

Enhanced kernel set promoted by synchronous pollination determines a tradeoff between kernel number and kernel weight in temperate maize hybrids

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Abstract

Maize (*Zea mays* L.) grain yield is strongly related to the number of harvested kernels, where kernel number can be increased by synchronously pollinating silks rather than allowing them to be progressively pollinated as they naturally appear from the husks. However, there is scarce evidence on how this practice affects kernel weight (KW) and plant grain yield (PGY), and no report exists on its effects when combined with treatments aimed to reduce apical dominance, like male sterility and detasseling. Field experiments were conducted in two growing seasons (Exp₁ and Exp₂) using two hybrids, cropped at contrasting stand densities (3 and 9 plants per m²) and including (i) male-fertile and male-sterile versions, (ii) tasseled and detasseled plants, and (iii) natural (NP) and synchronous pollination (SP; pollen added manually to ears bagged 5 days after initial silking) systems. Tassel growth of sterile and fertile versions was also evaluated in a separate experiment (Exp₃). Detasseling increased the number of ears per plant reaching silking ($P < 0.001$) of NP plants, but this beneficial effect of reduced apical dominance did not improve kernel number per plant (KNP) or PGY. Similarly, the early arrest of anther growth in male-sterile plants had no clear benefit on KNP. In contrast, KNP was enhanced by synchronous pollination (range between –13% and +71%; average of +15.4% in Exp₁ and +3.9% in Exp₂). However, this pollination system promoted a decreased in KW (range between –30% and +4%; average of –11.8% in Exp₁ and –7.8 in Exp₂) such that the treatment had no effect on PGY (range between –19% and +37%; average of +1% in Exp₁ and –4% in Exp₂). Because plant growth rate around flowering was not different between pollination treatments, assimilate availability per kernel was reduced from ovary fertilization onwards in synchronously pollinated plants when compared to open pollinated plants. This explains the reduced KW when increasing KNP by synchronous pollination. In summary, none of the imposed treatments allowed grain yield to be increased at the plant level.

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Keywords: Maize; *Zea mays*; Synchronous pollination; Apical dominance; Grain yield components

1. Introduction

Maize grain yield is strongly related to the number of grains harvested at maturity (Otegui, 1995; Chapman and Edmeades,

1999), and research on the effects of breeding on maize grain yield demonstrated that enhanced kernel numbers translated into grain yield increases because of no tradeoff by individual kernel weight (Luque et al., 2006). Consequently, breeding and production practices have targeted increased kernel number as a means of increasing grain yield. Since kernel number per plant (KNP) and ear growth rate during the period bracketing flowering were shown to be related (Andrade et al., 1999), traits for improving KNP should represent improved resource capture for increasing plant biomass production (Andrade et al., 2002) and enhanced biomass partitioning to the ear during this period (Echarte et al., 2004; Luque et al., 2006).

Ear growth around flowering, however, is not the only determinant of final kernel number. For the same ear growth, kernel number can be substantially increased by synchronous

Abbreviations: ASI, anthesis-silking interval; ASI_{ip}, ASI of individual plants; ASI_{pp}, ASI of the plant population; E₁, apical ear; E₂, second ear; ESI, ear silking interval; ESI_{ip}, ESI of individual plants; ESI_{pp}, ESI of the plant population; Exp_n, experiment *n*; KNP, kernel number per plant; KW, kernel weight.; NP, natural pollination; PGY, plant grain yield; SP, synchronous pollination; TT, thermal time.

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pollination of exposed silks (Cárcova et al., 2000). This practice consists of adding pollen manually on *ca.* silking + 5 days to all silks exposed from ears bagged before silking. The KNP increase obtained with this pollination system suggested that kernel set per unit of biomass allocated to the ear at silking could be improved, a promising result for increasing grain yield. Reports on this topic, however, are contradictory. The first documented experiment using synchronous pollination (Sarquís et al., 1998) indicated a positive effect on final grain yield of a tropical maize population, but with no reference to changes in grain yield components. Contrarily, Schussler et al. (2002) reported on possible tradeoff effects between KNP and kernel weight (KW) for different commercial hybrids, but results were limited to one year of data. Possible causes of this tradeoff were partially clarified in recent research on KW determination (Gambín et al., 2006), which demonstrated that potential kernel weight was strongly related to plant growth per kernel around flowering. Consequently, increased KNP obtained with synchronous pollination could be counter-balanced by reduced assimilate availability per kernel from ovary fertilization onwards, which results in reduced KW due to a decreased potential weight. There are also evidences of feedback effects on photosynthesis or shoot biomass production caused by total removal of the reproductive sink (Sadras et al., 2000) or artificial reduction of kernel set (Borrás and Otegui, 2001), but there is no evidence of the converse in cereals (Sinclair and Jamieson, 2006).

Reduced tassel size, male sterility, and detasseling are traits/conditions reported to reduce apical dominance exerted by the tassel on axillary-positioned organs (Russell, 1991; Cline, 1991), and assumed to improve growth of the latter. Assessing reductions in apical dominance have always been indirect, and usually quantified as an increase in the number of vegetative (*i.e.* tillers) or reproductive (*i.e.* earshoots) growing branches (Motto and Moll, 1983). Enhanced reproductive growth has been represented as improved prolificacy (number of grained ears per plant), and determined at final harvest. There are, however, early indicators of decreased apical dominance, like reduced time to silking and the anthesis-silking interval (ASI; Edmeades et al., 1993; Borrás et al., 2007). These traits are useful for assessing changes in apical dominance promoted by reduced tassel size, but not by detasseling and male sterility. When there is no pollen-producing organ because of removal or sterility, early indicators of a shift in apical dominance could be the silking interval between ears (Cárcova et al., 2000) or the number of silked ears per plant (Uribebarrea et al., 2002). Changes in silking dynamics are a consequence of changes in biomass accumulation at the ear level (Borrás et al., 2007), and can be promoted by altered apical dominance and biomass partitioning. Further effects on KW and grain yield are yet to be determined.

In the present article, we tested the combined effects on final grain yield of agricultural practices aimed at reducing apical dominance (*e.g.* male sterility and detasseling) with those aimed at improving kernel set per unit ear growth rate (*e.g.* synchronous pollination). There is no evidence on how these agricultural practices might affect the relationship between

KNP and KW when silks are synchronously pollinated. We hypothesized that kernel number per plant is maximized in male sterile or detasseled plants exposed to synchronous pollination, with no negative effect on KW. For testing this hypothesis, we conducted a number of field experiments including hybrids differing in prolificacy (*i.e.* grained ears per plant), male fertility (fertile and sterile versions), tassel presence (plants with and without tassel removal), and pollination systems (natural and synchronous), all cropped under contrasting stand densities (3 and 9 plants per m²) aimed at altering total plant growth. Flowering dynamics were used as early indicators of changes in apical dominance resulting from treatments. Differences in tassel development and growth between male fertility versions were also evaluated.

