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Maize Pollen Longevity and Distance Isolation Requirements for Effective Pollen Control

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ABSTRACT

Pollen is an important vector of gene flow in maize (*Zea mays* L.). Experiments were conducted to investigate the duration of pollen viability and the effectiveness of isolation distance for controlling gene flow. Pollen longevity was tested by collecting pollen at dehiscence and exposing it in a thin layer in the open air and sunshine for prescribed time periods before assessing pollen viability by measuring seed set after pollination and scoring visual appearance. Isolation distance efficacy was evaluated by growing 12.8-m^2 plot of maize at various distances from a 4000-m^2 pollen source. The pollinator contained either a genetic leaf or seed marker that allowed pollen flow to be measured. Pollen maintained viability for 1 to 2 h after dehiscence depending on atmospheric water potential. The theoretical, maximum distance viable pollen could move was 32 km, assuming pollen was transported linearly at the maximum average afternoon windspeeds for our location, viability was maintained for 2 h, and pollen settling rate was ignored. Cross pollinations occurred at a maximum distance of 200 m from the source planting, and only a limited number of cross pollinations occurred at the shortest distance (100 m). No cross pollinations occurred at 300 m from the source planting. The results are consistent with conclusions that maize pollen is desiccation intolerant and has a high settling rate. The results indicate isolation distance can be a useful tool for controlling gene flow via pollination in research scale plantings.

Considerable research has been conducted on the effects of gene flow from improved cultivars of maize into the landraces and teosinte that grows in the ancient farming areas of Mexico (Cervantes, 1998; Serratos et al., 1997). This research has focused primarily on conventionally derived, improved cultivars and their impact on germplasm diversity in farmers' fields. Maize production in these ancient farming areas can be thought of as peasant farmers' management of the maize genome within its Mesoamerican region of domestication (Bellon and Brush, 1994). Crop evolution in this context is the interaction of the plant genome and the accumulated experience of the cultivators with their crop. The process of local selection over the past 7000 to 10 000 yr has resulted in many distinct landraces of maize in south-central and southwestern Mexico (Goodman, 1988).

Those who conserve maize genetic resources are concerned that the introduction of improved varieties into these regions will result in the loss of genetic diversity. Iltis (1974) proposed the creation of in situ conservation of germplasm to complement ex situ conservation strategies. In situ conservation refers to the preservation of an entire agrosystem within a biological reserve. Controversy generated by such proposals led to research on the level of germplasm knowledge and its selection by farmers in the states of Chiapas and Jalisco (Bellon and Brush, 1994; Louette and Smale, 1996; Louette et al., 1997). This research indicated that present diversity in maize is the result of relatively controlled introductions of genetic material and not of geographical isolation. Farmers maintain cultivars through seed selection. Spatial and temporal separation are also used to maintain desired cultivars. When thought of in this manner, local cultivars constitute genetic systems that are open and evolving. The openness is a reflection of the curiosity and willingness of traditional farmers towards using new cultivars to solve their requirements for sustenance. Over the millennia, this approach has likely been a significant causal factor in the creation of modern maize (Louette et al., 1997).

More recently, concern has developed about the potential flow of transgenes from commercial cultivars into landraces and wild relatives of maize (Serratos et al., 1997). The two principal fears surrounding the introgression of transgenes into wild relatives and land races are possible genetic erosion and increased weediness of maize or teosinte plants containing a transgene (Rogers and Parkes, 1995). Two important vectors of introgression are seed and pollen. Farmer exchanges of commercial seed obtained from previous plantings can result in the transfer of transgenes into another area (Serratos et al., 1997). This process has been slowed by a moratorium on movement of maize seed containing transgenes into Mexico declared by regulatory officials.

