Rev. FCA UNCUYO. 2017. 49(2): 51-66. ISSN impreso 0370-4661. ISSN (en línea) 1853-8665.

Sorghum (*Sorghum bicolor*) pollen availability and seed set under different proportion male:female plants in Mexican highlands

Disponibilidad de polen y producción de semilla en sorgo (*Sorghum bicolor*) bajo diferente proporción de plantas macho y hembra en valles altos de México

María E. Cisneros-López [1], Alberto J. Valencia-Botín [2], Yokiushirdhilgilmara Estrada-Girón [3]

Originales: *Recepción:* 28/05/2014 - Aceptación: 04/12/2015

Abstract

The availability of pollen in sorghum (*Sorghum bicolor* [Moench] L.) production is generally not considered a restrictive factor due to the sorghum self-pollination process. However, during the cross pollination process restrictions could play a role that depends on the distance of the pollen source, the proportion of male to female plants and the sowing date. The objective of this experiment was to establish the relationship between pollen production, deposition and season variations in seed set under different proportion male:female rows. The A9/B9-line was sowed on May 3, June 10, 2005, with the proportions: 2:4, 2:6 andon April 3, 2006 with proportions of 2:12 and 2:16 in Montecillo, State of Mexico. In isolines, number of flowers per panicle was measured at the ending of flowering. During all the flowering period, pollen production was quantified in male line and pollen deposition on female line rows using passive traps. During harvest the length of panicle was measured, quantified by the number of seeds per panicle and seed set was estimated. The pollen availability, synchrony floral, duration of phenological stage and seed production were influenced by sowing date and varied with the male and female rows. Precocity was observed in A9 female isoline more than B9-male line in three sowing dates. Number of pollen grains and seeds declined proportionally with increasing distance from the pollen source, but the rate was different for each proportion. The pollination was associated more to the size of pollen source and its dispersion than with population of female plants.

Keywords

Sorghum bicolor • floral synchrony • pollen production • pollen availability

1 INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias), Campo Experimental Rio Bravo, Tamaulipas, México.

2 Universidad de Guadalajara, Centro Universitario de la Ciénega. Av. Universidad 1115, Ocotlán, Jalisco, México. botin77@gmail.com (Corresponding author's).

3 Departamento de Ingeniería Química, Universidad de Guadalajara, Blvd. M. García Barragán 1451, Guadalajara, Jalisco, México.

Resumen

La disponibilidad de polen en la producción de sorgo (*Sorghum bicolor* [Moench] L.) generalmente no se considera un factor restrictivo debido al proceso de auto polinización; sin embargo durante la polinización cruzada diversas restricciones como la distancia de la fuente del polen, proporción de plantas macho y hembra y la fecha de siembra, podrían jugar un papel importante. El objetivo de este experimento fue establecer la relación entre la producción, deposición y cambios estacionales de polen en la producción de semilla; utilizando diferentes proporciones de surcos masculinos:femeninos. La línea A9/B9 se sembró el 3 de mayo y 10 de junio de 2005 con las proporciones 2:4, 2:6 y el 3 de abril de 2006 con los proporciones 2:12 y 2:16 en Montecillo, Estado de México. El isolíneas, se midió el número de flores por panícula al final de la floración. Durante todo el período de floración, se cuantificó la producción de polen en línea masculina y la deposición de polen en surcos femeninos, utilizando trampas pasivas. En la cosecha se midió la longitud de la panícula, cuantificado por el número de semillas por panícula y la producción estimada de semillas. La disponibilidad de polen, sincronía floral, duración del estado fenológico y la producción de semillas fueron influenciadas por la fecha de siembra y varió por los surcos masculinos y femeninos. Se observó precocidad en la isolínea femenina A9 más que en la línea masculina B9 para las tres fechas de siembra. El número de granos de polen y semillas declinó proporcionalmente según se incrementó la distancia de la fuente de polen, pero la tasa fue diferente para cada proporción. La polinización se asoció más con el tamaño de la fuente de polen y su dispersión más que con la población de plantas femeninas.

Palabras clave

Sorghum bicolor • sincronía floral • producción de semilla • disponibilidad de polen

Introduction

The pollination and floral biology of sorghum [*Sorghum bicolor* (L.)Moench] are important factors for hybrid seed production (33) and relate to the incidences of sorghum midge [*Contarinia sorghicola* (Coquillet)] and sorghum ergot (*Claviceps africana* Frederickson, Mantle and de Millano) (16, 18). In anemophilous species the floral morphology, synchrony and weather conditions affect the pollen shedding (24).

The level of cross pollination varies with genotype of the male and female lines (31). The pollen emission is linked to the fraction of plants emitting pollen, pollen concentration in the air and pollen deposition within the crop (23). Successful pollination in seed plots depends on the pollen dispersion, space, and time restrictive factors (4, 35).

Temperatures under 12°C reduce the quantity of viable pollen in susceptible genotypes of sorghum. Low temperature could also affect stigma receptivity (26, 32) and the viability of pollen as measured by the seed set (9, 22).