2. Materials and methods

2.1. Crop husbandry and experimental design

Field experiments were conducted in Argentina during 1997–1998 (Exp₁) and 1998–1999 (Exp₂) at the experimental station of Dekalb-Monsanto at Salto (34°33'S, 60°33'W), on silty clay loam soils (Typic Argiudol). Treatments were a factorial combination of two stand densities (3 and 9 plants per m²), two single-cross hybrids (non-prolific DK752 and prolific DK 664) in their male-fertile and male-sterile versions (*i.e.*, four hybrids), and two levels of tassel presence (tasseled and detasseled plants). Treatments were arranged in a split split-plot design with two replicates, with stand densities in the main plots, hybrids in the sub-plots, and tassel presence in the sub sub-plots (herein termed plots). Pollination treatments (natural and hand-pollinated) were performed on plants distributed at random in all treatment combinations (*i.e.*, stand density per hybrid per tassel presence). Each subplot had 25 rows, 0.7 m apart, and 25 m length. Detasseling took place when tassels appeared within the whorl of the uppermost leaves and always immediately before anthesis. Tassels were cut carefully to minimize leaf removal and plant damage. The destasseled area covered 20 rows, and detasseling was performed on alternate rows (each two or four rows) in order to have a variable amount of pollen for the experiment described in Uribebarrea et al. (2002). Plants used in the present research were always within the lowest detasseling level (*i.e.*, each two rows) in order to ensure pollen availability for adequate kernel set of open pollinated plants. For the same purpose, alternate (each two rows) tasseled rows of male-fertile versions were included in the subplots of the male-sterile hybrids. Two border rows were left at each side of the subplots, and no plant was sampled within these rows.

Sowing took place on 7th November (Exp₁) and on 26th September (Exp₂), both within the range regularly used by farmers in this region. Plots at all stand densities were over planted at a rate of three seeds per hill, and thinned to one plant per hill at the three-ligulated leaf stage (V₃). Experiments were side-dressed with 150 kg of N ha⁻¹ applied as urea at V₈. Weeds were controlled with a pre-emergence application of 2.2 kg a.i. ha⁻¹ alachlor (2-chloro-*N*-(2,6-diethylphenyl)-*N*-

(methoxymethyl acetamide) and 3.3 kg a.i. ha⁻¹ cyanazine (2-(4-chloro-6-ethyl-amino-1,3,5-triazin-2-ylamino)-2-methyl-propionitrile), and by hand weeding after the crop was established. Crops were kept free of pests and diseases, and water stress was prevented by means of furrow irrigation, with the uppermost 1 m of soil held near field capacity throughout the growing season.

Daily values of incident global solar radiation, mean air temperature, and rainfall were obtained from a weather station installed at the experimental field. Solar radiation was converted into photosynthetically active radiation (PAR) by multiplying by 0.45 (Monteith, 1965), and accumulated thermal time (TT, base temperature 8 °C) was computed from mean daily air temperatures from sowing onwards (Ritchie and NeSmith, 1991). Mean daily temperatures were computed from hourly registered records.

2.2. Measurements

Details on measurement of flowering dynamics are described in Uribelarrea et al. (2002). To briefly summarize, at least 20 (Exp₁) or 40 (Exp₂) plants were tagged at random within each plot before tasseling. Each group of plants was distributed evenly between natural and hand pollination treatments (for additional details on this treatment see Cárcova et al., 2000). In plants destined for hand pollination, both uppermost (E₁) and second (E₂) ears were bagged before silking, and all exposed silks were pollinated simultaneously on 5 days after silks first appeared from the husks of E₁. The dates of silking (*i.e.*, at least one silk visible after extruded from the husks) of E₁ and E₂ were registered for each tagged plant, and the ear silking interval of individual plants (ESI_{ip} = E₂ silking date – E₁ silking date, in days) was calculated. Anthesis (*i.e.*, at least one extruded anther visible at the tassel) date was also registered for plants tagged in the non-detasseled rows, and used to compute the ASI of individual plants (ASI_{ip} = anthesis date – E₁ silking date, in days). The cumulative totals of tagged plants that had anthesed or silked were recorded daily, and the dates of 50% anthesis, 50% silking of E₁ and 50% silking of E₂ were registered. These data were used to calculate the ASI and the ESI of the plant population (ASI_{pp} and ESI_{pp}, respectively), which may differ from estimates obtain by averaging records from individual plants (Uribelarrea et al., 2002). The date of 50% anthesis in non-detasseled rows of male-fertile plots was used for calculating ASI_{pp} in male sterile and detasseled treatments, in order to have an estimate of the effect of these treatments on this attribute and to allow for ANOVA computation. The number of silked ears per plant was recorded in each plot.

Grain yield and grain yield components were registered for each tagged plant at physiological maturity, totaling at least 640 plants in Exp₁ and 1280 plants in Exp₂. Prolificacy was computed as the number of grained ears per plant. Grained ears of each plant were oven dried at 60 °C until constant weight (*ca.* 6% moisture). Ears were hand shelled separately, grains were weighed for the determination of plant and crop grain yields, and the number of harvestable kernels per ear was counted

manually. KW was computed as the quotient between plant grain yield (PGY) and KNP.

2.3. Development of male fertile and male sterile tassels

An additional experiment was performed during 2000 (Exp₃) at the experimental field of the University of Buenos Aires (34°25'S, 58°25'W) on a silty clay loam soil (Vertic Argiudol). This experiment was conducted in order to provide a detailed analysis of tassel development in male-fertile and male-sterile plants of hybrids DK 752 and DK 664 under no resource competition. For this purpose plants were cropped at a very low stand density (3.5 plants per m²) in a completely randomized design with three replicates. Plots were three rows, 0.7 m apart, and 10 m length. Sowing took place on 14th January, which is at the limit for late sown or second-planted maize (*e.g.* after wheat harvest) in this region but has no effect on plant development up to flowering (*i.e.*, in total number of leaves or TT requirements). Plots were side-dressed with 150 kg of N ha⁻¹ at the four-ligulated leaf stage. The experiment was kept free of weeds, plants and diseases, and was frequently irrigated in order to keep the soil near field capacity during the whole cycle. Weather data were collected from a nearby automatic station and TT computed as described above. The dates of anthesis and silking were recorded on five successive plants tagged in the central row of each plot.

One plant was collected from each plot every three days between tassel initiation (Bonnett, 1966) and anthesis. Plant height from ground level to the uppermost collar was measured on all harvested plants, which were subsequently dissected for tassel and apical ear observation under a binocular microscope. The length of both reproductive structures was determined, and tassel development was characterized according to the stages described in Table 1.

