



Responses of Maize Hybrids to Twin-Row Spatial Arrangement at Multiple Plant Densities

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ABSTRACT

Twin-row planting systems in maize (*Zea mays* L.) have been proposed as an alternative spatial arrangement that should theoretically decrease plant-to-plant competition, alleviate crop crowding stress and improve yields. Uncertainty remains, however, as to whether twin rows are a feasible option to increase plant densities and improve grain yields. Three hybrids (DKC62-54, DKC61-19, and DKC57-66) were grown from 2009 to 2011 to evaluate the individual and interacting effects of plant density (PD1 = 69,000; PD2 = 81,000; PD3 = 93,000; and PD4 = 105,000 plants [pl] ha⁻¹) and spatial configuration (conventional single 76-cm row width vs. 20-cm twin rows spaced 76-cm between paired-rows) on dark prairie soil in West-Central Indiana. The primary research objectives were to determine (i) whether the twin-row spatial arrangement permits higher optimum plant densities, (ii) whether hybrids vary in their response to a twin-row arrangement, and (iii) diverse morphophysiological trait responses to density and spatial treatments. Twin rows never yielded significantly more than single rows at any plant density or hybrid combination in any year of this study. Furthermore, there was no evidence that grain yield-optimizing plant densities were any higher with twin vs. single rows in any hybrid. Twin rows slightly increased leaf area index (LAI) at silk emergence stage in 2010 (mean LAI = 4.8) and 2011 (mean LAI = 4.0), but not in 2009 (mean LAI = 4.4). Despite higher plant spacing variation, radiation interception was initially favored by earlier canopy closure with twin-row planting, but the relative radiation-interception advantage declined as plant density increased and at a later vegetative stage.

THE CONTINUOUS INCREASE in maize grain yield in the world's primary growing areas during the last decades was mainly driven by the development of crowding stress tolerant hybrids that allowed for dramatic increases in plant population and, therefore, in production per unit area (Russell, 1984; Tollenaar and Wu, 1999; Duvick, 2005). Maize grain yields in the United States have also increased due to earlier planting dates (Kucharik, 2008) and more extensive use of irrigation (Cassman, 1999). Sustaining maize grain yield increases into the future requires continued reconsideration of current agronomic practices.

Decreasing row spacing at equal plant density promotes more equidistant plant spacing, theoretically reducing plant-to-plant competition, while improving plant resource capture and utilization (Duncan, 1984; Andrade et al., 2002; Barbieri et al., 2008) and decreasing weed competition through earlier canopy closure (Bullock et al., 1988). Nonetheless, sharply contrasting conclusions have been reported regarding grain yield response to narrow rows (Nielsen, 1988; Porter et al., 1997; Barbieri et al.,

2000; Farnham, 2001; Ma et al., 2003; Andrade et al., 2002; Shapiro and Wortmann, 2006; Yilmaz et al., 2008), and the grain yield benefit from the implementation of this practice may not warrant the additional machinery investment required.

The spatial configuration known as twin rows (Karlen and Camp, 1985) is not a new concept. Twin-row planting systems have proven to be advantageous to soybean [*Glycine max* (L.) Merr.] yields vs. the single-wide-row alternative of 76-cm spacing (Janovicek et al., 2006) and have gained renewed interest for U.S. maize production in the past decade. Theoretically, twin-row maize planting systems appears to be an opportunity to derive the benefits of narrow rows without need of major changes in harvest, nutrient, or pest application equipment. While the distance between consecutive maize plants within a row at around 85,000 pl ha⁻¹ is around 15 cm for 76-cm planting row widths, in a precisely distributed twin-row arrangement with a 20-cm distance between paired rows, plants ought to be approximately 25 cm from their closest neighbors. Twin-row research has been performed across the United States with varying success, but recent studies showed no consistent grain yield benefit from twin-row over single-row configurations at the same plant densities in the states of Alabama, Iowa, Missouri, or Nebraska (Elmore and Abendroth, 2007; Nelson and Smoot, 2009; Balkcom et al., 2011; Novacek, 2011).

In conditions without major nutrient or water limitations, maize grain yield depends most on radiation interception and radiation-driven photosynthetic conversion efficiencies around

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Abbreviations: ASI, anthesis-silking interval; BM, total aboveground biomass; CMR, comparative relative maturity; CV, coefficient of variation; HI, harvest index; LAI, leaf area index; PAR, photosynthetically active radiation, PH, plant height; pl, plants; SD, stalk diameter.

the critical period bracketing silking (Andrade et al., 1993, 2002). A very strong association, following an exponential function, between the LAI and the proportion of radiation intercepted by maize plants is documented in the scientific literature (Hippes et al., 1983; Jones and Kiniry, 1986; Trápani et al., 1992). Following these fundamental principles, a critical LAI threshold (close to $4.0 \text{ m}^2 \text{ m}^{-2}$) was defined after which further increases in LAI are not reflected in increases in the proportion of the radiation intercepted by maize (Maddonna and Otegui, 1996). Overall, benefits from narrowing rows are expected in situations where the crop is not likely to achieve the critical value of LAI at silking (Barbieri et al., 2000; Maddonna et al., 2001; Andrade et al., 2002). Increases in plant density generally have a large positive impact on the incident solar radiation intercepted (%) and, as a consequence, on crop growth rate around silking as well as final grain yield (Tollenaar and Aguilera, 1992; Andrade et al., 1999). Conceivably, decreased plant-to-plant competition due to narrow or twin rows could also benefit maize production via greater early-season radiation interception (Nielsen, 1988) and via assertions of higher biomass production and improved root growth (Sharratt and McWilliams, 2005; Great Plains, 2012).