Moreover, the abundant pollen is critical for genetic purity during cross pollination (1, 15). The combination of male per female rows and their densities for maximum yield per female is often based on practical experience rather than quantitative information on the flowering biology of crop (39). There is a general lack of information about the effectiveness of cross pollination in seed set of sorghum. Recently, studies have been conducted for evaluating the risk of GM flow (34), as well as the adverse effects of global climate change in pollen production, pollen viability and seed set (29, 32).

Objectives

a) Evaluate the changes of pollen availability by quantifying the pollen production of male plant and pollen deposition on passive pollen traps in female rows during flowering period,

b) Measure the floral synchrony in the pair A9/B9 isolines by flowering dynamics,

c) Measure the effect of distance to pollen source and female population in seed set of A9/B9 sorghum line using five proportions male:female rows (MFR) under field condition.

Materials and methods

Experiment settings

Experiments were conducted in Montecillo, State of Mexico (18°56'48" N; 97°49'54" W; 2240 m altitude). The male-sterile A9-line (A9 x B9) was sowed in May 3 and June 10, 2005. The proportion male:female rows (MFR) were 2:4 and 2:6. The number of female rows was increased in the year 2006 to 2:12 and 2:16 and the sowing date was April 3. In all the cases, harvest was completed by October. Orientation row plot was according to the dominant wind. The rows were of 20 meters long, with a spacing 0.90 m and 0.10 m among plants in both parents. Final plant population in female rows were 74,000 (2:4); 83,000 (2:6), 95,000 (2:12) and 99,000 (2:16). Each proportion represents male number rows *versus* female rows.

Ten rows of tall (3.0 m) maize (*Zea mays* L.) were used to wall and separate each subplot and boot stage in order to eliminate tassel and to tramp maize pollen during pollination. The plots were maintained as isolated for sowing date in order to avoid alien pollen on tramps.

The experiment was free of weeds and of watering. Air temperature (°C), relative humidity (%), rain (mm), direction and wind speed (m/s) at 2 m height were measured using a meteorological station located at 150 m from the experimental site.

Pollen production and viability

At the beginning of flowering; a branch from the middle of the panicle of five male-fertile plants was fixed in FAA (3.6% formaldehyde, 5% acetic acid and 50% ethanol in distilled water).

The pollen viability (PV) was determined in ten anthers per branch (five 20 grains fields per anther, on average) using acetocarmine with five repetitions. Pollen was considered fertile when it had a cytoplasm density of at least 75% (9).

In each sowing date at the beginning of flowering, 15 male plants were bagged for collection and quantified pollen production (PP) per plant per day until ending of flowering. The samples were dried at 75°C during 24 h and were weighed (Acculab Balance Mod. VI-3, precision = 0.001 g).

Pollen deposition

The pollen concentration was quantified during all the flowering period using passive traps of adhesive strip of paper tape (15 x 4 cm), attached to a metallic structure. The traps were orientated in a North and South direction (dominant winds). The first structure was placed inside the male row (0.0 m) and, subsequently each female row from 0.90 to 7.2 m, at the panicle high (1.50 m). At the pollination beginning, the pollen was collected from female rows until the ending of flowering of the male B9-line. The strips were placed since the morning (0900 h) until afternoon (1800 h).

Afterwards, they were taken and kept on plastic Petri dishes (8 cm). The number of pollen grains was counted in an area of 7 x 4 cm (28 cm^2) using a light microscope (Swift® No. 815944 in 10X). The variable was reported as the number of pollen grains cm^2 per day (PG), the total - pollen grains cm^2 per day of each proportion (TPG) and the pollen grains cm^2 per day per row of each proportion (PGR).

Floral synchrony

In each sowing date and proportion, 60 male and female plants at flag-leaf stage and complete competence were identified for measuring days to the beginning, (DB), days to 50% flowering or anthesis(DA) and ending of flowering (DE); flowering period (FP) (days from when the first plant began to flower and the last plant ended flowering). The flowering progress was evaluated as the percentages of florets which were exposed in the panicle (25, 50, 75 and 100%); the results were shown in the graphics. The total flowers per panicle in both lines (FFP) was quantify in 30 plants per proportion at the flowering ending.

Seed production and seed set

At harvest, in 30 plants per female row was quantified length of panicle (LP), the seed yield production per panicle (YP), 100 seeds weight (W100S), seeds number per panicle (SP = YP/W100S x 100). In this experiment, only SP was reported and the seed set was estimated by SS = SP/FFP

Data analysis

In all comparisons between the male and female lines (A9 *vs*. B9), sowing dates (D1 *vs*. D2, D1 *vs*. D3, D2 *vs*. D3) and the proportions male: female rows (2:4, 6.2, 2:8, 2:12 and 2:16) were made according to the Student "t" test. Simple linear regression analyses were conducted using the relationships to determine the relationship between PGR and SP and the distance to pollen emission source (0.9, 1.8, 2.7, 3.6, 4.5, 5.4, 6.3, 7.2 m.). The results were only shown by the third sowing date (April 3, 2006.) A simple linear regression was used between number of female rows (4, 6, 8, 12 and 16) and the variables LP, FFP, TPG, SP and SS (data average per proportion) and Pearson's correlations were calculated among these variables between PP and PG (daily data).