2.4. Statistical analysis

Year and treatment effects and their interactions were evaluated by ANOVA (Analytical Software, 2000), and a *t*-test was used to determine differences among means. Regression analysis was applied to the relationship between variables (*e.g.*, grain yield and grain yield components) and between pollination treatments for each variable.

Table 1
Stages of tassel development in a male-fertile maize plant

Stages	Description
1	Double ridge
2	Spikelet primordia on main branch
3	Start of branch primordia differentiation
4	Spikelet primordia visible on branches
5	Start of active tassel elongation
6	Tassel elongation completed
7	Tasseling
8	Anthesis

3. Results

3.1. Weather conditions

Weather conditions at Salto differed between experimental years (Fig. 1). The strong *Niño* event registered in 1997–1998 brought increased rainfall, accompanied by reduced solar radiation (ca. 36% below the regional average for the December–January period) and mean air temperatures (ca. 12% below the regional average for the same period). Weather conditions during the second growing season were close to available records (Hall et al., 1992). Daily maximum air temperatures during the period around flowering averaged 25.9 °C in Exp₁ and 29.8 °C in Exp₂, and records ≥ 35 °C were registered for 3 days during this period in Exp₂ but never in Exp₁.

The late sowing date of Exp₃ at Buenos Aires exposed the crop to high temperatures early in the cycle, and to mild conditions around flowering. Mean air temperatures averaged 25.3 °C between sowing and tasseling, and daily maximum values averaged 30.1 °C for the same period, with only one record above 35 °C. Mean air temperatures of 24.3 °C and maximum air temperatures of 28.6 °C corresponded to the period of active ear growth and kernel set, with no record above 35 °C.

3.2. Flowering dynamics

Differences in environmental conditions between experiments affected flowering dynamics. Overcast skies and rainy days around flowering of Exp₁ promoted an early silking and

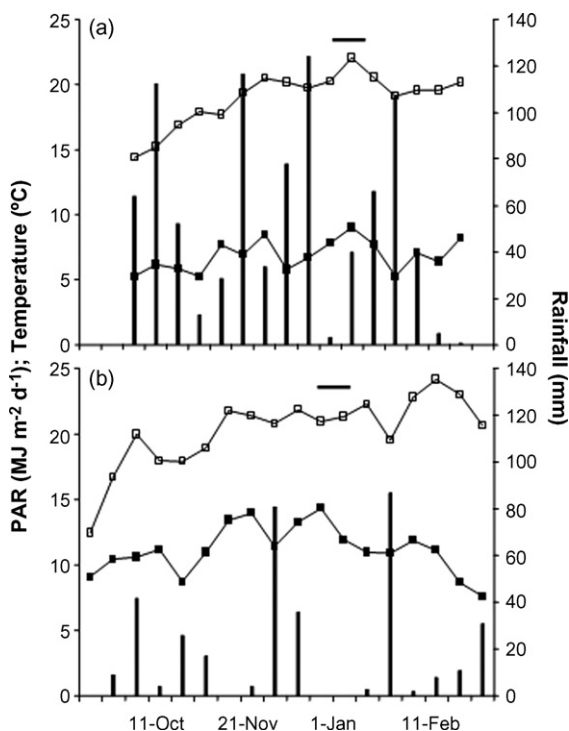


Fig. 1. Weather conditions of experiments developed at Salto during 1997–1998 (a) and 1998–1999 (b). Closed squares correspond to photosynthetically active radiation (PAR), and open squares to temperature. Vertical bars represent rainfall records and horizontal bars the flowering periods.

reduced ASI ($P = 0.011$) in the male-sterile versions of both hybrids (Table 2). This response was accompanied by an increase in the number of ears per plant that reached silking for these genotypes ($P < 0.054$). There was, however, a significant interaction among all treatments (*i.e.*, stand density \times hybrid \times tassel presence) for this trait ($P < 0.045$). The smallest number of ears per plant that reached silking corresponded to the male-fertile versions of both hybrids that remained with fertile tassels at high stand density. The number of ears that reached silking, however, was not different among hybrids and tassel presence treatments at the high growth condition of 3 plants per m².

The silking interval between ears (ESI) was always smaller in Exp₁ than in Exp₂ (Table 2), but the number of plants from each treatment that showed silking at the secondary ear was too variable to permit an ANOVA from individual plant data (ESI_{ip}). Moreover, 50% silking of secondary ears (*i.e.*, event reached on 50% of tagged plants in a plot) was not registered for any hybrid cropped a 9 plants per m² in Exp₂. This also avoided the ANOVA computation for this attribute at the plant population level (ESI_{pp}). There was, however, a clear trend toward reduced ESI values at low as compared to high stand densities. Differences in ESI between stand densities were unambiguously evidenced at the population level (ESI_{pp}). Lack of silking from E₂ of most plants cropped at 9 plants per m² (Table 2) is equivalent to an infinite ESI, a situation never registered at 3 plants per m² (*i.e.*, all plots reached 50% silking of E₂ at the low plant population). Similarly, there was a clear trend towards a higher number of secondary ears reaching silking because of detasseling, based on mean and standard deviation values obtained for ESI_{pp}. Except for male-fertile plots of DK664 cropped at low stand density in Exp₁, mean ESI_{pp} values of detasseled plots were always smaller than those of tasseled plots (Table 2). Detasseling also reduced the variability in time to silking between ears, as shown by smaller standard deviations when compared to the control condition.

Detasseling soon before anthesis had no effect on estimated ASI_{pp} (Table 2). In contrast, hybrids differed significantly for this trait ($P = 0.012$), and a reduced estimated ASI_{pp} was registered for male-sterile versions at all stand densities during Exp₁ but not during Exp₂. No clear trend was detected between male fertility versions for ESI.

Variations in the number of ears per plant that reached silking were related to the observed ASI, but the relationship differed ($P < 0.05$) between stand densities. Fitted models indicated that almost all secondary ears (94%) will reach silking when there is perfect synchrony between anthesis and silking of E₁ (*i.e.* ASI = 0) at 3 plants per m² (Silked ears per plant = $1.94 - 0.055$ ASI; $r^2 = 0.53$; $P = 0.0012$; ASI range between -1.5 and 2.75 d). Contrarily, only 35% of the plants will present silking of E₂ when ASI = 0 at 9 plants per m² (Silked ears per plant = $1.35 - 0.069$ ASI; $r^2 = 0.62$; $P < 0.001$; ASI range between -2 and 3.5 d).