Although it is expected that narrow or twin rows would allow planting at higher densities without potentially showing a detrimental effect in grain yield, there are inconsistencies in the literature regarding this interaction (Porter et al., 1997; Farnham, 2001; Yilmaz et al., 2008). Karlen et al. (1987) and Balkcom et al. (2011) reported a yield advantage from twin rows at the higher range of plant densities evaluated (up to approximately $85,000 \text{ pl ha}^{-1}$), while Novacek (2011) did not find significant benefits from the implementation of twin rows at any plant density (ranging from $65,000$ – $105,000 \text{ pl ha}^{-1}$). Additional research is needed to better understand possible row configuration by plant density interactions.

Optimum plant density varies among hybrids due to intrinsic differences in biomass production, biomass allocation to reproductive structures (i.e., harvest index) (Echarte and Andrade, 2003; Sarlangue et al., 2007), leaf growth and orientation (Maddonna et al., 2002), and the response of these traits to fluctuations in ground space available per plant. The latter suggests that hybrids differ in their ability to use the available soil-light environment, and the two-way hybrid by row spacing, and three-way hybrid by row spacing by plant density interactions are, therefore, worthy of study. Evidence of greater response to narrow rows has been documented for early vs. late maturing hybrids, primarily because individual plants of early maturity hybrids are smaller, with reduced LAI (Tollenaar, 1977; Dwyer et al., 1994) and might more frequently have challenges in achieving 95% radiation interception.

This research study compared three different hybrids at two different spatial arrangements (twin rows vs. the conventional single 76-cm row width) across four plant densities to (i) determine whether the twin-row spatial arrangement is a feasible management practice to achieve higher optimum plant densities for maize, (ii) evaluate whether hybrids vary in their response to a twin-row arrangement, and (iii) understand the response of diverse morpho-physiological traits to the different plant densities and spatial arrangements. We hypothesize that maize crops can experience a positive response to twin rows if this

configuration contributes to the achievement of a LAI above 4.0 at the silking stage in situations where this critical LAI would not be achieved with single rows.

MATERIALS AND METHODS

Cultural Practices, Experimental Design, and Treatments

A research study was conducted at the Purdue University Agronomy Center for Research and Education (ACRE) ($40^{\circ}28'07'' \text{ N}$, $87^{\circ}00'25'' \text{ W}$) near West Lafayette, IN, during the 2009, 2010, and 2011 growing seasons. The soil was a Chalmers (fine-silty, mixed, superactive, mesic Typic Endoaquoll) silty clay loam with approximately 4.0 to 4.5% organic matter content in the topsoil layer (0–30-cm soil depth). The location has an average annual precipitation of 960 mm. Further details regarding the specific climatic conditions for each growing season are given in Fig. 1.

The studies were arranged as a split-split plot design with three replications. The treatments evaluated involved the combination of three Monsanto (Monsanto Company, St. Louis, MO) hybrids [DKC62-54 (Comparative Relative Maturity-CRM = 112), DKC61-19 (CRM = 111) and DKC57-66 (CRM = 107)] as main plots, four plant densities (PD1 = $69,000$; PD2 = $81,000$; PD3 = $93,000$; and PD4 = $105,000 \text{ pl ha}^{-1}$) as subplots, and two spatial row configurations [76-cm row width, single rows (S) and twin rows (T)] as sub-subplots. Individual sub-subplots were 3.05 m wide (i.e., four single rows or four pairs of twin rows) and 30 m in length.

These maize field experiments followed soybean in 2009 and 2010, and followed maize in the 2011 season. Nitrogen fertilizer was pre-plant applied as NH_3 at a rate of 225 kg N ha^{-1} in 2009 and 2010, and 250 kg N ha^{-1} in 2011. Conventional tillage practices involved full-width spring cultivation after NH_3 application and before planting. Weed control was ensured using residual pre- and post-emergence herbicides. The experiments were planted using precision guidance on 22 May 2009, 7 May 2010, and 18 May 2011. In 2009, all plots (whether single or twin-row configurations) were seeded with a Great Plains YP425-TR planter with finger-pickup metering. In both 2010 and 2011, all plots were seeded with a Great Plains YP425A-TR with a positive-air seed metering system. The same planter was used in both row-width systems to avoid any possible row-width treatment confounding with unequal seeding depths, unequal seed placements, unequal seed closing, or unequal tractor wheel-track compaction. Seed drive sprockets were adjusted to achieve near identical seed populations for each pair of twin-row and single-row sub-sub-treatments, and planting speeds were approximately 6 km h^{-1} . Each year, planter unit seed drives were adjusted at planting to try to synchronize the seed drop at planting for the adjacent twin rows. Final plant densities were measured in four 5.3-m long positions in the center single or twin rows of each plot.

Individual plant spacing relative to adjacent plants within the single and twin rows was estimated during early vegetative stages each year. A 2-m tape measure (2009) or a 2.5-m tape measure (2010–2011) was randomly placed in each of the two center rows for single rows, and in each of the two center pairs of twin rows, and the position for each plant within that length of row (s) was recorded. Differences in plant spacing variation for the

row width treatments were estimated for each hybrid, population density, and row width combination. The coefficient of variation (CV) of the per-plant spacing was calculated as an intended measurement of the uniformity of plant distribution within contrasting spatial arrangements.

Morpho-Physiological Measurements

Duration of the different phenological stages was expressed in days or in growing degree days (thermal time). Growing degree days for a specific period were calculated as the sum of daily average temperatures above 8°C (Ritchie and NeSmith, 1991), starting the thermal time accumulation at crop emergence stage.

