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Quantitative assessment of kernel set and risk of out-crossing in maize based on flowering dynamics

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Quantitative assessment of kernel set and risk of out-crossing in maize based on flowering dynamics

by

Agustin Ezequiel Fonseca

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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For the Major Program
To my family and Gema Grau.
TABLE OF CONTENTS

LIST OF FIGURES vi
LIST OF TABLES x
ABSTRACT xii

CHAPTER 1. GENERAL INTRODUCTION 1
Introduction 1
Thesis Organization 7
References 8

CHAPTER 2. APPLICATION OF FLUORESCENCE MICROSCOPY AND IMAGE ANALYSIS FOR QUANTIFYING DYNAMICS OF MAIZE POLLEN SHED 13
Abstract 13
Introduction 13
Materials and Methods 15
Results and Discussion 17
References 20

CHAPTER 3. TASSEL MORPHOLOGY AS AN INDICATOR OF POTENTIAL POLLEN PRODUCTION IN MAIZE 29
Abstract 29
Introduction 30
Materials and Methods 32
Results and Discussion 36
Conclusions 42
References 43

CHAPTER 4. PREDICTING POTENTIAL KERNEL SET IN MAIZE FROM SIMPLE FLOWERING CHARACTERISTICS 56
Abstract 56
Introduction 57
Materials and Methods 59
Results 66
Discussion 71
References 75

CHAPTER 5. SIMULATING POTENTIAL KERNEL PRODUCTION IN MAIZE HYBRID SEED FIELDS 90
Abstract 90
Introduction 91
Materials and Methods 93
Results and Discussion 100
LIST OF FIGURES

Chapter 2
Figure 1. Direct measure of fluorescing pollen grains.
Figure 2. Correlation between actual and measured pollen density.
Figure 3. Distribution of pollen counts on pollen traps collected at three pollen shed densities.
Figure 4. Pollen density estimated using an increasing number of images taken from pollen traps collected on different days during pollen shed.
Figure 5. Digital images of fluorescing pollen taken from pollen traps collected at varying pollen shed densities.
Figure 6. Seasonal pattern of pollen shed relative to days after anthesis (50% of plants at maximum pollen shed) for 2 commercial maize hybrids (P3394 and P3489).

Chapter 3
Figure 1. Tassel development scale.
Figure 2. Changes in tassel weight during development for three maize genotypes with contrasting tassel morphologies.
Figure 3. Relationship between pollen weight and tassel weight loss (Stage 4 to Stage 8) for the three genotypes shown in Fig. 2.
Figure 4. Relationship between pollen production and tassel weight loss (Stage 4 to Stage 8) for 22 inbreds representing six heterotic groups.
Figure 5. Variation in pollen grain dry weight for two hybrids grown at 2.5, 7.5, or 12.5 plants per m².
Figure 6. Correlations between the total number of pollen grains produced by an individual tassel and selected morphological characteristics.
Figure 7. Correlations between the total number of pollen grains produced per tassel and main stem length, total branch length, main stem diameter and Tassel Area Index measured on individual plants at Stage 6.
Figure 8. Coefficient of variability for main stem length (cm), total branch length (cm), main stem diameter (mm), Tassel Area Index, and pollen production per tassel (grains per plant) for two hybrids grown in 2001 and 2002 at three population densities, and 22 inbreds grown at commercial densities in 2002.

Figure 9. Pollen grains per tassel (a) and Tassel Area Index (b) for 22 commercial inbred lines.

Chapter 4
Figure 1. Dynamics of silk exsertion in apical and subapical ears of Asgrow 740 as influenced by plant population density.
Figure 2. Seasonal dynamics of pollen shed intensity for hybrids P3978 (a,c) and P3925 (b,d).
Figure 3. (a,b) Normalized percentage of plant population at three stages of pollen shed: beginning shed, maximum shed, and end shed for P3978 (a) and P3925 (b).
Figure 4. Seasonal progress of silk emergence for the plant population and individual apical ears.
Figure 5. Seasonal dynamics of silk exsertion on an area basis for hybrids P3978 (top) and P3925 (bottom).
Figure 6. Relationship used to convert daily pollen shed density into potential kernel set adopted from Bassetti and Westgate (1994).
Figure 7. Seasonal dynamics of pollen shed and potential kernel set for P3978 (a,c) and P3925 (b,d) at two levels of male fertility (MF).
Figure 8. Kernel set expressed relative to the number of exposed silks at four levels of male fertility (MF) for P3978 and P3925.

Chapter 5
Figure 1. Quantification of the pollen shed dynamic in Field F determined on the basis of male flowering dynamics.
Figure 2. Silk exsertion dynamic in Field F determined on the basis of observations of ear development.

Figure 3. Simulated kernel production in six hybrid seed fields (A-F) planted to various combinations of male and female inbreds varying in flowering characteristics.

Figure 4. Correlation between measured and simulated kernel production for six hybrid seed fields.

Figure 5. Change in simulated kernel production because of variation in pollen production per tassel.

Figure 6. Change in simulated kernel production because of synchrony in male-female flowering dynamics.

Figure 7. Change in simulated kernel production because of the interval between anthesis dates for two male pollen sources.

Figure 8. Response of simulated kernel production to uniformity of silking for the female population.

Figure 9. Response of simulated kernel production to rate of silk exsertion per ear.

Chapter 6

Figure 1. In vitro germination of maize pollen grains.

Figure 2. Pollen moisture content measured immediately after grains are released from the anthers and collected at different times of the day.

Figure 3. Moisture content of pollen grains exposed for one hour to a range of vapor pressure deficits (VPD) provided by a combination of temperature (25, 32.5 and 40°C) and relative humidity (15, 50 and 85%).

Figure 4. Percentage of viable pollen grains as a function of its moisture content of Inbred B.

Figure 5. Change in pollen moisture content (PMC) with time for pollen grains exposed to different VPDs.

Figure 6. Relationship between pollen moisture content and the vapor pressure deficit time index (VPDT).
Figure 7. Simulated dispersal patterns of viable pollen from four corn production environments in 2003.

Chapter 7
Figure 1. Correlation between measured and simulated kernel production for 13 hybrid seed fields representing two growing seasons.
Figure 2. Simulated daily kernel production in four hybrid seed fields that differed in seed yield and RI.
Figure 3. Simulated change in RI and kernel yield due to variation in the anthesis silking interval in four fields.
Figure 4. Simulated change in RI and kernel yield due to variation in the amount of tassel pollen production in four fields.
Figure 5. Simulated percentage of out-crossed kernels assuming an arrival of 1 adventitious pollen grain per cm$^2$ on a single day in four selected fields.
Figure 6. (A) Simulated genetically-pure and self-pollinated kernel production in Field L. (B) Simulated percentage of self-pollination for a range of female anthesis-silking intervals.
LIST OF TABLES

Chapter 3
Table 1. Linear correlations between pollen production and various tassel morphological characteristics for three contrasting genotypes grown at 2.5, 7.5, and 12.5 plants per m².
Table 2. Mean values for pollen production per tassel and tassel morphological characteristics for 22 inbred lines from six heterotic groups.

Chapter 4
Table 1. Parameters used in Eq. [4] to predict daily silk exsertion for subapical ears of P3925.
Table 2. Observed and predicted number of florets and kernels per unit land area produced at various levels of male fertility (MF) for P3978 and P3925.
Table 3. Predicted kernel number at decreasing levels of pollen viability for the 20% male fertile (MF) treatment for P3978 and the 50% MF treatment for P3925.

Chapter 5
Table 1. Inputs required to simulate potential kernel production for a given pair of male and female inbreds.
Table 2. Comparison of simulated and measured kernel production for six seed production fields having various combinations of male and female inbred flowering characteristics.

Chapter 6
Table 1. Pollen viability data for 11 genotypes grown in the field.
Table 2. Regression parameters and vapor pressure deficit time index (VPDT) values for 11 genotypes grown in the field.
Table 3. Simulated time (min) to 50 and 0% pollen viability (PV_{50} and PV_{0}) at four locations in 2003.
Chapter 7
Table 1. Flowering characteristics, simulated and measured kernel production, and RI for 13 hybrid seed production fields examined in 2002 and 2003.
ABSTRACT

Pollination in maize can occur only if airborne pollen shed by the staminate flowers on the tassel is captured by the stigmas of the pistillate flowers borne on the ear. Since maize domestication, and especially with the introduction of hybrid seed production, many attempts have been made to control pollination to ensure maximum kernel set and high levels of genetic purity. The central hypothesis of this dissertation is that pollen shed and silk emergence are predictable processes, which can be simply quantified and used to characterize the capacity of the male inbreds to produce pollen and the ability of the females to extrude silks. This information, coupled with an assessment of pollen viability, could be used to model kernel set under varying conditions and to predict pollen drift and the risk of genetic contamination from one field to another. Chapter 2 presents a novel method to quantify the timing and intensity of pollen shed using passive pollen traps and capitalizing on the capacity of pollen to fluoresce. Chapter 3 provides another novel method of relating genetic differences in pollen production to the morphological characteristics of plant tassels. Chapter 4 describes a mathematical model developed to simulate potential kernel set from seasonal dynamics of pollen shed and silk exsertion. Chapter 5 successfully applies the model to hybrid seed production fields and demonstrates its utility to define management strategies that would optimize seed production. Chapter 6 presents an indirect approach to assess loss of pollen viability during its transport in air. Quantitative relations between environmental conditions during pollen dispersal (i.e. temperature, relative humidity) and pollen desiccation that can be used to assess pollen viability are shown. Finally, chapter 7 presents a quantitative approach to assess out-crossing and self-pollination risks based exclusively on the flowering characteristics of a given field.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Pollination in maize (Zea Mays L.) can occur only if airborne pollen shed by the staminate flowers on the tassel is captured by the stigmas (silks) of the pistillate flowers borne 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 (Bassetti and Westgate, 1994; Cárcova et al., 2000). Since maize domestication, and especially with the introduction of hybrid seed production, many attempts have been made to control pollination to ensure maximum kernel set and high levels of genetic purity. In hybrid seed production fields, seed companies utilize several practices, including crop rotations, high purity parent seed, mechanical detasseling of the female parent, temporal or spatial isolation from corn in nearby fields, and inclusion of border rows of the male parent around the field (Burris, 2001). Although expensive and laborious, these strategies are not always successful. With the recent release of transgenic corn hybrids, pollen dispersion has also become an important issue in grain production fields, due to the potential flow of transgenes from commercial cultivars into landraces and wild relatives of maize (Luna et al., 2001), as well as into conventional hybrids harvested for seed. For these reasons, a better understanding of the factors that affect pollen production, pollen dispersion and silk emergence under varying field conditions is needed. To that end, simple methods for quantifying these processes are highly desirable.

Corn is monoeic and the flowers are physically separated. Therefore, the female flowers are naturally cross-pollinated. Due to this uncontrolled pollination, corn often is referred to as an open-pollinated crop (Poehlman, 1995). Corn pollen is 70 to 100 μm in diameter and spherical in shape (Wodehouse, 1935; Jones and Newell, 1948), and among the largest particles that are commonly airborne (Raynor et al., 1972). Pollen weight is approximately 250 ng (Goss, 1968). Pollen shed from an individual tassel usually begins after the tassel is fully expanded, beginning on
the main rachis close to the apex and continuing in both directions. Branches start to shed one or two days after the beginning of the main rachis and follow the same pattern. The typical tassel will shed pollen for 2-10 days, varying with genotype and environment. Daily pollen release will depend in some extent on moisture and temperature conditions, but it will generally last about 4-5 hours, starting one hour after sunrise (Flottum et al., 1984). Studies attempting to describe the quantitative patterns of pollen release from maize tassels are limited, generally because collecting and counting pollen is laborious. Flottum et al. (1984) determined the intensity of pollen shed by collecting samples on spinning rods and counting adhering pollen visually. Sadras et al. (1985) collected pollen shed daily within tassel bags, and counted the pollen with a light microscope. Bassetti and Westgate (1994) monitored pollen shed intensity with passive pollen traps, and counted the pollen grains by computer-aided video imaging. None of these techniques readily distinguished pollen from similar-sized debris in the measurement. For this reason, a simple, rapid and more reliable technique for quantifying the dynamics of maize pollen shed under field conditions is needed. Recent advances in instrumentation for acquiring, processing and analyzing fluorescence signals have made it possible to capitalize on natural auto-fluorescence of pollen grains and tubes to quantify pollen germination in *Nicotiana tabacum* L. (Tirlapur and Cresti, 1992; Keller and Hamilton, 1998), *Tradescantia paludosa* ES Anderson & Woods (Keller and Hamilton, 1998) and *Pennisetum ciliare* (L.) Link. (Shafer et al., 2000). To our knowledge, pollen fluorescence has not been used to characterize any aspect of maize pollen development or shedding dynamics.

There is considerable information on tassel development and pollen shed, but it has not been used to predict pollen production per tassel. Total pollen production per plant can vary considerably. Reported values of 20-42.2 million grains for old cultivars (Hall et al., 1982; Sadras et al., 1985), contrasts with more recent values of 9.6 to 11.3 millions grains (Uribelarrea et al., 2002) observed for modern hybrids. This decrease in pollen production reflects the smaller tassel size of today's maize hybrids (Galinat, 1992; Duvick, 1997). This trend is also true for male inbreds, and
the higher incidence of out-crosses in modern hybrid seed production may be associated with reduced pollen capacity of modern male inbred parents. The average pollen yield for male inbreds ranges from 200,000 to more than 3 million pollen grains per tassel (Grass, unpublished data). The general relationship between tassel size and pollen production suggests that a quantitative estimate of pollen production can be obtained from tassel morphological characteristics. To our knowledge, no satisfactory results have been obtained in this regard.

Other approaches for assessing pollen production have been explored. Westgate et al. (2003) developed an indirect method of predicting pollen shed in the field from male flowering dynamics. They calculated a 'population index' for a daily pollen shed considering the progression of pollen shedding in a population of tassels, from the initiation of shed through maximum shedding to the end of shedding. This index, coupled with an estimate of the average pollen production per tassel, predicted pollen shed dynamics very accurately. While this technique provides a link between tassel development and pollen shed, it requires an accurate estimate of pollen production per tassel.

For simulation purposes, the developmental events that determine kernel number per plant can be divided in three consecutive processes. In the first stage, male (tassels) and female (ears) reproductive structures are initiated and differentiated. The second stage involves functional maturation of flowers and pollination. Synchrony in floral development is critical to ensure that pollen shed coincides closely with silk exsertion and that tassels produce enough pollen to ensure pollination of exposed silks. During the third stage, pollination is followed by fertilization and kernel formation. Current models for simulating maize yield, such as CERES-Maize (Jones and Kiniry, 1986), focus exclusively on the third stage. Most efforts have attempted to associate the final kernel number per plant with the current supply of photosynthate, or related characteristics such as light interception or plant growth rate around the time of silking (Edmeades and Daynard, 1979; Tollenaar et al., 1992; Andrade et al., 1993; Kiniry and Knievel, 1995; Otegui, 1997; Andrade et al., 1999; Andrade et al., 2000). They implicitly assume that neither flower initiation,
differentiation, nor pollination limit kernel set. These assumptions might apply to
typical commercial corn production in some cases, but the number of fertilized
ovules can limit kernel yield in a wide array of circumstances. As 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 (Bassetti and
Westgate, 1994; Cárcova et al., 2000).

Environmental conditions can affect pollen availability by modifying the
synchrony between pollen shedding and silk emergence, by affecting how long
pollen remains viable, or by changing the amount of pollen produced per tassel (Hall
et al., 1982; Bolaños and Edmeades, 1993). Distinguishing between these
possibilities has important implications for yield stability across environments, as well
as germplasm selection and genetic purity. Yet, no simple means is available to
provide a quantitative measure of floral synchrony (number of grains per exposed
silk) under field conditions. Defining the quantitative relationships between pollen
shed, silk emergence, and kernel set under contrasting field conditions is an
essential prerequisite for managing floral synchrony in maize. Bassetti and Westgate
(1994) showed that pollen shed rates follow a 'normal' distribution and that pollen
shed densities at 100 grains cm$^{-2}$ d$^{-1}$ or greater reaching the plane of exposed silks
were sufficient to achieve maximum kernel number ear$^{-1}$. In further studies (Bassetti
and Westgate, 1993a and b) also described the dynamics of silk emergence and
senescence on individual ears of maize as a progressive process that varies by
genotype and environmental conditions. These few reports on pollen shed and silk
emergence indicate the processes of male and female flowering in maize progress
in a fairly predictable manner. As such, it should be possible to develop a
mechanistic description of kernel set on a field scale based on a quantitative
evaluation of synchrony between pollen production and silk exsertion.

In maize seed production field pollination could be less than desired for
several reasons. First, pollen shed density is much less than in a grain field since
inbreds typically produce less pollen than do their hybrid counterparts. Second, only
a fraction of the field population is permitted to shed pollen, i.e., male inbred. A
major goal in hybrid seed production, in fact, is to reduce the area dedicated to male rows as much as possible without decreasing the number of kernels harvested per area (Wych, 1988). Third, the level of pollen viability could be less than required for optimum pollination of receptive silks (Schneider, unpublished 2003). Finally, pollen shed and silk exsertion on physically separated plants increases the probability that floral asynchrony can lead to poor kernel set. Together these biological and physical factors create conditions in which kernel numbers could be limited primarily by the number of pollinated flowers. Since achieving the optimum seed yield per unit land area often is based on limited information about the quantity of pollen shed by the male and practical experience synchronizing pollen shed by the male inbred with silk emergence by the female inbred, a simple mechanistic description of the flowering dynamics of male and female could be useful to define management practices for a given inbred combination.

Over the last few years there has been increasing interest in pollen dispersal, particularly in relation to gene flow from transgenic crops and the maintenance of seed quality (Luna et al., 2001; Jarosz et al., 2003). But information regarding maize pollen travel and environmental effects on pollen dispersal and viability is still limited (Garcia et al., 1998; Luna et al., 2001; Aylor, 2003). Three general approaches for evaluating pollen quality are used: in vivo fertilization, in vitro pollen germination and pollen tube growth, and histochemical staining (Heslop-Harrison et al., 1984; Abdul-Baki, 1992). The in vivo approach involves placing pollen grains on silks and noting their ability to germinate and to cause seed production. Although only one pollen grain actually fertilizes the ovule, many usually arrive on each silk. Placing only one pollen grain on each silk to test viability is a task that is time-consuming and practically impossible (Goss, 1968). Furthermore, seed set may depend not only on fertilization, but also on the post-pollination development of the ovary, pistil receptivity, and incompatibility reactions (Stanley and Linskens, 1974). The in vitro approach has been widely attempted. Under appropriate incubation conditions, pollen grains germinate on an artificial liquid or semi-solid nutrient medium, producing normal pollen tubes. Media usually contain a carbon source and trace
minerals, especially boron and calcium salts (Zhang, 1998). However, in vitro germination tests must be carefully controlled because pollen germination and tube growth can be altered by many environmental factors. The germination medium required for consistent results for in vitro tests has not been found in maize. Histochemical approaches are based either on the ability of specific constituents or activity of specific enzymes of the pollen grain to stain. Tetrazolium salts are vital stains that are commonly used to detect enzymatic activity of pollen viability. Among these salts, 2,3,5-triphenyl tetrazolium chloride (TTC) has been successfully tested in maize (Zhang, 1998). The development of a reliable technique is still required for assessing pollen quality among different genotypes and environments.

Depending upon species, the duration of pollen viability may range from a few hours to one day (Barnabas, 1985). Using an in vivo approach, Luna et al. (2001) reported that corn pollen exposed to “hot and dry” field conditions (San Jose del Valle, Nayarit, Mexico) decreased to 20% viability in one hour and was completely nonviable within two hours. Pollen viability can be greatly reduced by high temperatures (Johnson and Herrero, 1991) but apparently is not affected by plant water deficits (Hall et al., 1982; Westgate and Boyer, 1986; Schoper et al., 1987). There is general agreement among investigators that functional life for pollen is longer at relatively low temperatures and high relative humidities (Johri and Vasil, 1961). After pollen is released from the anthers, both temperature and relative humidity have a direct effect on its desiccation rate. Roeckel-Drevet and Digonner (1995) found that maize pollen usually has relatively high water content at anthesis (about 60%) and that after anthesis pollen dehydrates and loses viability. Thus, establishing a temporal relationship between loss of pollen viability and environmental conditions (e.g. temperature, relative humidity) should be possible. Particular attention must be placed in genotypic effects as different genetic materials might differ in the response of their pollen to the environmental conditions (Herrero and Johnson, 1980; Vidal-Martinez, 1997).

Corn produces maximum grain yield when pollination occurs between plants or genotypes. This benefit of out-crossing, however, conflicts with the need to limit
genetic drift and control genetic purity of harvested seeds. It also limits the success of seed producers striving to fulfill market demand for genetically pure products. Therefore, assessing the risk of out-crossing events is critical. It must consider the complex interactions between the biology of flowering and physical aspects of the pollination processes. Based on the success of prior studies to simulate kernel set, it should also be possible to determine the out-crossing risk for a given field from a quantitative description of the parental inbreds flowering characteristics.

**Thesis Organization**

This dissertation includes six manuscripts that were written according to current requirements for scientific journals publication. Chapter 2 presents a completely novel method to quantify the timing and intensity of pollen in the field (Crop Science, 2002). Chapter 3 provides another novel method of relating genetic differences in pollen production to the morphological characteristics of plant tassels (Crop Management, 2003). Chapter 4 describes a mathematical model that simulates potential kernel set from seasonal dynamics of pollen shed and silk exsertion developed by Jon Lizaso et al. (Crop Science, 2003). My participation in this manuscript involved data collection and analysis. Chapter 5 successfully applies the model to simulate kernel set in hybrid seed production fields and to define management strategies that would optimize seed production (Crop Science, 2004). The last two chapters are ongoing projects that will represent future improvements to the model. Chapter 6 establishes quantitative relations between environmental conditions during pollen shed (i.e. temperature, relative humidity) and pollen desiccation that can be used to assess loss of pollen viability (submitted to Field Crops Research). Chapter 7 presents a quantitative approach to assess out-crossing and self-pollination risks. A 'risk index' that depends exclusively on the flowering characteristics of a given field is presented. This index is useful for determining management practices that maximizes genetic purity of harvest seed (for a given inbred pair), regardless the amount of adventitious pollen. References
are cited separately for the general introduction, and each of the manuscripts. Following the manuscripts a general conclusion is presented.

References


CHAPTER 2. APPLICATION OF FLUORESCENCE MICROSCOPY AND IMAGE ANALYSIS FOR QUANTIFYING DYNAMICS OF MAIZE POLLEN SHED

A paper published in Crop Science \(^1\)

Agustin E. Fonseca, Mark E. Westgate, and Robert T. Doyle

Abstract

The objective of this study was to develop a simple, rapid and accurate technique to quantify maize (Zea Mays L.) pollen shed under field conditions, capitalizing on the capacity of pollen to fluoresce and recent improvements in microscopic methods for acquiring, processing and analyzing fluorescence signals from biological systems. Pollen shed naturally by the tassels was captured daily on passive pollen traps placed at apical ear level. Fluorescence microscopy was used to generate digital images of the trapped pollen, and pollen density per unit area was counted using commercial imaging software. Visual confirmation of fluorescing pollen grains indicated high measurement accuracy \((r^2=0.99)\) for the entire range of pollen shed densities typically encountered in the field. Pollen was randomly distributed across the surface of the pollen trap, and six to eight images per trap provided greater than 95% confidence for the mean trap value. The entire process of sample preparation, image capture, and image counting required less than 6 minutes per trap. The accuracy and ease of use of this technique make it ideal for characterizing the pattern of maize pollen production and dispersal under field conditions.

Introduction

In maize, pollination can occur only if airborne pollen shed by the staminate flowers on the tassel is captured by the stigmas (silks) of pistillate flowers borne on the ear. Because the durations of pollen shed and silk receptivity are limited, close

\(^1\) Reprinted with permission of Crop Science, 2002, 42:2201-2206.
synchrony between pollen shed and silk emergence is required for high kernel set in
the field (Bassetti and Westgate, 1994; Cârcova et al., 2000). Environmental
conditions can affect pollen availability by modifying the synchrony between pollen
shedding and silk emergence, or by changing the amount of pollen produced per
tassel (Hall et al., 1982; Bolaños and Edmeades, 1993). Distinguishing between
these possibilities has important implications for germplasm selection as well as
ensuring genetic purity. To do so, however, requires a simple, rapid, and reliable
technique for quantifying the dynamics of maize pollen shed under field conditions.

Studies attempting to describe the quantitative patterns of pollen release from
maize tassels are limited, generally because collecting and counting pollen is
laborious. Flottum et al. (1984) determined the intensity of pollen shed by collecting
samples on spinning rods and counting adhering pollen visually. Sadras et al.
(1985a) collected pollen shed daily within tassel bags, and counted the pollen with a
light microscope. More recently, Bassetti and Westgate (1994) monitored pollen
shed intensity with passive pollen traps, and counted the pollen grains by computer-
aided video imaging. They found that accuracy of the counting procedure depended
almost entirely on the quality of the video image, which was difficult to standardize
with bright-field optics. None of these techniques readily distinguished pollen from
similar-sized debris in the measurement field.

Fluorescence microscopy has great potential for qualitative and quantitative
studies on the structure and function of plant cells, since fluorescence from a single
cell can be detected both as an image and as a photometric signal (Wang and
Taylor, 1989; Fricker et al., 1997). Recent advances in instrumentation for acquiring,
processing and analyzing fluorescence signals have made it possible to capitalize
on natural auto-fluorescence of pollen grains and tubes to quantify pollen
germination in *Nicotiana tabacum* L. (Tirlapur and Cresti, 1992; Keller and Hamilton,
and *Pennisetum ciliare* (L.) Link. (Shafer et al., 2000). To our knowledge, pollen
fluorescence has not been used to characterize any aspect of maize pollen
development or shedding dynamics.
The objective of this study was to develop a simple, rapid and accurate technique to quantify the dynamics of maize pollen shed under field conditions, capitalizing on the capacity of pollen to fluoresce. Such a technique would have immediate application for relating pollen production to staminate flowering characteristics, assessing patterns of pollen dispersal outside of shedding source fields, and providing true quantitative measures of pollen production by male inbreds parents in seed production fields.

Materials and Methods

Plant culture

Maize hybrids, Pioneer Brand 3394 and 3489, were planted in the field (Aquic hapludoll) near Ames, IA, on 10 May 2000 in 76-cm rows in eight-row plots (6.10 by 9.15 m) at a density of 80,000 plants ha\(^{-1}\). Three replicated plots were randomly distributed within a larger experiment occupying approximately 0.47 ha. Plots area received 168 kg N ha\(^{-1}\) as anhydrous ammonia before planting. Rainfall before to anthesis was adequate to ensure a normal pattern of pollen shed.

Pollen sampling

Pollen shed was monitored daily using passive pollen traps placed horizontally in the center of the plant canopy at the apical ear level, about 120 cm above the ground. The trap was supported on a clear plastic base (10.6 by 10.6 cm) mounted on a plastic coated metal stake. The pollen traps were constructed on a base of white opaque high impact polystyrene (HIPSP) sheeting (approximately 8 by 9 cm). Two bands of 1.9-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 = 34.2 cm\(^2\)). The black tape was covered with 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 field.

Pollen traps were placed in the center of each plot at about 1600 h each day throughout the pollen shed period, and remained in place for 24 h. Upon collection,
anthers and other debris were removed by hand, and the traps were covered with an acetate sheet (Highland Brand, 3M, Austin, TX) to prevent contamination with additional pollen or other debris. Pollen traps were stored in the lab under ambient conditions, about 20°C and 50% RH, until counted by image analysis. There was no evidence of sample deterioration in term of pollen fluorescence after 7 mo of storage under these conditions.

**Image analysis**

Fluorescence and bright field 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 (Fryer Company, Huntley, IL). For fluorescing images, preliminary results showed that excitation illumination at 488 nm and monitoring emission at 510 nm provided the strongest signal. Digital images of the pollen were captured using a Hamamatsu CCD C4742-95 camera (Fryer Company, Huntley, IL) controlled with acquisition software from Prairie Technologies (Middleton, WI).

Typically, ten 0.25-cm² images were collected from each trap. Camera exposure time was 1.07 ms, and gain (image sensitivity) was set at 100. The system was automated to collect an image every 4 s. The images were saved in tif file format. In most cases, all 10 images from different positions on a trap were captured and saved in less than 1 min.

To determine whether a portion of the pollen grains failed to fluoresce, images from identical 1-cm² areas were collected for a wide range of pollen densities using both fluorescence and bright field microscopy. Images from both sources were counted by eye and compared.

Pollen counting was performed with the Metamorph Imaging System (Universal Imaging Corporation, West Chester, PA). Image contrast and threshold were adjusted to differentiate between fluorescence of the background and that of pollen grains to be counted. Typically, maize pollen grains were 60 to 90 pixels in size, so the software was set to ignore objects smaller than 50 pixels. At high pollen shed densities, the occurrence of touching pollen increased. The software identified
these occurrences as large objects and reported them as multiples of single pollen grains. They were easily accounted for by visual inspection of the image.