3.3. Grain yield determination

Stand density had a large effect ($P < 0.001$) on PGY (mean reduction of -54% due to increased stand density), and

Table 2

Flowering data obtained for male-fertile (MF) and male-sterile (MS) versions of two hybrids (H), cropped at two stand densities (SD) and two tassel presence (TP) levels (ND, non-detasseled; D, detasseled) during two experimental years (Y)

Stand Density	Hybrid	Tassel presence	Silked ears per plant	ASIpp	ESIpp	ASIip	ESIip
				days			
1997–1998							
3 Plants per m ²	664 MF	ND	2.00	0.25	0.75 ± 0.35	–0.05	1.05 ^a ± 0.94
		D	1.93	0.50	1.50 ± 0.00		1.25 ± 0.81
	664 MS	ND	2.00	–1.50	1.25 ± 0.35		1.10 ± 0.64
		D	1.93	–0.75	0.75 ± 0.35		0.84 ± 0.96
	752 MF	ND	1.95	0.00	2.75 ± 1.06	–0.10	2.32 ± 1.49
		D	1.92	1.00	1.00 ± 0.00		1.26 ± 0.89
	752 MS	ND	1.95	–0.75	1.00 ± 0.71		1.53 ± 1.02
		D	1.90	–0.50	0.75 ± 0.35		0.94 ± 0.54
9 Plants per m ²	664 MF	ND	1.15	1.75	–	1.17	1.67 ± 2.52
		D	1.38	1.75	–		1.86 ± 0.66
	664 MS	ND	1.35	–1.00	4.00 ^b ± –		1.00 ± 1.00
		D	1.54	–0.75	2.50 ± 0.00		1.17 ± 1.03
	752 MF	ND	1.10	0.00	–	–0.02	3.50 ± 0.71
		D	1.21	–0.50	–		1.11 ± 1.36
	752 MS	ND	1.35	–1.50	3.00 ± –		1.56 ± 1.24
		D	1.71	–2.00	3.50 ± 0.71		2.13 ± 1.45
1998–1999							
3 Plants per m ²	664 MF	ND	1.90	1.50	4.00 ± 1.41	1.36	3.61 ± 2.38
		D	1.93	1.50	2.50 ± 0.71		2.76 ± 2.15
	664 MS	ND	1.75	3.00	2.50 ± 0.71		2.13 ± 1.31
		D	1.80	2.50	2.50 ± 0.71		2.28 ± 1.35
	752 MF	ND	1.80	0.50	3.50 ± 0.71	0.50	2.84 ± 1.74
		D	2.00	0.50	2.00 ± 0.00		2.48 ± 1.85
	752 MS	ND	1.85	0.00	3.00 ± 1.41		2.45 ± 1.79
		D	1.93	0.00	3.00 ± 0.00		2.78 ± 1.75
9 Plants per m ²	664 MF	ND	1.02	2.50	–	2.67	3.81 ± 2.96
		D	1.18	2.50	–		4.00 ± 2.31
	664 MS	ND	1.13	3.50	–		4.40 ± 0.55
		D	1.15	3.00	–		4.00 ± 1.26
	752 MF	ND	1.13	2.50	–	2.50	4.20 ± 1.30
		D	1.13	2.00	–		4.00 ± 2.24
	752 MS	ND	1.13	3.50	–		3.80 ± 0.45
		D	1.35	2.50	–		3.15 ± 1.41
		Y	ns ^c	ns			
		SD	0.003	ns			
		Y × SD	ns	ns			
		H	0.006	0.012			
		Y × H	0.011	0.003			
		SD × H	<0.001	ns			
		Y × SD × H	ns	0.022			
		TP	<0.001	ns			
		Y × TP	ns	ns			
		SD × TP	0.001	ns			
		Y × SD × TP	0.002	ns			
		H × TP	ns	ns			
		Y × H × TP	ns	ns			
		SD × H × TP	0.046	ns			
		Y × SD × H × TP	ns	ns			

The anthesis-silking interval (ASI) and the silking interval between apical and second ears (ESI) were computed from data obtained at the plant population (ASIpp and ESIpp) and the individual plant (ASIip and ESIip) levels.

^a Computed from a variable number of plants that exhibited silking in the second ear in each treatment (evidenced in the number of silked ears per plant), a restriction that did not allow for ANOVA computation.

^b 50% silking of second ears reached only in one replicate.

^c Significance level for main factors and their interactions; ns (not significant) represents *P* values larger than 0.10.

significant interactions were detected among treatments (Table 3). Detasseling promoted a significant reduction of PGY for synchronously pollinated plants (average reduction of –1.9% for NP and –5.9% for SP; $P < 0.019$). This detasseling

effect was larger in Exp₁ than in Exp₂, and differed ($P < 0.007$) among hybrids (Table 3). In contrast, detasseling had no effect on grain yield of plants under natural pollination. A significant ($P < 0.062$) hybrid per stand density interaction was detected

Table 3

Plant grain yield and grain yield components of male-fertile (MF) and male-sterile (MS) versions of two hybrids (H), cropped at two stand densities (SD) and two tassel presence (TP) levels (ND, non detasseled; D, detasseled) during two experimental years (1997–1998 and 1998–1999)