Each year, 20 plants located near the center of each plot in three replications were marked following emergence to perform nondestructive measurements throughout the growing season. Plant measurements followed similar procedures as previously described by Boomsma et al. (2009) and Ciampitti and Vyn (2011). Plant height (PH) was recorded at 155 and 540°C d⁻¹ [~V3 and V8 phenological stages (Ritchie and Hanway, 1982)] in 2009, at 415 and 730°C d⁻¹ (~V5 and V12 stages) in 2010, and at 405 and 600°C d⁻¹ in 2011 (~V8 and V10 stages), measured as the distance from the stem base (at the soil surface) to the uppermost developed leaf tip. Stalk diameter (SD) was measured at silking in 2009 and at physiological maturity in 2010, using a Mitutoyo ABSOLUTE 12 Digimatic caliper (Mitutoyo America Corp., Aurora, IL). The indirect estimation of chlorophyll content was taken through the single-photon avalanche diode (R1 and R2 stages in 2009, and R2 and R4 stages in 2010) on the ear leaves using the Konica Minolta SPAD-15 502 Chlorophyll Meter (Konica Minolta Sensing Americas, Inc., Ramsey, NJ). The SPAD values were averaged for the same 20 plants on successive dates within a year. The temporal separation between anthesis and silking [i.e., anthesis silking interval (ASI)] was determined for each plot every year. Anthesis and silking were respectively defined as the beginning of pollen shed from the tassel and the appearance of the first pollen receptive silk (Borrás et al., 2007). Throughout the flowering period, measurements of anthesis and silking were taken on a daily basis by counting the number of silked or tasseled plants out of 20 per plot and expressing it in percent terms.

During early vegetative stages in 2009 and 2011, radiation interception (%) was calculated as $(1 - I_t/I_0) \times 100$ where I_t is the incident photosynthetically active radiation (PAR) just below the lowest layer of photosynthetically active leaves and I_0 is the PAR at the top of the canopy. The values of I_t and I_0 were obtained with an AccuPAR radiometer (Decagon Devices, Pullman, WA). The sensor was placed diagonally between rows with each end of the sensor in a row or between the two rows in the twin row configuration. This measurement was performed at the 461 and 555°C d⁻¹ (~V8 and V10 stages) in 2009 and at 466 and 688°C d⁻¹ (~V8 and V14 stages) in 2011.

Leaf area index was estimated every year at the silking (R1) stage. In 2009, destructive evaluations were performed of individual leaf areas from representative plants of each hybrid following the procedures previously reported by Ciampitti and Vyn (2011). In 2010 and 2011, LAI was estimated indirectly using the Li-Cor LAI-2200 Plant Canopy Analyzer (PCA, LI-COR, Lincoln, NE).

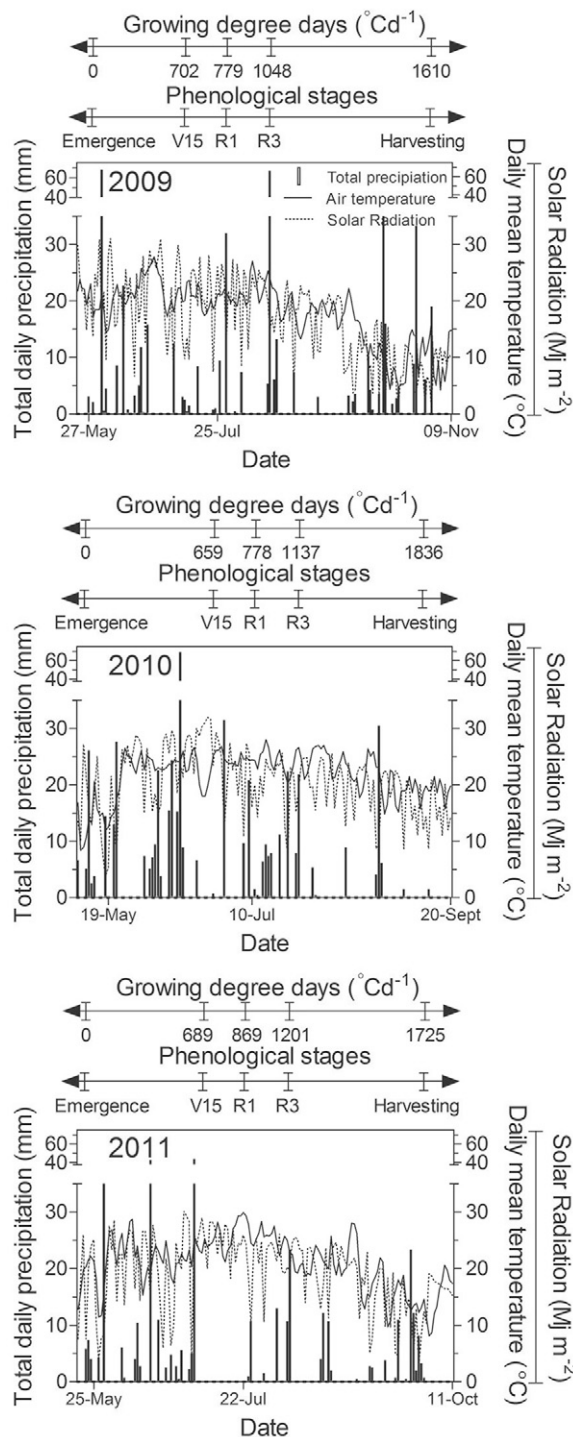


Fig. 1. Climatic information associated with the total daily precipitation, daily mean air temperatures, and solar radiation at the Purdue University Agronomy Center for Research and Education during three growing seasons (2009, 2010, and 2011). Timing of seedling emergence, harvesting, and the phenological stages V15, R1, and R3 (Ritchie and Hanway, 1982) are indicated, expressed in both days and thermal time units, for each growing season.