Results and Discussion

Accuracy

The accuracy of the fluorescing image technique requires that all pollen grains autofluoresce when exposed to the actinic light. Therefore we examined a large number of pollen traps for grains that did not fluoresce. Figure 1 shows that nearly every pollen grain observed in a bright field image fluoresce when exposed to excitation illumination of 488 nm, even after being stored in the lab for up to 7 mo.

Predicted results from the Metamorph software also were compared with the actual image values (visually counted) for a wide range of pollen densities (Fig. 2). Predicted values for individual images were very accurate, especially at pollen grain concentrations less than 200 grains cm$^{-2}$. At greater densities, the prediction tended to underestimate actual values slightly. This was due to two or more grains touching, which was increasingly common at high pollen densities. This counting error was easily corrected by a quick visual check on the computer screen for grains not counted (the software uses color to identify objects that are counted).

To determine if pollen deposition on the surface of the pollen traps was random, pollen traps with contrasting amounts of pollen were analyzed at forty 0.25-cm$^2$ positions across each trap surface. As expected, some variability in pollen density across the pollen trap was observed (Fig. 3). But there was no obvious bias in pollen deposition at any pollen shed density. Therefore, a practical way to obtain a representative value for each pollen trap was simply to divide the surface into uniform sections, collect an image from each section, and average the values from these images.

A running average approach was used to determine the minimum number of images necessary to achieve a reliable estimate of the pollen density observed on pollen traps collected at various times during pollen shed. Figure 4 shows that averaging counts from about eight images typically was sufficient to achieve a value
within the 95% confidence interval of the overall trap average (derived from 10 or more images). But the numerical differences in pollen density between traps were evident even among single images. Thus, the number of images taken from each trap depends largely on the accuracy required and amount of time and effort available for data analysis.

The fluorescence image presents pollen grains as bright spots on a dark field (Fig. 5). Accuracy of counting these bright spots depends almost entirely on the quality of the image. Consequently, particular attention must be paid during sample preparation and illumination to optimize image contrast and uniformity. Cleanliness of pollen trap will, of course, improve the image quality. On windy days, foreign debris often contaminates the traps. Large pieces, such as anthers or insects, are readily removed upon trap collection. Smaller particles also can be eliminated from the counting routine using the size-range option in the software program. Any dubious cases can be checked rapidly on the monitor and subtracted or added to the count as appropriate. In the 130 pollen grains image of Fig. 5, for example, the two long-narrow objects were counted as "pollen grains" and subsequently subtracted manually from the image count.

Irregularities on the pollen trap surface, such as small bubbles, tape edges, or even a slight curvature in the pollen trap itself make it more difficult to achieve the uniform image background required for accurate pollen counting. Curvature of the pollen trap, for example, caused pollen grains to move in and out of the focal plane as the trap was repositioned for each successive image. Because pollen scatters the fluorescent light to some extent, pollen grains that are out of focus appear slightly larger in the digital image. This variation in pollen size is evident among the images in Fig. 5. In large part, focusing and background problems related to trap curvature are overcome by keeping the pollen traps perfectly flat on the microscope stage. Image problems associated with bubbles and roughness of the pollen trap surface were avoided only by careful attention to trap construction.
Ease of use

With experience, the time required to obtain six to eight images from a pollen trap can be reduced to less than 1 min. If the original image is correctly focused, the auto-threshold function in the Metamorph software readily differentiates the pollen grains from background without operator adjustments. Small particles, such as dust, are eliminated easily from the count using a size limit option. And large objects, such as pieces of anthers or debris that were not previously removed, are easily detected and avoided. Once an operator is familiar with the imaging software, two to three images can be counted per minute. So the whole procedure to capture, process and count each image requires only about 40 s. Relative to techniques that rely on light microscopy (Bassetti and Westgate, 1994) or scanners to generate digital pollen images, the fluorescence technique is more accurate, more rapid, and considerably easier to use.

Finally, another positive feature of our approach is that pollen traps can be stored for extended periods without affecting the counting results. We have observed that pollen stored on traps autofluorescence effectively even after 2 yr of storage in the lab. In this study, fluorescent images were prepared 7 mo after pollen traps were collected from the field. This represents a significant advantage over techniques such as coulter counting (Carre and Tasei, 1997), which requires collecting and storing pollen in an isotonic solution that has a limited shelf life.

Field application

The accuracy observed across a broad range of pollen shed densities (Figs. 1 and 2) indicates this technique would be well suited for documenting variations in pollen production due to tassel size or male sterility, and characterizing pollen production in seed production fields. A practical application of this pollen measurement technique is shown in Fig. 6. We quantified pollen shed in the field for two hybrids, grown at typical commercial plant densities. Both hybrids exhibited a similar pattern of pollen shed, which lasted about 2 wk and peaked about 2 d after anthesis (50% of the plants at maximum shed). Differences between hybrids for the maximum rate of pollen shed and total amount of pollen produced per square meter
are readily apparent. Taking the area under the pollen shed curve as an estimate of the total amount of pollen shed by these hybrids, P3394 produced approx. $26 \times 10^6$ pollen grains m$^{-2}$, while P3489 produced about $18 \times 10^6$ pollen grains m$^{-2}$. These values correspond to $3.25 \times 10^6$ and $2.25 \times 10^6$ grains per plant, for P3394 and P3489, respectively. Total pollen production for these hybrids is considerably less than the $69 \times 10^6$ grains m$^{-2}$ reported by Hall et al. (1982) and the 40 to $96 \times 10^6$ grains m$^{-2}$ reported by Sadas et al. (1985b), who collected pollen in bags from selected plants and counted pollen by eye under a microscope. The lesser values for P3394 and P3489 in our study likely reflect the smaller tassel size of today’s maize hybrids (Galinat, 1992; Duvick, 1997), some loss of pollen carried by the wind out of the plot area (Westgate et al., 2000), and the greater inherent accuracy of our pollen counting technique. In any case, our fluorescence-based measurements of daily and seasonal pollen shed density obtained with passive pollen traps represent pollen production by the local plant population that actually reaches the plane of exposed silks.

Because this method of pollen quantification is simple, accurate and convenient, it has immediate application for relating staminate flower development to pollen release, documenting production conditions where pollen amount is limiting, and quantifying pollen dispersal downwind of corn fields. Future modifications of this technique will focus on the possibility of using fluorescent images to distinguish pollen from different genetic sources (Abdul-Baki, 1992; Aronne et al., 2001).

References


Figure 1. Direct measure of fluorescing pollen grains. Images from identical 1-cm² areas were taken by fluorescence and bright field microscopy were counted by eye. The actual number of pollen grains per square centimeter is taken from the bright field images. The line represents the 1:1 relation.
Figure 2. Correlation between actual and measured pollen density. Actual pollen density on the digital image was counted by eye, and predicted pollen density was provided by the Metamorph imaging software. The thick line represents the 1:1 relation, and the thin line is the linear regression. Each point is data from a single image.
Figure 3. Distribution of pollen counts on pollen traps collected at three pollen shed densities. Each value is the number of pollen grains per square centimeter. Average, standard deviation and coefficient of variation are shown for each density. Values more than one standard deviation greater than the mean (> mean +1S.D.) are shown in black. Values more than one standard deviation less than the mean (< mean −1S.D.) are shown in white.
Figure 4. Pollen density estimated using an increasing number of images taken from pollen traps collected on different days during pollen shed. The average value for each trap was calculated from 10 images and is represented by the horizontal line. Triangles indicate values significantly different from the average value for the pollen trap ($P<0.05$).
**Figure 5.** Digital images of fluorescing pollen taken from pollen traps collected at varying pollen shed densities. Contrast and threshold intensity were adjusted to distinguish pollen from background fluorescence and foreign objects prior to counting. Numbers in the left top corner indicate the number of pollen grains in each image. Each image is 0.25 cm$^2$. 
Figure 6. Seasonal pattern of pollen shed relative to days after anthesis (50% of plants at maximum pollen shed) for 2 commercial maize hybrids (P3394 and P3489). The data are the average ± standard error for three replicate plots. Note that pollen shed lasts about 14 d and peaks 2 to 3 d after anthesis for both hybrids.
CHAPTER 3. TASSEL MORPHOLOGY AS AN INDICATOR OF POTENTIAL POLLEN PRODUCTION

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Abstract

Adequate pollen production is an essential prerequisite for achieving high yields in commercial corn (Zea mays L.) production and for insuring high levels of genetic purity in the production of hybrid seed. Documenting the timing and intensity of pollen shed are fundamental to these goals, but methods to describe patterns of pollen release from maize tassels are limited and laborious. Our objective was to explore characteristics of tassel morphology that could be used as simple and indirect measures of pollen production per plant under field conditions. The progress of tassel development was documented using a nine-stage scale based on easily-quantified morphological characteristics. Genetic variation among hybrids and inbreds as well as environmental variation across planting densities and years was correlated with levels of pollen production. This analysis revealed that a change in tassel dry weight during pollen shed was not an accurate measure of pollen production per tassel. Likewise, no single morphological characterization captured all the genetic and environmental variation in pollen production per tassel. But a combination of morphological traits incorporated into a Tassel Area Index (TAI) accounted for up to 89% of the variation in pollen production among hybrids in response to population density, and 64% of the variation in pollen production among inbred heterotic groups. Because data collection is simple, quick, and non-destructive, the Tassel Area Index approach is well suited for distinguishing genetic variation in pollen production and relative responses to treatments under field conditions.

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conditions. The accuracy of the technique could be increased, if necessary, by incorporating additional information about flower density or pollen production per anther. But this would entail a much greater investment of time and resources.

Introduction

Pollination in maize (Zea mays L.) can occur only if pollen shed by the tassel is captured by the stigmas (silks) on the ear. Since the introduction of hybrid seed production, much effort has been directed towards managing this process to ensure maximum kernel set and high levels of genetic purity. Current practices include crop rotations, high-purity parent seed, mechanical and hand detasseling of the female parent, temporal or spatial isolation from corn in nearby fields, and use of male border rows around the field (Burris and Lauer, 2001). These strategies are laborious and not always successful. Managing pollen dispersion also is an important consideration in seed production, particularly for managing genetically-modified (GMO) materials, due to the potential for flow of transgenes into landraces, wild relatives of maize, and non-GMO commercial hybrids (Luna et al., 2001). Managing for maximum kernel set and 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.

Environmental conditions can affect pollen availability by modifying the synchrony between pollen shedding and silk emergence, by affecting how long pollen remains viable, or by changing the amount of pollen produced per tassel (Hall et al., 1982; Bolaños and Edmeades, 1993). Distinguishing between these possibilities has important implications for germplasm selection as well as designing seed production practices to ensure genetic purity. Attempts to describe the quantitative patterns of pollen release from maize tassels are limited, generally because collecting and counting pollen are laborious. Flottum et al. (1984) determined the intensity of pollen shed by collecting samples on spinning rods and counting adhering pollen visually. Sadras et al. (1985) collected the pollen shed daily within tassel bags, and counted the pollen with a light microscope. Bassetti and
Westgate (1994) monitored pollen shed intensity with passive pollen traps, and counted the pollen grains by computer-aided video imaging. More recently, Fonseca et al. (2002) used passive traps to collect pollen in the field and capitalized on its capacity to fluoresce to quantify pollen shed intensity. Although simple and accurate, this latter technique can be time consuming.

There is considerable information on tassel development and pollen shed, but it has not been used to predict pollen production per tassel. Pollen shed from an individual tassel usually begins after the tassel is fully expanded, beginning on the central rachis close to the apex and continuing in apical and basal directions (Kiesselbach, 1999). Branches start to shed one or two days after the beginning of the central rachis and follow the same pattern. An individual tassel may shed pollen for 2 to 10 days, depending on genotype and environmental conditions. Daily pollen release will depend in some extent on moisture and temperature conditions, but it will generally last about 4 to 5 hours, starting one hour after sunrise (Flottum et al., 1984; Westgate unpublished data). Corn pollen is 70 to 100 mm in diameter and spherical in shape (Wodehouse, 1935; Jones and Newell, 1948; Fonseca unpublished data) and among the largest particles that are commonly airborne (Raynor et al., 1972). The dry weight of individual pollen grains has been estimated at 250 ng (Goss, 1968). Total pollen production per plant can vary considerably. 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. This decrease in pollen production apparently reflects the smaller tassel size of today’s maize hybrids (Galinat, 1992; Duvick, 1997). This trend is also true for male inbreds, and the higher incidence of out-crosses in modern hybrid seed production may be associated with reduced pollen capacity of modern male parents. The average pollen yield for male inbreds ranges from 200,000 to more than 3 million pollen grains per tassel (Grass, unpublished data). The general relationship between tassel size and pollen production suggests that a quantitative estimate of pollen production can be obtained from tassel morphological
characteristics. To our knowledge, however, no satisfactory method has been established to provide this estimate.

Westgate et al. (in press) developed an indirect method of predicting pollen shed in the field from male flowering dynamics. They calculated a "population index" for daily pollen shed considering the progression of pollen shedding in a population of tassels, from the initiation of shed through maximum shedding to the end of shedding. This index, coupled with an estimate of average pollen production per tassel, predicted pollen shed dynamics very accurately (Westgate et al., in press). While this technique provides a link between tassel development and pollen shed, it requires an accurate estimate of pollen production per tassel.

Therefore, our objective was to explore characteristics of tassel morphology that could be used as indirect measures of pollen production under field conditions. If successful, this approach would have immediate utility as a rapid, simple, and inexpensive way to characterize inbred "maleness," provide a basis for inbred selection, define optimum male/female planting patterns, and estimate pollen shed in models to predict kernel set and pollen dispersal.

**Materials and Methods**

A series of related experiments was conducted to identify simple measures of tassel morphology and development that might be suitable for predicting the pollen production per tassel. The logic of these experiments progressed from an evaluation of the "dry weight difference" method, to the development of a morphometric measure of pollen production, and an evaluation of this approach for predicting pollen production from commercial inbred lines.

**Experiment 1**

An inbred (CM105), a grain hybrid (Pioneer 3893) and a sweet corn hybrid ('Delectable') were planted in the field at Swan Lake, Minnesota, on 13 May 1997 in 76-cm rows in four-row plots (3.05 by 12 m). Three population densities were tested: 2.5, 7.5, and 12.5 plants per m². The statistical design was a split-plot, with
population as main plots and genotypes as subplots, replicated four times. Plots were fertilized with 150 kg N per ha as anhydrous ammonia prior to planting.

The progress of tassel development was documented using a nine-stage scale, based on easily-identifiable morphological characteristics. Key elements of the scale are shown in Fig. 1. Tassels are at Stage 1 when the tip of the main branch is visible. At Stage 2, tassel is expanding rapidly and the peduncle at the base of the tassel is visible. Stage 3 indicates when the tassel is fully expanded and has entirely emerged from the whorl. Stage 4 is the beginning of pollen shed. The first anthers have begun to shed pollen, which generally occurs in center of the main tassel branch. Stages 5, 6, and 7 document the progress of pollen shedding on the lateral branches: Stage 5, anthers on one branch shedding; Stage 6, half of the branches are shedding pollen; Stage 7, all branches are involved in pollen shedding. At Stage 8, pollen shed from all anthers is complete. No new anthers are exposed. Stage 9 occurs 7 days after pollen shed is complete and indicates when most of the anthers and other floral structures have been shed from the tassel. Stages 4 through 8 are of particular interest in this study since they bracket the period in which pollen shed occurs. Presumably, the change in weight during this time reflects pollen liberated from the tassel. We defined Tassel Weight Loss (TWL) as the difference in tassel weight between Stage 4 and Stage 8.

Five plants per plot were tagged and observed daily to determine the progress of tassel development. Five additional tassels per plot were removed and weighed at each of the Stages 1 through 8. The amount of pollen produced per tassel was quantified by covering 5 tassels per plot with tassel bags at Stage 3, i.e., immediately before they started to shed pollen. Bags were sealed at the base with expanding foam to prevent pollen loss, and left in place until pollen shed was complete. After pollen shed, bagged tassels were excised from the plant and taken to the laboratory for dissection. Bags were carefully removed, anthers and debris removed by hand, and all the shed pollen was collected, dried at 60°C, and weighed. Main stem length, number of branches, main stem, and branch weight also were recorded after drying at 60°C.
Experiment 2

Two hybrids, Dekalb 611 and Holdens LH198 × LH185, were planted in the field at the Bruner Research Farm near Ames, Iowa, on 8 May 2001 in 76-cm rows in sixteen-row plots (6.10 by 15 m) at three population densities (1, 8 and 18 plants per m²). The statistical design was a split-plot with population as main plots and genotypes as subplots, replicated three times. Plots were fertilized with 168 kg N per ha as anhydrous ammonia prior to planting.

Five representative tassels per plot were selected for sampling when 40% of the population had begun to shed pollen. Pollen was collected daily in clear bags (Pantek, Montesson, France) designed to exclude moisture but allow gas exchange around the tassel. Prior to pollen shed and continuing for 4 to 7 days, bags were placed over the tassels, wrapped tightly around the peduncle to prevent pollen loss, and locked in place with a clip. Bags were replaced at about 1900 hours each day and carefully removed to avoid losing pollen or damaging the tassel. Collected bags were slowly compressed to exclude air and carried to the lab for pollen collection. Bags were allowed to air-dry prior to processing. Pollen was harvested from the bags by washing with approximately 30 ml of Isotone II solution (Coulter Corporation, Florida, USA). The solution containing pollen was filtered through a stainless steel mesh to remove anthers and large debris, brought to 60 ml, and stored at room temperature.

The number of pollen grains ml⁻¹ in triplicate 0.5-ml aliquots were quantified using a Coulter Multisizer II (Coulter Electronics Limited, Luton, Beds, England), which was calibrated to detect particles between 60 and 100 mm in diameter. Pollen grains per tassel were calculated from the total sample volume used to wash the pollen collection bags. The remaining solution was dried and weighed to estimate individual pollen grain weight. The weight added by solutes in the Isotone II solute was insignificant. In contrast to Experiment 1, tassel morphology was measured non-destructively on the same plants from which pollen was collected and quantified. Main stem length, effective main stem length, main stem diameter, number of branches, total branch length, and effective branch length were recorded when
tassels reached maximum pollen shed (Stage 6). These measurements were taken prior to replacing the clear collection bags. Effective main stem length and effective branch length were defined as the length in which anthers were exposed. Main stem length was measured from the insertion of the first branch on the stem to the tip of the main tassel branch (Fig. 1). Total branch length was calculated as the sum of all branch lengths. Main stem diameter represented the minimum diameter measured immediately below the insertion of the first branch.

Tassel Area Index (TAI) was calculated to integrate these parameters as follows:

\[
\text{TAI} = \pi \times \text{main stem diameter (mm)} \times \text{main stem length (cm)} + \\
\pi \times 0.5 \times \text{main stem diameter (mm)} \times \text{total branch length (cm)}
\]  

This calculation provides an estimate of the effective area of pollen production per tassel by assuming a uniform distribution of flowers on the main tassel branch, and a uniform, but lower density of flowers on the lateral branches. A coefficient of 0.5 for the lateral branches provided the best fit between TAI and pollen per tassel.

When pollen shed was complete (Stage 8), tassels were cut just below the insertion of the first branch and transported to the lab. Main stem length, main stem diameter, number of branches, and total branch length were measured immediately to minimize changes due to desiccation. Total dry weight was recorded after tassels were dried in oven at 60°C for 24 h.

**Experiment 3**

Hybrids Dekalb 611 and Holdens LH198 × LH185 were planted in the field at the Bruner Research farm near Ames, Iowa, on 6 May 2002 in 76-cm rows in 20-row plots (15.2 by 15 m) at three population densities (1, 4, and 8 plants per m²) and replicated three times. Crop management was as described for Exp. 2. Pollen production per tassel was quantified as in Exp. 2, except that the collection bags were replaced every third day instead of on a daily basis. Main stem length, total
branch length, and main stem diameter were recorded at Stage 6 and TAI calculated from Stage 6 morphology data.

**Experiment 4**

Pollen production and tassel morphology of 22 inbreds from Syngenta Seeds Inc. were characterized in 2002. The inbred lines were planted in a randomized complete block design, replicated three times at Washington, IA on 23 May 2002 in 4-row plots (6.1 by 3 m) at a population of 8.6 plants per m\(^2\). Pollen production per tassel was quantified as in Experiment 2 using clear tassel bags. Main stem length, number of branches, total branch length, and main stem diameter were measured at maximum pollen shed (Stage 6), and TAI calculated from these values. Additionally, tassel weights at the beginning (Stage 4) and end of pollen shed (Stage 8) were recorded on 5 representative tassels in each plant population. All observations were recorded on plants in the two center rows of each plot.

**Results and Discussion**

**Change in tassel weight as measure of pollen production**

Tassel morphology changes dramatically as it emerges from the whorl, sheds pollen, and senesces. Thus, attempts to relate tassel morphology with pollen production must consider this phenology and document the stage in which tassels are sampled. A simple visual scale was developed for that purpose (Fig. 1). Data from the three genotypes evaluated in Experiment 1 showed that tassel weight was increasing as tassels emerged from the whorl, reached their maximum as they began to shed pollen, decreased as pollen was shed, and continued to do so after pollen shed was complete (Fig. 2). The fact that tassel weight continued to decrease after pollen shed was completed indicates that not all of the weight loss could be attributed to pollen. Other floral structures such as anthers, palea, lemma, and glumes also are shed from the tassel and can account for a significant portion of the weight loss. While this general pattern is typical of all hybrids and most inbreds we have measured, there may be exceptions. In an unrelated study, tassels of one inbred continued to increase in weight during the early stages of pollen shed.
This observation underscores the difficulty in relating changes in tassel weight directly to pollen shed.

In Experiment 1, Total Weight Loss values for plants grown at the commercial density of 7.5 plants per m$^2$ were 0.5, 1.18, and 6.26 g for the inbred, grain hybrid, and sweet corn hybrid, respectively (Fig. 3). The accuracy of this approach for estimating pollen shed was tested by comparing TWL values with the weight of pollen collected from individual tassels of these three genotypes grown at three population densities. On average, the inbred, grain hybrid, and sweet corn hybrid grown at 7.5 plants per m$^2$ produced 0.44, 0.99, and 2.54 g of pollen. Pollen shed accounted for 89% of the weight loss in the inbred, 84% in the hybrid, and 41% in the sweet corn hybrid (Fig. 3). These results clearly demonstrated that TWL was not an accurate way of comparing genotype maleness, even when weight loss was measured only during pollen shed (Stage 4 to 8). For this particular inbred (CM105), we overestimated pollen production per tassel by about 11%, or 200,000 pollen grains per tassel (assuming an average weight of 250 ng per grain). This overestimation might not be significant in terms of seed production for a male that produces 3 million pollen grains per tassel, but it is certainly a concern for inbreds considered "poor males" because they produce much less pollen. Similarly, TWL overestimated pollen shed by 16% for the grain hybrid and 59% for the sweet corn hybrid, corresponding to 633,600 and almost 6,000,000 pollen grains per tassel, respectively. The error in estimating pollen production increased with the size of the tassel, which probably reflects a greater proportion of anthers and other floral structures contributing to the weight loss during pollen shed.

Comparing these three genotypes at extreme population densities produced similar results (Fig. 3). The sweet corn hybrid showed similar TWL values of 6.16 and 6.26 g when grown at 2.5 and 7.5 plants per m$^2$, but pollen production decreased from 3.74 to 2.54 g (about 15,000,000 to 10,160,000 pollen grains). The fact that contrasting amounts of pollen production were obtained with a similar TWL is additional evidence that the "weight loss" approach is imprecise at best. The overestimation of pollen production using TWL increased as plant population density
increased. This could be due to an increase in the number of sterile anthers per tassel and/or a decrease in the number of pollen grains per anther with increasing plant population densities. No data were collected to distinguish between these possibilities.

Analysis of 22 inbred lines used in commercial seed production led to the same conclusion (Fig. 4). In this case, however, the actual number of pollen grains shed per tassel was measured with a flow-cytometer. The expected change in tassel weight due to pollen loss was calculated assuming 250 ng per pollen grain and compared to the measured TWL. In every case, TWL overestimated measured pollen production. And more importantly, there was large variation in TWL that could not be accounted for by variation in pollen weight loss.

In this analysis, tassels were sampled at well-defined stages of development to ensure TWL was measured from the beginning to the end of pollen shed. It is likely that even greater uncertainty would have resulted if TWL were measured using tassels collected some time after pollen shed has ended since genotypes obviously differ in weight loss during pollen shed and after pollen shed is complete (Stage 8 to 9, Fig. 2).

The inaccuracy of the TWL method for estimating pollen production prompted us to search for a more accurate measurement system. We attempted to relate total pollen shed (by weight) with individual components of tassel morphology. The genotypes from Experiment 1 were examined for main stem length, number of branches, main stem weight, branches weight and total tassel weight (Table 1). These morphological characteristics failed to explain more than 56% of the variation in pollen production per tassel. Furthermore, the correlations between these characteristics and pollen production per tassel were not consistent across genotypes. Leaving tassel bags in place throughout pollen shed might have affected pollen production by modifying air temperature and moisture content around the tassel. Presumably, the effect of an altered environment around the tassel during pollen shed would depend on tassel size, which varied dramatically by genotype in Experiment 1, and by population density (Fig. 3). The numbers of grains per tassel
estimated for the inbred (1,300,000 to 1,760,000) and the hybrid (2,440,000 to 4,920,000), however, were similar to values obtained using clear bags (Experiment 2 and 3) and for other genotypes using passive pollen traps (Fonseca et al., 2002; Westgate et al., in press). We do not have prior experience with sweet corn hybrids, but our estimates of pollen production per tassel of 8,200,000 to 14,960,000 pollen grains are consistent with their much larger size.

Direct comparison between genotypes, environments or individual plants using the TWL approach assumes that weight per pollen grain is uniform and remains fairly constant. To examine this issue, we measured the average weight of pollen grains shed by the two hybrids examined in Experiment 2, in which pollen production per tassel varied from 28,000 to 1,900,000 grains per day. Figure 5 shows pollen grain weight typically varied between 250 and 350 ng with no obvious differences between hybrids or among population density treatments. Greater variability in weight per grain at low levels of pollen production probably reflects the greater measurement error for these small pollen samples. While this analysis confirms that the average weight of pollen was fairly stable across a wide range of population densities for these two hybrids, measurement errors alone could mask the relationship between tassel morphology and pollen production, and the amount of pollen production were determined only by a change in tassel weight.

**Tassel morphological characteristics related to pollen production**

In Experiment 2, we related the number of pollen grains shed per tassel to the morphological characteristics of the same tassel. We minimized the effects on the tassel environment by using clear bags that allowed for gas exchange during pollen collection. In this case, we adopted a simplified development scale to define when tassel morphology was measured relative to the beginning, maximum and end of pollen shed. These stages correspond to Stages 4, 6, and 8 in Fig. 1. Of the morphological variables examined, main stem length and main stem diameter at Stage 6 were closely correlated with pollen production, explaining 62 to 80% of the variation in pollen production per tassel (Fig. 6). All morphological parameters were more closely correlated with pollen production when measured during intense pollen
shed (Stage 6), rather than after pollen shed was complete (Stage 8). Number of branches, effective main stem length, and effective branch length accounted for less than 2%, 49% and 46% of the variation in pollen production among treatments (data not shown). For this reason, we did not evaluate these characteristics in Experiments 3 and 4.

Reasoning that the number of flowers per tassel determined total pollen production, and that this number should be distributed over the total tassel surface area, we combined main stem length, total branch length, and main stem diameter to calculate a tassel area index (TAI) for each plant. This index explained 89% of the variation in pollen production per tassel in 2001 (Fig. 6). This index assumes a lower density of flowers on the lateral branches and that they contribute half as much pollen per unit area relative to the main stem. More detailed information about the density of fertile flowers per cm of tassel main stem and branches or the amount of viable pollen grains produced per flower could be used to refine this relationship. The additional resources required to obtain this detailed information, however, would be considerable and negate the primary benefit of estimating pollen production by individual tassels from simple measures of their morphology and development in the field.

We tested whether main stem length, total branch length, main stem diameter, or TAI would vary similarly with pollen production for the same hybrids grown in a different environment (Experiment 3). We also examined the possibility of using these parameters to distinguish the capacity of inbred lines to produce pollen (Experiment 4). Considering the data from all three experiments together, the relationships between the morphological indices and pollen production were positive, but they explained a smaller portion of the variability in pollen production than observed in 2001 (Fig. 7). Main stem length was the most consistent and robust parameter, explaining 67% of the variation in pollen production across years and genotypes. There was considerably more variation in the TAI values for the inbreds, which generally produced 0.5 to 3 million pollen grains per tassel. Also, the extremely high plant density (18 plant per m²) was not included in the second
environment, which decreased the range of tassel size, and therefore the capacity to resolve the relationship between pollen production and tassel morphology for the two hybrids. These results indicated that the TAI approach could provide a useful indication of the relative response of pollen production to growth conditions that cause significant variation in tassel size. It was not sufficiently sensitive, however, to resolve variation in pollen production among individual plants or genotypes with similar tassel morphologies grown at the same population density. As suggested earlier, additional information on floral or anther density per unit length of tassel could be required to increase the resolving power of this technique.