Stand density	Hybrid	Tassel presence	Grained ears per plant			Kernel number per plant			Kernel weight			Plant grain yield			
			NP	SP	SP NP ⁻¹	NP	SP	SP NP ⁻¹	NP	SP	SP NP ⁻¹	NP	SP	SP NP ⁻¹	
									mg			g			
1997–1998															
3 Plants per m ²	664 MF	ND	1.75	1.74	0.99	772	876	1.13	261	230	0.88	201	201	1.00	
		D	1.87	1.54	0.82	817	708	0.87	248	231	0.93	202	163	0.81	
	664 MS	ND	2.00	2.00	1.00	755	869	1.15	256	255	1.00	193	221	1.15	
		D	1.90	2.00	1.05	878	859	0.98	206	211	1.02	181	180	0.99	
	752 MF	ND	1.35	1.75	1.30	773	1006	1.30	253	217	0.86	195	217	1.11	
		D	1.72	1.85	1.08	844	1012	1.20	239	208	0.87	201	210	1.04	
	752 MS	ND	1.75	1.85	1.06	810	907	1.12	273	209	0.77	219	189	0.86	
		D	1.90	1.80	0.95	910	865	0.95	255	252	0.99	232	217	0.94	
	9 Plants per m ²	664 MF	ND	1.00	1.00	1.00	410	536	1.31	236	166	0.70	96	88	0.92
			D	1.00	1.00	1.00	410	455	1.11	196	150	0.77	80	68	0.85
		664 MS	ND	1.00	1.00	1.00	367	468	1.28	214	211	0.99	77	98	1.27
			D	1.00	1.00	1.00	323	355	1.10	260	224	0.86	83	79	0.95
752 MF		ND	1.00	1.00	1.00	493	589	1.19	195	174	0.89	96	101	1.05	
		D	1.00	1.00	1.00	481	476	0.99	196	172	0.88	94	81	0.86	
752 MS		ND	1.00	1.00	1.00	477	513	1.08	227	212	0.93	108	108	1.00	
		D	1.00	1.00	1.00	309	528	1.71	246	198	0.80	76	104	1.37	
1998–1999															
3 Plants per m ²		664 MF	ND	2.00	1.83	0.92	822	831	1.01	277	236	0.85	228	196	0.86
			D	1.90	1.88	0.99	839	880	1.05	273	249	0.91	229	218	0.95
		664 MS	ND	1.88	1.93	1.03	822	883	1.07	285	268	0.94	234	235	1.00
	D		1.93	1.90	0.98	847	860	1.02	277	259	0.94	234	222	0.95	
	752 MF	ND	1.68	1.87	1.11	884	997	1.13	228	212	0.93	203	210	1.03	
		D	1.38	1.93	1.40	882	1023	1.16	246	217	0.88	217	222	1.02	
	752 MS	ND	1.80	1.80	1.00	934	933	1.00	262	246	0.94	244	229	0.94	
		D	1.80	1.63	0.91	871	774	0.89	264	245	0.93	228	188	0.82	
	9 Plants per m ²	664 MF	ND	1.00	1.00	1.00	431	408	0.95	236	238	1.01	101	97	0.96
			D	1.00	1.00	1.00	424	421	0.99	234	243	1.04	99	102	1.03
		664 MS	ND	1.00	1.00	1.00	437	448	1.03	255	237	0.93	111	109	0.98
			D	1.00	1.00	1.00	429	447	1.04	257	230	0.89	109	102	0.94
752 MF		ND	1.00	1.00	1.00	518	486	0.94	213	198	0.93	110	96	0.87	
		D	1.00	1.00	1.00	470	544	1.16	221	179	0.81	103	97	0.94	
752 MS		ND	1.00	1.00	1.00	505	575	1.14	209	191	0.91	105	109	1.04	
		D	1.00	1.00	1.00	515	547	1.06	206	190	0.92	105	103	0.98	
Y ^a			ns ^b	0.015		0.091	ns		ns	ns		ns	0.083		
SD			0.001	0.001		0.002	<0.001		0.001	0.005		<0.001	<0.001		
Y × SD			ns	ns		ns	ns		0.057	ns		ns	ns		
H			<0.001	0.088		0.008	0.005		0.009	<0.001		0.077	ns		
Y × H			ns	ns		ns	ns		0.019	<0.001		0.052	ns		
SD × H			<0.001	0.088		ns	0.100		ns	ns		0.063	ns		
Y × SD × H			ns	ns		ns	ns		ns	<0.001		ns	ns		
TP			ns	ns		ns	0.022		ns	ns		ns	0.019		
Y × TP			0.012	ns		ns	0.069		ns	ns		ns	ns		
SD × TP			ns	ns		0.030	ns		0.021	ns		ns	ns		
Y × SD × TP			0.013	ns		0.047	ns		0.016	ns		ns	ns		
H × TP			ns	ns		ns	ns		ns	0.077		ns	ns		
Y × H × TP			0.017	ns		ns	0.055		ns	ns		ns	0.007		
SD × H × TP			ns	ns		ns	ns		0.007	0.004		ns	ns		
Y × SD × H × TP			0.017	ns		ns	ns		0.022	0.004		ns	0.098		

Data correspond to natural (NP) and synchronously (SP) pollinated plants.

^a Y, year; SD, stand density; H, hybrid.

^b Significance level for main factors and their interactions; ns (not significant) represents P values larger than 0.10.

for PGY under this pollination system, and the highest grain yield corresponded to male-sterile plants of hybrid DK752 at 3 plants per m².

The above-mentioned trends in PGY were promoted by the particular effect of treatments on grain yield components (Table 3). Stand density produced the largest changes in both yield components (mean reductions of –46.7% in KNP and of –13.2% in KW due to increased stand density), but significant interaction effects were detected for most treatments (Table 3). Prolificacy values were higher for the male-sterile (1.43) than for male-fertile versions (1.37) when averaging across all treatments, but differences were significant ($P \leq 0.088$) only at the low stand density (1.87 for male-sterile and 1.75 for male-fertile). This interaction effect (stand density \times hybrid) on prolificacy expression (Table 3) was more significant under natural ($P < 0.001$) than under synchronous pollination ($P = 0.088$), but did not translate into similar differences in KNP. This result could not be attributed to defective pollination due to lack of pollen in male-sterile plants under natural pollination because it was also evident in hand-pollinated plants from both male-fertility types. In contrast to KNP, male-sterile plants produced larger kernels than male-fertile plants (mean kernel weight of 237 mg for the former and 221 mg for the latter; $P \leq 0.008$), but stand density and tassel presence introduced significant interactions ($P \leq 0.022$) for this attribute (Table 3). Detasseling effects were mainly evident as a reduction in final KNP of some hybrids under synchronous pollination (e.g. male-fertile DK 664 in Exp₁ or male-fertile DK 752 in Exp₂, both at 3 plants per m²), which translated into the above-mentioned decrease in grain yield (Table 3). Detasseling effects were less clear for the other grain yield components. It promoted differences in prolificacy only for natural-pollinated plants, which were evidenced by all versions of hybrid DK752 but not DK664, and only at the low stand density. Similarly, significant interaction effects were detected between tassel presence and other treatments on KW ($P \leq 0.022$), but we were not able to establish any clear response pattern.

Synchronous hand-pollination of all exposed silks on 5 days after silking led to an increase in KNP (Fig. 2a), which averaged +15% in Exp₁ (range between –13% and +71%; Table 3) and +4% in Exp₂ (range between –11% and +16%; Table 3). This increase was not related to an increased prolificacy. For KNP, the intercept of the linear model fitted between natural and hand-pollinated plants (parameter *a*) differed significantly ($P < 0.05$) from 0 and the slope (parameter *b*) of the relationship did not differ significantly from 1. This determined an average gain of 76 kernels per plant for synchronous pollination when compared to the natural pollination across the whole range of KNP. The few situations where synchronous pollination reduced KNP usually corresponded to detasseling treatments (Table 3 and Fig. 2a). There was a clear reduction in the KW of hand-pollinated plants when compared to the open pollinated ones (Fig. 2b). This reduction averaged –12% in Exp₁ (range between –30% and +2%; Table 3) and –8% in Exp₂ (range between –19% and +4%; Table 3). This decrease in KW of synchronously pollinated vs. natural pollinated plants