Results

Weather conditions

During all the flowering period were registered differences in quantity of rainfall among sowing dates. In the first and third sowing dates, the total rainfall was similar in duration and intensity: 18 d; 100±2 mm, whereas, in the second sowing date it was registered as 13 d with 52 mm. During all of the floral period the rainfall was recorded in the afternoon. The values of wind speed were 0.8-2.2; 0.5-1.5 and 0.5- 2.2 m/s (D1, D2 and D3, respectively). The average temperature fluctuated between 19±2°C in August, 2005 (D1), 17±2°C in September 2005 (D2), and 18.5±1.5°C in July, 2006 (D3). Averages of relative humidity were from 70-95%, 65-80% and 70-85% (D1, D2 and D3, respectively) with variations < 10 points.

Pollen production, viability and deposition

The variation in pollen production of line-B9 among dates was significant ($p < 0.01$) followed by a normal distribution during flowering period in three sowing dates. Curve shape varied with respect to quantity and distribution of pollen produced in each sowing date (figure 1, page 56). On average, the total pollen production per sowing date was of 169, 194 and 401 mg and pollen production per day was of 8, 9 and 18 mg in first May 3, 2005 second June 10, 2005 and third April 3, 2006, respectively. The results of pollen viability showed significant differences among sowing date 89±5.5, 80±6.8 and 76±9.5 (D1, D2 and D3, respectively).

Pollen grains were deposited on female panicle rows during the pollination; some peaks were formed during tramping period (figure 1d, e, f, page 56). The maximum quantity of pollen deposited (cm^{-2} d^{-1}) did not correspond with the maximum of pollen production (mg) in all dates. Pollen production was delayed by four and three days in first and third sowing dates on May 3, 2005 and on April 3, 2006 and it was speeded up for four days during the second day on June 10, 2005. Among sowing dates, the average pollen trapped was significantly different ($p < 0.01$) to the total of pollen deposition (868, 512 and 836 cm^2) and the counting per day was 42, 33, and 27 cm^2 (D1, D2 and D3, respectively).

Floral synchrony

At the anthesis, the female line showed more precocity than the male line of 2 until 9 d and its floral period was shorter than male line from 3 and 5 d. There were significant differences ($p < 0.01$) among dates in the floral behavior. On average, both parents flowered earlier in the second sowing date June 10, 2005 than the first date of the same year, for 9 days and 17 days, respectively, to the third sowing date April 3, 2006. On average, the floral period was similar among dates (20 d). The number of flowers per female panicle was 2003, 2155, and 1969 in average 2042 and 2228, 2301 and 2146 per male line in average 2225(D1, D2, D3, respectively). All differences were significant ($p < 0.01$).

The female parent had 5% (183) less flowers than the male line. In the field, both lines flowered heterogeneously in each proportion and sowing date. In figure 1 (page 56), it showed the flowering pattern in plant population in both line, in base on flowering opening of panicle in female (a, b, c) and male (g, h, i). On average, only 15% of female population began to flower near to 10% of plants was delayed (figure 1a, b, c, page 56) and the male line had similar response (figure 1g, h, i, page 56). A fraction of the female population was completely coincident with the maximum quantity of pollen production of male line (mgper day) during the pollination (figure 1d, e, f, page 56); the differences varied with the sowing date and this response was observed at the beginning of flowering by precocity of the female line (figure 1a, b, c, page 56). Thus, the average of flowers per panicle of the female line and its flowering progress between 25 and 50% of population had near to 400, 1000 and 1500 flowers exerted before pollination (D1, D2 and D3, respectively).