To determine which tassel characteristics provided the most consistent measure of pollen production, we compared the Coefficient of Variability (CV) for each morphological characteristic and compared them to the CV for pollen production for each of the three treatment groups. Figure 8 shows that main stem length, total branch length, and main stem diameter (except for the inbreds) varied much less than did pollen production in each group of plants. As such, these tassel parameters were relatively stable in response to genetic and environmental treatments that cause pollen production to vary considerably (Figs. 6 and 7). The CV for TAI, however, was of similar magnitude to that of pollen production per tassel for each treatment group. Therefore, this tassel characteristic captures the inherent variability in pollen production associated with relatively large changes in tassel morphology. Refinements may be needed to account for the seasonal variation and plant-to-plant variability observed in this study.

**Tassel area index for inbred lines**

We observed earlier that pollen production and tassel morphological characteristics varied considerably among the inbred lines (Fig. 7). Therefore, it was of interest to determine whether TAI or other characteristics could be used to categorize inbred males as poor, average, or good pollen shedders according to common practice in the hybrid seed industry. Figure 9a shows levels of pollen production for each of 22 inbred lines organized by heterotic group. Variation in
pollen production per tassel was as great within groups (notably D and E) as it was between groups (compare A and F).

In general, tassel morphological characteristics were poorly correlated with pollen production per inbred lines when lines were considered independently ($r^2 = -0.01$ to 0.31, Table 2). The variation in TAI among inbreds, for example, shows little correspondence to observed levels of pollen production (Fig. 9b). No single morphological characteristic was sufficient to properly identify the inbreds destined to produce < 0.5 million grains (poor), 0.5 to 2 million grains (fair), or > 2 million grains (good).

The relationships between tassel characteristics and pollen production improved considerably, however, when values were averaged by heterotic group (Table 2). The number of tassel branches explained 80% of the variation in pollen production between groups. This was the only tassel parameter, however, to provide a better estimate of pollen production per tassel than simply measuring TWL during pollen shed ($r^2 = 0.57$).

The poor relationship between the TAI of inbred tassels and the amount of pollen they produce indicates that critical information about other important tassel traits is still missing from the TAI equation. Total number of flowers, flower density, amount of pollen per anther, and loss of floral structures will impact this relationship. It is quite conceivable that the reduction in tassel size that has accompanied selection for high grain yield (Duvick, 1997) has had a disproportionate impact on one or more of these morphological characteristics on the male inbred parents. Detailed measurements of inbred tassel morphology are needed to resolve this possibility.

**Conclusions**

Using the Tassel Area Index as an indicator of pollen production per plant emerges as an interesting alternative primarily due to its speed and simplicity. It is suitable for describing genotype response to increasing population density or for documenting large genetic differences among inbred lines, such as between
heterotic groups. Seed companies in particular might find this approach useful for characterizing male inbred response to population density or across a range of environmental conditions. The technique as currently defined, however, is not sufficiently accurate to distinguish "maleness" of inbreds within a heterotic group. A better characterization of flower density and pollen production per anther could improve estimates of pollen production based on simple morphological measures. But these additional measurements would reduce the speed and simplicity of characterization, which makes the TAI approach more attractive than other techniques. Current studies are aimed at refining this measurement technique to account for genetic variation in floral density and structure.

**References**


Table 1. Linear correlations between pollen production and various tassel morphological characteristics for three contrasting genotypes grown at 2.5, 7.5, and 12.5 plants per m$^2$. Morphological characteristics were measured on five representative plants per plot at the end of pollen shed. Pollen production is the average per tassel collected on the same plants. N = 12 plots.

<table>
<thead>
<tr>
<th></th>
<th>$r^2$</th>
<th>Main stem length (cm)</th>
<th>Number of branches</th>
<th>Main stem weight (g)</th>
<th>Branches weight (g)</th>
<th>Total weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred</td>
<td>0.21</td>
<td>ns</td>
<td>0.00</td>
<td>ns</td>
<td>0.06</td>
<td>ns</td>
</tr>
<tr>
<td>Grain hybrid</td>
<td>0.28 *</td>
<td>0.02 ns</td>
<td>0.44 **</td>
<td>0.56 **</td>
<td>0.56 **</td>
<td></td>
</tr>
<tr>
<td>Sweet corn hybrid</td>
<td>0.03 ns</td>
<td>0.00 ns</td>
<td>0.00 ns</td>
<td>0.08 ns</td>
<td>0.06 ns</td>
<td></td>
</tr>
</tbody>
</table>

* Indicates significance at $P = 0.10$  ** Indicates significance at $P = 0.05$  ns, nonsignificant at the 0.10 level of probability
Table 2. Mean values for pollen production per tassel and tassel morphological characteristics for 22 inbred lines from six heterotic groups. Regressions with pollen production are calculated for the 22 inbred lines as a group, and for mean values of each heterotic group. TAI = tassel area index; TWL = tassel weight loss. Values in columns followed by the same letter are not different at $P=0.10$.

<table>
<thead>
<tr>
<th>Heterotic group</th>
<th>Pollen grains per tassel</th>
<th>Main stem length (cm)</th>
<th>Number of branches</th>
<th>Total branch length (cm)</th>
<th>Main stem diameter (mm)</th>
<th>TAI</th>
<th>TWL (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>864,125</td>
<td>32.75</td>
<td>5.10</td>
<td>100.89</td>
<td>3.65</td>
<td>962.1</td>
<td>4.9</td>
</tr>
<tr>
<td>B</td>
<td>1,094,826</td>
<td>29.14</td>
<td>7.69</td>
<td>122.44</td>
<td>2.46</td>
<td>775.5</td>
<td>6.4</td>
</tr>
<tr>
<td>C</td>
<td>1,176,107</td>
<td>32.14</td>
<td>6.83</td>
<td>137.87</td>
<td>4.06</td>
<td>1299.4</td>
<td>7.3</td>
</tr>
<tr>
<td>D</td>
<td>1,523,005</td>
<td>31.11</td>
<td>8.90</td>
<td>144.31</td>
<td>3.01</td>
<td>995.2</td>
<td>4.9</td>
</tr>
<tr>
<td>E</td>
<td>2,425,577</td>
<td>31.25</td>
<td>8.69</td>
<td>130.48</td>
<td>4.95</td>
<td>1471.5</td>
<td>6.4</td>
</tr>
<tr>
<td>F</td>
<td>2,990,000</td>
<td>25.35</td>
<td>11.93</td>
<td>162.45</td>
<td>4.38</td>
<td>1468.7</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Means with different letters are significantly different at $P=0.10$, based on LSD.

<table>
<thead>
<tr>
<th>$r^2$</th>
<th>Individual lines</th>
<th>Heterotic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.01</td>
<td>-0.51</td>
</tr>
<tr>
<td></td>
<td>rs</td>
<td>rs</td>
</tr>
<tr>
<td></td>
<td>0.18 *</td>
<td>0.80 **</td>
</tr>
<tr>
<td></td>
<td>0.11 rs</td>
<td>0.55 *</td>
</tr>
<tr>
<td></td>
<td>0.31 **</td>
<td>0.43 ns</td>
</tr>
<tr>
<td></td>
<td>0.30 **</td>
<td>0.64 **</td>
</tr>
<tr>
<td></td>
<td>0.12 ns</td>
<td>0.57 *</td>
</tr>
</tbody>
</table>

* Indicates significance at $P = 0.10$  ** Indicates significance at $P = 0.05$  ns, nonsignificant at the 0.10 level of probability
Figure 1. Tassel development scale. Stage 4 represents the beginning of pollen shed with anthers exposed on the main tassel branch. Stage 8 is the end of pollen shed; no new anthers are exposed. Arrows on Stage 4 delimit the length of the main branch. Main stem diameter was measured at the lower arrow position.
Figure 2. Changes in tassel weight during development for three maize genotypes with contrasting tassel morphologies. Arrows indicated the beginning and end of pollen shed. Time intervals between tassel developmental stages (X-axis) are not uniform. Note difference in scale of the Y-axes. Each point is the mean ± SD for five plants.
Figure 3. Relationship between pollen weight and tassel weight loss (Stage 4 to Stage 8) for the three genotypes shown in Fig. 2. Solid lines show the tendency among genotypes grown at three plant densities: 2.5 (red), 7.5 (blue) and 12.5 (green) plants per m$^2$. Dashed line indicates the 1:1 relationship. The values under each genotype are the percentage of tassel weight loss due to pollen. Each point is mean ± SD for four plots of 5 plants each.
Figure 4. Relationship between pollen production and tassel weight loss (Stage 4 to Stage 8) for 22 inbreds representing six heterotic groups. Each point represents one genotype and is the average of 5 plants. Weight loss per tassel due to pollen was calculated from measured pollen shed assuming 250 ng per grain. Dashed line indicates the 1:1 relationship.
Figure 5. Variation in pollen grain dry weight for two hybrids grown at 2.5, 7.5, or 12.5 plants per m². Pollen was collected from individual tassels on daily basis. Pollen grain weight was calculated by dividing the total weight of pollen collected per tassel each day by the number of grains measured using a coulter counter.
Figure 6. Correlations between the total number of pollen grains produced by an individual tassel and selected morphological characteristics: main stem length, total branch length, main stem diameter and the Tassel Area Index. Morphological characteristics were measured at Stage 6. Data are for two hybrids grown in 2001.
Figure 7. Correlations between the total number of pollen grains produced per tassel and main stem length, total branch length, main stem diameter and Tassel Area Index measured on individual plants at Stage 6. Data corresponds to 2001 hybrids (Exp. 2), 2002 hybrids (Exp. 3), and 2002 inbreds (Exp. 4).
Figure 8. Coefficient of variability for main stem length (cm), total branch length (cm), main stem diameter (mm), Tassel Area Index, and pollen production per tassel (grains per plant) for two hybrids grown in 2001 and 2002 at three population densities, and 22 inbreds grown at commercial densities in 2002. Note that the CV for Tassel Area Index is consistent with the CV in pollen production for all three treatment groups.
Figure 9. Pollen grains per tassel (a) and Tassel Area Index (b) for 22 commercial inbred lines. Colors indicate different heterotic groups designated A to F. Horizontal lines indicate the mean pollen production per tassel or TAI for each group. Bars are the mean for 5 plants.
CHAPTER 4. PREDICTING POTENTIAL KERNEL SET IN MAIZE FROM
SIMPLE FLOWERING CHARACTERISTICS

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Jon I. Lizaso, Mark E. Westgate, William D. Batchelor, and Agustin E. Fonseca

Abstract

Accurate prediction of kernel number per plant is critical for yield simulation in maize (Zea mays L.). Model predictions of kernel number generally are based on empirical relationships between final kernel number and carbohydrate supply or plant growth at silking, without regard to flowering dynamics. The objective of this study was to develop a model for predicting kernel set from seasonal dynamics of pollen shed and silk exsertion. Hybrids were planted in isolated plots at four ratios of male fertile (MF) and male sterile (MS) plants. Dynamics of pollen shed in each plot was described by a Gauss curve and by a population index ($P_{ind}$) derived from the percentage of plants at beginning, maximum, and ending pollen shed. Percentage of plants at silking followed a sigmoid curve, whereas the number of silks exposed daily per ear followed a monomolecular model. Pollen shed and silk exsertion curves were translated to potential kernel set using a published relationship. Excellent agreement was observed between predicted and measured number of florets per hectare available for pollination. The model overpredicted kernel number per hectare at high pollen shed densities. Predicted kernels per hectare were within 5% of actual kernel numbers at low pollen shed densities when delayed development of subapical ears and asynchronous pollination within ears were considered. These results indicate kernel set in maize can be predicted from simple measures of the flowering process.


Abbreviations: IPAR, intercepted photosynthetically active radiation; MF, male fertile; MS, male sterile; $P_{ind}$, population index; RMSE, root mean square of error.
They confirm that kernel set is limited primarily by factors other than pollen amount when all plants in the population are MF.

**Introduction**

The developmental processes that determine kernel number per plant in maize can be divided into three consecutive stages for simulation purposes. In the first stage, the reproductive structures are initiated and differentiated into staminate (male) flowers on the apical inflorescence (tassel) and pistillate (female) flowers on one or more lateral inflorescences (ears). In the second stage, the flowers are functionally mature and engage in pollination; male flowers release pollen grains from the anthers and female flowers exert receptive stigmas (silks) to intercept the airborne pollen. Separate male and female flowers require that the timing of pollen shed coincide closely with silk exertion and that tassels produce an overabundance of pollen to ensure pollination of exposed silks. In the third stage, pollination is followed by fertilization and kernel formation (Kiesselbach, 1999). Current models for simulating maize yield do not consider the quantitative and dynamic nature of the first two of these flowering stages.

Most efforts to simulate kernel formation in maize have attempted to associate the final kernel number per plant with the current supply of photosynthate or related characteristics such as light interception or plant growth rate around the time of silking (Edmeades and Daynard, 1979; Tollenaar et al., 1992; Andrade et al., 1993; Kiniry and Knievel, 1995; Otegui, 1997; Andrade et al., 1999; Andrade et al., 2000). Edmeades and Daynard (1979) were the first to relate the number of kernels per ear with a calculated rate of photosynthesis per plant at anthesis. They used a rectangular hyperbola to describe this relationship, which was the basis for predicting kernel number per plant in the original version of CERES-Maize (Jones and Kiniry, 1986).

Tollenaar and colleagues (1992) found that kernel number per plant was related to the rate of dry matter accumulation 1 wk before to 3 wk after silking. They observed also that modern hybrids tended to produce kernels on a second
(subapical) ear at low plant population densities. Therefore, they proposed a double-hyperbola function to describe the relationship between kernel number per plant and plant growth rate. Otegui (1995), however, reported that among Argentinean hybrids, only prolific types tended to produce kernels on subapical ears at commercial plant densities.

Because canopy photosynthesis is controlled primarily by solar radiation (Christy et al., 1986), a similar relationship between kernel number and light interception would be expected. Andrade et al. (1993) reported that the number of kernels per plant was curvilinearly related to the IPAR per plant during a 31-d period centered on silking. Kiniry and Knievel (1995) used a linear equation to relate kernels per plant with IPAR during 10-d period following silking, as did Otegui (1997), for IPAR during a 30-d period around silking. Andrade et al. (2000) and Lizaso et al. (2001), however, observed that curvilinear functions predicted kernel numbers from IPAR more accurately than did a simple linear function over a broad range of plant growth rates.

There is evidence that kernel number per ear is not simply a function of assimilate supply. Struik et al. (1986) associated the effects of temperature and photoperiod on kernel number with asynchrony between pollen shed and silk emergence. Because these environmental variables are known to alter floral development and function (Herrero and Johnson, 1981; Hall et al., 1982; Struik et al., 1986; Bassetti and Westgate, 1993c), a mechanistic description of the floral biology of maize may be needed to improve forecasting of kernel number per plant across a wide range of environmental conditions. Few studies provide the quantitative information needed to relate floral development and kernel formation.

Bassetti and Westgate (1994) measured kernel number per ear formed at various intensities of pollen shed. In their study, seasonal pollen shed rates followed a normal distribution that peaked at ~500 grains cm$^{-2}$ d$^{-1}$ two days after anthesis (50% of population shedding pollen). They also determined that daily rates of pollen shed densities at 100 grains cm$^{-2}$ d$^{-1}$ or greater reaching the plane of exposed silks
were sufficient to achieve maximum kernel number per ear. Struik and Makonnen (1992) reported a similar pattern of pollen shed, but did not relate it to kernel number.

Bassetti and Westgate (1993a)(b) described the dynamics of silk emergence and senescence on individual ears of maize as a progressive process that varies by genotype and environmental conditions. This silk exsertion dynamic for individual plants provides the essential link between the population dynamics of silking (percentage of plants with silks exposed) and the number of silks exposed for pollination on an area basis, which ultimately determines the potential for kernel set.

These few reports on pollen shed and silk emergence indicate the processes of male and female flowering in maize progress in a fairly predictable manner. As such, it should be possible to develop a mechanistic description of kernel set on a field scale based on a quantitative evaluation of synchrony between pollen production and silk exsertion. Sadras et al. (1985a)(b) recognized this possibility in their work, but predicted kernel set only on a relative basis. They also lacked sufficient quantitative information on the flowering and pollination processes to predict kernel number when pollen amount was limiting. The objective of this study, therefore, was to describe mathematically the processes of floral anthesis (i.e., pollen shed and silk emergence) and pollination. In this initial effort, treatments were designed to alter kernel set by limiting pollen amount. The potential impact of environmental limiting conditions on floral synchrony and pollen viability were not considered, but procedures were developed to accommodate the effects of such constraints.

**Materials and Methods**

**Procedure for estimating kernel number per plant**

The procedure for estimating kernel number per hectare is based on a quantitative description of maize flowering characteristics on a field scale. Mathematical functions were fit to temporal profiles of plant population dynamics for pollen shed and silk exsertion measured in the field. The characteristics described are: (i) amount and temporal distribution of pollen shed, (ii) amount and temporal
distribution of exserted silks; (iii) relationship between kernel set and daily pollen shed intensity reported by Bassetti and Westgate (1994). These components were linked mathematically to estimate the total number of receptive silks pollinated each day. This value represents the potential number of kernels that can be formed by the plant population.

Amount and temporal distribution of pollen

We used the field data of Westgate et al. (2003) for pollen shed in different MF treatments to generate a seasonal distribution of pollen production per plant. Daily values for pollen shed (grains cm\(^{-2}\)) were normalized to daily pollen shed per fertile plant by correcting for the fraction of MF plants and the population density in each treatment. Gauss curves were fit to these normalized data to generate a genotype-specific pattern of seasonal pollen shed given by:

\[
PR = \frac{p}{W \times \sqrt{\pi/2}} \times e^{-2(t-t_x)^2/W^2},
\]

where PR is the rate of pollen shed on a fertile plant basis (grains per plant per day), \(p\) is the total amount of pollen produced per plant (grains per plant), \(W\) is the width of the pollen shed curve measured at half the maximum pollen shed rate (days), and \(t\) and \(t_x\) are the current day and the day of maximum pollen shed. Rates of pollen shed per plant predicted from the Gauss curves were scaled to rates of pollen shed on an area basis (grains cm\(^{-2}\) d\(^{-1}\)) using the population densities and fraction of fertile plants in each MF treatment.

The seasonal distribution of pollen shed also was predicted from field observations of male flowering characteristics at the population level. The three stages of tassel development identified in Westgate et al. (2003): beginning of pollen shed (start shed—first anthers visible on main tassel branch), maximum intensity of pollen shed (max shed—all branches of tassel are involved in pollen shedding), and ending of pollen shed (end shed—no new anthers visible) were expressed as a percentage of the plant population on a daily basis. The progress of each stage was described using a sigmoid logistic function:
where Pop and Pop\_x are the percentage of the plant population at each stage of pollen shed and the corresponding maximum percentage; \( t \) and \( t_m \) are the current day and the day when 50% of the plant population reaches each stage of pollen shed; \( k \) is a curve parameter governing the slope of the function.

Using the predicted percentage of plant population at each stage of pollen shed (Pop), a \( P_{\text{ind}} \) was calculated to simulate the daily rate of pollen produced in the field:

\[
P_{\text{ind}} = \frac{\text{Start shed} + \text{Max shed}}{2} - \text{End shed}.
\]

where \( P_{\text{ind}} \) is the population index (%), and start shed, max shed, and end shed are the predicted percentages of the plant population that have begun to shed pollen, reached maximum shed intensity, or completed pollen shed. Calculated \( P_{\text{ind}} \) values were scaled to daily rates of pollen shed on an area basis (grains cm\(^{-2}\) d\(^{-1}\)) using the population density and the total pollen production per plant. Details of these procedures and calculations are provided by Westgate et al. (2003).

**Amount and temporal distribution of receptive silks**

The distribution of silks exposed for pollination in the field results from developmental processes occurring at the plant level and at the population level. At the plant level, silks emerge from the surrounding ear leaf sheaths (husks) during a period of days following a curvilinear pattern (Bassetti and Westgate, 1993a, 1994). We used a monomolecular model to describe the pattern of silk exsertion on each ear:

\[
\text{SN} = \text{SN}_x \times \left(1 - e^{-b(t-t_0)}\right).
\]
where SN is the cumulative number of exposed silks on one ear, SN is the potential number of silks per ear equal to the potential number of florets, \( t_0 \) is the time when the first silk is exserted (day of year), and \( b \) is a shape parameter controlling the slope of the curve.

At the population level, the percentage of plants with silks exposed progresses in a sigmoid fashion. We used a sigmoid logistic function, similar to Eq. [2], to characterize this developmental pattern. Daily percentages for each treatment were converted to number of plants silking by multiplying by the plant population density (plants ha\(^{-1}\)).

The number of newly exserted silks per unit land area was calculated each day by multiplying the pattern of silk exsertion by individual plants times the pattern of silk emergence for the population. The daily cohort of plants that began to exsert silks was calculated from the population dynamics curves for each treatment. Measurements of silk emergence patterns for the hybrids used in this study indicated silks continued to emerge for up to 9 d on unpollinated ears. Therefore, it was assumed each plant assigned to a daily cohort would exsert silks for the next 9 d (Bassetti and Westgate, 1993a) according to Eq. [4]. The same calculation was made for each subsequent day until 100% of the plants in each treatment were silking. The number of new silks exserted on an area basis was calculated each day as the sum of silks exserted by each of these nine cohorts.

An alternative mathematical approach used to describe the daily emergence of silks was a double Gauss function fit to the seasonal pattern of newly exposed silks in the field:

\[
S_{tot} = \frac{P_1}{W_1 \times \sqrt{\pi/2}} e^{-\frac{(t-t_0)^2}{w_1^2}} + \frac{P_2}{W_2 \times \sqrt{\pi/2}} e^{-\frac{(t-t_0)^2}{w_2^2}},
\]

where \( S_{tot} \) is the daily number of newly exposed silks per land area, \( P_1 \) and \( P_2 \) are the area of the Gauss Curves 1 and 2 whose addition represents the total number of
exposed silks (or viable flowers) during the season per unit area, $W_1$ and $W_2$ are shape parameters controlling the width of the curves (days), and $t_x_1$ and $t_x_2$ are the times when the peaks of Curves 1 and 2 are reached (days).

**Relationship between kernel set and daily pollen shed intensity**

We applied two linear functions in series to the published data of Bassetti and Westgate (1994) to calculate a percentage of kernel formed by flowers with exposed silks from the daily rate of pollen shed. The limits of pollen shed rate for each equation were:

$$\begin{align*}
\text{ks} &= 0.96 \times \text{pr} & 0 < \text{pr} \leq 100, \text{ and} \\
\text{ks} &= 96 & \text{pr} > 100.
\end{align*}$$

where $\text{ks}$ is the percentage of florets with exposed silks that formed kernels (%), and $\text{pr}$ is the daily rate of pollen shed (grains cm$^{-2}$ d$^{-1}$). In this formulation, 100 grains cm$^{-2}$ d$^{-1}$ is the critical rate of pollen shed below which $\text{ks}$ is less than the maximum 96%. Because Bassetti and Westgate (1994) used final kernel set in their analysis, their equations relating pollen shed density to percentage of kernel formed incorporate the natural level of pollen viability inherent in their experiment. Nonetheless, our approach provides the mathematical basis for testing the impact of pollen viability on kernel number per hectare by adjusting the critical rate of pollen shed required for maximum $\text{ks}$.

Daily kernel formation as measured by Bassetti and Westgate (1994) never reached 100% of exposed silks even at the highest rates of pollen shed (Eq. [6]). Kernel abortion was nil in their study since they measured kernel set only at highly receptive floral positions in the middle of the ear. Therefore, we assumed the remaining florets with exposed silks remained receptive. These silks were added to the next day's pool available for pollination. These unpollinated silks remained receptive to pollen for five additional days. Silks that were not pollinated by the sixth day were assumed to senesce and lose receptivity (Bassetti and Westgate, 1993b) and no longer contributed to kernel set.
Evaluation of the procedure for estimating kernel set

We compared the number of florets per hectare and kernels per hectare calculated by the model with measured floret and kernel numbers in eight isolated field plots that varied widely in pollen shed density. In 1986, MF levels of 100, 50, 20, and 0% were obtained by detasselling a 100% MF hybrid, Pioneer 3978 (hereafter P3978). In 1987, MF levels of 75, 50, 20, and 0% were obtained by mixing MS and MF isolines of Pioneer 3925 (hereafter P3925). P3978 was planted at 7.2 plants m\(^{-2}\), and produced an average of 658 silks per ear. P3925 and the pollen sterile isolate P3925S were planted at 5.7 plants m\(^{-2}\), and produced an average of 621 silks per apical ear. Details of the experimental design and plant culture conditions are provided in the companion paper by Westgate et al. (2003).

In the model, each MF treatment was corrected for plant population density and average number of ears per plant at harvest. Percentage of plants at each stage of pollen shed or reaching silking was recorded every other day by evaluating at least 80 plants in the four center rows of each plot.

Procedures used to document the daily rate of pollen shed and daily progress of silk exsertion are detailed in Bassetti and Westgate (1994) and Westgate et al. (2003). Briefly, passive pollen traps were placed within the canopy at ear level and collected every 24 h at \(\approx 1700\) h for counting. Pollen density on the trap surface was evaluated in the laboratory by image analysis according to Bassetti and Westgate (1994). Every 2 d beginning at first silk appearance, 1 to 2 cm of unpollinated silk tissue was collected from ears of 10 plants. Care was taken to collect all exposed silks and to prevent pollination at all other times by covering the ears with a glassine bag. Silk pieces were fixed in ethyl alcohol-glacial acetic acid-formalin-water (50:5:10:35 by volume) containing 10 g kg\(^{-1}\) aniline blue, and subsequently counted by hand.

Effect of asynchronous pollination: within-ear synchrony

Several studies indicate that kernel set at flower positions with late emerging silks increases dramatically if all exposed silks on the ear are pollinated synchronously (Freier et al., 1984; Cárcova et al., 2000). Evidently, both the timing of
pollination and the number of prior fertilization events affects kernel set by later pollinated flowers. Therefore, the effect of asynchronous pollination of florets on the apical ear was included following the hypothesis that earlier-formed kernels could have a detrimental effect on the success of kernel formation by later-fertilized flowers. The daily calculation of kernel set (Eq. [6]) was modified to account for the cumulative number of kernels set per ear (KS) and asynchronous pollination efficiency (EAP):

\[
E_{AP} = 1 - e^{c(KS - KS_0)}.
\]

where \(E_{AP}\) is a unitless function (0 to 1) that varies with the cumulative number of kernels per ear formed the previous day (KS). Parameter c controls the slope, and KS_0 is the intercept for kernel set. If \(E_{AP} = 1.0\), all pollinated flowers will set kernels. If \(E_{AP} = 0.0\), no pollinated flowers will set kernels, regardless of the pollen shed density.

Parameters in Eq. [7] (c = 0.013; KS_0 = 1.2 x SNk, for apical ear; KS_0 = 1.1 x SNk, for subapical ear) were calibrated to data in Cârcova et al. (2000). Asynchrony-adjusted kernels set was calculated daily using Eq. [8]:

\[
KS = SN \times ks \times E_{AP}.
\]

where KS is the adjusted kernel set (kernels ear^{-1}), SN is the cumulative number of silks exposed, ks is the fraction of kernel set estimated with Eq. [6] (%), and \(E_{AP}\) is the efficiency factor for kernel set calculated in Eq. [7].

**Effect of asynchronous pollination: between-ear synchrony**

We included the effects of asynchrony between apical and subapical ears of P3925 in terms of delayed silk exsertion and a decreased number of flowers per ear on subapical ears. P3978 was not included in this analysis because it did not produce second ears. Silk exsertion on subapical ears of P3925 was estimated from the pattern of silk exsertion measured on apical ears and the measured relationships between apical and subapical ear development in a similar prolific hybrid (Table 1). The experiment providing data on flowering of subapical ears was planted at the
Bruner Research Farm near Ames, IA, in 2000. Silk exsertion was evaluated on the prolific hybrid Asgrow 740 grown at three population densities (1, 8, and 16 plants m$^{-2}$) supplied with 168 kg N ha$^{-1}$ and replicated three times in a split-plot design. When 30% of the plants in each plot reached silking, five plants about to exsert silks were selected for sampling. Ears were covered with glassine bags to prevent pollination, and silks were sampled 2, 4, and 8 d after first silks appeared. Two-centimeter segments of silk tissue were cut at husk level, transferred to plastic bags containing 500 g kg$^{-1}$ ethanol, and stored at 4°C until counted manually.

