# Maize Grain Yield and Kernel Component Relationships to Morphophysiological Traits in Commercial Hybrids Separated by Four Decades

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#### ABSTRACT

Hybrid era and management practices like nitrogen (N) rate and plant density influence canopy traits and their correlations with grain yield, kernel number, and kernel weight in maize (Zea mays L.). A four site-year study was conducted employing two N rates (55 and 220 kg N ha<sup>-1</sup>) with three plant densities between 54,000 and 104,000 plants ha-1 for two newer hybrids (2005) and two older hybrids (1967 and 1975). Hybrids varied in anthesis-silking interval, specific leaf nitrogen (SLN), specific leaf area, and leaf greenness (soil plant analysis development [SPAD] readings), but not in leaf area index (LAI) at silking. Consistently higher kernel weight in newer hybrids (15% in 2012, 23% in 2013) across management treatments was related to their higher SLN at silking and green leaf number retention during grain filling. The threshold LAI at silking for maximum kernel number for 2005 hybrids (averaging 3917 kernels m<sup>-2</sup>) occurred at 4.0 m<sup>2</sup> m<sup>-2</sup>, compared with 3.28 m<sup>2</sup> m<sup>-2</sup> for the 1975 hybrid (3893 kernels m<sup>-2</sup>), and kernel number declined when LAI exceeded 3.43  $m^2\ m^{-2}$  for the 1967 hybrid. Higher leaf biomass, leaf N content, and therefore higher SLN at silking in newer hybrids contributed to greater green leaf retention during grain filling. Applying more N weakened correlations between grain yield and canopy attributes, including leaf retention in both years and yield correlations with leaf biomass, leaf N content, LAI, and SLN in 2013 (more favorable weather). Grain yields for all hybrids in 2013 had higher correlations with LAI at silking and during grain fill when grown at higher densities.

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**Abbreviations:** ACRE, Agronomy Center for Research and Education; APSIM, Agricultural Production Systems sIMulator; ASI, anthesis– silking interval; CGR, crop growth rate; GY, grain yield; KN, kernel number; KW, kernel weight; LAI, leaf area index; LB, leaf biomass at silking; LNCC, leaf nitrogen concentration at silking; LNCT, leaf nitrogen content at silking; PPAC, Pinney Purdue Agricultural Center; RUE, radiation use efficiency; SLA, specific leaf area; SLN, specific leaf nitrogen; SPAD, soil plant analysis development.

THE improvement in grain yield (GY) can be dissected into two grain components, kernel number (KN) and kernel weight (KW), which are both highly affected during the critical period around silking (Andrade et al., 1999; Borrás and Otegui, 2001). Potential KN is determined by crop growth rate (CGR) during the critical period (Andrade et al., 1999). Potential KW is determined at the end of lag phase, which is 12 to 15 d after the onset of grain filling (Borrás and Gambín, 2010). The key role of radiation interception and radiation use efficiency (RUE) in CGR has been demonstrated (Tollenaar and Aguilera, 1992; Barbieri et al., 2000). Direct traits like CGR and RUE are difficult to measure, whereas some secondary traits that are correlated with GY or its components are easier to collect. For instance, Hammer et al. (2010) described the usage of such indirect canopy variables in developing the Agricultural Production Systems sIMulator (APSIM) cropping system estimation model. Additionally, these secondary traits are widely used in offering selection suggestions for plant breeding under different environments (Bänziger and Lafitte, 1997; Cirilo et al., 2009). Secondary traits often include leaf area index (LAI), specific leaf area (SLA), specific leaf nitrogen

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(SLN), leaf chlorophyll concentration (represented as soil plant analysis development [SPAD] readings), transpiration rate, photosynthesis rate, leaf N concentration (LNCC), leaf N content (LNCT), leaf biomass (LB), and green leaf number or green leaf area during the grain-filling period.

Positive correlations between LAI and GY were already documented over 45 yr ago (Nunez and Kamprath, 1969). Subsequent studies explained that this correlation is through the progressive influences of LAI on radiation interception, CGR, and KN near the onset of the grain-filling stage (Uhart and Andrade, 1995a; Barbieri et al., 2000; Hammer et al., 2010). Muchow and Sinclair (1994) documented linear relationships between cumulative intercepted radiation with biomass at both low and high fertilizer levels. Specific leaf area (cm<sup>2</sup> g<sup>-1</sup>) quantifies plant capability in production of leaf area per unit of leaf dry matter. Leaf area index is partially affected by SLA because of the role of SLA in modifying leaf thickness by adjusting LB and leaf area (Hammer et al., 2010). The SLA parameter has been widely used in genetic selection of many species, such as maize (Zea mays L.), vegetable amaranth (Amaranthus spp.), and peanut (Arachis hypogaea L.), for advances in cold stress tolerance and water use efficiency (Liu and Stützel, 2004; Hund et al., 2005; Songsri et al., 2009). Genetic variation of SLA between maize hybrids is primarily caused by leaf thickness. In general, thicker leaves have superior palisade and spongy parenchyma structures in terms of CO<sub>2</sub> and water storage (Liu and Stützel, 2004). However, a higher SLA is not always a positive feature. For example, previous studies have shown negative correlations between SPAD readings and SLA in maize (Hund et al., 2005) and between water use efficiency and SLA in peanut (Nautiyal et al., 2002).

Leaf N concentration at silking is positively correlated with RUE in maize (Uhart and Andrade, 1995a). Muchow and Sinclair (1994) showed that leaf N content had a hyperbolic relationship with RUE. These correlations of RUE with both LNCC and leaf N content at silking (LNCT) can be attributed to the major role of leaf N in synthesizing rubisco, which is the main protein in photosynthesis. Further, leaf N can be modified by SLN, which reflects N partitioning to leaves versus the stem at the onset of grain filling (Lemaire et al., 2007; Hammer et al., 2010). Radiation use efficiency had a parabolic relationship with SLN when the range of SLN was large (Massignam et al., 2009), but the correlation behaved linearly when the range of SLN was small (Muchow and Davis, 1988). Lemaire et al. (2007) concluded that maximal RUE can be achieved when SLN is ~1.4 to 1.5 g N m<sup>-2</sup>. Because of the strong correlation of SLN with RUE and LNCT, DeBruin et al. (2013) used SLN as a marker of maize leaf N status in estimating GY, KW, and KN at maturity and observed that maximum GY, KW, and KN were achieved when SLN reached 1.5, 1.6, and  $1.3 \text{ g m}^{-2}$ , respectively.