Destructive Measurements

Total aboveground biomass (BM) and grain harvest index (HI) were determined in 2009 and 2010. At physiological maturity, six consecutive plants were cut from the center row of each plot in three replications at the lowest and the highest plant densities (PD1 and PD4) for all hybrid and row type

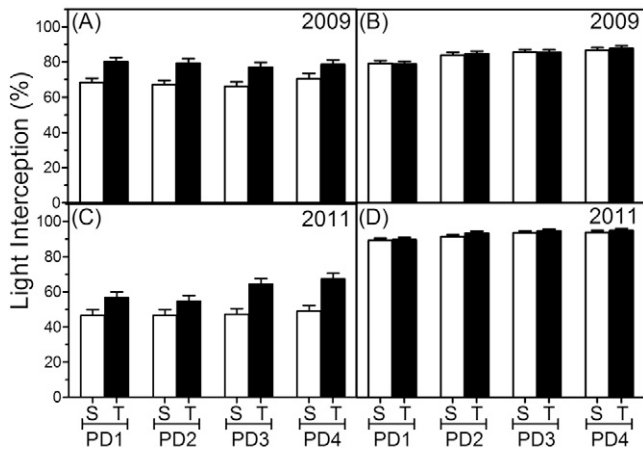


Fig. 2. Light interception expressed in relative terms determined at different phenological stages (A = 461°C d⁻¹ (~V8 stage), B = 555°C d⁻¹ (~V10 stage), C = 466°C d⁻¹ (~V8 stage), and D = 688°C d⁻¹ (~V14 stage)) for the different plant densities and row types across maize hybrids in 2009 (A and B) and 2011 (C and D). Black color bars refer to twin-row (T) spacing arrangements, and white bars represent the single-row (S) spacing combinations. For the plant density factor, PD1 = 69,000 plants (pl) ha⁻¹, PD2 = 81,000 pl ha⁻¹, PD3 = 93,000 pl ha⁻¹, and PD4 = 105,000 pl ha⁻¹.

combinations (totaling 12 treatments). The plants were cut at the stem base, chopped, dried to a constant weight at 60°C, and weighed. Harvest index was estimated as the ratio of grain weight and BM at harvest.

In all three growing seasons, maize grain yield was obtained with a Kincaid 8XP plot combine from the entire plot length of either single or twin rows in the center 1.5 m of each plot, and adjusted to 15.5% moisture.

Statistical Analyses

The experiments were arranged and analyzed as a split-split plot with three replications for all evaluated parameters. The number of treatment combinations evaluated came from the arrangement of three factors outlined previously (each one with different levels), with a consequent total of 24 treatments per replication. The different growing seasons (years) were analyzed separately, but individual parameters were averaged over hybrids if the hybrid interactions with plant density and row spacing were insignificant. The ANOVA for each parameter was executed using SAS PROC MIXED (SAS Institute, 2004). Least-squares mean tests were performed for fixed effects only when treatment effects were significant at different significance levels (P values = 0.1, 0.05, and 0.01).

The maize grain yield (expressed in Mg ha⁻¹), LAI (expressed in m² m⁻²), and light interception (expressed in relative terms, %) parameters were plotted with the GraphPad Prism 4 software (Motulsky and Christopoulos, 2003), using bar graphs to visualize the influence of the combination of different plant density and row spacing factors (Fig. 2).

RESULTS AND DISCUSSION

Final plant densities achieved were generally close to the target plant densities and the four plant density treatments within a year were always clearly different from each other (Table 1). There were no hybrid main treatment or interaction effects on the actual plant densities (data not shown). Nevertheless, in our

Table 1. Crop sequence and maize phenological data (dates of planting, emergence and harvesting, 15 leaves stage [V15], silking [R1], and milk stage [R3]), expressed in growing degree days after emergence (thermal time [TT]^{°C d⁻¹}), for each growing season.

Phenological data-date- (growing degree days)	Growing seasons		
	2009	2010	2011
Preceding crop	soybean	soybean	maize
Planting date	22 May	7 May	18 May
Emergence date	27 May (0°C d ⁻¹)	19 May (0°C d ⁻¹)	25 May (0°C d ⁻¹)
V15 stage	15 July (702°C d ⁻¹)	3 July (659°C d ⁻¹)	12 July (689°C d ⁻¹)
Silking date (R1)	25 July (779°C d ⁻¹)	10 July (778°C d ⁻¹)	22 July (869°C d ⁻¹)
Milk stage (R3)	14 Aug. (1048°C d ⁻¹)	1 Aug. (1137°C d ⁻¹)	10 Aug. (1201°C d ⁻¹)
Harvesting date	9 Nov.	20 Sept.	11 Oct.

experiments, final plant densities for twin and single rows were not significantly different in the majority of the plant density treatments over the years (Table 2).

Most of the assumed theoretical benefits from twin rows are related to the changes in the per-plant-available space, which is strongly linked to uniformity of within-row spaces between consecutive plants. Twin-row per-plant spacing was more uneven as compared to the single-row distribution (Table 2). We acknowledge that this CV comparison is perhaps overly simplistic in that it expresses the variation in plant spacing of consecutive plants in a similar fashion whether in single or paired rows (where the rows are 20 cm apart). Nevertheless, it serves to highlight the deviation of the actual twin-row planting from the theoretically desirable goal of the diagonal or "diamond" pattern for plant spacing in the paired rows.

Table 2. Final plant density achieved and per-plant spacing coefficient of variation (CV) for each plant density and row type (RT) (single [S] or twin [T] rows) combination averaged across hybrids during the three growing seasons evaluated (2009, 2010, and 2011). The value in parenthesis refers to the standard error of each treatment combination. Different letters indicate significant differences among treatment combinations at different P values evaluated (LSD test, $P < 0.05$).

Target plant density	RT	Maize growing seasons		
		2009	2010	2011
		Final plant density achieved		
plants ha ⁻¹		plants ha ⁻¹		
69,000	S	68,100 (644)f	74,700 (433)d	69,600 (1388)e
	T	67,100 (687)f	72,200 (472)e	69,800 (929)e
81,000	S	81,800 (438)de	85,400 (374)c	81,000 (235)d
	T	78,900 (494)e	83,400 (387)c	81,500(214)d
93,000	S	91,400 (717)c	94,400 (563)b	87,500 (593)c
	T	84,700 (585)d	96,200 (694)b	92,700 (269)b
105,000	S	111,500 (1079)a	105,100 (301)a	105,100 (1412)a
	T	102,600 (1116)b	105,900 (269)a	104,500 (276)a
		per-plant spacing CV, %		
69,000	S	31.5d	30.7d	23.1d
	T	61.2b	73.3a	48.6c
81,000	S	36.7cd	33.0d	21.2d
	T	69.1a	70.6a	63.0b
93,000	S	35.3cd	30.8d	27.3d
	T	64.5ab	54.2b	77.6a
105,000	S	41.9c	34.0cd	27.1d
	T	70.1a	42.5c	61.3b