Pollen management has begun to be investigated as a method for researchers to limit transgene flow in Mexico while allowing field research to continue. To date, research has focused on documenting the efficacy of detasseling as a method of controlling pollen shed as well as documenting cross pollination resulting from small scale plantings typical of research-scale plots (Garcia et al., 1998). Results indicated that routine breeding activities

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could be conducted with transgenic maize without danger of pollen dissemination and gene escape if transgenic plants were used as females and detasseled prior to anthesis. As might be expected, results also indicated that it is more difficult to control pollen dissemination if transgenic plants are used as pollinators. Precautions in addition to those described by Garcia et al. (1998) would be necessary to provide complete pollen control if 0.1% outcrossing to adjacent rows is deemed unacceptable for transgenic cross pollination.

Research on the use of spatial isolation to control maize hybridization can be difficult to evaluate. This difficulty is due to the variety of experimental conditions employed by researchers and, in some situations, the lack of essential information they provided in their reports. For example, Bateman (1947a,b) performed several experiments but, because the small source of pollen was surrounded by a 3.3-m-high wall, his results were biased by reduced air circulation. Jones and Brooks (1950), in contrast, planted a very large source of pollen and likely had much greater wind speeds in their prairie locations in Oklahoma. As a result, the isolation distance required for purity was larger. In a thorough analysis of pollen dispersion and deposition, Raynor et al. (1972) measured maize pollen flow in terms of meteorological theory. They showed that maize pollen was not transported as far by the wind as pollen from several other wind pollinated species. Additionally, the authors documented dispersal both horizontally and vertically and noted that much of the pollen settled to earth within the source itself. Pollen concentrations 60 m from the source in the downwind direction averaged 1% of those at 1 m. Variables identified as important in deciding effective isolation distance were the size of the source planting and windspeed. Collectively results such as these, combined with experience have resulted in the seed industry practice of using 185 m to isolate adjacent plantings of maize.

Various models of pollen dispersal by wind pollinated conifers have been investigated and reviewed (Di-Giovanni and Kevan, 1991). Important physical factors found to influence dispersal were gravity, wind speed and direction, turbulence, air density, and air viscosity. Biological parameters that influence the effect of these factors include pollen density, pollen radius, and sedimentation velocity. Subsequent research (Di-Giovanni et al., 1995) that included measurements on maize pollen along with pollen from various wind pollinated trees, found maize pollen settled at approximately an order of magnitude faster rate than pollen from all of the other plant sources. Maize pollen was also found to settle at an approximately 30% faster rate than predicted by Stoke's Law. However, the combined understanding of the physical and biological aspects of maize pollen movement such that one can predict how it will move and how long it will retain viability is incomplete.

Knowledge of maize pollen biology is helpful in understanding various methods of managing pollen flow. Maize pollen is large, 90 to 100 μm in diameter, and spherical in shape (Wodehouse, 1935; Jones and Newell, 1948). It is among the largest particles that are commonly airborne (Raynor et al., 1972). At anthesis, water comprises about 60% of the fresh weight of maize pollen (Kerhoas et al., 1987). This is one of the highest water contents among angiosperm pollens (Hoekstra, 1986). Despite having a relatively low water potential at dehiscence (Schoper et al., 1987a; Westgate and Boyer, 1986a), maize pollen is generally considered desiccation intolerant relative to pollen of other species since it loses water rapidly and viability decreases sharply if grains are dried to water contents less than 0.4 g g^{-1} (Buitink et al., 1996). After anthesis, pollen dehydrates as it moves through the atmosphere until it lands on a receptive stigma. Because the atmosphere is quite dry, the pollen grains must land on a receptive silk shortly after being shed. Upon landing on a receptive stigma, the pollen absorbs water from the stigma and proceeds to germinate (Heslop-Harrison, 1979). Other factors such as high temperature can also negatively affect pollen viability (Roy et al., 1995; Schoper et al., 1987a,b). In commercial maize production fields, these risks are usually overcome by the copious quantities of pollen that are shed (Kiesselbach, 1999).

The objectives of our studies were to develop an improved understanding of the factors that influence pollen longevity in the field and to determine the required isolation distances for complete pollen control in a region of Mexico that is particularly suitable for maize research activities.