Whether KW at maturity achieves potential KW depends on crop status during grain filling or on factors such as persistence of green leaf area (Hammer et al., 2010), source:sink ratio, as represented by postsilking dry matter accumulation per KN (Borrás and Otegui, 2001; Chen et al., 2016), and ear growth rate during the period from 50% silking to 50% milkline (Chen et al., 2016). Green leaf number or green leaf area during grain filling are normally recorded for purposes of estimating visual "stay green" (Rajcan and Tollenaar, 1999; Tollenaar et al., 2004; Peng et al., 2010). However, the accuracy of green leaf number measurement alone for recording leaf senescence and consequent loss in CGR has been questioned (Lee and Tollenaar, 2007; Antonietta et al., 2014). Although quantification of functional stay green (such as leaf photosynthesis rate) would be more precise than visual stay green in discussions of green leaf number or green leaf area consequences for maize yield, functional stay green is more difficult to measure. However, the addition of leaf SPAD measurements to estimate leaf chlorophyll status can provide a complementary indicator for leaf greenness. Nitrogen deficiency during vegetative growth can reduce GY by reducing LAI and RUE, which results in limited CGR and low KN (Uhart and Andrade, 1995a, 1995b). Nitrogen deficiency in later stages speeds leaf senescence and results in lower KW (Muchow, 1988). Some studies indicated that maize appears to preferentially maintain radiation interception instead of RUE under N deficiency; that response pattern would tend to retain LAI at the expense of a rapid decline in SLN as N stress increases (Massignam et al., 2009).

Much of the increase in maize GY in the last 50 yr is a consequence of increasing density and crowding stress tolerance in newer hybrids (Lee and Tollenaar, 2007). Average density is presently around 76,232 plants ha<sup>-1</sup> in the United States, which is more than 2.5-fold that in the 1930s (Nielsen et al., 2017). Given the higher densities now employed, modern hybrids have been adapted to achieve high yields by strategies including: (i) more kernels per unit of plant growth rate around silking (Echarte et al., 2000), and (ii) higher RUE and delayed leaf senescence during the grain-filling period, leading to greater dry matter accumulation. Many studies have shown that the increase of yield at high density is due to higher KN per unit area instead of KW (Echarte et al., 2004; D'Andrea et al., 2008). One potentially negative consequence of higher plant densities is the reduction in N availability per plant, which consequently lowers cumulative biomass production and plant growth rates during silking (D'Andrea et al., 2009). As a result, high densities can decrease leaf N status, such as SLN at silking (Ciampitti et al., 2013), which then can enhance leaf senescence and lower KW. The other negative consequences are that high densities could increase plant-to-plant variability and reduce

biomass transfer to ears around silking, which would further reduce KN per plant (Pagano and Maddonni, 2007).

There are relatively few reports of direct relationships between canopy variables and GY plus yield components (KN and KW) in maize (Bänziger and Lafitte, 1997; Cirilo et al., 2009), and those that exist are typically constricted to just a few canopy variables or genotype and management situations. Therefore, important questions to be explored include:

- 1. What is the response of multiple canopy variables to interactions of multiple plant densities and N rates in newer- versus older-era hybrids?
- 2. How are these canopy variables correlated with GY, KN, and KW when N input, plant density, and hybrid era factors are considered simultaneously?

The primary objectives of this study were (i) to evaluate the effects of N rate and plant density on canopy variables—SLN, SLA, leaf greenness (by SPAD readings), LAI, LNCC, LNCT, LB at silking, and green leaf number during the grain-filling period—in older versus newer hybrids, and (ii) to evaluate the impacts of those canopy variables on GY and kernel components under varied management conditions.

## **MATERIALS AND METHODS**

### **Experiment Design and Management**

A 2-yr (2012 and 2013) and two-location experiment was conducted at the Agronomy Center for Research and Education (ACRE; 40°28'07 " N, 87°00'25 " W), West Lafayette, IN, and the Pinney Purdue Agricultural Center (PPAC; 41°26'41" N, 86°56'41" W), Wanatah, IN. The soil type at ACRE was a Drummer silty-clay loam in 2012, and a Chalmers silty-clay loam in 2013 (both are fine-silty, mixed, superactive, mesic Typic Endoaquolls). The soil type at PPAC in both years was a Sebewa loam (fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Argiaquolls). Average soil pH, organic matter, Mehlich-3 P, and Mehlich-3 K were, respectively, 6.3, 4.6 g 100 g<sup>-1</sup>, 22.2 mg kg<sup>-1</sup>, and 94.5 mg kg<sup>-1</sup> at ACRE in 2012; 6.7, 2.9 g 100 g<sup>-1</sup>, 34.5 mg kg<sup>-1</sup>, and 106.3 mg kg<sup>-1</sup> at PPAC in 2012; 6.9, 3.7 g 100 g<sup>-1</sup>, 22.2 mg kg<sup>-1</sup>, and 105.7 mg kg<sup>-1</sup> at ACRE in 2013; and 6.7, 4.4 g 100 g<sup>-1</sup>, 17.2 mg kg<sup>-1</sup>, and 91.8 mg kg<sup>-1</sup> at PPAC in 2013. In both years, the crop rotation was maize after soybean [Glycine max (L.) Merr.] at ACRE, and first-year maize after maize at PPAC. All four experimental fields were chisel plowed in fall and field cultivated in spring. The experimental design was a split-split-plot design with six blocks. The main treatment was two N rates: 55 (N1) and 220 kg N  $ha^{-1}$  (N2). The subtreatment was three plant densities: 54,000 (D1), 79,000 (D2), and 104,000 plants ha<sup>-1</sup> (D3). The sub-subtreatment was three hybrids in 2012 and four hybrids in 2013. The three common hybrids used in both years were DKC61-69 (DeKalb, VT3, 2005), DKC61-72 (DeKalb, RR2, 2005), and XL72AA (DeKalb, Conventional, 1975). In 2013, an older hybrid, XL45 (DeKalb, Conventional, 1967), was added. The VT3 hybrid (DKC61-69) contained European corn borer (Ostrinia nubilalis), corn rootworm (Diabrotica spp.), and glyphosate resistance, whereas the companion RR2 hybrid (DKC61-72) contained only glyphosate resistance. Conventional hybrids (XL72AA and XL45) do not have resistance for all the traits mentioned above. The nomenclatures employed for these hybrids are: DKC61-69 as H1, DKC61-72 as H2, XL72AA as H3, and XL45 as H4. Nitrogen was sidedress applied as band-injected urea ammonium nitrate with a DMI Nutri-Placer 2800 at 5, 30, 17, and 38 d after planting for ACRE-2012, ACRE-2013, PPAC-2012, and PPAC-2013, respectively.