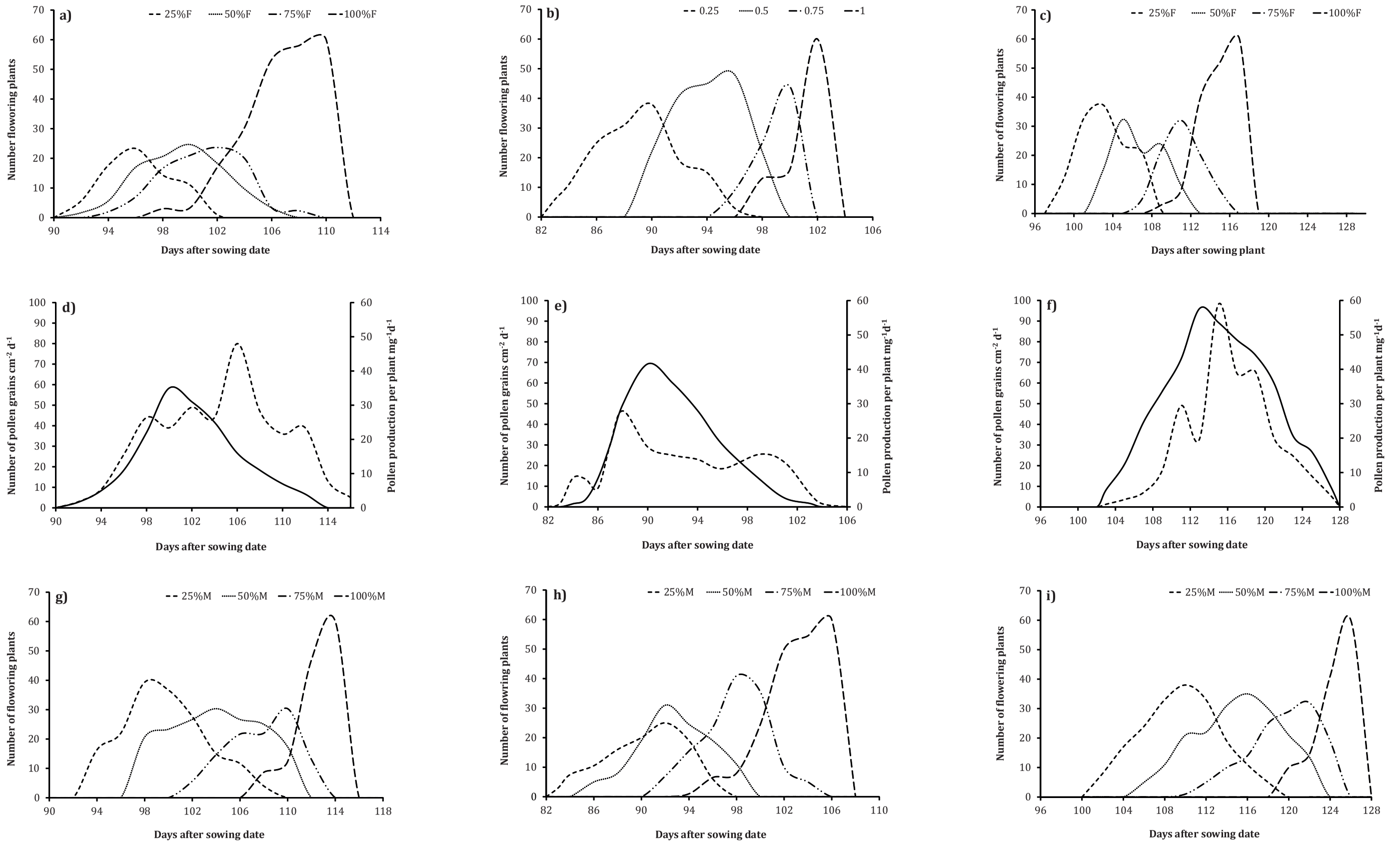

Figure 1. Progress flowering in population of female line (F= a, b, c) and male line (M= g, h, i), pollen production and deposition (d, e, f) in the sowing dates May 3, 2005 (a, d, g), June 10, 2005 (b, e, h) and April 3, 2006 (c, f, i).

Figura 1. Progreso de la floración de línea femenina (F= a, b, c) y línea masculina (M= g, h, i), producción de polen y deposición (d, e, f) en las fechas de siembra mayo 3, 2005 (a, d, g), junio 10, 2005 (b, e, h) y abril 3, 2006 (c, f, i).

Effect of distance in pollen deposition, seed production and seed set

The average of pollen grains trapped cm^2 per day (PGR) and seed per panicle (SP) for each proportion 2:8, 2:12 and 2:16 in year 2006 as a function of distance from the pollen source for each female row, based on simple regression analyses (figure 2). These results confirm the distance effect in the pollen deposition.

At 7.2 m of of distance from the pollen source, the number of pollen grains and seeds declined, but the rate of change was different for each proportion (figure 2).

The regression data showed that for each female row added (0.90 m) both variables in average diminished two times, in others words the tendency was similar (r=0.94).

Size source of pollen and seed set

Results from t-tests showed significant differences ($p < 0.01$) for the comparisons among sowing dates by floral and reproductive variables (table 1, page 58). On average, the second date (data of the proportions 2:4 and 2:6) was superior to the first (data of the proportions 2:4, 2:6 and 2:8) and third (data of the proportions 2:8, 2:12 and 2:16) dates in length of panicle (18.8±1.5 cm), flowers per panicle (2155±176), and seeds per panicle (1262±176) and seed set (0.62±0.20), which represented a difference of 11%.

Contrary to these results, the number of pollen grains cm^2 per day trapped during all of the flowering period was 40% less (512±22) than the first (868±25) and third (836±31) dates.

There were no significant differences among rows proportions, except for proportion 2:16 where the panicle was shorter than the average the others proportions; the panicle was shorter (16.7±0.1) with less flowers (1916±128) and seeds per panicle (969±146). These results represented 180 seeds (16%), however, there were no differences in seed set (0.51±0.07), in contrast to the proportions 2:8 and 2:12, although the proportion 2:16 had more female rows.

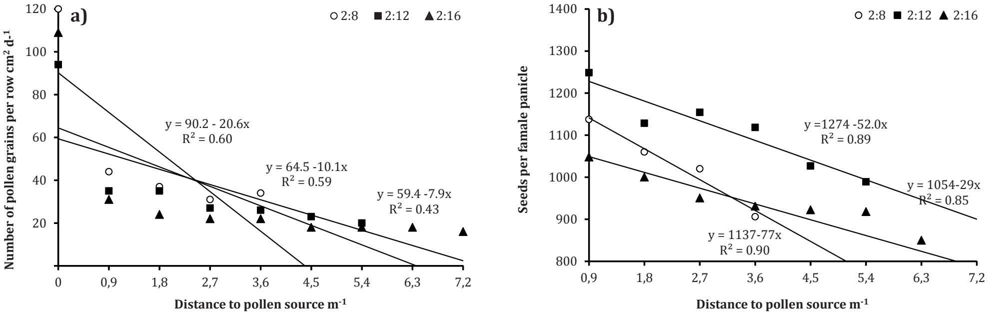

Figure 2. Changes in pollen grains cm^2 per day (a) and seeds per panicle (b) as the distance to the pollen source increases in female rows. Data only of sowing date of April 3, 2006.