MATERIALS AND METHODS

Experiments were conducted at the Pioneer Hi-Bred International, Inc. Research Center near San Jose del Valle, Nayarit, Mexico (20° 49′ North, 105° 55′ West, elevation 26 m). Environmental and topographical features of the area are described in Garcia et al. (1998).

Experimental Procedures

Cultural Procedures

Experiments were conducted during 1997 to 1999 and all plants were grown using standard, optimal cultural practices for maize production. Fertilizer was applied at a rate of 220, 92, and 30 kg ha^{-1} of N, P, and K, respectively. To control weeds, the herbicides metolachlor, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide, and atrazine, 6-chloro-*N*-ethyl-*N*′-(1-methylethyl)$^{-1}$,3,5-triazine-2,4-diamine, were applied at rates of 1.4 and 1.3 kg a.i. ha^{-1}, respectively. All plots were furrow irrigated 6 to 8 times following normal local practices. To control insects, the insecticides carbofuran, 2,3-dihydro-2,2-dimethyl-7-benzofuranylmethylcarbamate, and permethrin, (3-phenoxyphenyl)methyl (±)-cis,trans - 3 -(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate, were applied at a rate of 0.5 and 0.4 kg ha^{-1} at planting. Additional applications of foliar insecticides and fungicide were made as needed to maintain healthy plants and produce healthy seeds. All plots were grown at a density of 50 000 plants ha^{-1} and the spacing between rows was 0.8 m.

Pollen Longevity Experiments

The experimental design for measuring pollen viability as measured by seed set was a randomized complete block with four replications. The replications were blocked in time with

one complete replication of treatments being completed per day. Blocking in time provided insurance against inclement weather. No precipitation occurred on any of the days when treatments were applied so all replications of data were used in the analysis of variance. Seven pollen treatments were common to both years and one additional treatment was added to the experiment that was planted in 1998. Four samples or plants were pollinated per treatment per day. The planting dates were 27 Nov. 1997 and 30 Dec. 1998 and the pollination dates were 27, 28, 29, and 30 Jan. 1998 and 10, 11, 12, and 13 March 1999. The single cross hybrid P3394 was used and plot row length was 5 m.

The seven pollen treatments that were used to generate seed set data in both years were: control (immediate crossing) and crosses made after 0.25, 0.5, 1, 2, 4, and 6 h of exposure to ambient atmospheric conditions. The additional treatment added to the experiment planted in 1998 was a control designed to test the level of contamination by inadvertent pollinations when the shoot bag was removed to allow pollination. In this treatment the bag was removed from the earshoot in the same manner as the other treatments but no pollination was made and a new bag was placed over the earshoot. Contamination levels were 2 to 3 kernels per ear in the experiment planted in 1997 and 0 in the experiment planted in 1998.

Additional visual scores of pollen condition for all treatments were also made immediately prior to pollination only in the 1998 experiment. These data were analyzed in the same manner as the seed set data except there was no year replication. The visual scores included the percentage of pollen grains that were spherical in shape and white in color when placed on a black background (the appearance of freshly dehisced pollen), the percentage that developed an intense yellow color, and the percentage of collapsed pollen. The scoring of pollen appearance was done at ×25 magnification with a hand lens in the field.

At anthesis, approximately 10 mL of pollen was collected at the onset of dehiscence, which was between 1000 and 1100 h. All pollen was sieved to remove anthers and insects. The pollen was evenly distributed as a thin layer on a white plastic tray and allowed to dry in the open air and sunshine for the prescribed time for each treatment. Pollinations were made with a uniform volume of pollen (~2 μL) onto ears with similar silk lengths (8–9 cm). This volume of pollen is limiting to seed set on fully developed ears enabling us to measure fractional reductions in viability (Schoper et al., 1987a). Immediately before pollination the silks were trimmed to 2.5 cm of exposed silk tissue to facilitate more uniform pollen distribution over the silks.