Experiments were planted 17 May (ACRE-2012), 14 May (ACRE-2013), 12 May (PPAC-2012), and 1 June (PPAC-2013) with a four-row planter (Seed Pro 360, Almaco) with row spacing of 0.76 m and a plot length of 10 m.

### **Measurements**

Maximum temperature, minimum temperature and precipitation were obtained from the Purdue University–Indiana State Climate Office at station "ACRE-West Lafayette" and for PPAC at station "Wanatah 2 WNW, IN US."

Daily flowering measurements were taken from the same 20 plants in each plot to permit an anthesis–silking interval (ASI) calculation based on the difference in days from the date of 50% anthesis to 50% silked. A plant was considered "at silk-ing" when silks of the apical earshoot protruded at least 1 cm from the husk. A plant was considered "at anthesis" when at least 10 anthers had emerged from the tassel.

Leaf area index was measured at three plant stages: R1, R2, and R3 in 2012 and R1, R3, and R5 in 2013 (Abendroth et al., 2011). Five points above the canopy and five points below the canopy were taken for each plot using a Li-Cor 2200 (2014 LI-COR) with a 45° cap to avoid direct sunlight. Sampling points below the canopy followed a diagonal line between the center two rows of each plot, and the five points were evenly distributed along this diagonal line. The LAI measurements were conducted in at least three of the six blocks for each location-year.

Leaf chlorophyll content was estimated at R1 and R3 using a SPAD 502 Chlorophyll Meter (Minolta Company). For each plot, ear leaves of 20 plants were measured in approximately the middle of the leaf. The number of green leaves was recorded for 20 plants per plot at R1 and three plant stages during grain filling (R2, R3, and R5). All leaves retaining at least 50% green area on the leaf surface were counted as "green leaves."

Aboveground biomass was sampled at R1, which occurred on 23 July (ACRE-2012 and -2013), 18 July (PPAC-2012), and 13 August (PPAC-2013). The R1 sampling zones in 2012 ranged from 2.28 to 3.04 m<sup>2</sup> at both locations. In 2013, R1 sampling areas at ACRE and PPAC were consistently 3.04 m<sup>2</sup> in all plots. All plants in each predetermined harvest zone were cut at soil level and weighed. After weighing all of the plants from the harvest zone, a subsample of five plants per plot were selected and separated into leaves, stem, and ear shoot components within hours of removal from the field. The fresh and dry weight of each plant sample component was recorded before grinding to pass a 2-mm mesh. Samples were analyzed for N concentration by combustion (Method 990.03; AOAC International, 1995) at A&L Great Lakes Laboratories. Plant tissue was analyzed from all six replications from ACRE-2012, but only from three replications from other site-years due to resource constraints.

The R6 sampling areas ranged from 3.04 to 4.94 m<sup>2</sup> in 2012 for both locations, whereas they were consistently 3.04 m<sup>2</sup> in 2013. Procedures used at R6 were the same as at R1, with the exception that, in 2012, plants were partitioned into stover (leaves and stem) and ear only. Grain yield, calculated at 15.5% moisture, was determined from the same R6 harvest zones for the biomass weights above. All ears were removed from each zone and dried at 60°C until constant weight was achieved before shelling for the determination of GY per unit area. Five ears were chosen prior to drying for yield component analyses. These five ears were dried, weighed, and separated into grain and cob. After shelling, 200 kernels were counted and weighed to determine KW. Kernel number was estimated by the ratio of GY and KW. Grain and cob samples were ground and analyzed by A&L Great Lakes Laboratory for N concentration by combustion (AOAC International, 1995).

### **Calculations and Statistical Analysis**

Specific leaf N at silking is the ratio of leaf N uptake (kg ha<sup>-1</sup>) to LAI (m<sup>2</sup> m<sup>-2</sup>) at silking:

SLN (g m<sup>-2</sup>) = 
$$\frac{\text{Leaf N uptake at silking (kg ha-1)}}{\text{LAI at silking (m2m-2)}} \times 10^{-1}$$

Specific leaf area at silking is the ratio of LAI to LB at silking:

SLA (cm<sup>2</sup>g<sup>-1</sup>) = 
$$\frac{\text{LAI at silking (m2m-2)}}{\text{LB at silking (g m-2)}} \times 10^{\circ}$$

Statistical analysis was conducted with SAS 9.3 (SAS Institute, 2011). PROC MIXED was used for ANOVA with N rate, plant density, and hybrid as fixed factors and location or block as random factors. We combined data from two locations when the *F*-test based on mean squares between two locations had  $P (F > F_0) > 0.01$  for the majority of variables (Carmer et al., 1969). The interaction of N rate × block (location) was pooled when the majority of *F*-tests for this interaction had  $P (F > F_0)$ > 0.25 (Carmer et al., 1969). The model used was the same as that in Chen et al. (2015):

$$\begin{split} Y &= \mu + \alpha_i + \beta_j + \alpha \beta_{ij} + \gamma_k + \alpha \gamma_{ik} + \beta \gamma_{jk} + \alpha \beta \gamma_{ijk} + \tau_l \\ &+ \delta_{m(l)} + \alpha \beta \gamma \tau_{ijkl} + \varepsilon_{ijklm} \end{split}$$

where  $\mu$  was grand mean,  $\alpha_i$  was the main effect of N rate,  $\beta_j$  was the main effect of plant density,  $\alpha\beta_{ij}$  was the interaction of N rate  $\times$  plant density,  $\gamma_k$  was the main effect of hybrid,  $\alpha\gamma_{ik}$  was the interaction of N rate  $\times$  hybrid,  $\beta\gamma_{jk}$  was the interaction of Plant density  $\times$  hybrid,  $\alpha\beta\gamma_{ijk}$  was the interaction of N rate  $\times$  plant density  $\times$  hybrid,  $\tau_l$  was the random effect of locations,  $\delta_{m(l)}$  was the block effect that nested in locations,  $\alpha\beta\gamma\tau_{ijkl}$  was the interaction of location  $\times$  N rate  $\times$  plant density  $\times$  hybrid, and  $\varepsilon_{iiklm}$  was the error term.