Grain Yield

Overall maize yields were markedly greater during the 2009 growing season (~14.6 Mg ha⁻¹), as compared to 2010 and 2011 (~12.0 and ~9.0 Mg ha⁻¹, respectively). These substantial differences in mean yields among years are mostly related to different growing season climatic conditions (Fig. 1) and, to a lesser extent, to the preceding crop (Table 1). Maize grown in rotation with soybean yielded approximately 16% more than continuous maize during 2011 in nearby long-term rotation plots with a similar tillage system (Vyn, personal communication, 2011). An added crop stress in the 2011 growing season was a hail storm on 13 August (~R3 stage) which resulted in substantial loss of leaf area and a shortened grain-filling period.

Maize grain yield response to plant density is often described as matching a characteristic curvilinear pattern with an optimum value (Echarte et al., 2000; Tokatlidis and Koutroubas, 2004) strongly dependent on the environment (climatic conditions), hybrid (Cox, 1996; Farnham, 2001; Sarlangue et al., 2007), N supply (Boomsma et al., 2009; Ciampitti and Vyn, 2011) and/or water availability (Tokatlidis et al., 2011), among others. Currently recommended plant densities for modern hybrids in the U.S. Corn Belt range from 75,000 to 85,000 pl ha⁻¹ (Nielsen, 2012; Thomison et al., 2011). In the present study, plant density significantly affected grain yield in 2009 ($P = 0.019$), but the density effect was negligible during the 2010 and 2011 growing seasons (Table 3). The highest grain yield values were documented at 81,000 pl ha⁻¹ in 2009 and 2010, and 69,000 pl ha⁻¹ in 2011 (Table 3). It is therefore evident that the highest grain yields were not automatically associated with the highest plant density evaluated in these studies. In addition, in our research, the typical quadratic response was not evident. Nielsen (2012) reported virtually flat responses of maize grain yield to plant densities ranging from 55,000 to 110,000 pl ha⁻¹

in 27 on-farm trials across Indiana from year 2001 to 2011; the latter emphasizes the complexity involved in predicting optimum plant densities among different environments, hybrids, and years. Although less frequently observed, linear responses of maize grain yields to plant density have also been reported in the scientific literature. Novacek (2011) evaluated the same treatment combinations (hybrids, densities, and row types) in Nebraska under full irrigation during 2009 and 2010, and observed slightly positive linear responses to the range of plant densities evaluated.

Although the overall hybrid effect on maize grain yields was significant ($P < 0.05$) only in 2009 and 2011, hybrids DKC62-54 and DKC61-19 yielded consistently more than the short-season hybrid (DKC57-66) in all 3 yr (Table 3). The hybrid factor interacted with plant density ($P = 0.07$) for grain yield in 2010. In separate hybrid-specific statistical analyses for this season, DKC61-19 yields were not significantly different among plant density levels (12.0 Mg ha⁻¹), except for the 93,000 pl ha⁻¹, which resulted in the lowest yield (~11.3 Mg ha⁻¹) (data not shown). For DKC62-54, the highest yield values were observed at the 81,000 and 105,000 pl ha⁻¹ (~12.7 Mg ha⁻¹) as compared to the yields achieved at 69,000 and 93,000 pl ha⁻¹ (~12.1 Mg ha⁻¹). For DKC57-66, yields were highest at either 81,000 or 93,000 pl ha⁻¹ (~12.1 Mg ha⁻¹), but lower when this genotype was planted at lower and higher densities (69,000 and 105,000 pl ha⁻¹; ~11.4 Mg ha⁻¹). Balkcom et al. (2011) found differences in the maize yield response to the interaction of hybrids (conventional and glyphosate resistant) and plant densities (low, 40,000–44,000 pl ha⁻¹; medium, 59,000–64,000 pl ha⁻¹; high, 79,000–84,000 pl ha⁻¹), but reported higher yields at the highest plant density for specific situations.

Maize grain yields with twin-row spacing were never significantly different from the comparable yields in single rows,

Table 3. Maize grain yields at maturity, and leaf area index (LAI) measured at silking time for each individual treatment factor {plant density [PD], row type [RT](single [S] or twin [T] rows), and hybrids [hyb]} evaluated during the three growing seasons. Only significant single treatment effects resulting from the ANOVA analysis were analyzed for the means separation test (LSD); different letters indicate significant differences among treatment combinations at different P values evaluated ($P < 0.1$, $P < 0.05$).

Treatment factors			Maize growing seasons					
			2009		2010		2011	
PD	RT	Hyb	Grain yield	LAI RI	Grain yield	LAI RI	Grain yield	LAI RI
plants ha ⁻¹			Mg ha ⁻¹	m ² m ⁻²	Mg ha ⁻¹	m ² m ⁻²	Mg ha ⁻¹	m ² m ⁻²
69,000	-	-	14.30b	3.91c	11.90	4.47b	9.22	3.55
81,000	-	-	15.10a	4.00c	12.50	4.67b	9.07	4.07
93,000	-	-	14.60ab	4.76b	11.70	5.11a	9.10	4.01
105,000	-	-	14.40b	5.10a	11.90	5.11a	8.88	4.25
-	S	-	14.54	4.45	11.90	4.77b	9.17	3.82b
-	T	-	14.40	4.44	12.00	4.91a	8.96	3.95a
-	-	DKC62-54	15.00a	4.43	12.40	4.72	9.17a	3.70
-	-	DKC61-19	14.80a	4.43	11.81	5.06	9.71a	4.12
-	-	DKC57-66	13.90b	4.58	11.75	4.98	8.26b	4.08
ANOVA								
PD			*	*	ns†	*	ns	ns
RT			ns	ns	ns	‡	ns	*
PD × RT			ns	ns	ns	ns	ns	ns
Hyb			*	ns	ns	ns	*	ns
Hyb × PD			ns	ns	‡	ns	ns	ns
Hyb × RT			ns	ns	ns	ns	ns	ns
Hyb × PD × RT			ns	ns	ns	ns	ns	ns

* $P < 0.05$.