Distance Isolation Experiments

The experimental design for all experiments involved planting a square, 4000-m^2 block of an inbred pollen source that carried a genetic marker and surrounding it with 4 to 5 locations in each cardinal direction with the white seeded hybrid P3428 to assess the frequency of cross pollination. Each plot of the white seeded hybrid consisted of 4 rows, 4 m long. The distance of these plantings from the pollen source was 100, 200, 300, and 400 m in experiments with the exception of the addition of a 150-m plot in one of the experiments when it was repeated for the second year. This experiment therefore contained 20 locations of P3428.

Leaf Marker Experiments. Plants of a yellow seeded inbred line, AS44, carrying two dominant genetic markers (B, Pl) for intense purple leaf color were used as apollen source in an experiment planted on 27 Nov. 1997 and 30 Dec. 1998. A progeny test was performed to assess cross pollination with AS44 because other yellow seeded material was grown in the area and the purple leaf trait would be expressed in the F_1 generation. The progeny tests for purple leaf phenotype involved planting all yellow seeds collected from the ears of P3428, seeds from AS44, and a sample of the white colored seed from the P3428 ears. The seeds of AS44 and P3428 ears were included as controls to ensure the correct identification of phenotype. The progeny tests were planted in May of each year. The number of purple leafed plants were then counted.

Seed Marker Experiment. Seeds of a second, non-Pioneer coded inbred line were planted in the same design on 30 Dec. 1998. This inbred line was homozygous for the genetic markers R-r, C1, C2, A1, A2, Bz1, Bz2, and Pr. Collectively, the phenotype for this genetic combination was observable directly on the P3428 ears as purple colored F_1 seed.

Plots were monitored for synchrony to ensure that the pollen shedding of the inbreds containing the genetic markers was coincident with silk emergence of the white seeded hybrid. The 50% pollen shed and 50% silk emergence dates for the experiments involving AS44 were on the same day in the 1997 planting and 50% pollen shed of AS44 preceded 50% silk emergence of P3428 by 1 d in the 1998 experiment. In the experiment involving the purple seed genetic marker, 50% pollination preceded 50% silk emergence of P3428 by 2 d.

Atmospheric Water Status

Atmospheric water status was assessed by calculating atmospheric water potential (Ψatm) from temperature and relative humidity (Nobel, 1974). Temperature and relative humidity were measured by a hygrothermograph (Oakton, series 025028, Japan) located in the field where the experiment was conducted. Atmospheric water potential was calculated as follows:

$$\Psi\text{atm} = \frac{RT}{V}\ln\left(\frac{\%\text{RH}}{100}\right)$$

where Ψatm = atmospheric water potential (MPa), R = ideal gas constant = (0.0083 L MPa mol^{-1} deg^{-1}), T = absolute temperature (K), V = molar volume of water = (0.018 L mol^{-1}) and RH = percent relative humidity.

RESULTS

Atmospheric water status differed during the pollination treatments in the two years (Table 1). Temperatures were higher and the relative humidities were lower during pollen shed in March 1999 compared with January 1998. This combination of temperature and relative humidity resulted in a lower Ψ_{atm} in March 1999 relative to January 1998 by an average of 21 MPa from 1000 to 1700 h. These results contrasted with the typical 5 yr averages that show January as cooler and drier than March. The difference between the two months in Ψ_{atm} on the basis of 5-yr average temperatures and relative humidities for 1000 to 1700 h was 53 MPa with January having a lower Ψ_{atm} than March.

Viability of pollen as measured by seed set declined by an average of 80% after 1 h and 100% after 2 h of atmospheric exposure (Fig. 1). The results for the 1 h of atmospheric exposure treatment differed between the two years. Pollen viability decreased by 96% within 1 h of dehiscence in the year with lower Ψ_{atm}. In the more humid year, pollen viability was reduced by 58%

Table 1. Atmospheric conditions for the days on which pollinations were made in 1998 and 1999 and the 5-yr mean atmospheric condition for the same days.