The critical difference of mean separation at  $\alpha = 0.05$  level was calculated by using Fisher's LSD. The principle component analysis was conducted in R using the FactoMiner package (R Development Core Team, 2014), and we plotted the results using SigmaPlot 13.0 (Systat Software, 2014). Principle component analysis was conducted under two N rates (55 and 220 kg N ha<sup>-1</sup>) and three densities (54,000, 79,000, and 104,000 plants ha<sup>-1</sup>) for 2012 and 2013 using singular value decomposition. Genotypes were presented in biplot and shown as dots. Data points of each genotype under each variable were standardized using mean and standard deviation: standard =  $[\mathbf{x} - \text{mean}(\mathbf{x})]/$ standard deviation $(\mathbf{x})$ , where  $\mathbf{x}$  is the data vector.

After centering, the independent dimension was reduced by one (Kroonenberg, 1995). Hence, two dimensions covered 100% of variance in 2012 because there were only three genotypes. The cosine of the angle between two variables is their correlation, with an acute angle leading to a positive correlation, a right angle leading to no correlation, and an obtuse angle leading to a negative correlation (Kroonenberg, 1995, Husson et al., 2010). The length and position of projection from the position of a genotype onto each variable represents its performance on that variable; a positive value of projection indicates a relatively good performance, and a negative value of projection indicates a relatively poor performance (Kroonenberg, 1995). Correlations were conducted by using Proc CORR and Proc REG with SAS 9.3 (SAS Institute, 2011). Dummy variables (no. of dummy variables = no. of categories -1) were generated to test whether lines are the same or not when multiple regressions (interested variable had more than one category) were presented in one plot. Bilinear function analysis was conducted in SPSS 23.0 (IBM, 2013). The equations used for bilinear function analysis were  $y = a_1 + bx$  at  $x < x_0$  and  $y = a_2$  at  $x \ge x_0$ .

Parameter estimation was based on the loss function, which is  $(y - \hat{y})^2$ .

### **RESULTS** Weather Conditions in 2012 and 2013

In general, temperature was higher and precipitation was much less before flowering in 2012 than in 2013 (Table 1). Mean temperatures were similar after silking at the four environments. The cumulative precipitation before flowering in 2012 was 63 and 104 mm at ACRE and PPAC, respectively, whereas it was 250 and 153 mm after flowering. Similarly, in 2013, the cumulative precipitation before flowering was 196 and 346 mm at ACRE and PPAC, respectively, whereas it was 165 and 179 mm after flowering (Table 1). For each site-year, at least 19 mm of rain was received in the 12-d period before flowering commenced, and at least 20 mm of rain fell during the 14-d period during which flowering took place (Table 1).

# Canopy Development at Silking Response to N Rate, Density, and Hybrid

Nitrogen rates had minor effects on LAI during grain filling in both years (Table 2–5), but the higher N rate permitted more LAI retention at the R3 stage in 2012 (Table 4). Plant density affected LAI in five of six comparisons across both locations and years (Table 2–5). In 2012, LAI gains with density treatments D1 to D2 were observed at R1 (an increase of  $0.26 \text{ m}^2 \text{ m}^{-2}$ ) and at R3 (a similar increase of  $0.27 \text{ m}^2 \text{ m}^{-2}$ ) but were not evident at R2 (Table 4). Further LAI gains associated with density increases from D2 to D3 were not realized at R1 and R2 but were evident at R3 in 2012 (Table 4). Plant density effects on LAI were more consistent in 2013 (Table 5). When density increased from D1 to D2 in 2013, LAI increased by ~0.50 m<sup>2</sup> m<sup>-2</sup> at both the R1 and R3 growth Table 1. Mean monthly maximum (max. temp.), minimum (min. temp.), and average temperature (ave. temp.) and precipitation for designated time periods at the Agronomy Center for Research and Education (ACRE), West Lafayette, IN, and the Pinney Purdue Agricultural Center (PPAC), Wanatah, IN, in 2012 and 2013.

		ACRE					PPAC		
Time period	Max. temp.	Min. temp.	Ave. temp.	Precipitation	Time period	Max. temp.	Min. temp.	Ave. temp.	Precipitation
		°C		mm			°C		mm
					2012				
May	28.6	14.0	21.3	15	May	26.8	11.9	19.3	9
June	29.1	15.0	22.0	42	June	27.9	14.0	20.9	89
1–12 July	34.0	18.8	26.3	6	1–11 July	34.0	18.9	26.4	6
13–25 July†	33.9	19.9	26.9	20	12–23 July†	32.9	18.5	25.7	107
23–31 July	32.8	16.9	24.8	1	24–31 July	29.7	16.8	23.2	42
Aug.	30.0	14.0	22.0	200	Aug.	27.9	12.5	20.2	82
Sept.	24.7	10.0	17.3	39	Sept.	24.4	7.0	15.7	28
Oct.	16.4	4.9	10.7	11	Oct.	20.7	6.1	13.4	1
					2013				
May	25.3	13.4	19.4	60	June	25.7	14.2	19.7	238
June	26.9	16.1	21.3	106	July	27.2	15.8	21.6	63
1–14 July	26.2	16.5	21.3	31	1–5 Aug.	25.2	13.3	19.1	46
15–23 July†	31.4	20.2	25.9	34	6–20 Aug.†	25.9	13.0	19.3	46
24–31 July	24.3	11.9	18.5	4	21–31 Aug.	29.2	16.3	22.7	21
Aug.	27.7	14.7	21.0	44	Sept.	22.7	9.2	15.7	78
Sept.	26.8	12.2	19.3	83	Oct.	19.5	6.6	12.7	80

+ Flowering period.

stages, but at R5, there was no difference in LAI between D1 and D2. Further LAI gains associated with density increases from D2 to D3 averaged 0.72, 0.55, and 0.22  $m^2 m^{-2}$  at R1, R3, and R5 stages, respectively (Table 5).