Figura 2. Cambios en los granos de polen cm^2 per day (a) y semillas por panícula (b) según el incremento de la distancia de la fuente de polen en surcos femeninos. Datos únicamente para la fecha de siembra abril 3, 2006.

Table 1. Differences among sowing dates in the variables studied.

Tabla 1. Diferencias entre fechas de siembra en las variables estudiadas.

Sowing date	LP	FFP	TPG	SP	SS
May 3, 2005 (D1)	17.6±1.9	2003±200	868±25	1108±245	0.51±0.18
June 10, 2005(D2)	18.8±1.5	2155±176	512±22	1262±139	0.62±0.20
April 3, 2006 (D3)	17.3±1.2	1969±142	836±31	1026±203	0.57±0.21
Comparisons					
D1 *vs.* D2	-1.2*	-151*	+178*	-154*	-0.11*
D1 *vs.* D3	+0.29 ns	+34 ns	+16 ns	+82*	-0.06*
D2 *vs.* D3	+1.5*	+185*	-162*	+236*	-0.04*

LP= Length of female panicle (cm), FFP= flowers per female panicle, TPG= total of pollen grains cm^2 per day, SP= seed per panicle per row and SS= seed set.* Significant according to Student's t-test.

LP= Longitud de la panícula femenina (cm), FFP= flores por panícula femenina, TPG= total de granos de polen cm^2 por día, SP= semillas por panícula por surco y SS= producción de semilla. * Significativo de acuerdo con la prueba t de Student.

Unexpected differences ($p < 0.01$) were found among proportions in panicle length (17.7±0.59) and flowers per panicle (2018±65), because these variables should not be associated with the increase of female rows (table 2, page 59).

However, the correlation of these two variables with number of female rows was high r = -0.91* and -0.87*, while the totals of pollen grains cm^2 per day, seed per panicle and seed set were less correlated (r = 0.63, -0.63 and -0.51).

With the increasing of number of female rows, the simple regression analyses showed progressive reductions in the panicle length and flowers per panicle (y = 18.7- 0.1x R^2= 0.83; y = 2125 - 11.6x R^2= 0.73) and decreased logarithm in seeds per panicle (y = 1608-235 in(x) R^2= 0.52) and seed set (y = 0.72 - 0.08ln(x), R^2= 0.34).

The total of pollen grains deposited followed a logarithmic tendency (y = 444 +160ln(x), R^2= 0.58) to be taken into account.

Discussion

Pollen production, viability and deposition

The real pollen production per plant is pollen grains per anther (26, 30). In this experiment, it was performed by estimating plants emitting pollen associated with tassel behavior of shedding pollen (39).

The pattern of pollen production followed normal distribution among sowing dates (figure 1, page 56) This tendency was also observed in maize (23, 37).

The difference among sowing dates was registered as the quantity in pollen produced. The major production was in third sowing date (400 mg), twice more than the other dates (figure 1, page 56).

The maximum production was registered 4, 2 and 3 days before male anthesis (figure 1, page 56).

In maize (*Zea mays* L.) the aptitude to produce pollen is primarily under genetic and physiological controls (13, 14). Another important factor affecting pollen production is tassel architecture.

Table 2. Mean and standard deviation among male:female rows proportions plants in the variables studied.

Tabla 2. Media y desviación estándar entre proporción de surcos macho:hembra en las variables estudiadas.

MRF	PL	FFP	TPG	SP	SS
4:2	18.2 a	2075 a	578 c	1419 a	0.68 a
6:2	18.0 a	2067 a	804 b	1014 c	0.49 c
8:2	17.9 a	1999 b	846 a	1050 c	0.53 b
12:2	17.8 a	2034 ab	854 a	1111 b	0.55 b
16:2	16.7 b	1916 c	824 b	969 d	0.51 b
Mean	17.7	2018	782	1113	0.55 b

MFR= male to female rows proportions, LP= Length of panicle cm, FFP= flowers per panicle, TPG= total of pollen grains cm^2, SP= seed per panicle per row and SS= seed set. Data of the proportions 2:4 and 2:6 were average of two sowing date of 2005 and the proportion 2:8 were average of sowing date May 3, 2005 and April 3, 2006. Data of 2:12 and 2:16 correspond to April 3, 2006. Means followed by the same letter in each column were statistically similar (Student's t-test, $p< 0.05$).