	Four-day mean†						Five-year mean					
	January 1998			March 1999			January 1994 to 1999			March 1994 to 1999		
Time	Temperature	Relative humidity	Ψatm	Temperature	Relative humidity	Ψatm	Temperature	Relative humidity	Ψatm	Temperature	Relative humidity	Ψatm
hour	°C	%	MPa	°C	%	MPa	°C	%	MPa	°C	%	MPa
0800	15	89	−15	17	94	−8	15	68	−53	17	87	−19
0900	18	70	−48	22	65	−59	18	48	−99	22	63	−63
1000	22	57	−77	25	52	−90	22	37	−135	26	53	−88
1100	25	49	−98	28	44	−114	25	31	−161	29	49	−99
1200	27	48	−102	29	45	−111	27	31	−162	29	50	−97
1300	28	58	−76	29	50	−97	28	38	−134	29	53	−91
1400	27	59	−73	28	50	−96	27	38	−134	29	53	−89
1500	27	59	−73	29	48	−102	27	40	−127	30	53	−89
1600	26	62	−66	28	52	−91	27	44	−114	29	55	−83
1700	26	70	−49	28	57	−78	27	51	−96	28	60	−71
1800	24	78	−34	24	69	−51	25	60	−70	26	74	−42

† The pollination dates were 27, 28, 29, and 30 Jan. 1998 for the experiments that were planted on 27 Nov. 1997. The pollination dates were 10, 11, 12, and 13 March 1999 for the experiments that were planted on 30 Dec. 1998.

after 1 h of atmospheric exposure. No viable pollen was detected after 2 h of atmospheric exposure in either year. A slight increase in seed set occurred in both years 15 to 30 min after pollen collection.

Visual characteristics thought to be potentially useful as easily estimated measures of pollen viability were scored immediately before pollination during the March 1999 pollinations (Fig. 2). All scores changed with time and appeared to be related to viability (compare Fig. 2 with Fig. 1). The percentage of spherical, white colored pollen grains decreased from 100% immediately after anthesis to 0% at 1 h after anthesis. The intense yellow color characteristic of aged or dead pollen correlated well with percent collapsed pollen.

The maximum distance over which out-crossing occurred from any of the 4000-m^2 genetic marker pollen source plantings was 200 m (Table 2). Two cross pollinations occurred when the purple seeded genetic marker was used in the genetic marker pollen source planting. Both were single kernel events, one at 200 m North and one at 150 m South of the pollen source. One cross pollination in the 100-m South trap was produced by the pollinator containing the purple leaf marker.

DISCUSSION

During the normal process of cross-pollination in maize, pollen is shed from the anther in the mid-morning hours. At anthesis, the pollen is partially dehydrated and it continues to dehydrate as it moves through the atmosphere until it intercepts a silk (Hoekstra, 1986; Kerhoas et al., 1987). Because dehiscence normally oc-