† ns = not significant.

‡ $P < 0.1$.

Table 4. Aboveground biomass (Mg ha⁻¹) and grain harvest index (HI, %) determined at physiological maturity, for each individual treatment factor {plant density [PD], row type [RT] (single [S] or twin [T] rows), and hybrid [hyb]} evaluated during the two growing seasons (2009 and 2011). Only significant single treatment effects resulting from the ANOVA analysis were analyzed for the means separation test (LSD); different letters indicate significant differences among treatment combinations at different P values evaluated ($P < 0.1$, $P < 0.05$).

Treatment factors			Maize growing seasons			
			2009		2010	
PD	RT	Hyb	Aboveground biomass	HI	Aboveground biomass	HI
plants ha ⁻¹			Mg ha ⁻¹	%	Mg ha ⁻¹	%
69,000	-	-	22.5b	56.9a	18.2b	55.0a
105,000	-	-	24.5a	55.5b	20.6a	52.8b
-	S	-	23.7	56.5	19.7	53.9
-	T	-	23.3	55.9	19.1	54.3
-	-	DKC62-54	24.4a	56.3ab	19.2ab	52.2b
-	-	DKC61-19	24.3a	55.1b	20.8a	53.5b
-	-	DKC57-66	21.9b	57.3a	18.2b	56.0a
ANOVA						
PD			*	*	*	*
RT			ns†	ns	ns	ns
PD × RT			ns	ns	ns	ns
Hyb			‡	*	*	*
Hyb × PD			ns	ns	ns	ns
Hyb × RT			ns	ns	ns	ns
Hyb × PD × RT			ns	ns	ns	ns

* $P < 0.05$.

† ns = not significant.

‡ $P < 0.1$.

and interactions of row width with hybrid and (or) density were also not significant (Table 3). In agreement with our findings, Nelson and Smoot (2009) reported no maize yield advantage with twin rows as well as similar optimum plant densities for twin or single row types. Similar results were documented by several other researchers (Elmore and Abendroth, 2007; Balkcom et al., 2011; Novacek, 2011). Previous findings suggested that grain yield responses to changes in planting arrangement are more likely to occur when restrictions to crop development are evident (Thelen, 2006). For the conditions explored in these research studies, no evidence was detected to support the claim that the twin-row spatial arrangement presents a yield advantage opportunity at higher plant densities (at least within our range from 69,000–105,000 pl ha⁻¹).

Leaf Area Index

Average LAI at silking resulted in values of 4.5, 4.9, and 4.0 m² m⁻² during the 2009, 2010, and 2011 growing seasons, respectively (Table 3). Weather conditions during 2010 growing season allowed for an optimum vegetative development (Fig. 1), but unusually high temperatures over a prolonged period after the silking stage affected kernel set, shortened the grain-filling period, and negatively impacted final yields. Plant density was the major factor driving LAI variations in this research study, although the effect was not significant in 2011 (Table 3). For all years, the highest LAI values were consistently observed at the highest plant density level (averaging 4.8 m² m⁻²; Table 3), but no differences were found between PD3 and PD4 in 2010. The influence of plant density on this trait is well known (Tetio-Kagho and Gardner, 1988; Tollenaar and Aguilera, 1992; Westgate et al., 1997; Maddonni et al., 2006; Ciampitti and Vyn, 2011); however, LAI responses for twin vs. single rows have not been extensively documented. In our study, significantly higher LAI was observed for twin rows in 2010 (~3% gain; $P = 0.069$)

and 2011 (~8% gain; $P = 0.056$). The 2-yr average LAI attained on the twin-row was ~4.4 m² m⁻² compared to ~4.2 m² m⁻² for the single-row treatment (Table 3). The hybrid factor was not significant for this trait in any of the experiment years (Table 3).

Because the achievement of 95% intercepted radiation during the critical period bracketing silking is essential to maize yield determination (Andrade et al., 2002), inadequate canopy development in specific genotype, environment, and (or) management situations suggest that alternate testing regimes might affect the outcomes with twin-row research. For illustration, if maize canopy development is limited by northern locations (Widdicombe and Thelen, 2002), short-season hybrids (Dwyer et al., 1994), or N deficient areas (Barbieri et al., 2000) narrow or twin-row treatments might respond differently than when canopy development and leaf area retention are already optimum. Nevertheless, plant density remains an important factor in any row spacing research. The higher LAI values achieved with the twin rows, as compared to the single-row system, during the 2010 and 2011 growing seasons were not translated into any benefit in grain yields (Fig. 2, Table 3). Within the range of densities used in our experiment, maize yield benefits from twin over single rows arising from an increase in LAI and PAR interception were not detected. We can tentatively conclude that, even with the lowest plant density in single rows, LAI values were above the critical level in all the environment-years explored. In contrast, Balkcom et al. (2011) reported LAI benefits from twin over single rows, but the LAI values were obtained by averaging measurements determined at different phenological stages (initial or late differences in LAI are thus misrepresented) in that situation. In this research, whether the overall influence of twin-row management on LAI at the R1 stage was neutral (2009) or positive (2010 and 2011), the LAI response to row type never significantly interacted with plant density and (or) hybrids (Table 3).