Monomolecular functions (Eq. [4]) were fit to measured silk exsertion data for the apical ear of P3925 and for apical and subapical ears of Asgrow 740 (Fig. 1). Parameters SN (maximum silk number), $b$ (shape factor), $t_0$ (initial day of silk emergence), and duration of silk emergence were generated from each curve. Parameters for subapical ears of P3925 were calculated relative to the apical ear using the ratio of apical/subapical values for the corresponding Asgrow 740 parameters. Subapical ear parameters for P3925 were adjusted to 5.7 plants m$^{-2}$ by linear interpolation. The delay in initial silk emergence for the subapical ear was calculated as $t_{c2} - t_{c1}$.

**Prediction evaluation**

Deviation of predicted and observed values were compared using the root mean square of error (RMSE):

$$\text{RMSE} = \left[ \frac{1}{n} \sum_{i=1}^{n} (P_i - O_i)^2 \right]^{0.5},$$

where $P_i$ and $O_i$ are predicted and observed values, and $n$ is the number of observations.

**Results**

**Dynamics of pollen shed**

The first step in the procedure to calculate the number of kernels formed on an area basis was to characterize the dynamics of pollen production in the field. The
evaluation of daily pollen production showed greater variability of daily rates for P3978 than for P3925 (Fig. 2a and 2b). Also, there was a greater amount of pollen drift into the 0% MF treatment for P3978. These results likely reflected differences in methodology used to reduce the levels of pollen production (detasselling in P3978 vs. MS isoleine in P3925). The difference in plant population density (7.2 plants m$^{-2}$ for P3978 vs. 5.7 plants m$^{-2}$ for P3925) contributed to the lesser maximum rates of pollen shed for P3925 as well. To compare the response of two hybrids to pollen shed density, pollen shed rates were normalized using plant population density and the fraction of MF plants. Normalized in this way, it was evident that the MF treatments did not affect the pattern or the timing of the pollen shed process (Fig. 2c,d). Pollen production per plant based on the seasonal pollen shed curves fit to the field data were very similar for the two hybrids: 4.2 million grains per plant for P3978 and 4.5 million grains per plant for P3925. The seasonal pattern of pollen shed, however, was 4 to 5 d longer for P3978. The normalized curves for pollen production per plant were scaled to pollen shed rates on an area basis (grains cm$^{-2}$ d$^{-1}$) using the population densities and relative seasonal pollen production for each hybrid-treatment combination (Fig. 2a,b). These scaled curves of pollen production accurately described the seasonal pattern of pollen shed for each treatment and were used to predict kernel number per hectare.

The pollen trap method accurately characterizes the dynamics of pollen shed in the field (Bassetti and Westgate, 1994; Fonseca et al., 2002). But, it is a labor and time-intensive process. Therefore, an alternative procedure to describe the seasonal pattern of pollen shed in the field was needed. Westgate et al. (2003) showed that the daily rate of pollen shed could be derived from simple observations of the male flowering process. Using their procedure, we fit sigmoid curves (Eq. [2]) to field observations of percentage of plants beginning pollen shed (anthesis), reaching maximum shed, and ending shed (Fig. 3a,b). Simulated percentages of population at beginning, maximum, and ending pollen shed were used to calculate a $P_{\text{ind}}$ of plants shedding pollen (Eq. [3]). To simulate the intensity of pollen shed, the $P_{\text{ind}}$ was scaled using the total pollen production per plant (parameter $p$ in Eq. [1]) and the
plant population density (Westgate et al., 2003). Figure 3c and 3d show that the rates of pollen shed computed from the $P_{ind}$ were in close agreement with rates measured in the field. Values of RMSE were similar to corresponding values obtained when rates of pollen shed were predicted with the scaled Gauss curves (Fig. 2a,b). These results indicate that the $P_{ind}$ procedure provides a promising alternative to the pollen trap method to characterize the dynamics of pollen shed in the field. If the average pollen production per plant is known, pollen production on an area basis can be distributed realistically in time using simple observations of male flower development.

**Dynamics of silk exsertion**

The second component required to calculate kernel number per hectare is a measure of the dynamics of silk numbers available and receptive to pollination. This dynamic requires a simultaneous calculation of silks exserted per plant and an estimate of the percentage of plants beginning to exsert silks. We calculated silk numbers per hectare first on a cumulative basis as depicted in Fig. 4a and 4b. The process is described by Eq. [4], which accounts for variations in the maximum number of florets per ear ($SN_x$) and the duration of silk exsertion on an ear (days). The hybrids examined in this study required 8 and 9 d to complete silk exsertion, which is in agreement with Bassetti and Westgate (1993a), who reported differences up to 4 d between hybrids to complete the silking process.

To calculate the number of newly emerged silks per hectare, we computed the daily components for each plant and for the population (Fig. 4c and 4d). Each day, a new group of plants begins to exsert silk simultaneously with a second group in its second day of silk exsertion, along with those in their third day, and so on. After calculating the number of silks exserted by each group based on their respective day of silking, the values are added to obtain a daily total of newly exposed silks (Fig. 5). A double Gauss model also described the dynamics of silk exsertion for each hybrid. The model explained >99% ($r^2 = 0.99$) of the seasonal variation in silk exsertion per hectare. The maximum rate of silk appearance occurred 2 to 3 d after silking for the population (50% of population with silks exposed), which corresponds closely with
the maximum rate of pollen shed for both hybrids. On a population basis, the two hybrids exposed silks during a period of 31 to 34 d (Fig. 5). By the day of silking, the populations had exerted 20 to 25% of their seasonal totals.

**Pollen and silks synchrony: potential kernel set**

Each exposed silk translated mathematically into a potential kernel, depending on its timely pollination. So the third component in our predictive procedure was to link the dynamics of silk exsertion with the dynamics and intensity of pollen production to generate a cumulative curve of kernel set for each treatment. We relied on two facts about the flowering biology of maize to estimate potential kernel set: (i) kernel set varies with pollen shed density in a manner defined by Bassetti and Westgate (1994); and (ii) each exposed silk remains receptive to pollen for at least 6 d (Bassetti and Westgate, 1993a).

Each day, a new group of silks was exposed for pollination, a certain rate of pollen shed was available to pollinate them, and the daily pollen rate was associated with an expected percentage of fertilized florets (Bassetti and Westgate, 1994). To calculate the number of kernels set each day, we multiplied the number of silks exposed that day times the percentage of florets that should be fertilized given the daily rate of pollen shed (Fig. 6). Figure 7 shows the daily progress of kernel addition for two levels of pollen availability provided to P3978 and P3925. The model predicted that hybrid P3978 would pollinate 96% of the exposed silks when 100% of the plants contributed to pollen shed (Fig. 7a). All exposed silks were successfully pollinated until the daily rate of pollen shed decreased to \(\approx 100\) grains cm\(^{-2}\). Reducing the number of plants shedding pollen to 20% MF decreased the seasonal production of pollen to 30% of the 100% MF rate. Yet, sufficient pollen was available to fertilize \(\approx 90\%\) of the flowers with exposed silks (Fig. 7c).

Hybrid P3925 apparently was not as efficient as P3978 in pollinating receptive silks. With 75% of the plants shedding pollen, only 86% of the florets with exposed silks set a kernel (Fig. 7b). When the fraction of MF plants decreased to 20% MF, seasonal pollen production was reduced to 24% of the maximum, and 71% of flowers with exposed silks produced kernels (Fig. 7d). This supports the notion that
pollen production is generally well in excess of pollination requirements for >90% of exposed silks. Maize canopies might fail to pollinate late emerging silks, however, even when all plants are MF.

Calculated values for silking florets per hectare and kernels per hectare were compared with field measured floret and kernel numbers. Our procedure predicted floret numbers with >99.5% accuracy in all treatments, when the specific plant population and average number of ears per plant were considered (Table 2). Predicted kernel numbers were overestimated, however, by up to 15% for P3978 and up to 20% for P3925 even in the 20% MF treatment, which was shown to limit kernel set (Westgate et al., 2003). This consistent overestimation of kernel number suggested that a systematic factor affecting kernel set was not considered in the model.

**Pollen viability**

The relationship of Bassetti and Westgate (1994) adopted to predict kernel set (Eq. [6] and Fig. 6) included an unknown level of pollen viability. By using 100 grains cm\(^2\) d\(^{-1}\) as the critical pollen density to assure 96% kernel set in Eq. [6], we tacitly assumed that the level of pollen viability in Bassetti and Westgate (1994) applied to P3798 and P3925. A lower level of pollen viability in our experiment might explain the overprediction of kernel numbers in Table 2. To test this possibility, pollen viability was decreased mathematically by increasing the critical pollen rate in Eq. [6] to values >100 grains cm\(^2\) d\(^{-1}\). For this evaluation, we selected MF treatments in which pollen production was similar for both hybrids and was low enough to limit kernel set: the 20% MF treatment for P3978 and the 50% MF treatment for P3925 (Westgate et al., 2003). Table 3 shows that decreasing pollen viability lowered kernel set in these treatments, but the impact was too small to account for the overprediction observed, even when pollen viability was decreased by 37%, which is typical of high temperature stress (Herrero and Johnson, 1980) or field aging (Luna et al., 2001).
Pollination synchrony within ear and between ears

The initial calculation of potential kernel set assumed that the opportunity of a receptive silk to be pollinated and develop into a kernel depended only on the rate of pollen shed. It also assumed that silk exsertion on apical and subapical ears were identical in terms of number and timing. Yet Cárcova et al. (2000) showed that naturally pollinated plants often set fewer kernels than did plants whose silks were pollinated synchronous by hand, particularly at low population densities. Also, silk emergence on subapical ears often is delayed relative to apical ears (Jacobs and Pearson, 1991; Otegui and Melon, 1997), with greater delays evident at higher plant population densities (Jacobs and Pearson, 1991; Cárcova et al., 2000).

To take these developmental effects into account, we modified the procedure for calculating kernel number to include the delay in silk exsertion by subapical ears and the effects of asynchronous pollination within and between ears on the same plant (Fig. 1, Table 1). Although these refinements are based on existing evidence in the literature, we did not measure these developmental effects directly in our data set. Therefore, they are considered as hypothetical effects in our analysis that will require experimental confirmation.

Figure 8a shows that our procedure correctly forecast the lower potential of P3925 florets to form kernels (83% maximum set) compared with those of P3978 (65% maximum set) at high pollen shed density. Including the effects of asynchronous pollination and the delayed development of subapical ears decreased kernel set (relative to the number of exposed silks) substantially in both hybrids (Fig. 8b). Predicted kernel set closely matched measured kernel set at all pollen shed densities for P3978, but the procedure only accounted for about half of the overestimate in P3925.

Discussion

The purpose of this work was to develop a model to simulate kernel set from the mathematical description of male and female flowering dynamics in a maize field. We attempted to generate general algorithms useful to improve existing models of
kernel number (Lizaso et al., 2001). The functions were developed for nonstress conditions, but can be adapted readily to accommodate a range of conditions known to alter flower development and function. Rates of pollen shed density measured at the level of exposed silks were accurately described with Eq. [1] (Fig. 2a,b). Maximum deviation between predicted and measured pollen rates (as expressed by the RMSE) was 92 grains cm\(^{-2}\) d\(^{-1}\) for P3978 at 100% MF. Similar rates of pollen shed were reported earlier (Hall et al., 1982; Struik and Makonnen, 1992; Bassetti and Westgate, 1994). We also calculated daily appearance of silks using Eq. [4] (Fig. 4) following patterns in previous reports (Bassetti and Westgate, 1994; Cárcova et al., 2000). These patterns and timing of pollen shed and silk appearance also can be modified readily to predict kernel set under stress conditions. High temperature from tassel initiation to kernel set, for example, shortens the duration of pollen shedding (Struik et al., 1986). Drought at or before tasseling delays silk emergence (Herrero and Johnson, 1981; NeSmith and Ritchie, 1992). Flooding during the early vegetative growth delays silking more than tasseling (Lizaso and Ritchie, 1997). These environmental responses can be simulated by altering parameters in Eq. [1] and [4].

Our calculations show that the seasonal duration of silk exsertion for the eight populations examined persisted for a period of 31 to 34 d (Fig. 5). The maximum duration of pollen shed was 24 d for P3978 and 17 d for P3925. Rates of pollen shed at or above that required for maximum kernel set (Fig. 6), however, occurred only on eight of these days for P3978 and on five days for P3925. Because silks remain receptive to pollen for 6 d after they first emerge (Bassetti and Westgate, 1994), most of the unpollinated silks exserted early in pollen shedding were pollinated eventually (Fig. 7). The beneficial impact of this prolonged silk receptivity was most evident in the 20% MF treatments in which a large reduction in pollen deposition early in silking resulted in only a 6% (P3978) and 15% (P3925) reduction in kernel set. Silks exserted after the rate of pollen shed was less than required for maximum kernel set constituted the vast majority of unpollinated silks. In this regard, the longer
duration of pollen shed for P3978 (Fig. 2c) contributed to its advantage over P3925 in terms of setting a larger proportion of kernels per exposed silk (Fig. 8).

In the treatments that were saturated for seasonal pollen deposition (≤50% MF), 83% and 65% of the pollinated florets developed into kernels for P3978 and P3925, respectively (Table 2). These percentages are in general agreement with reported values (Otegui and Melon, 1997). Yet, our calculations overpredicted the percentage of florets that set kernels both under pollen-abundant and pollen-limited conditions. When pollen is abundant, some degree of overprediction is expected since kernel set is limited by other factors, such as available assimilate supply (Andrade et al., 1999; Andrade et al., 2000; Lizaso et al., 2001). Under pollen-limited conditions (≥30% of maximum pollen shed for each hybrid), however, overprediction of kernels per hectare indicates that the model did not consider one or more important factors constraining kernel formation.

We tested the possibility that the level of pollen viability was lower than originally assumed in the Bassetti and Westgate (1994) relationship between pollen shed density and percentage kernel set. Decreasing the efficiency of kernel set at low pollen shed density by 37% (effectively decreasing pollen viability) decreased predicted kernels per hectare by <5% in MF treatments that were already marginal for pollen amount (Table 3). Therefore, it seems unlikely that this factor alone could account for the overestimation of kernel set. This exercise, however, demonstrates the utility of our modeling approach to quantify developmental and environmental effects on kernel set.

A second possibility was that the original calculation of kernel number did not consider the potential for kernel loss due to asynchronous pollination within and between ears. We tested two related components of asynchronous pollination for their impact on kernel set. First was the developmental dominance of basal kernels over apical kernels on the same ear (i.e., within-ear asynchrony). The second was the delayed development of subapical ears relative to apical ears (i.e., between-ear asynchrony).
Cárcova et al. (2000) showed that synchronously hand-pollinated ears set more kernels than did naturally pollinated ears at the same level of assimilate supply. On the basis of their results, we developed an asynchronous pollination efficiency factor \((E_{AP})\) to account for the dominance of early-formed kernels on predicted kernel number. Originally, the opportunity for a silk to be pollinated and for its floret to develop into a kernel depended only on the daily rate of pollen shed as dictated by Eq. [6]. In the modified procedure, the fraction of kernels set each day decreased as the number of previously set kernels increased (Eq. [7]). This approach is consistent with Freier et al. (1984), who observed that the success of later-formed kernels decreased as the number of prior pollinations on the same ear increased. After taking within-ear synchrony into account, the model predicted kernel numbers per hectare accurately for P3978 at all pollen shed densities (Fig. 8b). The modified procedure, however, still overestimated kernels per hectare for P3925 by \(\approx 10\%\).

A number of studies have reported a delay in subapical ear development relative to the apical ear as plant population density increases (e.g., Otegui, 1997). In this study, subapical ears produced kernels on a number of P3925 plants (1.2 ears per plant on average), but none formed on P3978 plants. Because we did not measure silk exsertion on these subapical ears, it was necessary to assume that their silk numbers and the timing of exsertion were identical to that of the apical ears. Measurements of silk exsertion on subapical ears on a similar prolific hybrid (Asgrow 740) grown at several plant densities (Fig. 1) indicated this assumption likely caused us to overestimate the contribution of the subapical ears to kernel set in P3925. When silk exsertion from the subapical ears of P3925 followed the same relative pattern as for Asgrow 740, predicted kernel numbers for P3925 decreased to within 5\% of measured values for the three lowest MF treatments (Fig. 8b).

It is important to emphasize the uncertainty associated with incorporating the potential impact of pollination asynchrony and delayed development of subapical ears. These functions likely are affected by population density and by the inherent prolificacy of each hybrid (Otegui, 1997; Cárcova et al., 2000). Yet our results provide evidence that asynchrony between ears and within ears must be taken into
account for accurate prediction of kernel numbers. This is particularly important when pollen amount limits kernel number.

Many authors have shown that kernel number per plant depends on IPAR around silking (Andrade et al., 2000; Lizaso et al., 2001). Such results support the view that the supply of carbohydrates around silking imposes the primary limitation on the number of fertilized ovules that ultimately develops into kernels. There are numerous field conditions such as drought (Hall et al., 1982; Edmeades et al., 1993), flooding (Lizaso and Ritchie, 1997), or hybrid seed production in which asynchrony of male and female flowering could impose a limit on pollen availability to fertilize exposed silks (Bassetti and Westgate, 1994). Kernel set under such conditions would be constrained by the number of fertilized ovaries, even though concurrent metabolic responses to stress also contribute to decreased kernel set. The procedure presented herein predicts kernel set based primarily on the synchrony of staminate and pistillate flowering, delayed development of subapical ears, and the detrimental effect of prior fertilization on the success of later-fertilized ovaries. A general procedure to forecast kernel numbers under both assimilate-limited (Lizaso et al., 2001) and pollen-limited conditions is forthcoming.

References


Table 1. Parameters used in Eq. [4] to predict daily silk exsertion for subapical ears of P3925. Silk exsertion curves were fit to measured field data for the apical ear of P3925 and for apical and subapical ears of Asgrow 740. Parameters for subapical ears of P3925 were calculated relative to the apical ear using the ratio of apical/subapical values for the corresponding Asgrow 740 parameters. Subapical ear parameters for P3925 were adjusted to 5.7 plants m$^{-2}$ by linear interpolation.

<table>
<thead>
<tr>
<th>Eq. [4] parameters</th>
<th>1 plant m$^{-2}$</th>
<th>8 plants m$^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apical ear</td>
<td>Subapical ear</td>
</tr>
<tr>
<td>$SN_0$ (max silk no.)</td>
<td>808</td>
<td>808</td>
</tr>
<tr>
<td>$b$, d $^{-1}$</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>$d$, day of year</td>
<td>195.1</td>
<td>195.1</td>
</tr>
<tr>
<td>duration, d</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Pioneer 3925</th>
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<tbody>
<tr>
<td></td>
<td>Apical ear</td>
<td>Subapical ear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$SN_0$ (max silk no.)</td>
<td>621</td>
<td>608.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$b$, d $^{-1}$</td>
<td>0.486</td>
<td>0.329</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$d$, day of year</td>
<td>199.81 n</td>
<td>200.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>duration, d</td>
<td>8</td>
<td>8.8</td>
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</table>

$^a$ $SN_0$, the potential number of silks per ear equal to the potential number of florets.
$^b$ $b$, shape parameter controlling the slope of the curve.
$^c$ $d$, time when the first silk is exserted.
Table 2. Observed and predicted number of florets and kernels per unit land area produced at various levels of male fertility (MF) for P3978 and P3925. Data in parenthesis indicate the floret or kernel value as a percentage of the observed number of florets in each MF treatment. Difference (Diff) = Predicted % – Observed %.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of florets</th>
<th>Number of kernels</th>
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<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Predicted</td>
</tr>
<tr>
<td>P3978</td>
<td></td>
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</tr>
<tr>
<td>100% MF†</td>
<td>4.51 x 10^7</td>
<td>4.51 x 10^7</td>
</tr>
<tr>
<td>50% MF‡</td>
<td>4.60 x 10^7</td>
<td>4.60 x 10^7</td>
</tr>
<tr>
<td>20% MF§</td>
<td>4.81 x 10^7</td>
<td>4.80 x 10^7</td>
</tr>
<tr>
<td>0% MF¶</td>
<td>4.67 x 10^7</td>
<td>4.66 x 10^7</td>
</tr>
<tr>
<td>P3925</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75% MF#</td>
<td>3.38 x 10^7</td>
<td>3.37 x 10^7</td>
</tr>
<tr>
<td>50% MF††</td>
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<td>3.14 x 10^7</td>
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<tr>
<td>20% MF‡‡</td>
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<td>2.59 x 10^7</td>
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<tr>
<td>0% MF§§</td>
<td>3.82 x 10^7</td>
<td>3.81 x 10^7</td>
</tr>
</tbody>
</table>

* 70676 plants ha⁻¹; 6.97 ears per plant.
† 71395 plants ha⁻¹; 6.90 ears per plant.
‡ 73791 plants ha⁻¹; 6.99 ears per plant.
§ 70916 plants ha⁻¹; 1.00 ears per plant.
# 52019 plants ha⁻¹; 1.07 ears per plant.
†† 54442 plants ha⁻¹; 1.09 ears per plant.
‡‡ 54445 plants ha⁻¹; 1.10 ears per plant.
§§ 54714 plants ha⁻¹; 1.15 ears per plant.
Table 3. Predicted kernel number at decreasing levels of pollen viability for the 20% male fertile (MF) treatment for P3978 and the 50% MF treatment for P3925. Kernel set was limited by pollen amount in both treatments. The critical pollen rate needed to achieve 96% kernel set in Eq. [6] was increased mathematically to simulate loss of pollen viability below the original level assumed from Bassetti and Westgate (1994). Predicted kernel numbers are presented as a fraction of the observed values. Note that a 37% decrease in relative pollen viability resulted only in a 3 to 5% decrease in kernel set.

<table>
<thead>
<tr>
<th>Critical pollen rate</th>
<th>Relative pollen viability</th>
<th>Relative kernel number predicted</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>P3978</td>
</tr>
<tr>
<td>grains cm$^{-2}$ d$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.00</td>
<td>1.19</td>
</tr>
<tr>
<td>110</td>
<td>0.91</td>
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<td>120</td>
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<tr>
<td>140</td>
<td>0.71</td>
<td>1.15</td>
</tr>
<tr>
<td>150</td>
<td>0.67</td>
<td>1.14</td>
</tr>
<tr>
<td>160</td>
<td>0.63</td>
<td>1.13</td>
</tr>
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</table>
Figure 1. Dynamics of silk exsertion in apical and subapical ears of Asgrow 740 as influenced by plant population density. Symbols are number of exposed silks per ear and lines are corresponding monomolecular functions fit to the data. Parameters SN, b, t₀ and duration in Eq. [4] to predict silk emergence from subapical ears of P3925 were generated from these curves (see Table 1). Vertical lines are standard errors for five plants. Plants grown at 16 plants m⁻² did not produce a second ear.
Figure 2. Seasonal dynamics of pollen shed intensity for hybrids P3978 (a,c) and P3925 (b,d). (a,b) Observed (symbols) and predicted (lines) rates of pollen shed at four levels of male fertility (MF). (c,d) Daily pollen shed rates normalized per fertile plant (symbols) and Gauss functions fit to the normalized data (lines). Vertical lines are standard errors for four measurements. Note that a single curve describes the seasonal pattern of pollen shed for each hybrid.
Figure 3. (a,b) Normalized percentage of plant population at three stages of pollen shed: beginning shed, maximum shed, and end shed for P3978 (a) and P3925 (b). (c,d) Measured pollen rates (symbols) and predicted pollen rates using the population index (lines) for hybrids P3978 (c) and P3925 (d). The population index was calculated using Eq. [3]. Vertical lines are standard errors of four measurements.
Figure 4. Seasonal progress of silk emergence for the plant population and individual apical ears. (a,c) P3978; (b,d) P3925. (a,b) Cumulative percentage of plant population at silking (solid lines) and cumulative number of silks exserted on the apical ear (broken lines). (c,d) Daily percentage of plant population that has started silking (solid lines) and daily number of silks exserted from the apical ear (broken lines).
Figure 5. Seasonal dynamics of silk exsertion on an area basis for hybrids P3978 (top) and P3925 (bottom). Calculated values (symbols) were generated from population and silk exsertion curves in Fig. 4a and b. Predicted curves (lines) are a double Gauss function fit to the seasonal pattern of newly exposed silks using Eq. [5].
Figure 6. Relationship used to convert daily pollen shed density into potential kernel set adopted from Bassetti and Westgate (1994). Data for pollen shed are from passive pollen traps placed at ear level, and kernel set was measured on ears exposed to pollen for one 1 d at the indicated densities. The broken line indicates the predicted kernel set if pollen viability were decreased to 63% of that in the Bassetti and Westgate (1994) study.
Figure 7. Seasonal dynamics of pollen shed and potential kernel set for P3978 (a,c) and P3925 (b,d) at two levels of male fertility (MF). Note that all florets pollinated before maximum pollen shed develop into kernels. At low pollen density, a greater proportion of late emerging silks fail to be pollinated.
Figure 8. Kernel set expressed relative to the number of exposed silks at four levels of male fertility (MF) for P3978 and P3925. Pollen shed is presented as a fraction of the total seasonal deposition expected in a field of 100% MF plants. Symbols are measured values; lines indicate predicted values. (a) Predicted kernel set is based on silk exsertion and pollen shed dynamics for each MF treatment. (b) Predicted kernel set incorporates potential kernel loss due to asynchronous pollination within ears and asynchronous development between ears.
CHAPTER 5. SIMULATING POTENTIAL KERNEL PRODUCTION IN MAIZE HYBRID SEED FIELDS

A paper published in Crop Science

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Abstract

In maize (Zea mays L.) hybrid seed production, achieving the optimum seed yield per unit land area often is based on limited information about the quantity of pollen shed by the male and practical experience synchronizing pollen shed by the male inbred with silk emergence by the female inbred. We recently reported that kernel production per hectare could be simulated fairly accurately under pollen-limited conditions from simple measures of pollen shed and silking dynamics. The objective of this study was to determine whether a simple mechanistic description of the flowering dynamics of male and female inbreds could be used to simulate and optimize kernel production in seed production fields. We estimated kernel production on the basis of flowering dynamics in six commercial seed fields located near Washington, IA in 2002, which differed in the quantity of pollen production and silk emergence. In all cases, the fields were managed and harvested by standard seed industry methods. Harvested kernel number varied from 8.4 to 23.1 million kernels per female hectare. Simulated kernel number was closely correlated with these measured values ($r^2 = 0.98$). This result indicates that relative differences in kernel production can be assessed directly from inbred flowering dynamics. Examples are provided to show how inbred management can be modeled to optimize harvested kernel number for a given inbred pair. Model simulations, however, overestimated harvested kernel number by 11%, on average, which implies that other plant factors, such as pollen viability, prolificacy, pollen capture by the canopy, or kernel abortion

in response to leaf removal during detasseling might have limited kernel production across the six seed fields. Information about these variables can be incorporated readily into the kernel set model to improve its accuracy. This study indicates that kernel production in a hybrid seed field can be simulated from simple measures of inbred flowering dynamics. The model is a useful tool for optimizing harvested kernels for an established inbred pair or for defining initial management protocols for new combinations of inbreds.

**Introduction**

Kernel number per plant is the yield component primarily responsible for variation in maize grain yield. For simulation purposes, the developmental events that determine kernel number per plant can be divided in three consecutive processes (Lizaso et al., 2003). In the first stage, male (tassels) and female (ears) reproductive structures are initiated and differentiated. The second stage involves functional maturation of flowers and pollination. Synchrony in floral development is critical to ensure that pollen shed coincides closely with silk exsertion and that tassels produce enough pollen to ensure pollination of exposed silks. During the third stage, pollination is followed by fertilization and kernel formation. Current models for simulating maize yield, such as CERES-Maize (Jones and Kiniry, 1986), focus exclusively on the third stage. They implicitly assume that neither flower initiation, differentiation, nor pollination limit kernel set. These assumptions might apply to typical commercial corn production in some cases, but the number of fertilized ovules can limit kernel yield in a wide array of circumstances.

When maize is stressed at flowering by water deficits (Westgate and Boyer, 1986), low light levels (Andrade et al., 1993), or nutrient deficiency (Lafitte and Edmeades, 1994a and b), ear growth slows in relation to tassel growth and the anthesis-silking interval (ASI) increases. This increase is well documented and negatively related to kernel set (Bolaños and Edmeades, 1993). Lack of pollen, reduction in pollen viability and silk receptivity are proposed as the main causes for the reduction in kernel production under these conditions.
Selection for improved hybrid performance under stressful conditions such as high population density has resulted in a decrease in tassel size among modern maize hybrids (Galinat, 1992; Duvick, 1997). This smaller tassel size is correlated with diminished capacity for pollen production (Fonseca et al., 2003). Low levels of pollen production also occur intentionally in hybrid seed production, with 50% male-sterile hybrid blends, and in top-cross schemes used to produce high-oil corn (Feil et al., 2003). Although the maize plant has traditionally been considered an overabundant producer of pollen relative to the number of ovaries available for pollination, such genetic, management, and environmental influences on pollen production and viability provide numerous opportunities for the timing and density of pollen shed to limit kernel production under field conditions (Westgate et al., 2003).