MFR= proporciones de surcos machos y hembras, LP= Longitud de panícula cm, FFP= flores por panícula, TPG= total de granos de polen cm^2, SP= semillas por panícula por surco y SS= producción de semillas. Datos de las proporciones 2:4 y 2:6 se obtuvieron del promedio de dos fechas de siembra en 2005 y la proporción 2:8 fueron el promedio de la fecha de siembra mayo 3, 2005 y abril 3, 2006. Los datos de 2:12 y 2:16 corresponden para abril 3, 2006. Medias seguidas de la misma letra en cada columna fueron estadísticamente similares (prueba t de Student, $p<0,05$).

The total of male flowers, flowers per branch, number of branches per tassel are the best morphological traits for estimating pollen production in maize (36). In previous report, B- and R-lines adapted to High Valley of México were diverse in pollen traits.

The B-lines produced less viable pollen (379 mg/75%), shorter panicle (22±2 cm) and minor flowers per panicle (1577±150) than R-lines and another difference was in anther size (9). In this experiment the B9-line on average had a similar number of flowers per panicle in the two sowing dates in the year 2005 (2200±330) and 6% more flowers than the third sowing date (2371±213).

The variations observed in the number of flowers did not explain the changes of pollen production between sowing dates. Neither did we expect relevant changes in anther size of B9-line.

A previous study among species of family Poaceae shows that this trait is conservative (30).

Another aspect was the flowering progress of male line (figure 1g, h, i, page 56.

The time and number of male plants that remained shedding pollen during flowering period in the third date was more than the others dates (figure 1i, page XXX) due to heterogeneous stand. In the field, the timing and pattern of tassel development of maize is not the same from plant-to-plant (3).

The pattern of pollen deposition (pollen grains cm^{-2} d^{-1}) varied among sowing dates. Pollen deposition registered some peaks and did not follow the tendency of pollen production of B9-line (mg^1 per day) (figure 1, page 56). In maize, pollen concentration in the air has a diurnal periodicity with a daily maximum in the morning and similar dynamics as pollen production (19).

The first peaks occurred at five and three days after a maximum quantity of pollen production in the first and third sowing dates (figure 1a, c, page 56), and in the second date pollen production peak appeared two days before (figure 1b, page 56).

Contrary to the graphic results, simple correlation between pollen production and deposition per day was significant for each date (r = 0.64*, 0.75* and 0.82*). In sorghum and maize, it was determined that pollen deposition (pollen grains cm^{-2} d^{-1}) could be related to effective pollen production per plant (pollen reaching the location of silks) and the shedding pollen curve of male line (26, 39). On the first date, the pollen deposition pattern was different in comparison to other dates; because the pollen dispersion was more uniform during all flowering period. The wind had daily oscillations from the lowest value 0.8 until 2.2 m/s and more frequently after the anthesis.

The wind speed on the third day had similar values to the first day, however, the oscillating wind occurred before anthesis and it was less frequently along a flowering period and the pollen deposition was not moved over the production curve.

The increases of wind velocity in the second day oscillated from 0.5 to 1.6 m s^{-1} during all of the flowering period and the dispersion pollen was lower than the others dates (figure 1d, page 56). In the three cases, the peaks appeared near to the increases of wind even under the presence of precipitation.

The maximum peak of rainfall (30 mm) was registered near anthesis in the third sowing date, and this may have influenced the minor dispersion of pollen. Normal presence of rainfall during the flowering was in the afternoon, the average values oscillated from 0 to 10 mm and none storms. When the rain falls near to the point of highest pollen concentration, it decreased by two to three times, in hybrid of sorghum MR Buster ® (33).

The velocity of wind determines the rate of pollen deposition (19). The most pollen grains in maize are dislocated from anther by wind speeds from 0.2 to 0.5 m/s (4). In this experiment, the velocity of wind was of 0.8-2.0 (1.3±0.32 m/s); 0.4-1.5 (0.7±0.30 m/s); 0.2-2.2 (0.7±0.45 m/s) (first, second and third sowing days, respectively) and these values were sufficient to remove sorghum pollen. Moreover, the pollen size of sorghum is two times smaller than maize pollen (8, 9). The wind velocity on the first day was sufficient to disperse pollen beyond the curve of pollen production (figure 1d, page 56) and this did not occur on the others days. Effect of the wind direction during pollination has been observed in Redlan A-line of sorghum; the most plants have more seeds on the front hemisphere than the backward-orientation hemisphere (34). Pollen level dynamics in the atmosphere is a complex process, because it is associated to pollen production, release and dispersion (6), in addition to the length of flowering time (30). Also, grass pollen concentration in the air is greatly influenced by weather conditions (17, 32).

The factors climate is closer related therefore, their effect on pollination is additive, and this result is related to temporal variations and specifically the atmospheric conditions (4), which includes a probabilistic event (39). Under field conditions the specific floral structure plays an important role in pollen concentration (24). There are differences between genotypes of sorghum for flowering diurnal hour (flowering stage of each spikelet, since beginning to open glumes to completely closing) (18).

The male B9-line or pollen donor in this experiment started shedding pollen from 0930 to 1000 h, and before this time the relative humidity was high (90%) and the temperature oscillated between 0 and 15°C. Its anthers exerted after pollination, in others words, there is a probability that some of pollen was not released to the environment or prevented anther aperture. Redlan B-line sheds pollen in the morning from 0600 to 1130 h and sometimes also in the afternoon from 1600 to 1800 h under field temperature of 21.3°C and relative humidity of 61.8% (34). More than 75% of pollen grains of commercial hybrid MR Buster® are trapped between 0300 h and 1000 h during the Australian mid-summer with 95% of relative humidity and near to 30°C of temperature (18). The decrease of relative humidity and increase of temperature are paired processes.