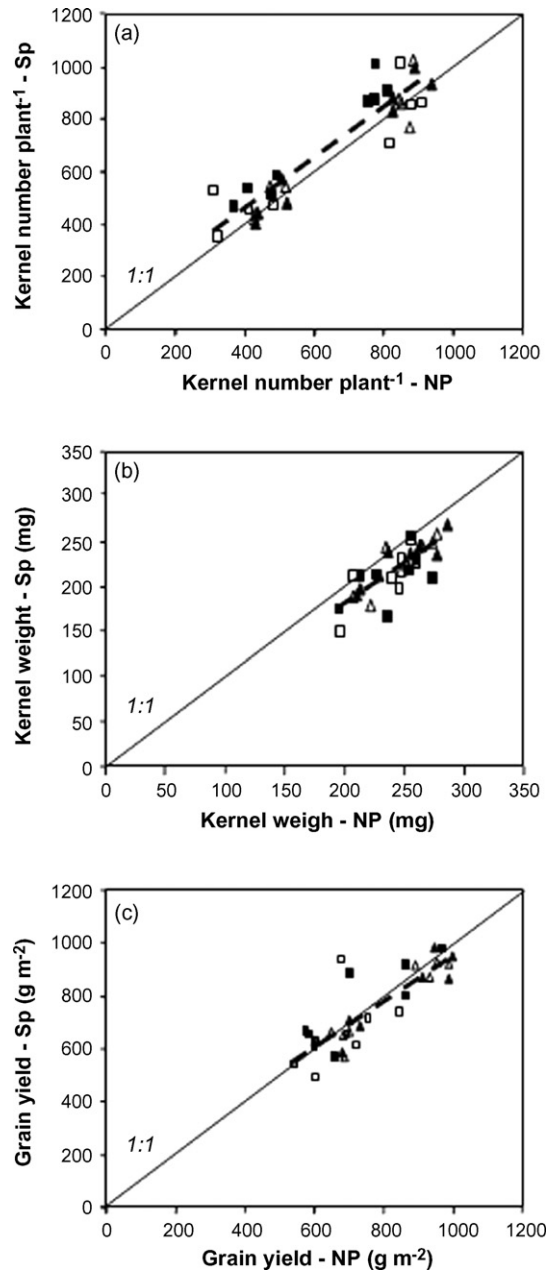


Fig. 2. Effect of pollination treatment (NP, natural pollination; SP, synchronous pollination) on (a) kernel number per plant, (b) kernel weight, and (c) plant grain yield. Data correspond to two hybrids and two male-fertility versions cropped during 1997–1998 (squares) and 1998–1999 (triangles) at Salto. Open symbols are for detasseled plants and closed symbols for non-detasseled plants. The solid lines represent the 1:1 relationship. The dotted lines represent the linear models fitted to the data: (a) $Y = 76 + 0.96 X$, $r^2 = 0.87$, $P < 0.001$; (b) $Y = 4.2 + 0.88 X$, $r^2 = 0.62$, $P < 0.001$; (c) $Y = 86 + 0.87 X$, $r^2 = 0.70$, $P < 0.001$.

was evidenced in a slope value significantly ($P < 0.05$) smaller than 1 and an intercept value not different from 0. As such, the divergent effect of the pollination system on KNP and KW resulted in an almost complete tradeoff between both grain yield components, so that this treatment had no effect on final grain yield (average effect of SP on PGY was +1% in Exp₁ and –4% in Exp₂; Fig. 2c).

3.4. Tassel development

At the low stand density used in Exp₃, there was no delay in silking date respect to anthesis date (*i.e.* ASI = 0) for any hybrid. Male-fertile tassels of DK 752 reached anthesis and silking slightly later than those of DK 664 (+66 °C), but the general pattern of tassel and ear development and growth was not different between hybrids (data not shown).

Final plant, tassel and ear lengths differed ($P < 0.05$) between hybrids, but male-fertility versions were not different within either hybrid. There was a trend towards enhanced tassel and ear elongation before flowering in male-sterile plants when compared to the male-fertile ones (Fig. 3), but differences were not significant at flowering. From silking –265 °C onwards, tassels from male-fertile plants continued the normal development described in Table 1, while anthers from male-sterile tassels collapsed and appeared visually reabsorbed. Active ear elongation started at the end of tassel growth and matched the period of tassel peduncle elongation (Fig. 3).

4. Discussion

4.1. Apical dominance

In general, the initial response of plants to tassel removal and male sterility evidenced the expected reduction in apical dominance and enhanced biomass partitioning to ears, verified in a reduced time to silking that promoted a decrease in the ASI_{pp} and ESI_{pp} as compare to the male-fertile controls. There are no reports in the literature on the effects of male sterility on maize flowering dynamics. However, a reduced time to silking in response to detasseling had already been informed by Grogan (1956). This positive response to reduced apical dominance, however, did not translate into the improved kernel set reported in previous research (Grogan, 1956; Chinwuba et al., 1961) for stand densities within the range explored in our work.

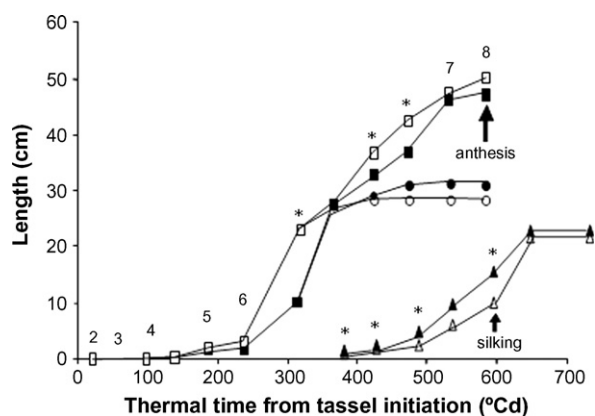


Fig. 3. Evolution of ear length (triangles), and tassel length with (squares) and without (circles) peduncle of male-fertile (close symbols) and male-sterile (open symbols) plants of hybrid DK 752 cropped in Exp₃. Numbers on the curves correspond to stages described in Table 1. Asterisks above symbols indicate significant ($P < 0.05$) differences between male-fertility versions. Arrows indicate the timing of 50% anthesis and 50% silking.

Early reports suggested that the beneficial effects of detasseling on kernel set and grain yield were due to improved assimilate availability for ear growth because energy costs associated with pollen production were eliminated (Grogan, 1956; Chinwuba et al., 1961). Benefits of detasseling could also be related to the reduction of light interception by the tassels (Duncan et al., 1967; Hunter et al., 1969). Evidences from our study and previous research (Horner and Palmer, 1995) minimize the value of tassel removal immediately before anthesis for reducing the use of assimilates in pollen production, because most pollen grains seemed already formed at this stage. Also, detasseling benefits on light interception may be strongly dependent on tassel size. The indirect selection for reduced tassel size performed in maize during the last decades (Fischer et al., 1987; Duvick and Cassman, 1999; Edmeades et al., 2000) may have diminished the positive effects of tassel removal on light interception, explaining results obtained in the present research with current commercial hybrids. This assertion is supported by recent findings (Echarte et al., 2004) on kernel number response to detasseling of a new (DK752) and an old (DKF880) maize hybrid, where improved kernel number in apical ears was only observed for the latter.