This seems particularly clear for hybrid seed production since pollination could be less than desired for several reasons. First, pollen shed density is much less than in a grain field since inbreds typically produce less pollen than do their hybrid counterparts (Fonseca et al., 2003). Second, only a fraction of the field population is permitted to shed pollen, i.e., male inbred. A major goal in hybrid seed production, in fact, is to reduce the area dedicated to male rows as much as possible without decreasing the number of kernels harvested per area (Wych, 1988). Third, the level of pollen viability could be less than required for optimum pollination of receptive silks (Schneider, unpublished 2003). Finally, pollen shed and silk exsertion on physically separated plants increases the probability that floral asynchrony can lead to poor kernel set. Together these biological and physical factors create conditions in which kernel numbers could be limited primarily by the number of pollinated flowers. This potential for pollination, of course, depends directly on the dynamics of male and female flowering within the seed field.

Westgate et al. (2003) recently developed quantitative descriptions for the daily progress of pollen shed and silk emergence under field conditions on the basis of simple measures of male and female flowering. When coupled mathematically to the pollination efficiency curve generated by Bassetti and Westgate (1994), these estimates of male and female flowering can be translated into daily values for kernel
set. Lizaso et al. (2003) showed that this mathematical approach was highly accurate at simulating kernel production for two maize hybrids across a wide range of pollen shed densities.

Our objectives were i) to determine whether the mechanistic model of Lizaso et al. (2003) could be used to simulate kernel production in commercial hybrid seed fields and ii) to demonstrate the potential of this approach for optimizing kernel production for current inbred pairs or for defining the optimum management strategy for new combinations of inbreds.

**Materials and Methods**

**Procedure for simulating potential kernel set**

The procedure for simulating kernel production begins with developing a temporal profile of pollen shed for the male population and a profile of silk exsertion for the female population. These floral dynamics are translated into daily values of kernel production by the female inbred using the procedures described by Lizaso et al. (2003), which rely on the quantitative relationship between daily pollen shed density (grains per cm$^2$) and percent kernel set published by Bassetti and Westgate (1994). Table 1 lists the crop inputs required to generate the curves for male and female flowering dynamics. Details for collecting these data are outlined briefly below.

**Amount and temporal distribution of pollen shed**

The seasonal distribution of pollen shed is simulated from field observations of male flowering characteristics at the population level. This calculation assumes that pollen density is distributed homogeneously among the female population. One hundred consecutive male plants at each sampling site are examined for the progress of tassel development each morning. Beginning Shed (Beg Shed) is recorded as the proportion of plants that have anthers exserted on the main tassel branch. Plants that have exserted anthers on main and side tassel branches are at Maximum Shed (Max Shed). And those with no new anthers on any tassel branch are recorded as having completed pollen shed (End Shed) (Westgate et al., 2003).
The progress of each population through Beg Shed, Max Shed and End Shed are readily described by a common set of sigmoid logistic functions separated in time by 1 to 5 d. The area between these curves provides a daily index of pollen shed for the population. Typically, the same group of plants is used to record the proportion of plants at Beg Shed, Max Shed, and End Shed to generate the population index curve. The actual rate of pollen shed (grains cm\(^{-2}\) d\(^{-1}\)) is calculated by multiplying this index value by the average pollen production per plant and the male population density.

However, seed production managers typically collect data only on the progress of Beg Shed for their male inbreds. This was the case in the six Syngenta seed production fields, which required us to assume the dynamics of pollen shed followed published patterns (Lizaso et al., 2003; Westgate et al., 2003). Lizaso et al. (2003) showed that the seasonal pattern of pollen shed followed a Gauss curve according to Eq. [1]:

\[
PR = \frac{P}{W \times e^{-\frac{(t-t_x)^2}{2W^2}}}
\]

where \(PR\) is the daily rate of pollen shed (grains cm\(^{-2}\) d\(^{-1}\)), \(P\) is the total seasonal amount of pollen produced by the male population (grains cm\(^{-2}\)), \(W\) is the width of the pollen shed curve measured at half the maximum pollen shed rate (d), and \(t\) and \(t_x\) are the current day and the day of maximum pollen shed. The total seasonal amount of pollen, \(P\), was defined by the average pollen production per plant and the plant population density. The day of maximum pollen shed, \(t_x\), typically occurs 2 or 3 d after anthesis (50% Beg Shed) (Bassetti and Westgate, 1994; Fonseca et al., 2003). The width of the pollen shed curve, \(W\), was determined empirically for each field by forcing the Gauss curve to start pollen shed at Beg Shed = 0% and end pollen shed at Beg Shed = 100% + 5 d. The addition of 5 d was based on prior studies (Westgate et al., 2003; Lizaso et al., 2003) indicating that the interval
between Beg Shed and End Shed for an individual tassel was typically 5 d. The daily value for pollen shed density was used in Eq. [2] and [3] to calculate daily percent kernel set.

**Amount and temporal distribution of exserted silks**

The temporal distribution of silks exposed for pollination is calculated from the progress of female flower development at the population level and at the plant level. At the population level, 100 consecutive plants are examined for silking each morning. The percentage of population with exposed silks progresses in a sigmoid fashion similar to the population curves generated for tassel development. At the plant level, silks emerge from the surrounding ear leaf sheaths (husks) over a period of days in a curvilinear fashion. The process is described by a monomolecular equation, using rate of silk exsertion and maximum silk number per ear as genotype-specific variables (Lizaso et al., 2003). When 30% of the population has started silking, ears on five plants about to exsert silks are covered with glassine bags to prevent pollination. Silks are sampled 1, 3, 5, 7 and 9 d after first silks appear. Two-centimeter segments of exposed silk tissue are cut at husk level, transferred to plastic bags containing 500 g kg\(^{-1}\) ethanol, and stored at 4 °C until counted manually.

The daily and cumulative number of silks exserted per unit land area were calculated by combining the daily dynamics of silking for the population, the daily rate of silk exsertion for each plant, and the female plant population density (plants female ha\(^{-1}\)).

**Relationship between kernel set and daily pollen shed density**

Exposed silks were pollinated at a rate determined by two consecutive linear functions on the basis of the pollination efficiency curve of Bassetti and Westgate (1994). Their pollination efficiency curve was generated by means of receptive florets in the middle of the rachis for which no abortion occurred. Therefore, this efficiency curve provides the expected percentage of kernel set when receptive silks are exposed to a known density of pollen shed for 1 d. The limits of pollen shed density for each equation are:
where \( ks \) is the percentage kernel set (%), and \( pr \) is the daily rate of pollen shed (grains cm\(^{-2}\) d\(^{-1}\)). These equations indicate that percent kernel set is linearly related to daily pollen shed density up to 100 grains cm\(^{-2}\) d\(^{-1}\) with an efficiency of 96% (i.e., at 50 grains cm\(^{-2}\) d\(^{-1}\), 48% of exposed silks will be pollinated). At pollen shed densities greater than 100 grains cm\(^{-2}\) d\(^{-1}\), 96% of the exposed silks are pollinated. The remaining unpollinated silks are added to the next day’s pool available for pollination. Exposed unpollinated silks were considered to remain receptive to pollen for five additional days. Silks that were not pollinated by the sixth day were assumed to lose receptivity and no longer contribute to kernel set (Bassetti and Westgate, 1993b).

**Effect of asynchronous pollination within the ear on kernel set**

Prior fertilization has been shown to decrease the percentage of kernels set by later pollinated flowers on the same ear (Frier et al., 1984; Cárcova et al., 2000). This effect of asynchronous pollination was incorporated into the calculation of kernel production for each ear by multiplying the daily value for kernel production by an efficiency factor, which varied with the cumulative number of florets pollinated on each ear (Lizaso et al., 2003). On the basis of the published data of Cárcova et al. (2000), this efficiency factor decreased from 100% of potential set for initial pollinations at the base of the ear to near zero set at the ear tip depending on population density.

**Application to commercial seed production fields**

We tested the procedure for simulating the number of kernels per hectare from flowering dynamics with measured values for kernel yield in six seed production fields in 2002 in Washington County, IA, managed by Syngenta Seeds Inc. (Washington, IA). The fields were chosen to provide a range of male and female flowering characteristics (Table 2). Descriptive terms commonly used in the seed industry such as “good”, “fair”, and “poor” for a male inbred, and “regular” or “small”
for female ear size are provided for the benefit of the reader and do not imply a quantitative basis for comparison between inbreds.

Female inbreds were planted between 4 and 10 May 2002. In most cases, male inbreds were planted within 1 or 2 d of the female. Exceptions were Field A in which the male was planted 7 d before the female and Field D in which the male was planted 6 d after the female inbred. The planting pattern was 4 female: 2 male rows in all cases. To extend the period of pollen shed, plant development was delayed in one of the two male rows by burning away exposed leaf area at the V4 to V5 stage. This management practice, commonly referred to as "flaming", effectively delayed pollen shed up to 3.5 d in the treated row.

Flowering dynamics for the male inbred populations and female inbred were assessed at one location in each field. The location was selected 2 wk before flowering to be representative of typical inbred development across the field. The sampling area was approximately 125 m² and at least 25 m from the field border.

Average pollen production per plant was documented by covering 10 representative tassels with clear bags (Pantek, Montesson, France) designed to exclude moisture but allow gas exchange around the tassel. Pollen was harvested from the bags and quantified with a Coulter Multisizer II (Coulter Electronics Limited, Luton, Beds, England) according to Fonseca et al. (2003). We used average pollen production per plant and approximate population densities (based on data collected by field managers) to calculate total pollen production for each male population.

The interval between Male 1 and Male 2 curves was taken as the difference in days between 50% Beg Shed for the two populations. Separate but identical pollen shed curves were generated for each male population. These curves then were combined to produce a total pollen shed curve for the field. The resulting pollen amount per unit area was adjusted for the female-male planting ratio for each field. The calculated rates of total pollen shed matched measured rates using passive traps according to Fonseca et al. (2002).

Approximately 1.3 ha of the female inbred were harvested from each field to estimate kernel number per female hectare. The yield was calculated in kilograms
per hectare and adjusted to 155 g kg\(^{-1}\) moisture. Average kernel weight was calculated from the number of kernels in a 454-g sub-sample, and adjusted to 155 g kg\(^{-1}\) moisture. Harvested kernel number per female ha was calculated as grain yield (kg ha\(^{-1}\)) / average kernel weight (kg kernel\(^{-1}\)). Information regarding prolificacy was not available and assumed to be one ear per plant.

The intent of this analysis was to test the robust nature of the model for simulating kernel set across a wide range of seed production fields and practices. Therefore, regression analysis was used to evaluate the relationship between observed and simulated kernel set across seed fields. Assessing the impact of within-field variation on simulated kernel set was beyond the scope of this study.

**Simulating possible seed production scenarios**

We used the kernel set model to examine how altering management variables or flowering kinetics might affect potential kernel set. Various scenarios were tested by altering the value of one input variable at a time, such as pollen production per tassel or rate of silk exsertion. A series of simulations generated a kernel set response curve for each input variable tested. Because these response curves were specific to the flowering characteristics of a given inbred pair, they provided a basis for comparing the potential impact of various management scenarios on kernel production for that pair.

Field F was chosen for this scenario analysis because simulated values for kernel production were within 1% of measured values, kernel yield was relatively high, but pollination efficiency was fairly low. The model indicated only 55% of florets set kernels. Amount of pollen per tassel, male-female synchrony, peak pollen shed interval, silking uniformity, and silk exsertion rate were adjusted relative to the initial conditions established for Field F. Variables that remained constant were approximate female plant density = 65,200 plants per female ha, approximate male density = 63,300 plants per male ha, maximum silks per ear = 505, and prolificacy = 1. For simplicity, only one variable was adjusted at a time, although multiple factors can be adjusted simultaneously to assess interactions between management and genetic factors.
Tassels in Field F produced about 1.5 million grains per tassel, on average. Kernel production response to altered pollen amount (maleness) was tested from 0 to 3.5 million pollen grains per tassel. Kernel production dynamics for a good male (about 2.0 million grains per tassel) and a poor male (about 0.5 million grains per tassel) are provided for comparison.

The anthesis-silking interval for Field F was –0.55 d. Since male planting was split in Field F, the anthesis-silking interval is measured relative to the first male planting (A1SI). We examined the impact of altering male-female synchrony over a wide range of A1SIs, from –8 to +14 d. For this example, the silk exsertion dynamic for the population was held constant and the timing of pollen shed was varied. An alternative approach would have been to alter A1SI by changing both the timing of silk exsertion and pollen shed. Kernel production dynamics for male anthesis 2 d ahead and 2 d after 50% silking are provided for comparison.

Flaming one of the paired male rows in Field F resulted in an interval of 1 d between peak pollen shed for the two pollen sources. We tested the response of kernel production to increasing this interval up to 11 d. Kernel set response to delaying peak pollen shed of the second male by 3 and 5 d is provided for comparison.

Silking of the female population (i.e., from 5 to 95% of plants) occurred over a period of 11 d in Field F. The uniformity of the female population for silking was varied from 6 to 21 d to test the impact on potential kernel set. Two scenarios, representing more uniform and more variable populations relative to Field F, are shown for comparison.

The average ear for the female inbred in Field F required about 6 d to exsert 95% of its silks. The effect of more rapid or slower rates of silk exsertion on kernel production was tested assuming the final number of silks exserted remained fixed at 505 silks per ear. Results for a faster silk exsertion (4 d to 95% of silks exserted) and a slower silk exsertion (9 d to 95% of silks exserted) are provided for comparison to initial conditions for Field F.
Results and Discussion
Simulating potential kernel production in seed production fields

The model developed by Lizaso et al. (2003) relies on quantitative measures of male and female flowering dynamics to calculate the potential number of kernels set on an area basis. Their approach was validated using hybrids under field conditions in which pollen shed density was varied by detasseling or by mixing male-fertile and male-sterile isolines (Lizaso et al., 2003; Westgate et al., 2003). In the current study, we examined whether the quantitative relationships developed for maize hybrids could be applied to inbred pairs used in hybrid seed production. In this case, pollen production often is limiting and floral synchrony between inbred pairs is critical. Therefore, the capacity to simulate kernel set for an assortment of inbred pairs that vary in pollen production, silking dynamics, and seed yield should provide a rigorous test of the underlying theory and the model's general utility.

The first component needed to simulate kernel production on a field scale was the temporal distribution of pollen shed. This parameter was estimated according to Westgate et al. (2003) from the flowering characteristics, plant density, and average pollen production per tassel for the male inbred population in each field (Fig. 1). Westgate et al. (2003) and Lizaso et al. (2003) showed that pollen shed dynamics calculated in this way closely reflect the seasonal pattern of pollen shed. Because the practice of flaming one of the male rows typically delayed pollen shed in the flamed row, pollen shed was quantified separately for each male population and then summed to generate a total pollen shed curve for the field.

The temporal distribution of silk exsertion per hectare provided the maximum number of florets that could be pollinated each day. This parameter was calculated from the daily dynamic of silk exsertion per ear, the silking dynamic for the female population, and the plant population density measured for each female inbred (Fig. 2). Silk exsertion per hectare occurred over a period of 6 to 12 d for the female inbreds examined in this study (Fig. 3).

We tested our approach for simulating kernel production in six commercial seed production fields (designated A-F), which included various combinations of
male and female flowering characteristics (Table 2). Male inbred in Field A was considered a poor pollen source. The inbreds in Fields B and C were fair pollen sources. And those in Fields D, E and F were designated as good pollen sources according to their measured field performance. Female inbreds in Fields B and C typically produced small ears; females that produced regular sized ears were used in Fields A, D, E and F, according to their measured exserted silks per ear (Table 2).

Field A combined a poor male inbred with a regular-eared female inbred (Fig. 3A). The average male tassel shed approximately 520,000 pollen grains, which provided only low daily pollen densities for about 5 d. The dense stand of female plants combined with an average silk exsertion of 577 per ear (Table 2) resulted in a cumulative number of about 33.5 million receptive silks per female hectare. On the basis of the temporal dynamics of pollen shed and silk exsertion, the model indicated that this combination of inbreds and field management would produce about 22.3 million kernels per female ha, or about 66.4% of the exposed silks. This simulated value assumes each pollinated silk results in ovary fertilization (Westgate and Boyer, 1986). Therefore, despite close synchrony between pollen shed and silk emergence, not all florets with exposed silks were fertilized during rapid pollen shed. The kernel set simulation also considers the effect of asynchronous pollination within the ear by decreasing the number of late-pollinated florets that produce kernels (Cárcova et al., 2000). However, this latter effect is small relative to the lack of pollination (Lizaso et al., 2003). These results confirm that pollen availability limited potential kernel production in Field A.

Field B combined a fair male inbred with a female inbred producing a small sized ear (Fig. 3B). Although pollen production per tassel was slightly greater than in Field A, the low population density for the male inbred resulted in a very low pollen density that peaked at about 40 pollen grains cm$^{-2}$ d$^{-1}$. Initial silk exsertion preceded pollen shed by 3 d, but 12 d were required to reach maximum silk exsertion for the population (about 16 million silks per female ha). The model simulated only about 64% of these receptive silks would be pollinated and set kernels. As in Field A, the
lack of complete pollination of exposed silks during pollen shed indicated pollen amount limited kernel set.

Field C combined a fair male inbred with a female inbred expected to produce a small ear (Fig. 3C). Both inbred populations were significantly higher than in Fields A and B. And pollen shed by Male 1 and Male 2 were nearly synchronous. About 23.0 million silks per female ha were exposed for pollination, but less than 70% of these were expected to set kernels. As in Fields A and B, the male inbred failed to pollinate a large fraction of newly exposed silks each day even during peak pollen shed.

In Field D, a good male, producing nearly 1.5 million pollen grains per tassel, was matched with a normal-eared female inbred (Fig. 3D). In this case, flaming of Male 2 effectively separated pollen shed for the two male populations. The expanded period of pollen shed coupled with close synchrony between pollen shed and silk exsertion minimized the number of silks left unpollinated during peak pollen shed. The model indicated nearly 77% of the 28.4 million exserted silks per female ha were pollinated. Only those florets with late emerging silks failed to set kernels.

Field E also combined a good male inbred with a normal-eared female inbred (Fig. 3E). Although the male inbred was planted at a relatively high density, the average male tassel produced more than 1.9 million pollen grains. As such, pollen production in Field E was the greatest among the fields examined. Silk exsertion began 4 or 5 d in advance of pollen shedding and was nearly completed when pollen shed ceased. The model simulated that this combination of protogynous silk exsertion and high pollen shed density would produce more than 25.6 million kernels per female ha, or about 84% of exposed silks. These values were the greatest kernel yield and pollination efficiency observed for the six production fields examined in this study.

In Field F, a good male inbred and a regular-eared female inbred were planted at high population densities (Fig. 3F). Unlike the other production fields, silk exsertion in Field F was delayed relative to pollen shed. As such, the model simulated that all early-emerging silks would be pollinated on the day they were
exserted from the husks. But a large fraction of late-emerging silks failed to be pollinated. Ultimately, only about 55% of the 32.4 million silks potentially available for pollination were expected to produce a kernel. In this case, pollen shed duration apparently posed a greater limitation on kernel production than did pollen amount.

**Simulated vs. measured kernel set**

Harvested kernel number varied from about 8.4 to 23.1 million kernels per female ha among the six seed production fields (Table 2). Values provided by our kernel set model were closely correlated ($r^2 = 0.98$) with these measured values (Fig. 4). This result clearly indicates that variation in kernel production can be assessed directly from the flowering dynamics of the inbreds used to produce hybrid seed.

Model simulations, however, overestimated kernel production by 11%, on average. Evidently, one or more plant or canopy factors that might have limited kernel were not taken into account. Barrenness, pollen viability, pollen capture by leaves, and slow silk growth on apical florets were not considered as variables in the initial analysis. Also, the process of mechanical detasseling itself can reduce the yield potential of the female inbred, a factor not considered by the model. Prolificacy was not documented and assumed to be 1 ear per plant for all female inbreds. This assumption is not likely to be valid, since there is usually a small percentage of barren plants at the female plant densities used in this study. Overestimating ears per plant will result in direct overestimation of potential kernel set, since it defines the number of ears per unit surface. Pollen viability also was not quantified for each male inbred. Viability was assumed equal to that reported by Bassetti and Westgate (1994) in their relationship between percent kernel set and daily pollen shed density for a maize hybrid. This relationship has proven to be very robust for estimating kernel production of hybrids across a wide range of pollen shed densities (Lizaso et al., 2003). Nonetheless, variation in pollen viability among male inbreds has been observed (Fonseca, unpublished 2003) and needs to be considered as a model refinement in the future. Finally, the amount of pollen available for pollination, as estimated from bagged tassels, likely overestimates the quantity of pollen reaching the plane of exposed silks. Leaves above the ear intercept some of the pollen...
released from the anthers. Values for pollen capture by the upper canopy are lacking, but they could be estimated if leaf numbers and leaf angles are known (Aylor et al., 2003).

Recent evidence indicates prior pollination of older florets can decrease kernel set by younger florets on the same ear either by inhibiting their silk growth (Cârcova et al., 2003) or by preventing kernel development (Cârcova et al., 2000). Silk exsertion rates used in this study are calculated from data collected on unpollinated silks, which likely overestimates the actual number of florets that become pollinated on an ear. However, on the basis of the work of Cârcova et al. (2000, 2003), the model does consider the impact of prior pollination on kernel set by younger (apical) florets by decreasing kernel set efficiency as kernel number per ear increases. Mechanical detasselling removes leaf area. The amount removed depends on the depth of the cutting and detasseling operations. Loss of sufficient leaf area will decrease light interception and photosynthetic production per plant, which has been shown to limit kernel production at high plant densities (Andrade et al., 1993, Lizaso et al., 2001). We are currently expanding the model to simulate kernel production under both pollen-limited and assimilate-limited conditions (Lizaso et al., 2002).

Nonetheless, the model in its current form simulated kernel production with consistent accuracy across a wide range of seed yields (Fig. 4). This result indicates that simulating kernel set based on inbred flowering dynamics is an entirely suitable approach for assessing potential kernel production for inbred pairs and for testing management options to optimize their productivity.

**Optimizing seed production**

Once the flowering dynamics for a given inbred pair are known, the model can be used to test alternative management scenarios to optimize their kernel production per hectare. Various genetic characteristics that might affect flowering dynamics can be tested as well. As examples, we examined the effect of altering pollen production per tassel ("maleness effect"), varying the timing between pollen shed and silk exsertion (A1SI), modifying the anthesis-interval between pollen
sources, varying silking uniformity within the female inbred population (homogeneous vs. non-homogeneous populations), and altering silk exsertion rates (fast vs. slow females).

These scenarios were simulated relative to the initial conditions for Field F. Only 55% of the exserted silks were expected to set kernels in this field, which implies there is ample opportunity to improve pollination efficiency. Inbreds were planted in a 4:2 (female:male) pattern at relatively high population densities (Table 2). One male row was flamed; and peak shed differed by 1.2 d for the two populations. The average tassel produced about 1.5 million pollen grains. The anthesis-silking interval (A-SI) was -0.55 d, which means 50% of the male inbred population began to shed pollen within 1 d of 50% silking for the female inbred population. Each female plant was assumed to produce 1 ear bearing up to 505 kernels.

In each case, kernel production was calculated over a wide range of values for each response variable (e.g., pollen production per tassel). The daily dynamics of flowering and kernel production were plotted for two of these values to illustrate the basis for the change in kernel production relative to the initial conditions in Field F.

**Pollen production (maleness)**

Pollen production per tassel varies among inbreds and typically decreases with increasing population density (Fonseca et al., 2003). As such, optimum male inbred management depends on a quantitative evaluation of kernel set response to pollen shed density. Figure 5 shows the response of kernel production in Field F to variation in pollen production per tassel. Model simulations indicated that harvested kernels per female hectare increased asymptotically with pollen amount. Increasing pollen production per tassel from 1.5 million grains (Field F) to 2.0 million grains (Example 5A) increased harvest to about 19.0 million grains per female ha, or 59.3% of the exserted silks. Thus, a 37% increase in pollen production in Field F would have improved potential kernel production by only 8%. As in the original condition within Field F (Fig. 3F), a large fraction of late-emerging silks remained unpollinated. If pollen production were limited to 0.5 million grains per tassel, only 10.9 million
(33.6%) of the silks would set kernels (Fig. 5B). Relative to the original situation, this 66% reduction in pollen availability resulted in a 39% reduction in potential kernel set.

Simulating the dynamics of potential kernel production for these scenarios (Fig. 5A and B) and comparing them to the original conditions (Fig. 3F) provides considerable insight into the yield limitations in Field F. It is clear that having a male inbred that produces 1.5 million pollen grains per tassel contributes to the efficient pollination of early-emerging silks. But the relative insensitivity of kernel set to large changes in pollen production per tassel indicates pollen shed density was not the most important factor limiting potential kernel production in Field F. Kernel production was limited to a greater extent by the large fraction of silks exposed after pollen shed density started to decline (Fig. 3F). Altering the synchrony between male and female flowering, rather than increasing pollen availability, would likely have a greater impact on improving pollination efficiency in this field.

**Anthesis-silking interval**

A short ASI typically is considered optimum for kernel set (Bolanos and Edmeades, 1993; Duvick, 1997; Edmeades et al., 2000). Limited pollen availability in seed production fields makes careful management of this kernel set factor especially important. In this case, anthesis is taken as the day 50% of the Male 1 population begins to shed pollen. Silking is taken as the day 50% of the female inbred plants have silks visible. Figure 6 shows the potential consequences of altering the A1SI on kernel production in Field F. Of all the genetic and management variable tested, variation in the A1SI had the greatest impact on harvested kernels per female hectare. Delaying silking by 1.5 d relative to the original condition in Field F to an A1SI = -2 would cause a 35% reduction in potential kernel production to about 11.5 million kernels per female ha (Fig. 6A). But if silking in field F were advanced to 2 d prior to anthesis (A1SI= +2), pollination efficiency would increase to nearly 76%, and potential kernel production would increase by 38% to 24.6 million kernels per female ha (Fig. 6B). There are two likely reasons for such a significant enhancement in kernel production. First and foremost, a much larger fraction of late-emerging silks
are pollinated when pollen shed is delayed relative to silking. Second, all the early emerging silks are pollinated as well because they remain receptive to pollen for several days after they appear (Bassetti and Westgate, 1993a, 1993b).

This example clearly demonstrates why floral synchrony between male and female inbreds is such an important management variable in hybrid seed production. A delay in silking relative to pollen shed of 1 or 2 d has a large impact on potential kernel production when pollen amount is limiting. It is important to note that perfect synchrony (i.e., A₁SI=0, silking and Male 1 anthesis on same day) did not correspond to optimum potential kernel production for Field F (Fig. 6). Maximum kernel yield was obtained by delaying anthesis for the male inbred population by about 5 d, relative to silking.

This evaluation does not take into account the risk of out-crossing from foreign sources of pollen. Delaying pollen shed to maximize kernel yield could expose the seed field to increased potential for out-crossing of the early-emerging silks. On the other hand, greater coverage of late-emerging silks by delaying pollen shed dramatically increases kernel yield and thereby decreases the potential for out-crossing for the later silkers in the female population. The best approaches to managing floral synchrony will depend on the specific risk of foreign pollen entry during silking. The model provides the framework to quantify such risks when coupled with an estimate of adventitious pollen entry.

**Interval between pollen sources**

It is common practice in seed production fields to flame the male inbred to delay pollen shed and expand pollen shed duration. In this study, one row in each pair of male rows was flamed for this purpose. This treatment resulted in anthesis intervals of 1 or 2 d between adjacent male rows (Fig. 3). In the initial condition for Field F, anthesis for the two male populations was separated by 1.2 d. We simulated the response of kernel harvest to an increase in the interval between anthesis for the two male populations up to 11 d (Fig. 7). For this set of simulations, the synchrony between anthesis for the first male population and silking for the female inbred population was not altered.
When anthesis for the second pollen source was delayed from the original 1.2 to 3 d relative to the first pollen source, the model indicated that nearly 68% of the silks would be pollinated and potential kernel yield would increase 23% to about 21.9 million kernels per female ha (Fig. 7A). If the interval between pollen sources were increased to 5 d, potential kernel yield would be increased about 38%, which represents an increase of about 6.8 million kernels over the initial conditions in Field F (Fig. 7B).

In this particular field, the optimum delay between pollen sources to maximize kernel yield was about 6 d. From our study of all six seed fields (Fig. 3), it was clear that there is great potential to improve potential kernel yield by carefully managing the anthesis interval between pollen sources to increase pollen shed duration. The number of sources, the timing between them, and the proportion of plants assigned to each source are management options that can be tested and optimized by the model.