The effect of anther behavior or the threshold necessary to start of anther dehiscence in sorghum has not been explored completely. The low levels of relative humidity prevent the anther dehiscence in *Oryza sativa* L. cv. Nipponbare (25).

The pollen release differs among rice genotypes depending on the response of anthers to changes in adverse factors such as drought stress (21).

On average, the seeds per panicle varied between 969 and 1419 and the pollen viability varied between 76 and 89%. This data was taken during the beginning of flowering before release and the pollen remained inside the anther, which is a wet environment and did not have an effect on the changes during the transportation (22). The drying process is the principal cause of loss of pollen viability in sorghum (20). Rainfall during the flowering period in the three sowing dates avoided drastically the changes of relative humidity. In commercial sorghum hybrids, pollen viability is from 20 to 65% and the seeds per panicle are from 100 to 350, and these increase from between 300 to 400, when pollen viability is from 70 to 80% (40).

Floral synchrony

The correspondence between the plants that shed pollen and plants silking is often a measure of floral synchrony, necessary for high seed set and for prevention of ergot incidence (7, 16). Significant differences ($p < 0.01$) were observed in floral behavior between both parents and among sowing dates. The female line showed more precocity during all the flowering period from day 1 to 9, and had on average 5% less flowers per panicle. In the third sowing date (April 3, 2006) the largest difference was registered between both parents -4, -8, -9 and -5 days (DB, DA, DE and FP, respectively).

In the field, the synchrony floral between pollen donor and the receptor had a large impact on cross pollination. Highest rates of cross-pollination were found in maize at synchrony of up +3 days, with decrease beyond this range (5). Theoretically, the isogonic pair of A9/B9-lines only differs in pollen fertility. Previous studies have shown significant variations in phenological and reproductive traits among six pairs of isogenics lines (9). Dahlberg *et al*. (2001) also reported differences 1 to 3 days in male/female floral syncrony in 12 A/B pairs. These results show that it is common to find variation in isogenic pairs of sorghum, even though, it is not known why this response occurs in sorghum.

The pair A9/B9-lines in the field flowered heterogeneously in each proportion and sowing date (figure 2, page 57).

The A9-female line had a floral period shorter ($p < 0.01$) than B9-male line at the three sowing dates (-3, -3 and -5 days for D1, D2 and D3 respectively). In a sorghum study, flowering progress was measured as the fraction of flowered plants in the population after eight days of the start of flowering later, sorghum A-lines were divided into three groups as fast (80-90%), moderate (75-79%) and slow (60-70%) (16).

A similar criterion was used in the present study to assess flowering in pair A9/B9-lines. On the first sowing date May 3, 2005 at the 102 days after sowing both lines had started to flower, but intra-panicle floret aperture was different between A9- and B9-lines; the female line had two times more exert florets (50%). In the second sowing date June 10, 2005 at 94 days after sowing day, 55% of female plants had flowered two times more than male plants, in this case both lines had similar proportion of exposed flowers (25%).

The flowering progress in the third sowing date April 3, 2006 was very contrasting between both parents, as the male plants delayed flowering; only 28% had begun pollen emission, when the 100% of female plants was in anthesis (figure 2, page 57). In this experiment the female line was more precocious than male line.

The biggest difference occurred in the third date; 12 days between both parents. In field is common, the delated during sorghum seedling establishment and then effect in later phenological stages.

One probably reason is differences in sowing deep. It no rule out any kind effect of temperature above all female line. Temperature is a major determinant of the rate of ant development. It is not known why this response occurs in this sorghum (32).

Effect of distance in pollen deposition and seed production

The distance from pollen source had an effect in pollen dispersal and seed production. The most direct way of assessing success of pollen dispersal is measured by the level of fertilization (4).

The simple regression analyses confirm that the greater the distance from the pollen source, the number of pollen grains and seeds declined proportionally with increasing distance, but the rate was different for each proportion. In maize the rate of cross-pollination between yellow donor and white receptor decreased exponentially and rates varied from year to next year (38).

In this experiment the first passive traps of pollen collection was placed on the row of fertile B-9 line (0.0 m) and was counted in an average of 108 pollen grains cm^2 per day per row (data of third sowing date). At a distance of 0.90 m from pollen source, only 34% of pollen arrived to the female A9-line and at a distance of 7.2 m, 15%.

Jarosz *et al*. (2003) in a commercial plot of maize, reported that the pollen concentration and deposition decreased with source distance and high < 10% for each 1 m and decrease above 2 m, respectively. After the pollen release, during transportation the pollen can modify water status, shape and density, therefore, affecting the quantity of viable pollen that arrives to the crop (2), although, during flowering *S. bicolor* releases more pollen than other species, such as annual grass (33).