Male-sterility effects on grain yield observed in the present work did not allow for definitive conclusions. In contrast to detasseling, male-sterility of hybrids included in our study affected tassel growth even before the start of active ear growth (Fig. 3). This condition, however, seemed not to improve biomass allocation to the earshoots at stand densities below the optimum for maximum grain yield, and consequently did not translate into enhanced kernel set. Our results on reduced kernel number improvement of male-sterile versions are in agreement with data reported by Sangoi and Salvador (1998), who included maize inbreds and hybrids cropped at stand densities comparable to those used in the present work. In turn, Chinwuba et al. (1961) reported significant differences in kernel number between male-fertility versions of hybrids cropped at 4 and 7 plants per m². Similarly, Andrade et al. (1996) indicated that the male-sterile version of one hybrid had a larger kernel number per unit area than its male-fertile counterpart when crop at the supra-optimal stand density of 11 plants per m². This apparent contradiction among different studies may be attributable to maize improved tolerance to increased stand density (Tollenaar and Lee, 2002), which promoted a shift in the optimal stand densities from *ca.* 4 to 7 plants per m² for hybrids cropped in the 1960s (Tollenaar, 1989) to more than 9 plants per m² in currently grown hybrids (Luque et al., 2006). This shift may also be partially related to the described reduced tassel size resulting from breeding, which have reduced differences between versions in biomass allocation to tassel growth. Thus, reports on improved kernel set of male-sterile versions seem mostly associated to supra-optimal stand densities, suggesting this form of reduced apical dominance may be a valuable trait when growing high input maize crops aimed at achieving potential grain yield. High stand densities are usually used in these cropping systems, and may become supra-optimal depending on the prevailing

weather conditions of each year. Already available blends of male-fertile and male-sterile versions are a good alternative for minimizing the risk of overcrowding stress associated to these systems.

4.2. Synchronous pollination

Synchronous pollination had a clear positive effect on final kernel number, due to improved kernel set in sub-apical ears at low stand density (data not shown) and in apical ears at high stand density. This trend in kernel number, however, did not translate into an improved grain yield, in contrast to the only well documented previous report (Sarquís et al., 1998). Sarquís et al. (1998) showed no evidences of reduced KW in response to improved kernel number due to synchronous pollination in their work. But evidences of possible tradeoff situations have been reported (Borrás and Otegui, 2001; Schussler et al., 2002). The observed reductions in KW can be attributed to a reduction in the resource available per fertile floret around flowering, caused by greater kernel set under synchronous pollination. Because plant growth rate during the flowering period was similar among pollination treatments (Cárcova, 2003), assimilate availability per established kernel was reduced in synchronously pollinated plants from ovary fertilization onwards (Cárcova et al., 2000; Borrás and Otegui, 2001). These changes in the number of kernels set by the plant for a similar plant growth rate around flowering surely modified the potential kernel weight by reducing the assimilate availability per individual kernel during the first stages of grain development (Gambín et al., 2006). These findings agree with the general hypothesis that maize plants set an individual kernel sink potential early in grain filling, which usually induces subsequent kernel growth to take place within a range of saturating assimilate availability for biomass accumulation during late kernel growth (Borrás et al., 2004). As such, increasing kernel number by modifying the number of kernels set by the plant for the same plant growth around flowering might not increase the final plant grain yield. It will depend on the capacity of the plant to increase the total source so as to maintain a similar source per established sink. Our findings show that an increased established sink (*i.e.*, KNP) do not usually increase source production (Sinclair and Jamieson, 2006), especially at periods when yield is known to be source limited (Egli and Bruening, 2003).

Finally, discrepancy with previous findings by Sarquís et al. (1998) may be attributable to genotypic differences (*e.g.*, tropical vs. temperate germplasm) and breeding effects on grain yield determination around flowering, from a sometimes sink-limited (tall plants of non prolific small-eared hybrids with large tassels; Duvick and Cassman, 1999) to a predominantly source-limited condition. For the post-green revolution period (*i.e.*, since the 1960s), Luque et al. (2006) indicated no breeding effects on leaf area index of temperate Argentine maize hybrids grown across a wide range of stand densities. Similar findings were reported for plant leaf area in studies performed with North American temperate hybrids (Duvick and Cassman, 1999). Lack of change in the size of the source was

accompanied by a steady increase in sink size represented by the number of harvestable kernels, particularly at high stand densities. A reduced anthesis-silking interval (Duvick and Cassman, 1999) and an enhanced biomass partitioning to the ear around flowering (Luque et al., 2006) allowed for improved kernel set. Nevertheless, these trends may have pushed source availability per kernel to a limit that promoted a complete tradeoff between grain yield components when kernel numbers were artificially augmented by means of synchronous pollination. Future studies on synchronous pollination effects on grain yield determination should compare tropical and temperate maize hybrids, because the former are supposed to be more sink limited than the latter (Hay and Gilbert, 2001).

5. Conclusions

Results obtained in our research gave new evidences on the effects of practices commonly used for improving kernel set and grain yield in commercial maize crops and seed production fields. In principle, some results seem controversial compared with earlier studies on reduced apical dominance, but differences may be attributed to improved stress tolerance obtained through breeding during the last decades. This trend may have reduced the beneficial effects observed in the past. Some aspects of apical dominance still remain unclear, like (i) the negative effects of detasseling on final kernel number in spite of the initial positive effect of this practice on female flowering dynamics, and (ii) the lack of response in kernel set to the early arrest of tassel growth in male-sterile plants at standard stand densities, in contrast to the positive effect always reported at supra-optimal plant populations. Finally, synchronous pollination usually improved kernel number, but kernel weight responded negatively to this practice, resulting in no yield increases.

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References

- Analytical Software, 2000. Statistix 7. Tallahassee, Fl. www.statistix.com.
- Andrade, F.H., Cirilo, A.G., Uhart, S., Otegui, M.E., 1996. *Ecofisiología del cultivo de maíz*. Editorial La Barrosa and DekalbPress, Buenos Aires, Argentina, pp. 292.
- Andrade, F.H., Echarte, L., Rizzalli, R., Della Maggiora, A., Casanovas, M., 2002. Kernel number prediction in maize under nitrogen or water stress. *Crop Sci.* 42, 1173–1179.
- Andrade, F., Vega, C.R.C., Uhart, S., Cirilo, A.G., Cantarero, M., Valentinuz, O., 1999. Kernel number determination in maize. *Crop Sci.* 39, 453–459.