**Silking uniformity**

The time required for the entire female inbred population to begin silking obviously has an impact on potential kernel yield. Therefore, we tested the response of kernel yield to variation in silking uniformity from 6 to 21 d to progress from 5 to 95% of plants silking. To simplify this analysis, the interval between pollen shed and silking remained constant at A₁SI=−0.55. This approach effectively spread silking for the population across a greater number of days, but advanced silk emergence for a proportion of the population (Fig. 8A and B). An alternative approach would have been to hold the beginning of silking constant. But decreasing the uniformity of population in this latter case would effectively increase the A₁SI.

Kernel yield decreased linearly with decreasing uniformity of silking for the population (Fig. 8). The impact of silking uniformity on kernel yield, however, was much smaller than that of other variables tested. Two scenarios representing a more uniform population (8 d to 95% silking) and a much less uniform population (20 d to 95% silking) than Field F revealed that improving the population uniformity by 12 d would set only about 2.4 million additional kernels per female ha. As such, effort to
improve the uniformity of silking in Field F from 11 to 8 d would likely have little impact on harvested kernels.

**Duration of silk exsertion**

Repeated sampling of silks on unpollinated ears indicated that the female inbred in Field F required about 7 d to exsert 95% of the silks on an average ear. A similar rate has been reported for maize hybrids with normal-sized ears (Bassetti and Westgate, 1993a; Lizaso et al., 2003). Since the availability of silks for pollination depends directly on the duration of silk exsertion, we tested whether altering silk exsertion on individual plants could improve harvested kernel yield. The amount of pollen, timing of pollen shed, and A1SI were held constant.

As was the case with silking uniformity for the population, decreasing the uniformity of silking on individual ears had a direct negative impact on kernel yield (Fig. 9). Simulated kernel yield, however, was much more sensitive to the duration of silk exsertion. A female inbred capable of exserting 95% of the silks within 4 d would increase potential kernel production about 17% to 20.8 million kernels per female ha (Fig. 9A). A female requiring 9 d to exsert 95% of the silks would produce 15% fewer kernels, and leave more than 50% of the exposed silks unpollinated (Fig. 9B).

This analysis confirmed that both the population and individual plant components of silking affected kernel yield. The duration of silking per ear, however, had a greater impact on potential kernel set. By analogy to the capacity for pollen shed by males inbred, more detailed characterization of female inbreds for their silking uniformity (fast vs. slow female; small vs. big sized ear), as well as their response to changing environments (i.e., population density), would be of considerable value for making management decisions based on these model simulations.

**Comparisons among seed fields**

The dynamic characterization of male and female flowering allowed us to simulate differences in potential kernel production among fields and to expose the primary causes for low pollination efficiency. Fields A and F, for example, exerted a similar number of silks per female hectare. The male inbred in Field F produced
nearly three times as much pollen, but Field A produced almost 17% more kernels per ha (Table 2). The kernel set model accurately simulated this outcome on the basis of the genotypic differences in flowering synchrony, pollen shed density, pollen shed duration, and silking duration. This comparison confirms that a poor male can be used to produce acceptable kernel yields if pollen shed is delayed relative to silk emergence and silking of the female population is uniform.

Fields A, B, C produced markedly different kernel yields (10.2 to 22.2 million kernels) despite very similar pollination efficiencies (64 to 70%). The female inbreds in Fields B and C produced about two-thirds as many kernels per ear as in Field A. The male inbred in Field C produced nearly twice as much pollen per tassel as did the male inbred in Field A. And the greatest number of pollen grains available per exserted silk occurred in Field B. A common feature of all three fields, however, was that pollen shed was delayed relative to silking (Fig. 3). Therefore, pollination of early–emerging silks was highly effective in these fields, even at the low pollen densities observed in Field A. This delay in pollen shed and the uniform exsertion of a large number of silks combined to produce the high kernel yield in Field A.

In nearly all fields, a larger interval between pollen sources has the potential to improve kernel production significantly as well as to reduce the risk of outcrossing from an adventitious pollen source. The exception was Field D in which anthesis for Male 2 was delayed about 3 d relative to Male 1. Pollen shed covered almost completely the entire period of silk exsertion (Fig. 3D). Pollination efficiency of exposed silks was nearly 80%, with a male producing less than 1.5 million pollen grains per tassel (Table 2).

Field F was the only one in which pollen shed began before silk exsertion. Model analysis indicated that potential kernel production in this field could have been 44% greater if pollen shed were delayed by as few as 3 to 4 d relative to silking. Affecting this delay, based on an understanding of the flowering dynamics of the male and female inbreds in this field, would have set an additional 7.8 million kernels per female ha.
Conclusions

We have applied the kernel set model of Lizaso et al. (2003) to simulate kernel production in hybrid seed fields from the flowering dynamics of the inbreds. This model provides an easy and reliable approach to assess the impact of seed production practices on kernel production and to define management strategies to maximize seed production per female hectare. With a minimum of information on the flowering dynamics of their male and female inbreds, the model can also be used to establish production requirements for new combination of inbreds.

For simplicity of demonstration, response to several genetic and management variables were examined one variable at a time. Of the variables tested, $A_7SI$ and the anthesis interval between pollen sources had the greatest impact on kernel number. But the model is structured to allow several variables to be adjusted simultaneously so that their additive and synergistic effects can be assessed. The approach for simulating kernel production also provides the mathematical basis for testing the impact of adventitious pollen entry on the potential for out-crossing events in the hybrid seed field.

The combination of inbreds examined in this study did not permit an evaluation of the kernel set model as a predictive tool. Predicting kernel set across locations for a given inbred pair requires more detailed information about environmental effects on female silking dynamics, male pollen viability and pollen shed dynamics. Seed companies interested in using the kernel set model for predictive purposes will need to collect this information as part of their inbred parent evaluation and development.

References


Table 1. Inputs required to simulate potential kernel production for a given pair of male and female inbreds.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Units</th>
<th>Data collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population density</td>
<td>Plants per male ha</td>
<td>Documented at flowering</td>
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<tr>
<td>Average pollen</td>
<td>Grains per tassel</td>
<td>Collect pollen from 10 representative tassels, quantify with Coulter Multisizer</td>
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<tr>
<td>production per tassel</td>
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<tr>
<td>Pollen shedding</td>
<td>Cumulative % of plants</td>
<td>Document dates at which 100 plants reach each stage</td>
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<tr>
<td>phenology</td>
<td>at beginning, maximum and</td>
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<td></td>
<td>end of shedding</td>
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<tr>
<td>Planting pattern</td>
<td>female / male rows ratio</td>
<td>Defined at planting</td>
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<tr>
<td>Population density</td>
<td>Plants per female ha</td>
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<tr>
<td>Silk ejection dynamic</td>
<td>Cumulative % of exerted</td>
<td>Collect and count exposed silks 1, 3, 5, 7 and 9 d after initial silk emergence</td>
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<tr>
<td></td>
<td>silks per ear</td>
<td>from 10 representative plants</td>
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<td>Silking phenology</td>
<td>Cumulative % of plants</td>
<td>Document dates at which 100 plants have exposed silks</td>
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<tr>
<td></td>
<td>silking</td>
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<tr>
<td>Prolificacy</td>
<td># ears per plant</td>
<td>Document # of ears harvested from 100 plants</td>
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Table 2. Comparison of simulated and measured kernel production for six seed production fields having various combinations of male and female inbred flowering characteristics.

<table>
<thead>
<tr>
<th>Field</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<tr>
<td></td>
<td>Approximate plant density (plants/ha)&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>MALE</td>
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<tr>
<td></td>
<td>Measured pollen grains per tassel</td>
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<tr>
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<td>912,976</td>
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<td>Calculated pollen shed (grains per ha x 10&lt;sup&gt;9&lt;/sup&gt;)</td>
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<td>27.8</td>
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<td></td>
<td>22.3</td>
<td>10.2</td>
<td>15.9</td>
<td>21.8</td>
<td>25.6</td>
<td>17.8</td>
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<td></td>
<td>Measured kernel number (kernels per ha x 10&lt;sup&gt;6&lt;/sup&gt;)</td>
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<td>20.5</td>
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<td>19.5</td>
<td>23.1</td>
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<td></td>
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<td>121%</td>
<td>114%</td>
<td>111%</td>
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<td>101%</td>
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Figure 1. Quantification of the pollen shed dynamic in Field F determined on the basis of male flowering dynamics. The pollen shed curves corresponding to each male row (Male 1 and Male 2, where Male 2 was flamed at V4-5) were calculated from the beginning of shedding population trend, the average number of pollen grains per tassel, and the male plant density.
Figure 2. Silk exsertion dynamic in Field F determined on the basis of observations of ear development. The cumulative number of emerged silks each day per hectare was calculated by combining the dynamic of silk exsertion per ear (top left) with the percentage of plants with silks exposed each day (top right).
Figure 3. Simulated kernel production in six hybrid seed fields (A-F) planted to various combinations of male and female inbreds varying in flowering characteristics. Black curves indicate simulated pollen shed by Male 1, Male 2 and total pollen shed for the field (pollen grains cm$^{-2}$ d$^{-1}$). The gray curve indicates cumulative silk emergence (silks female ha$^{-1}$). The dashed lines indicate cumulative kernel set. Values for simulated kernel production (KN ha$^{-1}$) and percentage of exserted silks that become pollinated are shown for each field.
Figure 4. Correlation between measured and simulated kernel production for six hybrid seed fields. Measured kernel numbers per female hectare were estimated from "green bushels yield"; simulated kernel numbers were generated by the model. The dashed line represents the 1:1 relation. Each point corresponds to one seed production field.
Figure 5. Change in simulated kernel production because of variation in pollen production per tassel. Cumulative kernel production for two situations, tassels shedding 2.0 million (A) or 0.5 million (B) pollen grains, are presented relative to kernel production for Field F. Black curves indicate simulated pollen shed by Male 1, Male 2 and total pollen shed for the field (pollen grains cm\(^{-2}\) d\(^{-1}\)); gray curves are cumulative silk emergence (silks female ha\(^{-1}\)); dashed lines indicate kernel numbers. Values for simulated kernel number and percentage of exserted silks setting kernels are shown for each situation. Note that increasing pollen production beyond 2.0 million grains per tassel had little impact on kernel production.
Figure 6. Change in simulated kernel production because of synchrony in male-female flowering dynamics. Cumulative kernel production for two situations, 2 d of protandry ($A_{SI} = -2$) (A) and 2 d of protogyny ($A_{SI} = 2$) (B), are presented relative to kernel production for Field F. Black curves indicate simulated pollen shed by Male 1, Male 2 and total pollen shed for the field (pollen grains cm$^{-2}$ d$^{-1}$); gray curves are cumulative silk emergence (silks female ha$^{-1}$); dashed lines represent simulated kernel set. Values for simulated kernel production and percentage of exserted silks setting kernels are shown for each situation. Note that maximum kernel production occurs when silk emergence proceeds pollen shed by about 4 d.

**Initial Scenario (Field F)**

Female population density: 65,200 pl Fha$^{-1}$
Silks exserted per ear: 505
Total Male population density: 63,300 pl Mha$^{-1}$
Pollen production per tassel: 1,456,094
$A_{SI}$: -0.55 days
Prolificacy index: 1
**Figure 7.** Change in simulated kernel production because of the interval between anthesis dates for two male pollen sources. Cumulative kernel production for two situations, 3 d delay (A) and 5 d delay (B) in anthesis are presented relative to kernel production for Field F. Black curves indicate simulated pollen shed by Male 1, Male 2 and total pollen shed for the field (pollen grains cm$^{-2}$ d$^{-1}$); gray curves are cumulative silk emergence (silks female ha$^{-1}$); dashed lines represent simulated kernel set. Values for simulated kernel production and percentage of exserted silks setting kernels are shown for each situation. Note that maximum kernel production occurs when anthesis for Male 2 follows anthesis for Male 1 by 6 d.
Female Population Uniformity

**Initial Scenario (Field F)**

Female population density: 65,200 pl Fha\(^{-1}\)
Silks exserted per ear: 505
Total Male population density: 63,300 pl Mha\(^{-1}\)
Pollen production per tassel: 1,456,094
\(A,SI\) -0.55 days
Prolificacy index: 1

**Figure 8.** Response of simulated kernel production to uniformity of silking for the female population. Cumulative kernel production for two situations, 8 d to increase from 5 to 95% silking (A) and 20 d to increase from 5 to 95% silking (B) are presented relative to kernel production for Field F. Black curves indicate simulated pollen shed by Male 1, Male 2 and total pollen shed for the field (pollen grains cm\(^{-2}\) d\(^{-1}\)); gray curves are cumulative silk emergence (silks female ha\(^{-1}\)); dashed lines represent simulated kernel set. Values for simulated kernel production and percentage of exserted silks setting kernels are shown for each situation. Greater uniformity of the female population is beneficial, but the impact on kernel production is relative small.
Silk Exsersion Rate

Initial Scenario (Field F)
- Female population density: 65,200 pl Fha⁻¹
- Silks exserved per ear: 505
- Total Male population density: 63,300 pl Mha⁻¹
- Pollen production per tassel: 1,456,994
- A,SI: -0.55 days
- Prolificacy index: 1

Figure 9. Response of simulated kernel production to rate of silk exservation per ear. Cumulative kernel production for two situations, 4 d to exserved 95% of silks (A) and 9 d to exserved 95% of the silks (B) are presented relative to kernel production for Field F. Black curves indicate simulated pollen shed by Male 1, Male 2 and total pollen shed for the field (pollen grains cm⁻² d⁻¹); gray curves are cumulative silk emergence (silks female ha⁻¹); dashed lines represent simulated kernel set. Values for simulated kernel production and percentage of exserved silks setting kernels are shown for each situation. Note that the rate of silk exservation has a direct and significant impact on cumulative kernel set.
CHAPTER 6. RELATIONSHIP BETWEEN DESICCATION AND VIABILITY OF MAIZE POLLEN

A paper submitted to Field Crops Research

Agustin E. Fonseca, and Mark E. Westgate

Abstract

Controlling pollination is necessary to ensure maximum kernel set and high levels of genetic purity in maize. Current approaches for measuring maize pollen production are fairly simple and accurate, but they do not evaluate pollen viability. A simple and reliable technique to assess loss of pollen viability during its transport in air is required to simulate the pollination process and the risk of out-crossing associated with pollen dispersal. Anecdotal evidence indicates that pollen shed from anthers remains viable longer at low temperature and high relative humidity (RH). But a direct temporal relationship between loss of pollen viability and these environmental conditions is lacking. We tested whether vapor pressure deficit (VPD) could be used to predict the rate of pollen desiccation and subsequent loss of viability. Fresh pollen was harvested from greenhouse and field-grown plants and exposed to a range of VPD generated using a factorial combination of temperatures and RH. After exposure from 0 to 2 hours to known VPD, viability of the treated pollen was tested in vitro. These tests indicated that pollen grains are released from anthers at about 55 to 60% moisture content, and that subsequent desiccation is a function of air temperature, RH and time. These factors were incorporated into a single relationship that described the rate of pollen desiccation for several genotypes. Pollen viability decreased linearly ($r^2$ ranged from 0.77 to 0.93) with pollen moisture content (PMC) to zero at PMC $\approx$ 30%. Together, these relationships

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1Abbreviations: RH, relative humidity; VPD, vapor pressure deficit; PMC, pollen moisture content; $PV_0$, pollen viability = 0%; $PV_{50}$, pollen viability = 50%; VPDT, vapor pressure deficit*time.
provide a simple approach to assess loss of maize pollen viability under field conditions.

Introduction

Maize (*Zea mays* L.) is primarily wind pollinated, and due to this uncontrolled pollination, often is referred to as an *open-pollinated crop* (Poehlman, 1995). Since its domestication, and especially with the introduction of hybrid seed production, many attempts have been made to control this process to ensure maximum kernel set and high levels of genetic purity. In hybrid seed production fields, seed companies utilize several practices, including crop rotations, high purity parent seed, mechanical detasseling of the female parent, temporal or spatial isolation from corn in nearby fields, and inclusion of border rows of the male parent around the field (Burris, 2001). With the introduction of transgenic maize, there has been an increasing interest in quantifying pollen dispersal, particularly in relation to gene flow from transgenic crops and the maintenance of seed quality (Luna et al., 2001; Jarosz et al., 2003). The impact of pollen dispersal requires an accurate assessment of pollen viability, which is not a constant. Maize pollen is considered desiccation intolerant relative to pollen from other species since it loses viability rapidly as water content decreases (Roeckel-Drevet and Digonner, 1995; Luna et al., 2001). Loss of pollen viability reportedly is faster at low RH (Aylor, 2003) and high temperatures (Arritt et al., 2003).

Ongoing approaches for assessing cross-pollination and hybrid seed production rely on an accurate quantification of adventitious and local pollen production (Lizaso et al., 2003; Fonseca et al., 2004). Pollen shed duration and pollen shed intensity have been quantified visually by counting pollen grains adhering to spinning rods placed in the field (Flottum et al., 1984). Pollen collected on passive pollen traps also has been quantified by computer-aided video imaging (Bassetti and Westgate, 1994) and fluorescence microscopy (Fonseca et al., 2002). Pollen production per plant can be accurately assessed by covering tassels with clear bags and quantifying harvested pollen using a flow-cytometer (Fonseca et al.,
These techniques are useful for evaluating inbreds pollen production and stability of pollen production across environments. But they do not distinguish viable from nonviable pollen at the point of collection.

Two approaches for evaluating pollen viability are in vivo fertilization and in vitro pollen germination. The in vivo approach involves placing pollen grains on silks and noting their ability to germinate and effect fertilization. In the field, many pollen grains might land and germinate on an individual silk, but only one pollen grain typically fertilizes the ovule. Therefore, assessing pollen viability with this technique requires that pollinations be made with a limiting number of pollen grains (ideally one grain per silk) (Schoper et al. 1987). Placing only one pollen grain on each silk to test viability, however, is a time-consuming and practically impossible task (Goss, 1968). Furthermore, seed set may depend not only on pollen viability but also on pistil receptivity, incompatibility reactions and post-pollination development of the embryo (Stanley and Linskens, 1974).

Numerous in vitro approaches have been attempted (Herrero and Johnson, 1980; Schoper et al., 1987; Broglia and Brunori, 1994; Buitnik et al., 1996; Aylor, 2003) yielding from as low as 13% to up to 75% pollen viability. Under appropriate incubation conditions, pollen grains germinate on an artificial liquid or semi-solid nutrient medium, producing normal pollen tubes. Media usually contain a carbon source and trace minerals, especially boron and calcium salts. In vitro germination tests, however, must be carefully controlled because pollen germination and tube growth can be altered by many factors such as incubation temperatures, air temperatures and humidity during sampling, concentration of chemical compounds in the media, and concentration of incubated pollen grains (Zhang, 1998).

Grass pollen loses viability rapidly under natural conditions. Depending upon species, the duration of pollen viability can range from a few hours to one day (Barnabas, 1985). Luna et al. (2001) reported that corn pollen exposed to hot and dry field conditions (San Jose del Valle, Nayarit, Mexico) decreased to 20% viability in one hour and was 100% nonviable within two hours. Pollen viability also was reported to be greatly reduced by high temperatures (Johnson and Herrero, 1981).
Schoper et al. (1986) showed that high temperature stress on the tassel at the time of anthesis cause a large reduction in pollen viability. However, pollen viability is not affected by plant water deficits (Hall et al., 1982; Westgate and Boyer, 1986). There is general agreement among investigators that functional life of pollen is longer at relatively low temperatures and high RH (Johri and Vasil, 1961). Roeckel-Drevet and Digonner (1995) showed that maize pollen usually has relatively high water content at anthesis (about 60%) and subsequent dehydration disrupts ATP formation with the consequent loss in viability. Aylor (2003) proposed that the relative water content of corn pollen plays an important role both in the viability and the aerodynamics of airborne pollen. Therefore, quantifying the dynamics of pollen desiccation is critical for relating pollen dispersal and the potential for out-crossing.

The objective of this research is to establish a simple, rapid and reliable procedure to assess the effect of environmental conditions (temperature and RH) on the temporal pattern of maize pollen desiccation and subsequent loss of viability. A combination of controlled and field environments was used to explore the potential for genotype by environment interactions on these temporal patterns.

**Materials and Methods**

**Plant culture**

Maize inbred Mo17, hybrid Mo17xB73, and commercial hybrid Dekalb 668 were planted in the greenhouse in August 2002. Two plants per 20-L pot were grown in a bark-based growing mix (SB300 Universal, Sungro). Plants were fertilized weekly with 3.6 g of 20%N-20%P-20%K water-soluble fertilizer (Plantex, Plant Products Co.) and irrigated as necessary to ensure robust plant growth. Fully exposed tassels were excised from the plant prior to pollen shed and placed with their cut peduncles in water inside controlled-environment growth chambers. Chambers were programmed to mimic light intensity and temperature patterns typical during pollen shed. Measured temperature within the chambers gradually increased from 20°C at 0600 to 30°C at 1400 hours, while RH dropped from 60 to 30% during the same period. Conditions returned gradually to nighttime values.
between 2000 and 2100 hours. Chambers were set to 15 h photoperiod. Tassels remained in the chambers until shed was complete (4 to 6 days).

In a second experiment, 2 public inbreds (B104 and B97) and 9 inbreds provided by Syngenta Seeds Inc. (hereafter named Inbred A through Inbred I) were planted in the field (Aquic hapludoll) in Ames, Iowa, on 16 May, 2003 in 76 cm rows in 4-row plots (3.04 m x 6 m) at a density of 80,000 plants ha\(^{-1}\). The plot area received 168 kg ha\(^{-1}\) anhydrous ammonia prior to planting. Rainfall prior to anthesis was adequate to ensure normal pollen production and pattern of pollen shed.

**Pollen collection**

Prior to pollen shed from newly exserted anthers, tassels were gently shaken to remove pollen remaining in anthers exserted the previous day. Fresh pollen was collected between 0930 and 1230 hours by gently tapping the main tassel branch above an aluminum tray, held below the tassel. A pollen sample was typically composed of pollen from 5 to 15 tassels collected over 2 to 3 min. Collection time was recorded and sub-samples of pollen were allocated immediately to temperature-RH treatments. Approximately 50 mg of pollen was taken to determine initial moisture content. This procedure was identical in both greenhouse and field experiments. In the field experiment, an additional sub-sample was used to assess initial pollen viability.

**Vapor pressure deficit treatments**

Pollen sub-samples were placed on an aluminum weigh pan in glass incubation chambers containing saturated salt solutions. The salts used and their target RH were LiCl: 15% RH, Ca(NO\(_3\))\(_2\): 50% RH, and NaCO\(_3\): 85% RH. Each chamber was equilibrated to a pre-determined treatment temperature prior to use, and maintained at this temperature during the incubation period within a temperature-regulating incubator (Lab-line Max Q4000, Barnstead International, IL, USA). Actual temperature and RH within each chamber was monitored at 5 min intervals using HOBO Pro series H08 data loggers (Onset corporation, MA, USA). A small brushless circulating fan (Cat. No. 273-240, RadioShack) was used to ensure uniform conditions within the chamber. Incubation chambers were opened at
prescribed intervals for a few seconds to sample pollen. Opening the chambers disturbed RH in the 15% and 85% RH treatments, but chamber RH typically returned to the target values within 1 to 2 min (data not shown). Treatments were a factorial combination of 25, 32.5 and 40°C at 15, 50 and 85% RH, providing a vapor pressure deficit (VPD) treatment range from 3.5 to 44.8 mmHg. These VPD values assume temperature equilibration between the pollen grain and the air, and that water vapor evaporating from surface of the pollen grain was close to 100% RH. Exposure times were 20, 40, 60, 90 and 120 min in the greenhouse experiment and 15, 30 and 60 min in the field experiment.

For the field experiment, the temperature incubators, in vitro germination chamber, and associated sampling equipment were housed in a shed adjacent to the plots. The entire process of collecting pollen and preparing samples for assigned temperature-RH treatments was accomplished in less than 5 min. To ensure these critical sampling procedures were accomplished in a timely manner, only a few genotypes were tested each day. Pollen from every genotype was sampled on at least two days during maximum shedding.

Average pollen production per plant for the inbred lines was documented by covering 5 representative tassels with clear bags (Pantek, Montesson, France) designed to exclude moisture but allow gas exchange around the tassel. Pollen was harvested from the bags and quantified using a Coulter Multisizer II (Coulter Electronics Limited, Luton, Beds, England) according to Fonseca et al. (2003).

Pollen moisture content and viability

Pollen moisture content (PMC) was determined immediately after pollen collection and following every treatment sampling period. A sub-sample of pollen was placed in a closed 1.5 mL micro centrifuge tube, weighed, freeze-dried and re-weighted. PMC was calculated as the change in weight, expressed as a percentage of sample fresh weight.

To estimate pollen viability of the same sample, sub-samples of pollen were sprinkled immediately on to Petri dishes (approximately 1000 grains per dish) containing a semi-solid nutrient medium composed of 300 mg L⁻¹ CaCl₂·7H₂O, 100
mg L\textsuperscript{-1} H\textsubscript{3}BO\textsubscript{3}, 120 g L\textsuperscript{-1} sucrose and 7 g L\textsuperscript{-1} agar (Cook and Walden, 1965). Open dishes were placed in an incubation chamber for 3 h at 28°C and 100% RH. Temperature and RH within the chamber were monitored at 5 min intervals using a HOBO Pro series H08 data logger (Onset corporation, MA, USA). Petri dishes were stored at 4°C until pollen viability was measured (within 10 h from collection). Viable grains were determined visually through a 4x objective lens of an Olympus CH-2 microscope (Olympus optical Co., Ltd.). A pollen grain was considered viable if the length of the germ tube was at least one diameter of the pollen grain (approximately 70 \(\mu\)m). Pollen viability was expressed as a percentage of 200 grains per Petri dish.

Pictures taken with an Olympus SC35 camera (Olympus optical Co., Ltd.) mounted on the microscope were used to document various levels of pollen viability (Fig. 1).

**Statistical analysis**

An ANOVA was performed to test temperature and RH effects on PMC for each genotype grown in the greenhouse using the General Linear Model procedure (SAS institute, 1985).

TableCurve 2D (SYSTAT Software Inc.) was used to fit piecewise bilinear regression models to pollen viability/pollen moisture content data to estimate the rate of pollen viability loss and PMC at 0% viability (PV\textsubscript{0}). A conditional model (de la Vega and Hall, 2002) was used with a first stage where:

\[
Pollen Viability = a + b \text{ PMC}
\]  \[1\]

for PMC > C, and a second stage where:

\[
Pollen Viability = a + b \ C
\]  \[2\]

where \(a\) and \(b\) are the intercept and the slope, respectively, of the linear regression corresponding to the first stage, PMC is % pollen moisture content, and \(C\) is the value of PMC in which viability equals 0%. Functions were fitted to data from all
genotypes and statistical differences among regression parameters determined based on 95% confidence interval of their slopes and intercepts.

Pollen desiccation in the air depended almost entirely on VPD. Assuming that pollen desiccation rate at a given VPD remained constant with time, we combined VPD and time into a single index (vapor pressure deficit x time, VPDT). Table Curve 2D was used to fit an exponential regression to relate individual PMC with VPDT, where:

\[
PMC = a e^{-b \cdot VPDT}
\]  

[3]

where \( a \) and \( b \) are the intercept and the slope, respectively, and VPDT is a calculated vapor pressure deficit time index (mmHg min) which affects pollen desiccation. Functions were fitted to data from all genotypes and statistical differences among regression parameters determined based on 95% confidence interval of their slopes and intercepts.

Results and Discussion

Pollen moisture content at shed

Moisture content of fresh pollen was determined immediately after release from the anthers for 14 genotypes (3 grown in the greenhouse + 11 grown in the field) collected at various times during the day. The initial PMC was fairly uniform, ranging from 54.2 to 60.2%, with an average of 57.3% across all 14 genotypes (Fig. 2). These values are consistent with those reported in the literature (Kerhoas et al., 1987; Roeckel-Drevet and Digonner, 1995; Buitnik et al., 1996; and Aylor, 2003). Figure 2 included PMC data for pollen collected under a range of environmental conditions, on different days, times of the day, from plants grown in the greenhouse and in the field. The fact that PMC was similar in all cases suggests that anthers buffered pollen grains from desiccation to a similar extent in all the genotypes examined. As the day progresses, air temperature typically rises and RH decreases. Yet PMC immediately after being shed from the anthers was largely independent of
the time of the day (Fig. 2). As such, it is reasonable to conclude that initial PMC is fairly constant, at about 57%, and that desiccation occurs primarily after pollen is shed from the anthers.

**Pollen desiccation is controlled by temperature and relative humidity**

Pollen collected from three genotypes grown in the greenhouse was exposed to controlled temperature and RH conditions for 1 h. PMC changed from fully hydrated (close to 60%) to highly dehydrated (<10%) within hours or minutes, depending almost entirely on RH and temperature of the air (Fig. 3). For example, hybrid Dekalb 668 pollen lost about half of its moisture content (from close to 60% at shed to 28.9%) after one hour at 32.5°C and 50% RH. At 25°C and 85% RH, however, PMC remained close to 54% during this time. The other two genotypes responded to temperature and RH in a similar fashion.