Hybrid differences in LAI were not significant in 2012 (Table 2) and were only significant at the R5 stage in 2013 (Table 3), when the two newer hybrids, H1 and H2, had a higher LAI than H3 and H4 (Table 5). The much

lower R1-stage LAI in 2012 versus 2013 (Tables 3 and 4) was primarily due to the much lower preflowering precipitation in 2012 (Table 1), but some leaf rolling during the R1 stage measurement in 2012 (especially at ACRE) may have accentuated the low "effective" LAI results from the LiCor 2200 in that year. We acknowledge that other direct methods of LAI determination are more reliable in estimating the "real" LAI in droughty conditions.

Table 2. Mixed model for ANOVA analysis for 2012 parameters under two N rates (55 and 220 kg N ha<sup>-1</sup>), three densities (54,000, 79,000, and 104,000 plants ha<sup>-1</sup>), and three hybrids varying in release from 1975 to 2005. Data from the Agronomy Center for Research and Education, West Lafayette, IN, and the Pinney Purdue Agricultural Center, Wanatah, IN, are combined.

Variable†	N rate (N)	Density (D)	Hybrid (H)	N  imes D	N  imes H	D  imes H	$N \times D \times H$
				$ P (F > F_0)$			
LB, g m <sup>-2</sup>	0.003	< 0.001	ns‡	ns	ns	ns	ns
LNCC, g 100 g <sup>-1</sup>	< 0.001	< 0.001	< 0.001	ns	ns	ns	ns
LNCT, g m <sup>-2</sup>	< 0.001	<0.001	ns	ns	ns	ns	ns
ASI, days	ns	ns	< 0.001	ns	ns	ns	ns
LAI <sub>B1</sub> , m <sup>2</sup> m <sup>-2</sup>	ns	0.031	ns	ns	ns	ns	ns
LAI <sub>B2</sub> , m <sup>2</sup> m <sup>-2</sup>	ns	ns	ns	ns	ns	ns	ns
LAI <sub>B3</sub> , m <sup>2</sup> m <sup>-2</sup>	< 0.001	< 0.001	ns	ns	ns	ns	ns
SLA, cm <sup>2</sup> g <sup>-1</sup>	ns	0.004	ns	ns	ns	ns	ns
SLN, g m <sup>-2</sup>	0.001	ns	ns	ns	ns	ns	ns
SPAD <sub>B1</sub>	0.001	<0.001	<0.001	ns	ns	ns	ns
GL <sub>B1</sub> , no. leaves plant-1	< 0.001	< 0.001	<0.001	ns	ns	ns	ns
GL <sub>B2</sub> , no. leaves plant <sup>-1</sup>	< 0.001	< 0.001	<0.001	ns	ns	ns	ns
GL <sub>B3</sub> , no. leaves plant <sup>-1</sup>	< 0.001	< 0.001	<0.001	ns	ns	0.023	ns
GL <sub>B5</sub> , no. leaves plant <sup>-1</sup>	< 0.001	< 0.001	<0.001	ns	ns	0.046	ns
KW, mg kernel <sup>-1</sup>	< 0.001	< 0.001	<0.001	ns	0.039	ns	ns
KN, kernels m <sup>-2</sup>	0.024	<0.001	<0.001	ns	ns	ns	ns
GY, kg ha <sup>-1</sup>	< 0.001	0.020	<0.001	ns	ns	ns	ns
HI, g g <sup>−1</sup>	ns	ns	<0.001	0.006	ns	ns	ns

+ LB, leaf biomass at silking; LNCC, leaf N concentration at silking; LNCT, leaf N content at silking; ASI, anthesis–silking interval; LAI<sub>R1</sub>/LAI<sub>R2</sub>/LAI<sub>R3</sub>, leaf area index at R1/R2/ R3; SLA, specific leaf area; SLN, specific leaf nitrogen; SPAD<sub>R1</sub>, soil plant analysis development at R1; GL<sub>R1</sub>/GL<sub>R2</sub>/GL<sub>R3</sub>/GL<sub>R5</sub>, green leaf number at R1/R2/R3/R5; KW, kernel weight; KN, kernel number; GY, grain yield; HI, harvest index.

‡ ns, not significant at the 0.05 probability level.

Table 3. Mixed model for ANONA analysis for 2013 parameters under two N rates (55 and 220 kg ha N ha<sup>-1</sup>), three densities (54,000, 79,000, and 104,000 plants ha<sup>-1</sup>), and four hybrids varying in release from 1967 to 2005. Data from the Agronomy Center for Research and Education, West Lafayette, IN, and the Pinney Purdue Agricultural Center, Wanatah, IN, are combined.