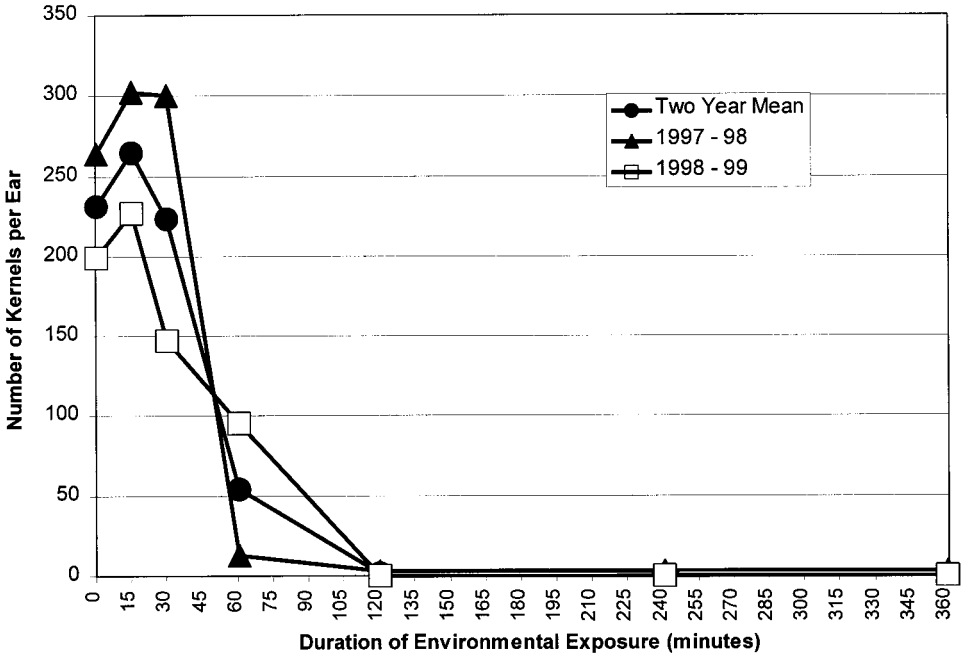

Fig. 1. Loss of pollen viability as measured by the ability to produce seed. Fresh pollen collected from shedding anthers was exposed to atmospheric conditions for various times prior to its use in pollination. The average standard error for the 2-yr mean kernel number is 22. The average standard error for individual means within each year was 38 in the 1997 experiment and 23 in the 1998 experiment.

Table 2. Cross pollination frequency at various distances between pollen from the planting of material containing a genetic marker and silks of the plantings of the white seeded hybrid P3428†. Purple plants and purple seeds indicate cross pollinations in the leaf and seed marker experiments, respectively.

Distance from the pollen source	Direction relative to the pollen source											
	North			South			East			West		
	Leaf marker experiments		Seed marker experiment	Leaf marker experiments		Seed marker experiment	Leaf marker experiments		Seed marker experiment	Leaf marker experiments		Seed marker experiment
	Purple plants 1997	Purple plants 1998	Purple seeds	Purple plants 1997	Purple plants 1998	Purple seeds	Purple plants 1997	Purple plants 1998	Purple seeds	Purple plants 1997	Purple plants 1998	Purple seeds
m	number/plot											
100	0	0	0	1	0	0	0	0	0	0	0	0
150	–	–	0	–	–	1	–	–	0	–	–	0
200	0	0	1	0	0	0	0	0	0	0	0	0
300	0	0	0	0	0	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0	0	0	0	0	0

† The pollen source containing a genetic marker was planted in a 4000-m^2 square block. The plots of P3428 were 12.8-m^2 square blocks.

curs in mid-morning when the temperature is typically increasing, relative humidity decreasing, and radiation load increasing pollen loses viability quickly. In our experiments, 100% was non-viable within 2 h in both years and within 1 h in the year with the drier atmosphere. The slight increase in seed set that occurred within the 15 to 30 min of pollen collection likely indicates additional silks had emerged from the husk cover on the ear and that all of the pollen was still viable in these treatments. Silk elongation occurs at a very rapid rate (2–5 mm h^{-1}) during the morning hours in well watered maize (Johnson and Herrero, 1981; Schoper and Martin, 1989).

These results are consistent with the observations that maize pollen is desiccation intolerant and loses water and viability because of desiccation rapidly after dehiscence as is found in Gramineae generally (Buitink et al., 1996; Hoekstra, 1986; Kerhoas et al., 1987). The environmental conditions during our experiments were typical for the region and many areas where maize is adapted, i.e., mean daily high temperatures of 28 to 30°C and mean daily minimum relative humidities of 31 to 53%. Maize pollen has a uniquely low water potential (Ψ_w) at dehiscence, about −2 to −3 MPa (Schoper et al., 1987a; Westgate and Boyer, 1986a) and seed has been produced when pollinations were made with pollen having a Ψ_w as low as −12.5 MPa (Westgate and Boyer, 1986b). Our calculations of minimum daily Ψ_{atm} during this experiment still exceed this very low pollen Ψ_w by 7 to 9×. The 5-yr average minimum daily Ψ_{atm} for January at −162 MPa is 13-fold lower than the lowest pollen Ψ_w measured by Westgate and Boyer (1986b). Given the arid atmosphere, one would expect that pollen would be desiccating very rapidly during our experiments and this