Biomass Production and Harvest Index

Similar to grain yield trends, BM at maturity was significantly higher in 2009 (~23.5 Mg ha⁻¹) as compared to 2010 (~19.4 Mg ha⁻¹) (Table 4). Both plant density and hybrid main effects were consistently significant (at different significance levels), but hybrid or plant density interactions with each other and (or) with row width were not significant for either BM or HI (Table 4). As anticipated, higher plant density resulted in increased BM but lower HI (Table 4). The same trend of lower HI values at higher plant density levels was previously observed by Tollenaar et al. (1997) and Vega et al. (2000). The short season genotype (DKC57-66) consistently resulted in the lowest BM values ($P < 0.05$), averaging 2.2 Mg ha⁻¹ less than the other two hybrids (Table 4). Consistent with the lack of grain yield response, row type had no significant influence on BM or HI (Table 4).

Light Interception

Row type significantly affected the proportion of the light intercepted by the maize canopy at the V8 stage ($P < 0.0001$) (Fig. 2). Twin rows resulted in relative advantages of 13 and 22% in the proportion of the radiation intercepted at the V8 stage during 2009 and 2011, respectively. However, differences in light interception between row types were less evident during the later V stage ($P > 0.05$) in 2009, although still significant ($P < 0.1$) in 2011. In the latter year, twin rows resulted in an average ~93% of light intercepted vs. ~91% for single rows (Fig. 2). Novacek (2011) also reported radiation interception advantages from twin-row configuration during initial vegetative stages. The lowest plant density treatment significantly lowered radiation interception only at V10 during both years, but otherwise plant densities had little impact on the proportion of light intercepted.

Morpho-Physiological Parameters

Diverse morpho-physiological parameters were measured to better understand the interacting and single effects of different treatment factors. Plant heights for twin rows were very similar to single rows and no significant PH differences were observed among plant density and hybrid treatments or among their interactions with row spacing (Table 5).

Normally, the anthesis-silking interval (ASI) variable is strongly dependent on the plant growth rate achieved around silking (Borrás et al., 2007). Typically, higher individual plant growth rates are achieved at low plant densities, and these lead to a greater synchrony between anthesis and silking (i.e., low ASI values). However, in these environments, ASI was not affected by variations in plant density. The latter can be attributed to relatively low stress during flowering and perhaps to high data variability which did not allow detection of small differences among treatments. Ear leaf SPAD values at R1 and R2 phenological stages in 2009 were primarily affected

Table 5. Morpho-physiological measurements determined at different phenological stages for each individual treatment factor (plant density [PD], row type [RT] (single [S] or twin [T] rows), and hybrid [hyb]) during the three growing seasons (2009, 2010, and 2011). Only significant single treatment effects resulting from the ANOVA analysis were analyzed for the means separation test (LSD); different letters indicate significant differences among treatment combinations at different P values.

Treatment factors	Maize growing seasons																	
	2009							2010							2011			
	PD plants ha ⁻¹	RT	Hyb	PHV3 cm	PHV8 cm	ASI °Cd ⁻¹	SPAD R1	SPAD R2	SD R2 mm	PHV5 cm	PHV12 cm	ASI °Cd ⁻¹	SPAD R2	SPAD R4	SD R6	PHV7 cm	PHV13	ASI °Cd ⁻¹
69,000	-	-	18.9	161	2.0b	48.9a	52.8a	27.5a	26.7	201	5.0	51.0	45.1	24.8a	81.9	158	-7.4	
81,000	-	-	19.3	165	5.5ab	48.0ab	51.5ab	25.8b	27.0	201	3.0	52.9	47.6	23.5b	82.6	158	-3.8	
93,000	-	-	19.2	164	11.5a	45.9b	50.7b	24.6b	27.5	207	5.0	52.6	48.3	22.7c	84.2	163	1.7	
105,000	-	-	19.3	164	3.5b	43.6c	47.9c	21.8c	26.7	193	3.0	49.8	45.4	21.7 d	85.0	160	8.8	
-	S	-	18.8b	162	5.5	45.9b	50.6	24.5b	27.0	200	2.0	51.5	46.7	23.0	83.5	159	-1.0	
-	T	-	19.5a	164	5.8	47.4a	50.8	25.3a	27.0	201	6.0	51.7	46.5	23.4	83.5	160	0.7	
-	-	DKC62-54	18.7	163	4.6b	45.8	49.4b	25.6a	26.6	204	0.7b	49.9	46.6ab	24.6a	83.1b	160ab	-3.5	
-	-	DKC61-19	19.1	164	1.2b	47.2	51.9a	24.2b	26.9	200	0.9b	53.8	49.5a	22.8b	80.3b	155b	-4.3	
-	-	DKC57-66	19.5	163	11.6a	46.9	51.2a	25.2a	27.4	197	12.0a	51.7	43.7b	22.1c	87.0a	164a	7.3	
ANOVA																		
PD			ns†	ns	*	†	*	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
RT			*	†	*	*	*	*	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
PD × RT			ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Hyb			ns	ns	*	*	*	*	ns	ns	*	ns	*	*	*	*	†	ns
Hyb × PD			ns	ns	ns	†	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Hyb × RT			ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Hyb × PD × RT			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

* $P < 0.05$.
† ns = not significant.
‡ $P < 0.1$.

by plant density, decreasing as plant density increased across the different hybrids and row types (Table 5). Boomsma et al. (2009) and Ciampitti et al. (2012) documented similar trends in their studies at the same location. For the row type factor, the SPAD values only significantly differed at R1 in 2009 (Table 5), when higher SPAD values were observed for twin rows. The latter suggests a more even light distribution within the canopy for the twin-row configuration in specific situations, which may be related to a more uniform leaf N profile within the canopy (Drouet and Bonhomme, 1999). The lack of significant effect on SPAD for the rest of the years evaluated may be due to the timing of the measurement (after the silking time), which suggests that early differences in SPAD values were not detectable later in grain filling (R2 and R4 stages). Hybrid differences were found for the SPAD values obtained during reproductive stages both years (Table 5), but a consistent trend was not discernible.