Temperature and RH effects on PMC were highly significant ($P < 0.001$), but their interaction was not (data not shown). Therefore, it was reasonable to merge both effects into a common factor to quantify the driving force for pollen desiccation, the VPD between the water vapor on the surface of the pollen grain and the ambient air. Aylor (2003) used a similar approach for pollen desiccation of one genotype exposed to a narrow range of VPD. Vapor pressure deficit had a significant effect on pollen desiccation for the three genotypes (Fig. 3). Inbred Mo17 tended to desiccate faster than the two hybrids tested, particularly at intermediate VPD between 11.6 and 26.4. It is possible that these results reflect differences among genotypes in the hydraulic and diffusive pathway for water movement through the plasma membrane and wall layers of the pollen grain. But information on these basic processes is not available for pollen, and our data set was not sufficient to resolve these differences statistically.

**Pollen viability depends on its moisture content**

There is general agreement among investigators that functional life for pollen is longer at relatively low temperatures and high relative humidities (Johri and Vasil, 1961; Johnson and Herrero, 1981; Luna et al., 2001). Because these factors determine the VPD between the pollen grain and air, their effects on viability could
be mediated through their influence on pollen water status. In other words, pollen viability decreases as a consequence of its desiccation. Roeckel et al. (1995) observed that in vitro germination percentage decreased steadily as PMC decreased from 45 to 20%. If true, it should be possible to estimate pollen viability directly from its moisture content. In order to describe this relationship quantitatively, we generated a range of PMC by exposing pollen grains to different VPDs for various periods of time. In vitro estimates of pollen viability were conducted following each treatment. Initial pollen viability was 79%, on average, and up to 90% in the case of Inbred E (Table 1). These viability percentages are higher than most published values for maize pollen. For example, viability percentages of up to 75% (Buitnik et al., 1996) and 65% (Aylor, 2003) were reported for individual genotypes. Herrero and Johnson (1980) tested several genotypes obtaining average pollen germinations of 22 and 53% for two consecutive years, respectively. Average viability values as low as 38% (Broglia and Brunori, 1994) and 13% (Schoper et al., 1987) also have been reported. We attribute the uniformly high level of pollen viability in our experiments to the consistent handling of pollen and the high RH maintained during incubation. Because the high germination percentages were obtained regardless of the pollen source (genotype and sampling time), we present these values as a reliable approximation of initial pollen viability.

There was a very close relationship between PMC and pollen viability. In the case of Inbred B, for example, pollen viability decreased about 3% for every 1% decrease in PMC, up to a certain content of water below which pollen was completely nonviable (Fig. 4). Statistical analysis indicated that the PMC at zero viability (PV₀) was 28.7% for this inbred. The reduction in PMC accurately described pollen viability loss for all genotypes examined (Table 1). The reduction in pollen viability for every 1% decrease in PMC ranged from 1.9% (B97 and Inbred C) to 3.6% (Inbred G), while PMC at which pollen became completely nonviable varied from 25.6% (Inbred C) to 35.3% (Inbred G). We screened for variation among genotypic responses by comparing the slope and the intercept of individual regressions. Based on their 95% confidence intervals, there were no significant
differences among the genotypes for these regression parameters. A single regression for all the genotypes tested in this study explained most of the variation in pollen viability ($r^2 = 0.80$; data not shown). On average, pollen viability decreased by 2.6% for every 1% decrease in PMC, and maize pollen became completely nonviable at 30.0% pollen water content (Table 1).

The reason pollen loses viability completely at this moisture content is not known, but the similarity among genotypes suggest a common mechanism. In the partially dehydrated grains, pollen viability could be determined by the relative stability of cellular membranes. Kerhoas et al. (1987) have shown that the majority of cellular water is tightly associated with cell constituents at 28% PMC. This value corresponds with the percentage of PMC at PV$_0$ obtained for all genotypes (Table 1). Pollen dehydration below this value might lead to membrane permeability defects and irreversible membrane damage during imbibition (Barnabas, 1985; Roeckel et al., 1995). If such changes in membrane structure were not reversible, pollen grains likely would not germinate upon rehydration (Shivanna and Heslop-Harrison, 1981). Pollen membrane stability and recovery from desiccation, however, were not assessed directly in our study.

PMC at 50% viability (PV$_{50}$) was calculated for each genotype to provide a common basis for comparing genotypes in terms of their susceptibility to desiccation after shedding (Table 1). For example, about half of the pollen grains released by Inbred A would be nonviable when PMC drops to 50.1%, which is likely to occur in less than one hour, even at relatively low VPD (Fig. 3). On average, PV$_{50}$ was 50.0% for the genotypes examined and ranged from 45.1% (Inbred B) to 53.6% (B104).

This rapid loss of pollen viability with decreasing moisture content could explain the lack of uniformity in pollen viability values reported for maize (Herrero and Johnson, 1980; Schoper et al., 1987; Broglia and Brunori, 1994; Buitnik et al., 1996; Aylor, 2003). The extent of moisture loss from the pollen will depend on the time elapsed between release from the anthers and placement on the incubation medium and on the VPD of the air during that time. For this reason, PMC should be documented when reporting pollen viability percentages. Because pollen viability
decreases linearly with PMC to $P_{V_0}$ (Table 1, Fig. 4), it also should be possible to normalize pollen viability values to a common PMC for direct comparison between genotypes and studies.

**An index to assess pollen moisture content**

These results showed that PMC is fairly uniform when pollen is released from the anthers (about 57% on average). Subsequent pollen desiccation depended almost entirely on the VPD between the pollen grain and the surrounding air. And that loss of pollen viability could be explained primarily by changes in PMC. As such, it is possible to estimate when pollen released from anthers becomes nonviable based on the VPD of the air to which it is exposed. Figure 5 shows the temporal dynamics of pollen moisture loss at VPDs ranging from about 5 to 17 mm Hg, which encompass conditions tested by Aylor (2003), our 2003 field study, and the four additional field locations listed in Table 3. In all cases, the rate of pollen desiccation was fairly constant at each VPD until PMC reached about 25%. An accurate assessment of pollen desiccation below 25% PMC, however, is of little biological interest because pollen of all genotypes tested was nonviable at that point (Fig. 4 and Table 1). Thus, for the purposes of calculating loss of viability, pollen desiccation rate can be considered constant with time at a given VPD.

Based on these results showing that pollen desiccation rate was constant with time at a given VPD, it was possible to combine the effects of time and VPD on pollen desiccation into a single model, which we refer to as vapor pressure deficit time index (VPDT). One equation adequately described changes in PMC as a function of VPDT for all genotypes (Fig. 6).

$$PMC = 63.2 e^{-0.0012 VPDT}$$  \[4\]

The VPDT index captured most of the variation in PMC ($r^2 = 0.86$) associated with exposure of known VPD for the genotypes examined in our study as well as the one tested by Aylor (2003). Based on 95% confidence intervals, there were no
significant differences between the slopes (b) or intercepts (a) for the regression equations generated for individual genotypes (Table 2).

Eq. [4] can be used to estimate the time required for pollen to become non-viable under various RH and temperature conditions. For example, the 'average' genotype in our study required 211.8 VPDT units to lose 50% viability, and 612.0 VPDT units to become completely nonviable (Table 2). Consequently, if pollen were released from this 'average' genotype at 28°C and 75% RH (VPD = 6.85 mmHg), its viability would drop to 50% in 31 min, and the pollen would be nonviable in about 89 min.

Application to pollen containment

This approach for quantifying the temporal dynamics of pollen viability has an immediate application in pollen dispersal models attempting to quantify pollen drift and risks of out-crossing (Arritt et al., 2003; Aylor et al., 2003). In general, these models predict pollen movement based on environmental and physical parameters, but do not differentiate between viable or nonviable pollen grains. Our results offer the framework to simulate pollen viability loss in any given environment. We selected four locations that represent a range of environmental conditions during maize flowering (Table 3). For each, we calculated the expected VPDs for a typical day of pollen shed in 2003 from a public database of average hourly temperature and RH values. Calculated VPDs at Constantine (MI), Ames (IA), Woodland (CA) and York (NE) at noon were about 7.7, 8.7, 15.2 and 16.7 mm Hg respectively. In this example, VPD was assumed to remain constant after pollen was shed. Pollen released at 1100 hours at Constantine would lose half of its viability in 25 min; pollen shed at the same time in York would require only 11 min for the same loss in viability. Pollen shed at this time would no longer be viable after 85 and 39 min at these two locations, respectively. Loss of pollen viability at Ames and Woodland in 2003 represented intermediate situations.

Given the same potential for pollen dispersal at all four locations during pollen shed, the Constantine environment would have supported the greatest risk for out-crossing in 2003. Assuming for simplicity constant VPDs and sufficient wind speed
to carry pollen grains in the air, the lower VPD at Constantine extended the risk of out-crossing because 'biologically active' pollen grains could travel farther from the source before they lost viability completely (Fig. 7). The actual distribution of viable pollen from the source at any location, of course, depends on the spatial and temporal variation in VPD, wind speed and direction, as well as turbulent elevation of pollen grains into the atmosphere. In our current investigations, we are coupling such atmospheric dynamics with the temporal patterns of pollen shed and viability to simulate the dispersal of viable maize pollen under field conditions.

Acknowledgments

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References


Table 1. Pollen viability data for 11 genotypes grown in the field. A bilinear model was used to relate pollen viability with moisture content (Fig. 4). Regression slopes and pollen moisture contents (PMC) at 0% viability (PV_0) are presented with their respective 95% confidence intervals.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$r^2$</th>
<th>Slope</th>
<th>Initial viability (%)</th>
<th>PMC (%) at PV_{00}</th>
<th>PMC (%) at PV_{1}</th>
<th>Pollen grains (tassel$^{-1}$ x 10$^6$)</th>
<th>Viable pollen grains (tassel$^{-1}$ x 10$^6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B 104</td>
<td>0.66</td>
<td>2.4 ± 0.45</td>
<td>76</td>
<td>53.6</td>
<td>32.4 ± 6.60</td>
<td>3.24</td>
<td>2.46</td>
</tr>
<tr>
<td>B 97</td>
<td>0.77</td>
<td>1.9 ± 1.06</td>
<td>59</td>
<td>53.5</td>
<td>27.4 ± 13.26</td>
<td>3.27</td>
<td>1.93</td>
</tr>
<tr>
<td>Inbred A</td>
<td>0.83</td>
<td>2.1 ± 0.44</td>
<td>79</td>
<td>50.1</td>
<td>25.9 ± 7.90</td>
<td>4.80</td>
<td>3.79</td>
</tr>
<tr>
<td>Inbred B</td>
<td>0.93</td>
<td>3.0 ± 0.55</td>
<td>89</td>
<td>45.1</td>
<td>28.7 ± 5.58</td>
<td>2.99</td>
<td>2.65</td>
</tr>
<tr>
<td>Inbred C</td>
<td>0.90</td>
<td>1.9 ± 0.62</td>
<td>64</td>
<td>52.3</td>
<td>25.6 ± 16.54</td>
<td>1.87</td>
<td>1.18</td>
</tr>
<tr>
<td>Inbred D</td>
<td>0.93</td>
<td>2.8 ± 0.50</td>
<td>85</td>
<td>51.0</td>
<td>33.3 ± 4.93</td>
<td>3.98</td>
<td>3.38</td>
</tr>
<tr>
<td>Inbred E</td>
<td>0.79</td>
<td>2.8 ± 0.82</td>
<td>90</td>
<td>48.5</td>
<td>30.3 ± 8.46</td>
<td>2.80</td>
<td>2.50</td>
</tr>
<tr>
<td>Inbred F</td>
<td>0.93</td>
<td>3.0 ± 0.99</td>
<td>83</td>
<td>44.7</td>
<td>28.1 ± 13.29</td>
<td>2.87</td>
<td>2.38</td>
</tr>
<tr>
<td>Inbred G</td>
<td>0.87</td>
<td>3.6 ± 2.17</td>
<td>84</td>
<td>49.0</td>
<td>35.3 ± 10.56</td>
<td>2.65</td>
<td>2.23</td>
</tr>
<tr>
<td>Inbred H</td>
<td>0.81</td>
<td>2.3 ± 0.85</td>
<td>81</td>
<td>50.9</td>
<td>28.8 ± 10.61</td>
<td>2.24</td>
<td>1.82</td>
</tr>
<tr>
<td>Inbred I</td>
<td>0.79</td>
<td>2.9 ± 0.71</td>
<td>83</td>
<td>51.7</td>
<td>34.2 ± 5.40</td>
<td>3.41</td>
<td>2.83</td>
</tr>
<tr>
<td>Average</td>
<td>0.86</td>
<td>2.6</td>
<td>79</td>
<td>50.0</td>
<td>30.0</td>
<td>3.10</td>
<td>2.47</td>
</tr>
</tbody>
</table>
Table 2. Regression parameters and vapor pressure deficit time index (VPDT) values for 11 genotypes grown in the field. Eq. [3] was used to translate pollen moisture contents at 50% viability (PV$_{50}$) and 0% viability (PV$_0$) from Table 1 into vapor pressure deficit time index (VPDT) values. Regression parameters a and b are presented ± 95% confidence intervals.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$r^2$</th>
<th>a ± 95% CI</th>
<th>b ± 95% CI</th>
<th>VPDT to PV$_{50}$</th>
<th>VPDT to PV$_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B 104</td>
<td>0.91</td>
<td>65.1 ± 3.83</td>
<td>-0.0012 ± 0.0002</td>
<td>180.1</td>
<td>615.0</td>
</tr>
<tr>
<td>B 97</td>
<td>0.88</td>
<td>61.9 ± 8.63</td>
<td>-0.0015 ± 0.0005</td>
<td>94.5</td>
<td>532.2</td>
</tr>
<tr>
<td>Inbred A</td>
<td>0.89</td>
<td>62.7 ± 4.07</td>
<td>-0.0013 ± 0.0002</td>
<td>171.1</td>
<td>674.2</td>
</tr>
<tr>
<td>Inbred B</td>
<td>0.89</td>
<td>64.3 ± 5.38</td>
<td>-0.0012 ± 0.0002</td>
<td>297.0</td>
<td>676.6</td>
</tr>
<tr>
<td>Inbred C</td>
<td>0.85</td>
<td>59.3 ± 10.40</td>
<td>-0.0015 ± 0.0006</td>
<td>84.2</td>
<td>566.6</td>
</tr>
<tr>
<td>Inbred D</td>
<td>0.92</td>
<td>69.8 ± 5.18</td>
<td>-0.0014 ± 0.0002</td>
<td>223.4</td>
<td>528.1</td>
</tr>
<tr>
<td>Inbred E</td>
<td>0.88</td>
<td>62.6 ± 5.94</td>
<td>-0.0013 ± 0.0003</td>
<td>203.3</td>
<td>575.3</td>
</tr>
<tr>
<td>Inbred F</td>
<td>0.82</td>
<td>58.4 ± 12.06</td>
<td>-0.0010 ± 0.0004</td>
<td>267.9</td>
<td>729.1</td>
</tr>
<tr>
<td>Inbred G</td>
<td>0.86</td>
<td>61.0 ± 6.01</td>
<td>-0.0009 ± 0.0003</td>
<td>234.6</td>
<td>568.2</td>
</tr>
<tr>
<td>Inbred H</td>
<td>0.85</td>
<td>59.9 ± 6.39</td>
<td>-0.0010 ± 0.0003</td>
<td>162.8</td>
<td>727.9</td>
</tr>
<tr>
<td>Inbred I</td>
<td>0.90</td>
<td>62.5 ± 3.77</td>
<td>-0.0011 ± 0.0002</td>
<td>166.1</td>
<td>529.2</td>
</tr>
<tr>
<td>Average</td>
<td>0.88</td>
<td>62.6 ±</td>
<td>-0.0012 ±</td>
<td>211.8</td>
<td>612.0</td>
</tr>
</tbody>
</table>
Table 3. Simulated time (min) to 50 and 0% pollen viability (PV$_{50}$ and PV$_{0}$) at four locations in 2003. Temperature and RH values are averages for hourly data obtained from The Weather Underground, Inc. (www.wunderground.com) for the indicated locations, dates, and times. Calculated VPDs assume uniform conditions during each hour.

<table>
<thead>
<tr>
<th>Location</th>
<th>Dates</th>
<th>Daytime</th>
<th>Temp (°C)</th>
<th>RH (%)</th>
<th>VPD (mmHg)</th>
<th>Time (min) to PV$_{50}$</th>
<th>Time (min) to PV$_{0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constantine, MI</td>
<td>23-29 Jul 2003</td>
<td>9-10 am</td>
<td>20.7</td>
<td>75.7</td>
<td>4.4</td>
<td>44</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-11am</td>
<td>22.1</td>
<td>71.7</td>
<td>5.5</td>
<td>35</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11-12 am</td>
<td>23.4</td>
<td>63.3</td>
<td>7.7</td>
<td>25</td>
<td>85</td>
</tr>
<tr>
<td>Ames, IA</td>
<td>10-16 Jul 2003</td>
<td>9-10 am</td>
<td>22.9</td>
<td>70.9</td>
<td>6.0</td>
<td>32</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-11am</td>
<td>24.1</td>
<td>65.6</td>
<td>7.5</td>
<td>25</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11-12 am</td>
<td>25.2</td>
<td>62.7</td>
<td>8.7</td>
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<td>Woodland, CA</td>
<td>05-11 Jul 2003</td>
<td>9-10 am</td>
<td>22.3</td>
<td>54.3</td>
<td>9.0</td>
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<td>10-16 Jul 2003</td>
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Figure 1. In vitro germination of maize pollen grains. Grains were germinated for 3 h at 28°C and 100% RH in open Petri dishes containing nutrient agar described in Methods. A pollen grain was counted as germinated if the length of the germ tube was at least one diameter of the pollen grain. Germination percentages are shown next to each image.
Figure 2. Pollen moisture content measured immediately after grains are released from the anthers and collected at different times of the day. Data represent 14 genotypes grown in the greenhouse or in the field. Note that initial moisture content did not vary significantly during the day, and was 57.3%, on average for all samples.
Figure 3. Moisture content of pollen grains exposed for one hour to a range of vapor pressure deficits (VPD) provided by a combination of temperature (25, 32.5 and 40°C) and relative humidity (15, 50 and 85%). Data are for three genotypes grown in the greenhouse. Data are the average ± standard error for three pollen samples.
Figure 4. Percentage of viable pollen grains as a function of its moisture content of Inbred B. The range in pollen moisture content was a consequence of exposing the grains to combinations of temperature (25 and 40°C) and relative humidity (15, 50 and 85%) environments for varying periods of time (15, 30 and 60 min). A general bilinear model adequately described this relation for the 11 genotypes tested in the field (Table 1). Viability was zero at 28.7% moisture content.
Figure 5. Change in pollen moisture content (PMC) with time for pollen grains exposed to different VPDs. Grey symbols are data acquired from Figure 4 of Aylor (2003) using Digitizelt® software (© I. Bormann 2001-2003). Tendency lines were drawn by hand. Dark symbols are for pollen samples collected from Mo17, Mo17 x B73 and Dekalb 668 grown in the greenhouse and exposed up to 120 minutes to VPDs between 5 and 17 mmHg (see Fig. 3). Note that pollen desiccation rate was fairly constant at PMCs greater than 30.0%, the average PV₀ for all genotypes examined in this study.
Figure 6. Relationship between pollen moisture content and the vapor pressure deficit time index (VPDT). Data from all 11 genotypes grown in the field are included. Dark symbols are data acquired from Figure 4 of Aylor (2003) using Digitizelt® software (© I. Bormann 2001-2003).
Relative distance from the source

Figure 7. Simulated dispersal patterns of viable pollen from four corn production environments in 2003. Relative distances are simulated based on time required to reach 0% viability (PV₀). VPDs for each location used in this simulation are listed in Table 3.
CHAPTER 7. RISK INDEX FOR OUT-CROSSING AND SELF-POLLINATION IN MAIZE HYBRID SEED PRODUCTION

A paper to be submitted to Crop Science

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Abstract

Ensuring maximum kernel set and achieving a high level of genetic purity are critical aspects of maize (Zea mays L.) hybrid seed production. We previously reported that kernel production per hectare could be simulated fairly accurately under pollen-limited conditions from simple measures of pollen shed and silking dynamics. The objective of this project was to expand the utility of the kernel set model to quantify the risk of out-crossing by adventitious pollen and self-pollination by female inbred pollen as management conditions are altered to optimize hybrid seed production. For this purpose, we distinguished between the inherent risk, determined solely by the flowering characteristics of the inbred pair, and the actual risk, which incorporates the amount of pollen that could drift into the field. The former is a function of the number of receptive silks available for pollination and the density of favorable pollen; the latter is associated with adventitious pollen drift, driven by factors such as isolation time or distance, climate, and longevity of pollen viability.

We simulated kernel production based on flowering dynamics in 13 seed production fields, which differed in the quantity of pollen production and silk emergence. Values provided by the kernel set model were closely correlated with the measured values \( r^2 = 0.88 \). A procedure for assessing the risk of out-crossing and self-pollination (RI) was developed and fields were classified on this basis. Examples are provided to show how inbred management can be modeled to minimize the RI. This analysis provides a logical basis for defining management strategies to maximize seed yield, minimize the risk of harvesting genetically impure seed, or achieve an acceptable
balance between both. If a temporal distribution of adventitious or female pollen shed is provided, the model simulates the actual amount of out-crossing or self-pollination.

**Introduction**

Maize is primarily wind pollinated, and due to this uncontrolled pollination, often is referred to as an *open-pollinated crop* (Poehlman, 1995). Thus, pollination typically occurs between plants or genotypes. Production of genetically pure hybrid corn seed depends on cross-pollination between male and female inbred parents and effective reproduction isolation of the female inbred from other sources of pollen. Obtaining pure seed has become essential in hybrid seed production as it limits the success of companies striving to fulfill the market demand for genetically pure products. Seed companies often have isolation standards based on practical experience and limited experimental investigation (Ireland, 2004). With the introduction of transgenic maize, there has been an increasing interest in quantifying pollen dispersal, particularly in relation to gene flow from transgenic crops and the maintenance of seed quality (Luna et al., 2001; Jarosz et al., 2003).

Genetically impure seed can result from cross-pollination of the female inbred with adventitious pollen or pollen produced by the female inbred itself, commonly referred as out-crossing and self-pollination, respectively. We recognize these hazards as a combination of local characteristics of a particular field (i.e. amount and timing of local pollen, silks receptivity) and the amount and timing of other pollen sources in the field. An overwhelming pollen production supplied by the male inbred, for example, will favor pollination by the desired parent. However, a high density of male pollen is hard to achieve since inbreds typically produce less pollen than do their hybrid counterparts (Fonseca et al., 2003) and only a fraction of the field population is permitted to shed pollen, i.e., male inbred. A major challenge in hybrid seed production, in fact, is to reduce the area dedicated to male rows as much as possible without decreasing the number of kernels harvested per area (Wych, 1988). In addition, pollen shed and silk exsertion on physically separated plants increases
the probability for floral asynchrony that can lead to poor kernel set. Together these biological and physical factors create conditions in which seed production could be reduced, while the risk of out-crossing and self-pollination increased.

Achieving the optimum seed purity and yield per unit land area often is based on limited information about the quantity of pollen shed by the male and practical experience synchronizing pollen shed by the male inbred with silk emergence by the female inbred. Developing simple quantitative descriptions of male and female flowering dynamics can provide a better understanding of the pollination process and assist in optimizing management practices, in particular for new combination of inbreds. Westgate et al. (2003) recently developed quantitative descriptions for the daily progress of pollen shed and silk emergence under field conditions based on simple measures of male and female flowering. When coupled mathematically to the pollination efficiency curve generated by Bassetti and Westgate (1994), these estimates of male and female flowering can be translated into daily values for kernel set. Lizaso et al. (2003) showed that this mathematical approach was highly accurate at simulating kernel production for two maize hybrids across a wide range of pollen shed densities. More recently, we applied simple mechanistic descriptions of male and female inbreds flowering dynamics to simulate kernel production in hybrid seed production fields (Fonseca et al., 2004). The resulting kernel set model has proven useful for optimizing harvested kernels for an established inbred pair, or for defining initial management protocols for new combinations of inbreds.

The goal of this project is to expand the model's utility to assess the risk of out-crossing and self-pollination as management conditions are altered to optimize seed production. We developed a risk index based exclusively on the flowering characteristics of the inbreds involved. This index could be used to design management practices for minimizing the risk of out-crossing and self-pollination, regardless the amount of 'non-desired' pollen (either adventitious or female pollen). Furthermore, if combined with pollen dispersal models currently under development (Arritt et al., 2003; Aylor et al., 2003), this approach will be useful for simulating actual out-crossing that has occurred in a hybrid seed field.
Materials and Methods

Procedure for simulating potential kernel set

The procedure for simulating kernel production begins with developing a temporal profile of pollen shed for the male population and a profile of silk exsertion for the female population. These floral dynamics are translated into daily values of kernel production by the female inbred using the procedures described by Lizaso et al. (2003), which rely on the quantitative relationship between daily pollen shed density (grains per cm$^2$) and percent kernel set published by Bassetti and Westgate (1994). Details for collecting these data are outlined briefly below. The mechanistic aspects of the model are described in Fonseca et al. (2004).

Amount and temporal distribution of pollen shed:

The seasonal distribution of pollen shed is simulated from field observations of male flowering characteristics at the population and at the plant level. This calculation assumes that pollen density is distributed homogeneously among the female population. One hundred consecutive male plants at each sampling site are examined for the progress of tassel development each morning. Beginning Shed (Beg Shed) is recorded as the proportion of plants that have anthers exserted on the main tassel branch (Westgate et al., 2003). The population progress through Beg Shed is readily described by a sigmoidal logistic function. At the plant level, pollen from twenty tassels is harvested at each sampling site and quantified to provide the average pollen production per tassel. Also, Beg Shed and End Shed dates are documented for each tassel to calculate the average duration of pollen shed per tassel. The model uses pollen production per tassel ($P$) and duration of pollen shed per tassel ($P_{dur}$) to simulate daily pollen shed using a Gauss function:

$$ pr = \frac{P}{w \sqrt{\frac{\pi}{2}}} e^{-\frac{(t-t_0)^2}{w^2}} $$

where $pr$ is the rate of pollen shed per fertile tassel (pollen grains per plant per day), $P$ is the total amount of pollen produced per tassel (pollen grains per tassel), $w$ is the
width of the pollen shed curve, measured at half the maximum pollen shed rate (days), and \( t \) and \( t_x \) are the current day and the day of maximum pollen shed. The value of \( w \) is calculated from \( Pdur \), duration of pollen shed on each tassel (days), as:

\[
w = 0.3 \, Pdur
\]

[2]

and the value of \( t_x \) as:

\[
t_x = 0.5 \, Pdur
\]

[3]

The actual rate of pollen shed (grains cm\(^{-2}\) d\(^{-1}\)) is calculated by combining the dynamic of pollen shed per tassel and the temporal distribution of plants reaching Beg Shed.

**Amount and temporal distribution of exserted silks:**

The temporal distribution of silks exposed for pollination is calculated from the progress of female flower development at the population level and at the plant level. At the population level, typically 100 consecutive plants are examined for exposed silks each morning. The percentage of population with exposed silks progresses in a sigmoid fashion similar to the population curve generated for tassel development. At the plant level, silks emerge from the surrounding ear leaf sheaths (husks) over a period of days. The process is described by a monomolecular function, using rate of silk exsertion and maximum silk number per ear as genotype-specific variables (Lizaso et al., 2003). When 30% of the population has started silking, ears on five plants about to exsert silks are covered with glassine bags to prevent pollination. Silks are sampled 1, 3, 5, and 8 d after first silks appear. Two-cm segments of exposed silk tissue are cut at husk level, transferred to plastic bags containing 500 g kg\(^{-1}\) ethanol, and stored at 4 °C until counted manually.

The daily and cumulative number of silks exserted per unit land area were calculated by combining the daily dynamics of silking for the population, the daily
rate of silk exsertion for each plant, and the female plant population density (plants female ha\(^{-1}\)).

**Application to commercial seed production fields**

We applied the procedure for simulating the number of kernels female ha\(^{-1}\) from flowering dynamics with measured values for kernel yield in 13 seed production fields managed by Syngenta Seeds Inc. through 2002 and 2003 in Washington County, IA. The fields were chosen to provide a range of male and female flowering characteristics (Table 1). Planting patterns included 4 female: 1 male and 4 female: 2 male rows. Descriptive terms commonly used in the seed industry such as 'good', 'fair', and 'poor' for a male inbred, and 'big', 'regular' or 'small' for female ear size are provided for the benefit of the reader and do not imply a quantitative basis for comparison between inbreds.

Flowering dynamics for the male inbred populations and female inbred were assessed at one location in each field. The location was selected 2 wk prior to flowering to be representative of typical inbred development across the field. The sampling area was approximately 125 m\(^2\) and at least 25 m from the field border. To extend the period of pollen shed, plant development was delayed in a fraction of the male population by burning away exposed leaf area at around V4 stage. This management practice, commonly referred as 'flaming', effectively delayed pollen shed up to 2.5 d in the treated fraction (Table 1). The flowering dynamics of the 'flamed' plants was monitored separately. We refer to these populations as Male 1 and Male 2.