Variable†	N rate (N)	Density (D)	Hybrid (H)	N  imes D	N  imes H	D  imes H	$N \times D \times H$
				$ P(F > F_0)$			
LB, g m <sup>-2</sup>	ns‡	< 0.001	< 0.001	ns	ns	ns	ns
LNCC, g 100g <sup>-1</sup>	0.002	<0.001	<0.001	ns	ns	ns	ns
LNCT, g m <sup>-2</sup>	0.013	<0.001	<0.001	0.037	ns	ns	ns
ASI, days	ns	0.020	<0.001	ns	ns	ns	ns
LAI <sub>R1</sub> , m <sup>2</sup> m <sup>-2</sup>	ns	<0.001	ns	ns	ns	ns	ns
LAI <sub>B3</sub> , m <sup>2</sup> m <sup>-2</sup>	ns	<0.001	ns	ns	ns	ns	ns
LAI <sub>R5</sub> , m <sup>2</sup> m <sup>-2</sup>	ns	0.012	0.012	ns	ns	ns	ns
SLA, cm <sup>2</sup> g <sup>-1</sup>	ns	<0.001	< 0.001	ns	ns	ns	ns
SLN, g m <sup>-2</sup>	0.013	<0.001	< 0.001	ns	ns	ns	ns
SPAD <sub>R1</sub>	0.022	<0.001	<0.001	ns	ns	0.001	0.001
SPAD <sub>R3</sub>	0.004	<0.001	0.006	0.004	ns	ns	ns
GL <sub>B1</sub> , no. leaves plant <sup>-1</sup>	0.002	<0.001	< 0.001	ns	ns	0.003	ns
GL <sub>R2</sub> , no. leaves plant <sup>-1</sup>	0.006	<0.001	0.003	0.002	ns	0.009	ns
GL <sub>R3</sub> , no. leaves plant <sup>-1</sup>	0.001	<0.001	0.010	ns	ns	0.017	ns
GL <sub>B5</sub> , no. leaves plant <sup>-1</sup>	0.001	<0.001	< 0.001	ns	ns	0.003	ns
KW, mg kernel <sup>-1</sup>	< 0.001	<0.001	<0.001	ns	ns	0.010	ns
KN, kernel m <sup>-2</sup>	ns	<0.001	<0.001	ns	ns	0.002	ns
GY, kg ha <sup>-1</sup>	0.005	ns	<0.001	ns	ns	0.002	ns
HI, g g <sup>-1</sup>	ns	ns	<0.001	ns	ns	ns	ns

† LB, leaf biomass at silking; LNCC, leaf N concentration at silking; LNCT, leaf N content at silking; ASI, anthesis–silking interval; LAI<sub>R1</sub>/LAI<sub>R3</sub>/LAI<sub>R5</sub>, leaf area index at R1/R3/ R5; SLA, specific leaf area; SLN, specific leaf nitrogen; SPAD<sub>R1</sub>/SPAD<sub>R3</sub>, soil plant analysis development at R1/R3; GL<sub>R1</sub>/GL<sub>R2</sub>/GL<sub>R3</sub>/GL<sub>R3</sub>, green leaf number at R1/R2/R3/ R5; KW, kernel weight; KN, kernel number; GY, grain yield; HI, harvest index.

‡ ns, not significant at the 0.05 probability level.

Average SLN was significantly affected by N rate but not by hybrid or density treatments in 2012 (Table 2), with a mean SLN gain of 0.42 g m<sup>-2</sup> at the higher N rate (Table 4). However, SLN responded significantly to N rate, density, and hybrid treatments in 2013 (Table 3). The higher N rate increased SLN by 0.25 g m<sup>-2</sup> in 2013 (Table 5). In addition, SLN was unaffected by density as the density changed from D1 to D2, but decreased by 0.29 g m<sup>-2</sup> from D2 to D3 (Table 5). Lastly, H1, H2, and H3 had similar SLN, whereas the oldest hybrid (H4) had 0.3 g m<sup>-2</sup> lower SLN than the average of the other hybrids (Table 5).

Table 4. Means for main effects: two N rates (N1, 55 kg N ha <sup>-1</sup> ; N2, 220 kg N ha <sup>-1</sup> ), three plant densities (D1, 54,000 plants ha <sup>-1</sup>	1;
D2, 79,000 plants ha <sup>-1</sup> ; D3, 104,000 plants ha <sup>-1</sup> ) and three hybrids (H1, DKC61-69; H2, DKC61-72; H3, XL72AA) in 2012.	

Variable†	N1	N2	D1	D2	D3	H1	H2	НЗ
LB, g m <sup>-2</sup>	262.5b‡	278.0a	235.7c	274.8b	300.3a	272.3ns	268.8ns	270.8ns
LNCC, g 100g <sup>-1</sup>	2.21b	2.48a	2.43a	2.37a	2.25b	2.27b	2.37a	2.41a
LNCT, g m <sup>-2</sup>	5.78b	6.87a	5.72b	6.54a	6.75a	6.18ns	6.34ns	6.50ns
ASI, days	1.09ns	0.81ns	1.11ns	1.09ns	0.63ns	0.10b	-0.20b	3.00a
LAI <sub>R1</sub> , m <sup>2</sup> m <sup>-2</sup>	2.66ns	2.69ns	2.51b	2.77a	2.74a	2.66ns	2.69ns	2.67ns
LAI <sub>B2</sub> , m <sup>2</sup> m <sup>-2</sup>	2.54ns	2.55ns	2.42ns	2.57ns	2.64ns	2.56ns	2.56ns	2.52ns
LAI <sub>R3</sub> , m <sup>2</sup> m <sup>-2</sup>	2.04b	2.36a	1.97c	2.24b	2.38a	2.18ns	2.22ns	2.19ns
SLA, cm <sup>2</sup> g <sup>-1</sup>	103ns	98ns	108a	102a	93b	99ns	102ns	101ns
SLN, g m <sup>-2</sup>	2.22b	2.64a	2.45ns	2.34ns	2.50ns	2.40ns	2.40ns	2.48ns
SPAD <sub>R1</sub>	50.9b	53.9a	55.1a	52.2b	50.1c	51.4b	51.2b	54.7a
GL <sub>R1</sub> , no. leaves plant <sup>-1</sup>	12.1b	12.9a	13.0a	12.2b	12.1b	12.9a	12.6b	12.3c
GL <sub>R2</sub> , no. leaves plant <sup>-1</sup>	11.1b	12.3a	12.2a	11.6b	11.3c	12.0a	11.7b	11.3c
GL <sub>R3</sub> , no. leaves plant <sup>-1</sup>	10.8b	12.3a	12.1a	11.5b	11.1c	11.9a	11.6b	11.2c
GL <sub>R5</sub> , no. leaves plant <sup>-1</sup>	9.6b	11.3a	11.1a	10.4b	9.9c	10.5a	10.4a	10.0b
KW, mg kernel <sup>-1</sup>	276b	306a	308a	288b	278c	307a	302a	265b
KN, kernel m <sup>-2</sup>	3,196b	3,496a	2,979b	3,432 a	3,627a	3,501a	3,459a	3,085b
GY, kg ha <sup>-1</sup>	10,529b	12,760a	10,974b	11,886a	12,108a	12,680a	12,401a	9,928b
HI, g g <sup>-1</sup>	0.57ns	0.57ns	0.57ns	0.57ns	0.57ns	0.58a	0.59a	0.55b

† LB, leaf biomass at silking; LNCC, leaf N concentration at silking; LNCT, leaf N content at silking; ASI, anthesis–silking interval; LAI<sub>R1</sub>/LAI<sub>R2</sub>/LAI<sub>R3</sub>, leaf area index at R1/R2/ R3; SLA, specific leaf area; SLN, specific leaf nitrogen; SPAD<sub>R1</sub>, soil plant analysis development at R1; GL<sub>R1</sub>/GL<sub>R2</sub>/GL<sub>R3</sub>/GL<sub>R5</sub>, green leaf number at R1/R2/R3/R5; KW, kernel weight; KN, kernel number; GY, grain yield; HI, harvest index.