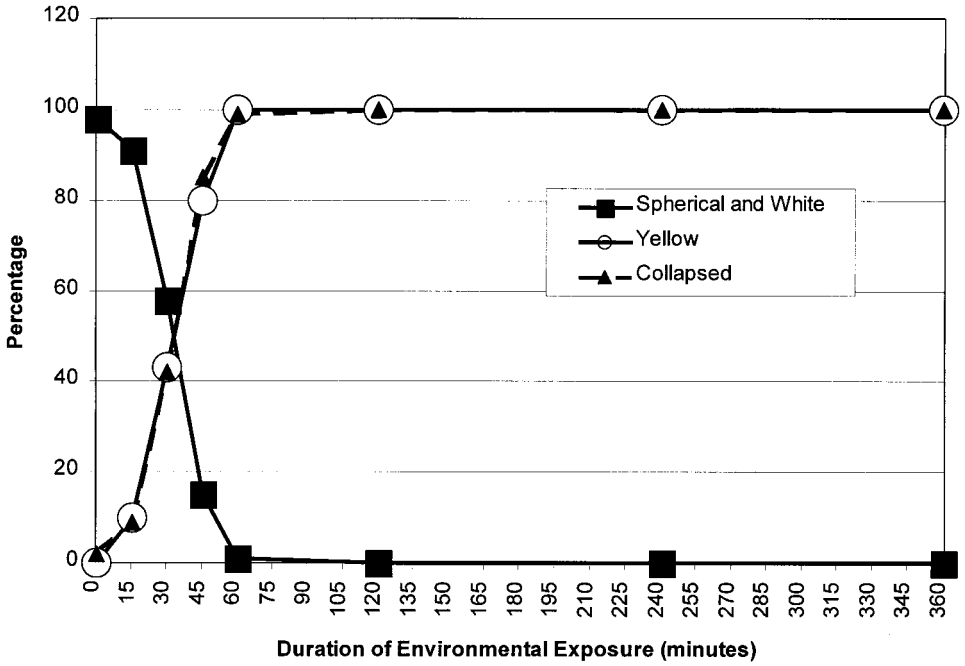

Fig. 2. Changes in pollen visual appearance after being exposed to atmospheric conditions for various amounts of time in the 1998–1999 experiment. All scores were made in the field immediately prior to pollination and at a ×25 magnification level. The average standard error of a mean for the yellow, collapsed, and spherical pollen scores was 11.

is supported by the collapse or loss of turgor of the pollen grains illustrated in Fig. 2.

Changes in the visual appearance of the pollen correlated well with losses in viability. Plump and spherical shaped pollen was viable. Maize pollen contains large amounts of starch and osmotically active solutes thus enabling turgor to be maintained at low Ψ_ws, e.g., −3 MPa (Goss, 1968; Schoper et al., 1987a). Even so, pollen with some degree of collapse likely remains viable. Our estimate of percent collapse was conservative in that great deformation of the pollen wall was needed to affect the score. The correlation of reduced seed set with the development of intense yellow coloration was also high and provides another means of estimating viability. Collectively, our results document that assessing pollen appearance in the field with a relatively low magnification hand lens (×25) is a quick and useful method for monitoring pollen viability. Our results also indicate that pollen exhibits fractional loss of viability as it dries. These results suggest that the use of nonlimiting amounts of pollen by Westgate and Boyer (1986b) may have caused them not to detect a decline in viability as pollen approached very low Ψ_w, e.g., –12.5 MPa.