Average pollen production per plant was documented by covering 10 representative tassels with clear bags (Pantek, Montesson, France) designed to exclude moisture but allow gas exchange around the tassel. Pollen was harvested from the bags and quantified using a Coulter Multisizer II (Coulter Electronics Limited, Luton, Beds, England) according to Fonseca et al. (2003). We used average pollen production per plant and approximate population densities (based on data collected by field managers) to calculate total pollen production for each male population.
The interval between the pollen shed curves for Male 1 and Male 2 was taken as the difference in days between 50% Beg Shed for the two populations. Separate but identical pollen shed curves were generated for each male population. These curves then were combined to produce a total pollen shed curve for the field. The resulting pollen amount per unit area was adjusted for the female - male planting ratio for each field.

Approximately 1.3 hectares of the female inbred were harvested from each field to estimate kernel number per female ha. The yield was calculated in kg·ha⁻¹ and adjusted to 155 g·kg⁻¹ moisture. Average kernel weight was calculated from the number of kernels in a 454-g sub-sample, and adjusted to 155 g·kg⁻¹ moisture. Harvested kernel number per female ha was calculated as grain yield (kg·ha⁻¹) / average kernel weight (kg·kernel⁻¹).

**Risk index for out-crossing and self-pollination (RI)**

In an open-pollinated crop such as maize, where the possibilities of adventitious pollen contamination are high, current knowledge is insufficient to simulate with accuracy the level of out-crossing or self-pollination that could be expected. Therefore, we distinguished between the *inherent* and the *actual* risk. The *inherent* risk depends exclusively on the flowering dynamics of the inbreds under study, while the *actual* risk requires a quantification of the amount and timing of 'non-desired' pollen. A quantification of the *inherent* risk could help design management practices that will minimize the risk of out-crossing and self-pollination for specific combinations of inbreds. Besides, the *inherent* risk does not depend on the amount of adventitious pollen, which is difficult to quantify and predict.

The procedure to calculate the *inherent* risk will be referred to as risk index for out-crossing and self-pollination (RI). RI is a seasonal integrated index with two components, a daily estimate of risk and a weighting procedure to appraise the relative significance of each day's risk over the flowering season.

The daily estimate of risk (DRᵢᵢ, %) is calculated as:
where \( UP_k \) is the number of unpollinated silks on each ear cohort (k), \( R_k \) is the number of potentially receptive silks per cohort, and \( m \) the number of cohorts simulated each day. This calculation assumes that silks remain receptive for 6 days (Bassetti and Westgate, 1993a, 1993b), thus \( m \) equals 6. The calculated number of receptive pollinated, and unpollinated silks follow the procedures described by Lizaso et al. (2003).

The Risk Index (RI) therefore will be:

\[
RI = \frac{\sum_{i=1}^{n} DR_i \times UP_i}{\sum_{i=1}^{n} UP_i}
\]  

In Equation 5, \( UP_i \) is today’s number of unpollinated silks. The procedure weights the daily risk by the relative number of unpollinated silks. Since RI formulation is based on daily count of silks that remain receptive after local pollen has fertilized silks, the basic assumption is that local pollen has priority over drifting adventitious pollen. It is also assumed that pollen is evenly mixed within the field. The RI is not an absolute value, but rather represents a ‘worst-case’ scenario for a particular seed field. It does, however, provide an approach to compare production options for their risk of out-crossing and self-pollination in relative terms.

**Simulating possible seed production scenarios**

The model can be used to examine how altering management variables or flowering dynamics might affect potential kernel yield and RI. As examples, we present the effect of altering the anthesis-silking interval (ASI) and the amount of pollen production per tassel. A series of simulations generated a kernel yield response curve and a RI response curve for each input variable tested. Because these response curves are specific to the flowering characteristics of a given inbred
pair, they provide a basis for comparing the potential impact of various management scenarios on seed production and purity for that pair. Therefore, strategies could be designed to optimize seed yield, minimize the Rl or achieve an acceptable balance between both.

Four fields were chosen for scenario analysis: B, G, L, and M (Table 1). These fields presented inbred combinations with contrasting flowering characteristics that led to a wide range of seed yields. Also, these fields presented different levels of calculated Rl. Male-female synchrony and the amount of pollen per tassel were adjusted relative to the original measured conditions for each field. Female and male plant densities, maximum silks per ear, silks exsertion rate, pollen shed duration, silking and shedding population dynamics, and prolificacy were held constant. The model assumes that, when altering one management variable, the environmental response is not different from the original situation. It also assumes no confounding interactions between management variables. For simplicity, only one variable was adjusted at a time, although multiple factors can be adjusted simultaneously.

The anthesis-silking interval was -2.7 d in Field L, -0.3 d in Field B, 1.6 d in Field G and 3.6 d in Field M. Since a fraction of the males was delayed in all fields, the anthesis-silking interval was measured relative to anthesis for the first male population (A_S_I). We examined the impact of altering male-female synchrony over a wide range of A_S_I values, from -6 to +6 d.

Tassels produced an average of 3.1, 1.9, 1.5 and 0.8 million grains in Fields B, G, L and M respectively. Kernel production and RI responses to alter pollen amount (maleness) were tested from 0.5 to 3.5 million pollen grains per tassel.

Simulating out-crossing and self-pollination

If provided an estimate of adventitious pollen drifting into the field, the model simulates the actual amount of out-crossing. If provided an estimate of pollen produced by the female population instead (i.e. female plants that escaped detasseling or broke male sterility), the model also provides an opportunity to simulate the amount of self-pollination. In either case, an additional pollen curve,
describing the daily density of either adventitious or female pollen, must be input.
The model assigns the same probability of fertilizing a silk to every pollen grain, regardless of its source. The new pollen curve is added to the local pollen sources (i.e. male 1 and male 2) and the total is coupled with the female information to simulate kernel set as previously described. The simulated amount of kernels set each day is divided into genetically pure and out-crossed/self-pollinated kernels based on the daily fraction of local and adventitious/female pollen.

We simulated the expected amount of out-crossing in fields B, G, L and M for a known amount of foreign pollen arriving each day. Ireland (2004) measured pollen depositions between 0.4 and 5 grains cm$^{-2}$ 100 m down wind from their origin, when wind speed fluctuated between 1 to 3 m s$^{-1}$. Most frequently, the daily pollen drift was about 1 grain cm$^{-2}$. Therefore, it seemed reasonable to assume an adventitious presence of 1 grain cm$^{-2}$ d$^{-1}$ in a hybrid seed production field. In our analysis we quantified the consequence of receiving that amount of foreign pollen on different days.

Also, we simulated the expected amount of self-pollination in Field L. We chose that field because it presented a high RI. To do so, it was necessary to develop a temporal profile of pollen shed by the female. In other related study, we observed that female inbreds typically produce as much pollen as a 'poor' male, which usually produces about 1 000 000 pollen grains in a period of 5 days (Schneider, unpublished). Therefore, we calculated the temporal distribution of female pollen shed considering this profile of pollen production. We also assumed that only 0.5% of the female population would shed pollen. This percentage represents the maximum tolerance usually employed by seed companies; fields are hand-detasseled whenever higher percentages are observed (Schneider, personal communication). Note that, it now becomes essential to assess precisely the synchrony between silking and pollen shed dynamics of the male inbred and that of the female. In addition to the A$_1$SI for the male and female inbreds, the synchrony between pollen shedding and silking within the female population itself needs to be documented. We refer to this synchrony as female ASI. In the example presented,
self-pollination was initially simulated for a female ASI = 0. But female inbreds can display protandry or protogyny. For that reason, we simulated self-pollination for a range of female ASI.

Results and Discussion

Simulating potential kernel production in seed production fields

The model developed by Lizaso et al. (2003) relies on quantitative measures of male and female flowering dynamics to calculate the potential number of kernels set on an area basis. The approach was validated with hybrids under field conditions in which pollen shed density was varied by detasseling or by mixing male-fertile and male-sterile isolines (Lizaso et al., 2003; Westgate et al., 2003), and with inbreds in seed production fields (Fonseca et al., 2004).

In this study, thirteen commercial seed production fields (designated A-M) were evaluated, which encompassed wide variation in male and female flowering characteristics (Table 1). The male inbred in Field I, for example, was considered a 'poor' pollen source; male inbreds from Fields F and M were considered 'fair', and those from other fields were designated as 'good' pollen sources according to their measured field performance. The average pollen shed among the male inbreds ranged from approximately 520 000 pollen grains (Field I) to 3 980 000 pollen grains (Field A). The total amount of available pollen per unit surface is a combination of the quantity of pollen produced per plant and the number of plants in that area. Field I produced the lowest density of pollen grains among all fields (27 800 million grains per ha), as it combined the 'poorest' male with a low population density. In contrast, Field A produced the highest density of pollen (247 400 million grains per ha), which resulted from having a 'good' male at a high population density. The rest of the fields presented intermediate situations. A detailed characterization of the response of male inbreds to population density would be of considerable value for making management decisions on how to maximize pollen density per area.

As for the female inbreds, Fields F and M typically produced 'small' ears, Fields E, G, and H produced 'big' ears, while the rest of the fields presented 'regular-
sized' ears. The average ear size among these inbreds ranged from approximately 350 silks (Field F) to 750 silks (Field G). As in the case of pollen density, the final number of receptive silks per unit area resulted from combining silks exserted per ear and the number of plants in that area. Field M presented the lowest density of silks among all fields (17.0 million per ha), while Field H presented the highest silk density (51.4 million per ha).

Other characteristics that varied widely among the fields under study were the synchrony between male and female inbreds and the time interval between male 1 and male 2 shedding (Table 1). The anthesis-silking interval (A<sub>s</sub>-SI) ranged from -2.7 d (Field L) to 3.6 d (Field M), while the time interval between male 1 and male 2 shedding varied from 0.4 d (Field F) to 2.5 d (Field D). While variable, these fields represent the typical range of flowering dynamics observed in hybrid seed production.

**Simulated vs. measured kernel set**

Harvested kernel number varied from about 8.4 to 30.3 million kernels per female ha among the seed production fields (Table 1). Values provided by the kernel set model were closely correlated ($r^2 = 0.88$) with the measured values (Fig. 1). As observed by Fonseca et al (2004), model simulations overestimated kernel production by 12% in average (Table 1). It is evident that one or more plant or canopy factors that might have limited kernel were not taken into account. Pollen viability and pollen capture by the leaf were not considered in our analysis. It is also possible that the process of mechanical detasseling itself reduces the yield potential for the female inbred, which would result in a loss of kernels after the initial kernel set is established. Nonetheless, the model in its current form simulated kernel production with consistent accuracy across a wide range of seed yields (Fig. 1). This result indicates that variation in kernel production can be assessed directly from flowering dynamics of the parental inbreds.

**Risk index for out-crossing and self-pollination (RI)**

We have developed a procedure to quantify the risk of out-crossing or self-pollination in a hybrid seed field based on the flowering dynamics of the inbreds...
involved. We calculated the Risk Index (RI) for all 13 seed production fields based on the flowering dynamics of the parental inbreds (Table 1). These fields were annotated A through M from least to greatest RI. Fields A and B, which produced the highest pollen density, presented the lowest RI values. Likewise, the field with the highest risk, Field M, produced a fairly low amount of pollen. But RI was not always associated with pollen production. Field C and D, for example, produced a much lower amount of pollen per hectare than did Field E, but presented a lower RI.

Analysis of male and female flowering dynamics help to understand situations with contrasting RI. We simulated their flowering dynamics and 'silks at risk' for fields B, G, L and M, that presented contrasting seed yield (Fig. 2). The RI varies dramatically among these fields (Table 1). Field B presented a RI of 48.1, the lowest risk among them. The low risk of out-crossing was due to a high production of pollen \((228.4 \times 10^9\ \text{grains ha}^{-1})\) and close synchrony with silk exsertion \((\text{ASI} = -0.3)\). Nonetheless, there was still a small fraction of 'early' and 'late' silks that would present some risk. Field G presented an intermediate RI of 56.8. In this case, pollen production was significantly lower than in Field B, and some silks were at risk of being out-crossed throughout the entire silking period. This is a clear example of how risk of out-crossing can exist even in a field with very high yield. Fields L and M presented RIs of 74.9 and 79.0 respectively, the greatest among all analyzed fields. In Field L, the male started shedding too early \((A_5 \text{SI} = -2.7\ \text{d})\) and there was a large fraction of late emerging silks at risk. In Field M, however, male shedding occurred too late \((A_5 \text{SI} = 3.6\ \text{d})\) and the majority of the early silks were at risk of out-crossing.

In general, low RI is associated with conditions that favor high kernel set. For example, Field A produced 30.3 million kernels \(\text{Fha}^{-1}\) with the lowest RI. Field M presented the worst situation, as low seed yield was coupled with a high risk of out-crossing (Table 1).

**Simulating seed production scenarios**

Based on the flowering dynamics of a given inbred pair, the model can be used to test alternative management scenarios to optimize kernel production per hectare or/and minimize RI. Various genetic characteristics that might affect flowering
dynamics can be tested as well. By way of example, we present the effect of varying
the timing between pollen shed and silking (A1-SI) and altering pollen production per
tassel (maleness effect) in fields B, G, L and M.

Anthesis-silking interval:

Limited pollen availability in seed production fields makes careful
management of A1-SI especially important. In this case, anthesis is taken as the day
50% of the Male 1 population has begun pollen shed. Silking is taken as the day
50% of the female inbred plants have visible silks. Figure 3 shows the potential
consequences of altering the A1-SI on RI and kernel production. Altering A1-SI had the
greatest impact on harvested kernels per female ha among several management
variables tested (Fonseca et al., 2004). As such, it is reasonable to expect this
variable would have the greatest impact on RI as well.

The inbreds in Field B had an A1-SI = -0.3 d, and the model simulated a
production of 26.8 million seeds per female ha. If pollen shed were delayed 3.3 d (or
silking were advanced 3.3 d), kernel set would have been optimized to 28.9 million
seeds per female ha (about an 8% increase). But, this adjustment in A1-SI would
have increased RI from 48.1 to 76.8 (close to 60% increase) (Fig. 3). If seed purity
were the main concern in this field, the original synchrony between female and male
inbreds should not be altered (note ‘silks at risk’ curve in figure 2). It is still possible,
of course, to reduce RI further by modifying other management variables.

In the case of Field G, the model simulated a production of 31.1 million seeds
per female ha at the measured A1-SI of 1.6 d (Fig. 3). While seed production could
have been improved slightly by increasing this interval, the minimum RI occured at
an A1-SI around 1 d. The fraction of ‘early’ silks that were at risk could have been
reduced by better synchronizing their exsertion with pollen shed (Fig. 2).

Field L simulated seed yield and RI are far from optimum. The male inbred
started shedding pollen well before receptive silks were available (A1-SI = -2.7 d).
Both seed production and RI could have been dramatically improved by delaying
shedding relative to silking (Fig. 3). A maximum of 36.8 million seed per female ha
(112% increase from the original situation) would have been obtained at +2 d A1-SI;
while a minimum Rl of 60.7 (19% reduction from original situation) would have been achieved at A_tSI = 0 d. The 'silks at risk' curve showed that a large fraction of silks were exserted after pollen shed was almost complete (Fig. 2).

Field M exhibited the opposite situation. Both, seed production and Rl could have been improved by advancing pollen shed relative to silking, although with less striking results (Fig. 3). In this case, low pollen production was the primary variable limiting seed production. This is evident in the 'silks at risk' curve (Fig. 2) which shows a large fraction of silks were exserted before pollen was available.

In general, an A_tSI = 2 to 3 was optimum for maximizing seed production, while an A_tSI = 0 to 1 was ideal for minimizing Rl. Negative A_tSI values (i.e. protandry) had an unfavorable impact on both values. When pollen shed is delayed relative to silking, a much larger fraction of late-emerging silks can be pollinated. The early emerging silks also are pollinated, as they remain receptive to pollen for several days after they are exserted (Bassetti and Westgate, 1993a, 1993b). On the other hand, early emerging (and unpollinated) silks are at risk of exposure to adventitious pollen. The optimum A_tSI for the field depends on the management goals for each inbred pair. A_tSI that maximized seed yield or minimized Rl were not necessarily identical for all fields (Fig. 3). Therefore, recommendations should be specific for each field situation, depending on the flowering characteristics of the inbreds involved and the potential for adventitious pollen entry into the field.

**Pollen production (maleness):**

Pollen production per tassel varies among inbreds and typically decreases with increasing population densities (Fonseca et al., 2003). A high pollen production supplied by the male inbred will favor pollination by the desired parent and reduce the risks of genetic contamination. Figure 4 shows the potential consequences of altering pollen production per tassel over Rl and kernel production in the four fields selected.

The average male tassel in Field B shed approximately 3,083,000 pollen grains at a population density of 74,100 plants per female ha, providing a high pollen daily density during most of the silking period (Table 1 and Fig. 2). Our analysis
indicated that pollen density was sufficient to maintain a low amount of 'silks at risk', and that additional increases in pollen production would not have impact over Rl (Fig. 4). Also, pollen availability was not a limiting factor for seed production.

Field G presented a 'good' male that shed in average 1,960,000 pollen grains per tassel at 69,700 plants per female ha. Pollen shed was fairly high (136,500 million pollen grains per hectare), still significantly lower than in the previous case (Table 1). The model revealed that both Rl and seed production could be further improved if male pollen production were increased (Fig. 4). For example, a 12% increase in pollen production would have resulted in a 4% decrease in Rl and a 21% increase in seed production.

The average male tassel in Field L shed about 1,456,000 pollen grains at a population density of 63,300 plants per female ha. This combination resulted in a total of 92,100 million pollen grains per female ha (Table 1). Seed purity and yield improvement due to increasing pollen production would have been minimal (Fig. 4). Evidently there was some other limiting factor rather than the amount of pollen produced, most likely male-female synchrony. The anthesis-silking scenario analysis showed that seed production could have been dramatically improved by delaying shedding relative to silking (Fig. 3).

Field M presented a 'fair' male that shed in average 800,000 pollen grains per tassel at 49,900 plants per female ha. In consequence, total pollen production per female ha was dramatically lower than in the other three cases (Table 1 and Fig. 2). Also, it had the higher Rl and lower seed set among all fields. In part it was due to a bad synchrony between male and female flowering, since a considerable fraction of silks were exserted before pollen was available (Fig. 2). But the low pollen production per tassel had a negative impact as well. A 25% increase in pollen production would have resulted in a 6% decrease in Rl and a 25% increase in seed production (Fig. 4).

The model indicated that increasing the amount of pollen production per tassel was beneficial for reducing Rl and increasing seed yield. However, it showed
that, for each combination of inbreds, there was an optimum level of pollen production beyond which no further improvement in either parameter was obtained.

**Simulating out-crossing and self-pollination**

The amount of out-crossing generated by adventitious pollen will depend on the day it occurs. Days in which adventitious presence will be more or less detrimental to seed purity are determined by the flowering dynamics for each inbred pair, which can be simulated by the model (Fig. 5). We simulated the impact of 1 adventitious grain cm\(^{-2}\) arriving to seed production fields B, G, L and M each day during silking. The 'worst' day for adventitious pollen to enter was July 27 for Field B (0.29% simulated out-crossing), July 28 for Field G (0.54% out-crossing), July 31 for Field M (1.40% out-crossing), and August 3 for Field L (0.95% out-crossing). The magnitude of 'worst day' out-cross values explained most of the variation in our calculated RIs for each field (\(r^2 = 0.99; P<0.002\)).

We also tested the consequence of having 0.5% of the female population escaping detasseling (or breaking tassel sterility) in Field L. We developed a temporal distribution of pollen shed for the female population, assuming that the average female tassel would shed 1 000 000 pollen grains in 5 days and a female ASI = 0. The female pollen shed dynamic and calculated self-pollination are shown in figure 6A. The model indicated this combination of flowering dynamics would produce about 17 811 900 genetically pure seeds and 157 700 self-pollinated seeds per female ha (0.88% self-pollination). Since female inbreds also can exhibit asynchrony between their pollen shed and silking, we tested how a change in female ASI would affect self-pollination in Field L (Fig. 6B). A more negative female ASIs (protandry) reduced the amount of self-pollination, since a fraction of the pollen shed by the female occurred before silks were exserted. But a delay in female pollen shed relative to silking (positive ASIs) increased the potential for selfing. This likely resulted because pollen shed by the male inbred was early relative to silk exsertion by the female and there would be a greater chance of selfing of the late emerging silks (Fig. 6A). We expect the potential for selfing in the other 12 fields of this study would vary based on the synchrony of silking, male pollen shed and female pollen...
shed dynamics. Information on female inbred flowering dynamics and their response
to population density should be available from parent testing.

Differences in pollen viability between male inbred, female inbred and
adventitious sources were not considered in this analysis. The model assigns all
pollen grains the same probability of fertilizing an ovary, regardless of their source.
In general, adventitious pollen will travel further than local pollen to arrive at a silk,
and will be exposed for a longer time to environmental conditions that might lead to
desiccation and viability loss. It is possible to estimate pollen viability loss based on
an index that incorporates the effects of temperature, relative humidity and time of
exposure (VPDT) (Fonseca et al., unpublished). However, considering the speed of
pollen drift, those issues are probably irrelevant (e.g. it will only take a pollen grain
60 s to travel 120 m with a 2 m s$^{-1}$ wind). Also, male, female and adventitious pollen
might present different compatibility with female inbred silks. Since current
knowledge is not sufficient to fully address this issue, it is conservative to assign all
pollen sources the same opportunity to affect pollination of exposed silks as a first
approximation of a worst case.

**Summary**

Our model can be used to simulate kernel production in hybrid seed
production fields based on simple mechanistic descriptions of male and female
flowering dynamics. Values provided by the kernel set model were closely correlated
with the measured values ($r^2 = 0.88$). Also, it provides an approach for assessing the
risk of out-crossing and self-pollination. It represents a logical basis for defining
management strategies to maximize seed yield, minimize the risk of harvesting
genetically impure seed, or achieve an acceptable balance between both. If a
temporal distribution of adventitious or female pollen shed is provided, the model
simulates the actual amount of out-crossing or self-pollination.

The value of RI procedure was tested under field conditions. We deliberately
generated a range of out-crossing risk situations by varying the female inbred
planting date in different sections of a hybrid seed production field, and arranging
stations at different distances from a potential source of adventitious pollen (i.e. commercial cornfield). The actual percentages of out-crossed and self-pollinated kernels in each field station were compared to our simulations. These results will be presented in a forthcoming paper.

Acknowledgments
We express our gratitude to Juan Astini and Sebastian E. Schneider for their excellent technical assistance.

References


Table 1. Flowering characteristics, simulated and measured kernel production, and Risk Index for 13 hybrid seed production fields examined in 2002 and 2003.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
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<tr>
<td>Approximate plant density (plants per male ha)</td>
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<td>74 100</td>
<td>55 600</td>
<td>57 300</td>
<td>74 600</td>
<td>59 800</td>
<td>69 700</td>
<td>65 900</td>
<td>53 400</td>
<td>66 200</td>
<td>69 700</td>
<td>63 300</td>
<td>49 900</td>
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<td>3.98</td>
<td>3.08</td>
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<td>97.4</td>
<td>84.7</td>
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<td>116.1</td>
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<td>126.8</td>
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<td>92.1</td>
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<td></td>
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<tr>
<td>Approximate plant density (plants per female ha)</td>
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<td>61 700</td>
<td>59 800</td>
<td>51 900</td>
<td>74 100</td>
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<td>58 800</td>
<td>69 700</td>
<td>65 200</td>
<td>44 000</td>
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<td>602</td>
<td>608</td>
<td>560</td>
<td>678</td>
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<td>750</td>
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<td>19.5</td>
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<td>61%</td>
<td>59%</td>
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<td>74%</td>
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<td>49%</td>
</tr>
<tr>
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<td>26.8</td>
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<td>15.9</td>
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<td>27.2</td>
<td>22.3</td>
<td>25.6</td>
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<td>10.2</td>
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<td>Simulated / Measured (%)</td>
<td>105%</td>
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<td>103%</td>
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<td>135%</td>
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<td>55.6</td>
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<td>60.9</td>
<td>61.8</td>
<td>74.9</td>
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Figure 1. Correlation between measured and simulated kernel production for 13 hybrid seed fields representing two growing seasons. Measured kernel numbers per female hectare were approximated from 80 ears; simulated kernel numbers were generated by the model. The dashed line represents the 1:1 relation. Each point corresponds to one seed production field.
Figure 2. Simulated daily kernel production in four hybrid seed fields that differed in seed yield and RI (Fields B, F, H and L). The black curve indicates total pollen shed for the field (pollen grains cm$^{-2}$ d$^{-1}$). The gray curve indicates cumulative silk emergence (silks female ha$^{-1}$). The dashed gray line indicates cumulative kernel set. The dashed black line represents the temporal dynamic of silks at risk (silks female ha$^{-1}$ d$^{-1}$). Values for simulated seed production (seed number female ha$^{-1}$) and calculated RI are shown for each field.
Figure 3. Simulated change in RI (dashed line) and kernel yield (solid line) due to variation in the anthesis silking interval (A1SI) in the four selected fields. Vertical lines represent the actual A1SI for each field.
Figure 4. Simulated change in RI (dashed line) and kernel yield (solid line) due to variation in the amount of tassel pollen production in the four selected fields. Vertical lines represent the actual pollen production per tassel for each field.
Figure 5. Simulated percentage of out-crossed kernels in the four selected fields assuming an arrival of 1 adventitious pollen grain per cm$^2$ each day.
Figure 6. (A) Simulated genetically-pure and self-pollinated kernel production in Field L. The solid and dashed black curves indicate male and female inbreds pollen shed curves respectively (pollen grains cm$^{-2}$ d$^{-1}$). The solid gray curve indicates cumulative silk emergence (silks female ha$^{-1}$). Dashed gray lines indicate genetically pure (above) and self-pollinated (below) cumulative kernel set. Total genetically-pure and self-pollinated seeds per female ha are indicated. In this analysis, the average female tassel was considered to shed 1,000,000 pollen grains in a period of 5 days. Only 0.5% of the female population was assumed to escape detasseling (or to break tassel sterility). (B) Simulated percentage of self-pollination for a range of female anthesis-silking intervals. The arrow indicates the female ASI represented in A.
CHAPTER 8. GENERAL CONCLUSIONS

Since the introduction of hybrid seed production, much effort has been directed towards managing pollination to ensure maximum kernel set and high levels of genetic purity. Managing pollen dispersion also is an important consideration in seed production, particularly for managing genetically-modified (GMO) materials, due to the potential for flow of transgenes into landraces, wild relatives of maize, and non-GMO commercial hybrids. Managing for maximum kernel set and 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 laid the groundwork for achieving both high kernel set and levels of genetic purity. It provides enhanced understanding of the dynamic nature of flowering and pollination processes, and a quantitative assessment of kernel set and risk of out-crossing based on flowering dynamics.

Two novel methods to quantify pollen shed are presented. The first one offers a simple way of quantifying the timing and intensity of pollen shed in a field scale. The accuracy observed across a broad range of pollen shed densities indicates this technique would be well suited for characterizing pollen production in seed production fields and quantifying pollen dispersal downwind of corn fields. Another positive feature of this approach is that pollen traps can be stored for extended periods without affecting the counting results. The second method provides a way of relating genetic differences in pollen production to the morphological characteristics of plant tassels. The Tassel Area Index is an interesting alternative to assess pollen production per plant primarily due to its speed and simplicity. The index as currently defined, however, is not sufficiently accurate to distinguish 'maleness' of inbreds within a heterotic group. Current studies are aimed to refining this measurement technique to account for genetic variation in floral density and structure.

These techniques are useful for evaluating maize pollen production and stability of pollen production across environments. But they do not distinguish viable from nonviable pollen at the point of collection. An original technique to assess loss
of pollen viability during its transport in air based on environmental conditions (i.e.
temperature and relative humidity) is included in this dissertation. It proved to be
simple and reliable.

A mathematical model developed to simulate potential kernel set from
seasonal dynamics of pollen shed and silk exsertion is described. This model can be
applied to simulate kernel production in hybrid seed fields from the flowering
dynamics of the inbreds with great success. It provides an easy and reliable
approach to assess the impact of seed production practices on kernel production
and to define management strategies to maximize seed production per female
hectare. The approach for simulating kernel production also provides the
mathematical basis for testing the impact of female or adventitious pollen entry on
the potential for out-crossing and self-pollination events in the hybrid seed field. A
distinction between the 'inherent risk', determined solely by the flowering
characteristics of the inbred pair, and the 'external risk', which incorporates the
amount of female or adventitious pollen that could drift into the field, is proposed. An
ongoing investigation focuses on evaluating the usefulness of the 'inherent risk'
index as a simple quantitative approach for assessing actual out-crossing and self-
pollination in commercial seed fields.
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