‡ Means followed by the same letter within a variable and treatment class are the same (*P* > 0.05). Critical difference is determined by Fisher's LSD; ns, not significant at the 0.05 probability level.

Table 5. Means for main effects: two N rates (N1, 55 kg N ha <sup>-1</sup> ; N	2, 220 kg N ha <sup>-1</sup> ), three plant densities (D1, 54,000 plants ha <sup>-1</sup> ;
D2, 79,000 plants ha <sup>-1</sup> ; D3, 104,000 plants ha <sup>-1</sup> ) and three hybrids	(H1, DKC61-69; H2, DKC61-72; H3, XL72AA; H4, XL45) in 2013.

Variable†	N1	N2	D1	D2	D3	H1	H2	H3	H4
LB, g m <sup>-2</sup>	256.0ns‡	260.6ns	221.6b	267.8a	285.5a	274.2a	280.2a	268.9a	210.4b
LNCC, g 100g <sup>-1</sup>	2.57b	2.76a	2.78a	2.66b	2.56c	2.63b	2.61b	2.68ab	2.74a
LNCT, g m <sup>-2</sup>	6.21b	6.92a	6.04b	6.75a	6.90a	6.96a	6.89a	6.89a	5.52b
ASI, days	0.10ns	-0.03ns	-0.23ns	-0.08ns	0.42ns	-0.80b	-0.80b	1.10a	0.60b
LAI <sub>R1</sub> , m <sup>2</sup> m <sup>-2</sup>	3.56ns	3.57ns	2.99c	3.50b	4.22a	3.61ns	3.67ns	3.54ns	3.45ns
LAI <sub>R2</sub> , m <sup>2</sup> m <sup>-2</sup>	3.16ns	3.29ns	2.72c	3.21b	3.76a	3.27ns	3.30ns	3.17ns	3.17ns
LAI <sub>R3</sub> , m <sup>2</sup> m <sup>-2</sup>	1.32ns	1.41ns	1.30b	1.29b	1.51a	1.39a	1.49a	1.30b	1.29 b
SLA, cm <sup>2</sup> g <sup>-1</sup>	151ns	144ns	140b	142b	161a	139b	139b	139b	171a
SLN, g m <sup>-2</sup>	1.74b	1.99a	2.02a	1.93a	1.64b	1.92a	1.91a	1.98a	1.64b
SPAD <sub>R1</sub>	52.6b	55.2a	57.1a	53.0b	51.6b	53.3b	53.6b	55.3a	53.4b
SPAD <sub>R3</sub>	51.8b	55.9a	57.2a	53.5b	50.7c	52.8b	52.7b	56.3a	53.5b
GL <sub>R1</sub> , no. leaves plant <sup>-1</sup>	12.2b	12.6a	13.0a	12.3b	11.9c	12.7a	12.5a	12.5a	12.0b
GL <sub>R2</sub> , no. leaves plant <sup>-1</sup>	11.5b	12.2a	12.5a	11.7b	11.3c	12.1a	12.0a	11.7b	11.6b
GL <sub>R3</sub> , no. leaves plant <sup>-1</sup>	10.6b	11.3a	11.6a	10.8b	10.4c	11.1a	11.1a	10.9ab	10.7b
GL <sub>R5</sub> , no. leaves plant <sup>-1</sup>	9.1b	9.8a	10.2a	9.3b	8.9c	9.9a	9.9a	9.5b	8.6c
KW, mg kernel <sup>-1</sup>	264b	281a	293a	269b	256c	299a	303a	252b	237c
KN, kernel m <sup>-2</sup>	3,579ns	3,620ns	3,370b	3,724a	3,705a	3,663a	3,633a	3,824a	3,282b
GY, kg ha <sup>-1</sup>	11,373b	12,188a	11,781ns	11,950ns	11,614ns	13,072a	13,184a	11,579b	9,318c
HI, g g <sup>-1</sup>	0.51ns	0.52ns	0.51ns	0.52ns	0.51ns	0.53a	0.52ab	0.50b	0.51b

† LB, leaf biomass at silking; LNCC, leaf N concentration at silking; LNCT, leaf N content at silking; ASI, anthesis–silking interval; LAI<sub>R1</sub>/LAI<sub>R2</sub>/LAI<sub>R3</sub>, leaf area index at R1/R2/ R3; SLA, specific leaf area; SLN, specific leaf nitrogen; SPAD<sub>R1</sub>/SPAD<sub>R3</sub>, soil plant analysis development at R1/R3; GL<sub>R1</sub>/GL<sub>R2</sub>/GL<sub>R3</sub>/GL<sub>R3</sub>, green leaf number at R1/R2/R3/ R5; KW, kernel weight; KN, kernel number; GY, grain yield; HI, harvest index.

‡ ns, not significant at the 0.05 probability level. Means followed by the same letter within a variable and treatment class are the same (P > 0.05). Critical difference is determined by Fisher's LSD.

Nitrogen rate had no significant effect on SLA in either year (Tables 2 and 3), but SLA was significantly affected, albeit inconsistently, by density treatments in both years. Overall SLA decreased as density increased in 2012 (Tables 2 and 4), whereas SLA increased in response to density in 2013 (Tables 3 and 5). This inconsistent response of SLA was caused by a differential response of LB and LAI to density at silking in those years. In 2012, the rate of LB gain was greater than that of LAI when density increased from D1 to D3, leading to a smaller SLA at D3. In contrast, in 2013, the relative gain of LB was smaller than that of LAI when density increased from D1 to D3, leading to a higher SLA at D3. The limited incremental gain of LAI from D1 to D3 in 2012 was likely due to relatively low precipitation that prevented further leaf expansion. The oldest hybrid (H4) had 32 cm<sup>2</sup> g<sup>-1</sup> higher SLA than the average of the other three hybrids in 2013 (Table 5).

