dotted lines).
Figure 9: Examples of population silking time courses (B, D, F) resulting from our proposed framework when testing specific combinations. A and B- Example of changes in the plant-to-plant variability within the population for 15, 30 and 60% coefficient of variation (black triangles, grey circles and white rhombus symbols, respectively) at the same mean plant growth rate. C and D- Example showing two genotypes differing in the base plant growth rate (PGR_b) at which higher plant growth rates give positive ear growth rates (0.5 and 1.5 g pl⁻¹ day⁻¹, triangle and circle symbols, respectively) in two growth environments (PGR₅₀: 2 g pl⁻¹ day⁻¹ white symbols; PGR₅₀: 6 g pl⁻¹ day⁻¹ black symbols). The CV of the populations was set to a constant 30% for both genotypes and environments. E and F- Example of two genotypes differing in their PGR_b (modern: 0.5 g pl⁻¹ day⁻¹, triangle symbol; old: 1.5 g pl⁻¹ day⁻¹, circle symbol), coefficient of variation (CV, modern: 25%; old: 35%) and in the attenuation in the relation between EGR_g and PGR_g (C, modern: 0.2; old: 0.1), growing in two growth environments (PGR₅₀: 2 g pl⁻¹ day⁻¹ white symbols; PGR₅₀: 6 g pl⁻¹ day⁻¹ black symbols)