Lowest stalk diameters were associated with the highest plant densities (Table 5) in both years investigated (2009 and 2010) at two different reproductive-stages (R2 and R6 stages, respectively) consistent with previous findings by Boomsma et al. (2009) and Ciampitti et al. (2012). Additionally, a significantly higher stalk diameter value was observed at the R2 stage in the 2009 season for the twin row type (~25 mm) as compared to the single row arrangement (~24 mm); a similar but weaker trend was detected at the R6 stage in the 2010 season.

Plant Uniformity and Relative Yield Advantages for Twin vs. Single Rows

Because simple within-row spacing CVs of consecutive plants along each single or paired row were approximately double in the twin-row vs. the single-row planting systems in all 3 yr (Table 2), we investigated the possible contribution of plant spacing uniformity to the relative yield benefits of twin rows. A simple analysis was performed in which the difference in per-plant grain yields (determined after adjusting bulk yield in each plot by the measured plant density in each year) between the twin vs. single row type (ΔGY_{RT}) was compared with the difference in the per-plant coefficient of variation (CV%) for plant spacing between the twin- and single-row arrangement ($\Delta CV\%_{RT}$) in individual reps for each hybrid and plant density combination (Fig. 3). This calculation was focused on the row type because the hybrid and plant density main treatments, and their interacting effects, were nonsignificant for both ΔGY_{RT} and $\Delta CV\%_{RT}$ (data not shown). The $\Delta CV\%_{RT}$ ranged from -13 to 66%, and the ΔGY_{RT} ranged from -2.6 to 2.8 Mg ha⁻¹ (-37 to 47 g per plant; 103 data points representing row-type paired plot means from individual replications). From the analysis, it was possible to detect, as a general trend, that mean paired-plot comparisons with lower $\Delta CV\%_{RT}$ (i.e., more spacing uniformity in the twin-row type) were correlated to positive ΔGY_{RT} (higher twin row yields relative to single rows). The yield advantage of the twin-row configuration over the single averaged close to 0.7 Mg ha⁻¹ (6.8 g per plant at an average plant density of 9.5 pl m⁻²) for 18 individual-paired-plot data points between the range from 0 to 21% $\Delta CV\%_{RT}$ while only 5 individual-paired-plot data points demonstrated higher grain yields with single rows vs. the twin rows (Fig. 3). The general trend was one of increasing yield benefit with the twin-row arrangement as the $\Delta CV\%_{RT}$ was lower or closer to 0 (i.e., similar CV% in both row types).

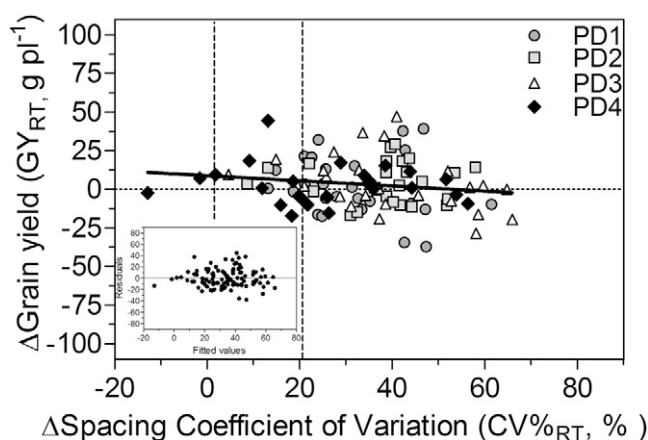


Fig. 3. Mean per-plant grain yield differences between twin vs. single row types (ΔGY_{RT}) relative to the corresponding differences in per-plant spacing coefficient of variation ($CV\%_{RT}$) between twin vs. single row types for paired individual plots of each hybrid and plant density combination from 2009 to 2011. For the plant density factor, PD1 = 69,000 plants (pl) ha⁻¹ (dark gray circles), PD2 = 81,000 pl ha⁻¹ (light gray squares), PD3 = 93,000 pl ha⁻¹ (white triangles), and PD4 = 105,000 pl ha⁻¹ (black diamonds). The sub-figure shows the residuals distribution of the evaluated relationship over the fitted values. Vertical broken lines embrace the variation range from 0 to 21% $CV\%_{RT}$.

The relative spacing uniformity achieved in twin-row planting, therefore, appears to be an influencing factor in the possible yield differential between arrangements.

Although more research should be conducted, these simple analyses documented that one of the possible explanations for the lack of yield advantage from the twin over the single row types may be a substantially less uniform per-plant arrangement in the twin row type, a consequence of the inability to achieve the theoretically optimum diagonal pattern of plant spacing within each pair of rows at all plant densities evaluated. The higher CV values reported for the twin row arrangement (Table 2) may have limited the expression of one of the potential benefits attributed to the twin-row spatial arrangement (i.e., that of reducing the per-plant competition). Nevertheless, specific per-plant physiological measurements at different phenological stages (such as stalk diameter, light interception, and plant growth at different timings, and leaf senescence scoring during the grain filling) are all essential phenotypic measurements to analyze beyond yield differences alone. These and other detailed individual plant measurements are required to better understand physiological mechanisms of individual plant behavior under different spatial arrangements as plant density increases.

CONCLUSIONS

Twin rows never resulted in any significant yield benefit relative to single rows across the hybrids and plant density levels evaluated for a 3-yr period in this environment. To some extent, the lack of response may have been related to LAI levels during early reproductive growth (around silking time), which were already consistently above critical levels for efficient radiation interception. Narrow-row production systems are known to be more likely to benefit maize yields in situations where the target intercepted radiation (~95%) is not achieved around the period bracketing silking (e.g., northern locations, short-season hybrids, and when nutrient/water deficiencies have occurred).

Although earlier canopy closure was evident in the twin-row system, no noticeable gain in resource use efficiency was observed in our relatively high-yielding environment. In our research, the lack of response to twin rows may also have been influenced by the inability to achieve the theoretically optimum spacing arrangement.

Although no positive yield benefits were observed with twin rows in this study, future research should explore the physiological consequences of alternate planting arrangements with comparable or superior spacing precision to traditional practices in different environments.

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