According to the number of flowers per panicle of female line the rate of out-crossing was of 56% at 0.90 m of pollen source and of 42% to 7.2 m (figure 2, page 57). For small fields of maize (< 2 h), the majority of cross-pollination occurs within the first 6 m, irrespective of donor size (5).

In many self-pollinated crops, floral morphology controls the cross-pollination (31). *Sorghum bicolor* and its relatives are self-pollinated that can be used in open-pollination. Pedersen *et al*. (1998) found that in four B- and R-lines there were differences in out-crossing on average the values ranging from zero to 10%, but it is possible that some individual plants inside the population reached 26%.

Size source of pollen and seed set

The mean comparison among sowing dates showed significant differences in floral and reproductive variables, although there were no differences between D1 *vs.* D3 in length of panicle and flowers per panicle. We expected less seed production per panicle in the second date (D2) because the total pollen deposition diminished by 40% compared with other dates and the panicle was larger with more flowers (2155±176) (table 1, page 58).

The results, in part, could be explained by confounded effect of the proportions between male and female rows. On the second date the proportions were established with less female population 2:4 and 2:6, the floral synchrony and the flowering progress was more uniform (figure 2, page 57).

Pollen production generally is not considered a limiting factor in maize production. However, Westgate *et al*. (2003) demonstrate that a minimum pollen shed density per exposed silk is required to achieve maximum kernel set and grain yield. In maize hybrids the grain yield remained stable, decreasing only until 20% of pollen remains (39).

The size of female population had a significant effect on panicle size, total pollen deposition, seed per panicle and seed set. Unexpected low values in seed per panicle and seed set were recorded in the proportion 2:6, due to heterogeneous plants stands. Widely varying field size proportions of donors to receptors in maize of approximately 4:1-1:8 influenced the cross-pollination rate at distances of 0-20 m from the pollen donor (5).

The sizes of pollen source were 33, 25, 20, 14 and 11% of the field population (2:4, 2:6, 2:8, 2:12, and 2:16, respectively). Pollen production per panicle is influenced by size panicle and floral behavior. In field during increases hybrid seed, it is possible chance the proportion male: female rows. This depends on male ability for pollen production (12). In this study, the proportion 2:4 had the best seed production (1419 seeds) and seed set (0.68) with a maximum distance from pollen source of 1.8 m.

The panicle size (length and flowers per panicle) diminished almost proportionately (r = 0.80) with increase of female plants. The largest reduction was observed in the proportion 2:16. In this experiment is confounding effect between proportions and sowing dates, the different panicle size, also attributable to sowing date. When the female population increased four times (2:16) until maximum distance of 7.2 m, the reduction in length panicle and flowers per panicle was 8% (160 flowers), but the reduction of seeds per panicle and seed set was of highest magnitude (32 and 25%).

The variation in the number of spikelets per panicle could be explained by genotypic variation and panicle length (28) and flowers per panicle which are components of yield (36). Using average data of proportions male to female rows the correlation between flowers per panicle and seeds per panicle and seed set was not significant (r = 0.61 and 0.46). This implies that the success of pollination was associated more with the size of pollen source and its dispersion than with the density of female plants.

Theoretically, nearly 2000 viable pollen grains are required to pollinate all flowers of female line per panicle (38).

Only one pollen grain is required to fertilize the sorghum ovule, but sometimes higher quantity raises the probability of a successful pollination, although restrictions could be considered during the interaction between pollen-stigma (10, 32).

The average pollen grains cm^2 counting was 782. Moreover, as less than 50% (578 pollen grains cm^2) were sufficient to produce 1419 seeds per panicle in proportion 2:4, while in the proportions 2:6, 2:8, 2:2 and 2:16 near 800 pollen grains cm^2 were needed to produce around 1000 seeds per panicle. These results suggest a minimum level of pollen by successful pollination with low levels of pollen shed density, using male sterile and male fertile isolines. The prediction model of maize pollen flux at silk height with typical proportion 4:1 in seed plot, has only 23% of expected amount, considering the pollen release duration of ±6 h (3).

With passive pollen traps it was not possible to estimate this threshold in this study. This methodology has limitations of accuracy, but in the field it was adapted to experimental conditions. Pollen shed estimation by pollen deposition using passive traps presents restrictions as pollen can disperse out of the field and it underestimates pollen production per tassel, because some pollen remains in the foliage (39).

Conclusions

The largest production of pollen was 400 mg on April, 2006, twice more than other dates. Seeds per panicle varied from 969 to 1419 and the pollen availability from 76 to 89%.

The A9/B9 isolines flowered heterogeneously in each proportion and sowing date. The A9 female isoline had a floral period shorter (precocity) than B9-male line. Number of pollen grains and seeds declined proportionally with increasing distance from the pollen source, but the rate was different for each proportion.

Finally, the success of pollination was associated more to the size of pollen source and its dispersion than with population of female plants. Five hundred and seventy eight pollen grains were sufficient to produce 1419 seeds in proportion 2:4, while in the proportions 2:6, 2:8, 2:2 and 2:16 at least 800 pollen grains were need to produce 1000 seeds per panicle.

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Acknowledgments

The first author gratefully acknowledges the scholarship granted by the National Council of Science and Technology of México (CONACYT). Also, we thank Ph. D. Matthew Copley for critical review of the English style.