- Bonnett, O.T., 1966. Development of the staminate and pistillate inflorescences of maize. In: *Inflorescences of maize, wheat, rye, barley and oats: their initiation and development*. Univ. of Illinois Agric. Exp. Stn. Bull. 721.
- Borrás, L., Otegui, M.E., 2001. Maize kernel weight response to post-flowering source–sink ratio. *Crop Sci.* 41, 1816–1822.
- Borrás, L., Slafer, G.A., Otegui, M.E., 2004. Seed dry weight response to source–sink manipulations in wheat, maize and soybean. A quantitative reappraisal. *Field Crops Res.* 86, 131–146.
- Borrás, L., Westgate, M.E., Astini, J.P., Echarte, L., 2007. Coupling time to silking with plant growth rate in maize. *Field Crops Res.* 102, 73–85.
- Cárcova, J., 2003. Características reproductivas y fijación de granos en maíz. Fundamentos para incrementar y estabilizar el rendimiento. PhD Thesis. University of Buenos Aires. p. 186.
- Cárcova, J., Uribelarrea, M., Borrás, L., Otegui, M.E., Westgate, M.E., 2000. Synchronous pollination within and between ears improves kernel set in maize. *Crop Sci.* 40, 1056–1061.
- Chapman, S.C., Edmeades, G.O., 1999. Selection improves drought tolerance in tropical maize populations: II. Direct and correlated responses among secondary traits. *Crop Sci.* 39, 1315–1324.
- Chinwuba, P.M., Grogan, C.O., Zuber, M.S., 1961. Interaction of detasseling, sterility, and spacing on yields on maize hybrids. *Crop Sci.* 1, 279–280.
- Cline, M.G., 1991. Apical dominance. *Bot. Rev.* 57, 318–358.
- Duncan, W.G., Williams, W.A., Loomis, R.S., 1967. Tassels and the productivity of maize. *Crop Sci.* 7, 37–39.
- Duvick, D.N., Cassman, K.G., 1999. Post-green revolution trends in yield potential of temperate maize in the north-central United States. *Crop Sci.* 39, 1622–1630.
- Echarte, L., Andrade, F.H., Vega, C.R.C., Tollenaar, M., 2004. Kernel number determination in Argentinean maize hybrids released between 1965 and 1993. *Crop Sci.* 44, 1654–1661.
- Edmeades, G.O., Bänziger, M., Ribaut, J.M., 2000. Maize improvement for drought-limited environments. In: Otegui, M.E., Slafer, G.A. (Eds.), *Physiological Bases for Maize Improvement*. Food Products Press, The Haworth Press, Binghamton, NY, pp. 75–111.
- Edmeades, G.O., Bolaños, J., Hernández, M., Bello, S., 1993. Causes for silk delay in a lowland tropical maize population. *Crop Sci.* 33, 1029–1035.
- Egli, D.B., Bruening, W.P., 2003. Increasing sink size does not increase photosynthesis during seed filling in soybean. *Eur. J. Agron.* 19, 289–298.
- Fischer, K.S., Edmeades, G.O., Johnson, E.C., 1987. Recurrent selection for reduced tassel branch number and reduced leaf area density above the ear in tropical maize populations. *Crop Sci.* 27, 1150–1156.
- Gambín, B.L., Borrás, L., Otegui, M.E., 2006. Source–sink relations and kernel weight differences in maize temperate hybrids. *Field Crops Res.* 95, 316–326.
- Grogan, C.O., 1956. Detasseling responses in corn. *Agron. J.* 48, 247–249.
- Hall, H.J., Rebella, C.M., Ghera, C.M., Culot, J.Ph., 1992. Field-crop systems of the Pampas. In: Pearson, C.J. (Ed.), *Field Crop Ecosystems*. Elsevier, Amsterdam, pp. 413–449.
- Hay, R.K.M., Gilbert, R.A., 2001. Variation in the harvest index of tropical maize: evaluation of recent evidence from Mexico and Malawi. *Ann. Appl. Biol.* 138, 103–109.
- Horner, H.T., Palmer, R.G., 1995. Mechanisms of genic male sterility. *Crop Sci.* 35, 1527–1535.
- Hunter, R.B., Daynard, T.B., Hume, D.J., Tanner, J.W., Curtis, J.D., Kannenberg, L.W., 1969. Effect of tassel removal on grain yield of corn (*Zea mays* L.). *Crop Sci.* 9, 405–406.
- Luque, S.F., Cirilo, A.G., Otegui, M.E., 2006. Genetic gains in grain yield and related physiological attributes in Argentine maize hybrids. *Field Crops Res.* 95, 383–397.
- Monteith, J.L., 1965. Radiation and crops. *Exp. Agric.* 1, 241–251.
- Motto, M., Moll, R.H., 1983. Prolificacy in maize: a review. *Maydica* 28, 53–76.
- Otegui, M.E., 1995. Prolificacy and grain yield components in modern Argentinean maize hybrids. *Maydica* 40, 371–376.
- Ritchie, J.T., NeSmith, D.S., 1991. Temperature and crop development. In: Hanks, J., Ritchie, J.T. (Eds.), *Modelling Plant and Soil Systems*. Agronomy Monograph 31. American Soc. of Agron. and Crop Sci. Soc. of America, Madison, WI, pp. 5–29.
- Russell, W.A., 1991. Genetic improvement of maize yields. *Adv. Agron.* 46, 245–298.
- Sadras, V.O., Echarte, L., Andrade, F.H., 2000. Profile of leaf senescence during reproductive growth of sunflower and maize. *Ann. Bot.* 85, 187–195.
- Sangoi, L., Salvador, R.J., 1998. Effect of maize plant detasseling on grain yield, tolerance to high plant density and drought stress. *Pesquisa Agropecuaria Brasileira* 33, 677–684.
- Sarquís, J.I., Gonzalez, H., Dunlap, J.R., 1998. Yield response of two cycles of selection from a semiprolific early maize (*Zea mays* L.) population to plant density, sucrose infusion and pollination control. *Field Crops Res.* 55, 109–116.
- Schussler, J.R., Edmeades, G.O., Campos, H., Wink, B., Ibañez, M., 2002. Use of synchronous pollination to investigate kernel set in drought stressed maize. ASA-CSSA-SSSA, Madison, WI (Abstract CSSA, CDROM).
- Sinclair, T.R., Jamieson, P.D., 2006. Grain number, wheat yield, and bottling beer: an analysis. *Field Crops Res.* 98, 60–67.
- Tollenaar, M., 1989. Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959 to 1980. *Crop Sci.* 29, 1365–1371.
- Tollenaar, M., Lee, E.A., 2002. Yield potential, yield stability and stress tolerance in maize. *Field Crops Res.* 75, 161–169.
- Uribelarrea, M., Cárcova, J., Otegui, M.E., Westgate, M.E., 2002. Pollen production, pollination dynamics, and kernel set in maize. *Crop Sci.* 42, 1910–1918.