The amount of outcrossing between the block of material carrying the genetic marker and the plots of the white seeded hybrid was very low. Only three cross pollinations were observed across all experiments, one in each of the 100-m South, 150-m South, and 200-m North plots. Pollen source strength should have been ample because the size of the pollinator blocks was large enough (4000 m^2 or approximately 20 000 plants each) to generate considerable pollen, approximately 40 000 million pollen grains, if one assumes 20 million pollen grains are liberated per tassel as calculated by Kiesselbach (1999). Tassel size was large on this material so this may be a reasonable assumption. The theoretical number of receptive silks was also large for each plot of P3428. If the number of 1000 silks per developing ear is representative (Kiesselbach, 1999), there would have been 64 000 emerged silks available to intercept pollen per planting of P3428. Even though the numbers of pollen grains and silks were large it is important to note that these experiments were intended to investigate research scale plantings. Pollen source strength has been found to be an important variable in modeling pollen dispersal (Di-Giovanni and Kevan, 1991) and dispersal dynamics could be different on commercial scale plantings.

Our cross pollination data were consistent with the prevailing wind speeds and previous experiments related to isolation distance and settling rate of maize pollen. Our experimental area is relatively calm with an average maximum wind speed of <16 km h^{-1} during the afternoon and lower wind speeds during the morning hours when pollen is shed (Julio Nava, 1997, personal communication). Jones and Brooks (1950) measured outcrossing from maize at 503 m from the source pollen in Oklahoma, which is a relatively windy state. Raynor (1972) noted 60 m as the maximum distance for outcrossing in New York, which is less windy and cooler than Oklahoma. In Cuzalapa, Jalisco, Mexico and Iguala, Guerero, Mexico, Cervantes (1998) determined the maximum distance for outcrossing in maize to be 32 m. Both these Mexican locations typically have low wind speeds during the time of year when the experiments were conducted. In the experiments of Raynor (1972) and Cervantes (1998), 63 and 54% of the pollen shed was deposited within the source planting itself. The relatively high settling velocity of maize pollen (31 cm s^{-1}, measured by Di-Gionvanni et al., 1995) combined with the range of wind speed experienced in these investigations confirms a relatively short dispersal distance for maize pollen. These results also illustrate the very low probability that viable pollen shed into the air will intercept a receptive silk in a non-target field at some distance.

Collectively, our results provided useful information for managing pollen flow via isolation distance. It was clear maize pollen does not remain viable in the atmosphere for longer than 2 h near San Jose del Valle, Nayarit, Mexico. Theoretically, viable pollen could move 32 km in this time if the wind speed during the morning pollen shed period was equivalent to the average maximum windspeed during the afternoon, pollen movement was linear, and the pollen did not settle. Actual measurements of pollen movement as measured by outcrossing from research scale plantings of material, indicated that viable pollen was barely detectable at 200 m and nonexistant at 300 m from the pollen source. The difference between the theoretical and actual measurements is likely a function of pollen settling and dessication combined with variations in the local physical factors controlling pollen movement, e.g., wind speed and air turbulence is typically lower during the morning than in the afternoon, etc.

Decisions regarding what might constitute adequate pollen control to protect landraces and teosinte in Mexico will depend on biological and nonbiological factors (Alvarez-Morales, 1999). Our research demonstrates and documents that distance isolation is an effective tool for managing pollen flow in research scale plantings. Its use in conjunction with other pollen management tools, e.g., detasseling (Garcia et al., 1998), temporal isolation, monitoring of known teosinte populations (Sanchez et al., 1998), and managing the scale of pollen release allows considerable management of gene flow. The extent to which precautions need to be applied to pollen flow depends ultimately on the implications of the flow of novel genes. If the consequences of novel gene flow are biologically significant, more precaution will need to be exercised than if experiments demonstrate no significant biological impact of the novel genes beyond that of traditional breeding activities.

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