Leaf greenness (measured by SPAD) responses to N rate, density, and hybrid treatments were significant at the R1 stage in 2012 (Tables 2 and 4) and at the R1 and R3 stages in 2013 (Tables 3 and 5). As expected, the higher N rate increased leaf greenness in both years, whereas higher density decreased leaf greenness (Tables 4 and 5). Hybrid treatment differences in leaf greenness were primarily caused by a higher leaf greenness in H3 than in other hybrids, which was perhaps a consequence of the slightly later silking date (5 d delayed silking, compared with other hybrids in both years).

Both N rate and density treatments affected green leaf number during grain filling in both years, with a

higher green leaf retention generally occurring at high N and lower densities (Table 2–5). The older hybrids (H3 and H4) had significantly lower green leaf numbers than newer hybrids (H1 and H2) during grain filling in 2012 and 2013 (Table 5).

## Grain Yield, Kernel Number, and Kernel Weight Response to N Rate, Density, and Hybrid

The higher N rate increased average GY (across hybrid and plant density treatments) compared with N1 by 2231 kg ha<sup>-1</sup> in 2012 and by 815 kg ha<sup>-1</sup> in 2013 (Table 2–5). A GY response to increased density occurred only in 2012, when average yields increased by 912 kg ha<sup>-1</sup> from D1 to D2 (Tables 2 and 4). Newer hybrids (H1 and H2) averaged 2613 kg ha<sup>-1</sup> higher GY compared with H3 in 2012, whereas in 2013, they averaged 1549 kg ha<sup>-1</sup> higher than H3 and 3810 kg ha<sup>-</sup> higher than H4 (Tables 4 and 5). Harvest index did not respond to increases in N rate or density in both years (Tables 4 and 5). Newer hybrids consistently had higher harvest index than older hybrid(s) in 2012 and 2013 when averaged over N rates and densities.

Both N rate and plant density treatments had substantial influences on KW and KN (Tables 2 and 3). The higher N rate increased KW by 30 mg kernel<sup>-1</sup> in 2012 and by 17 mg kernel<sup>-1</sup> in 2013 (Tables 4 and 5). Mean KW decreased 30 and 37 mg kernel<sup>-1</sup> from D1 to D3 in 2012 and 2013, respectively (Tables 4 and 5). However, KN rose by 1050 kernels m<sup>-2</sup> in 2012 and by 1342 kernels m<sup>-2</sup> in 2013 as density increased from D1 to D3 (Tables 4 and 5). Newer hybrids had at least 37 mg kernel<sup>-1</sup> higher final KW than older hybrids in both years (Tables 4 and 5). In 2012, KW of H1 and H2 was 41 and 37 mg kernel<sup>-1</sup> higher than that of H3, respectively. In 2013, KW of H1 was 48 and 63 mg kernel<sup>-1</sup> higher than that of H3 and H4, respectively. Similarly, the KW of H2 was 51 and 66 mg kernel<sup>-1</sup> heavier than those of H3 and H4, respectively. Newer hybrids averaged 395 kernel m<sup>-2</sup> higher KN than H3 in 2012 (Table 4) and averaged 425 kernel m<sup>-2</sup> higher in the other three hybrids compared with H4 in 2013 (Table 5). The relatively high KN of H3 in 2013 may have resulted from its reduced ASI that year, which was <1 d compared with 3 d in 2012 (Tables 4 and 5).

An N rate × hybrid interaction effect on KW was only observed in 2012 (Table 2, P = 0.04) when N2 increased KW by about 35 mg kernel<sup>-1</sup> in newer hybrids (H1 and H2) but only increased KW in the older hybrid (H3) by 18 mg kernel<sup>-1</sup>. The density and hybrid interactions were also observed for KW, KN, and GY in 2013. Two newer hybrids had higher KW regardless of densities; however, as density increased from D1 to D2, KW was reduced by an average of 23 mg kernel<sup>-1</sup> in newer hybrids, compared with 30 mg kernel<sup>-1</sup> in the 1975 hybrid and 16 mg kernel<sup>-1</sup> in the 1967 hybrid. The two 2005 hybrids experienced further reductions in KW of 17 mg kernel<sup>-1</sup> when density increased from D2 to D3, compared with 14 mg kernel<sup>-1</sup> of reduction for the 1975 hybrid and no reduction for the 1967 hybrid.

For KN, the 1975 hybrid had the highest KN at D1 and D2, but the two 2005 hybrids had the highest KN at D3 compared with the older hybrids. The two newer hybrids increased KN by an average of 492 kernel  $m^{-2}$  from D1 to D2, compared with 141 kernel  $m^{-2}$  for the 1975 hybrid and 293 kernel  $m^{-2}$  for the 1967 hybrid. The two newer hybrids realized a further KN increase of 149 kernel  $m^{-2}$  as density increased from D2 to D3, whereas the 1975 hybrid was reduced 49 kernel  $m^{-2}$  and the 1967 hybrid was reduced 299 kernel  $m^{-2}$ .

Grain yield was always higher in the two 2005 hybrids, regardless of densities or N rates. When density increased from D1 to D2, the two newer hybrids gained an average of 800 kg ha<sup>-1</sup>, whereas the 1975 hybrid experienced a GY reduction of 870 kg ha<sup>-1</sup> and the 1967 hybrid did not change in GY. However, both newer hybrids maintained their GY level when density increased further from D2 to D3, but the 1975 hybrid lost 430 kg ha<sup>-1</sup> and the 1967 hybrid lost 560 kg ha<sup>-1</sup>. Hybrids H1 and H2 achieved higher GY at both N rates compared with H3 in 2012 (Fig. 1a and 1c) and compared with H3 and H4 in 2013 (Fig. 1b and 1d).

## **Correlations among the Secondary Traits and Grain Yield**

Kernel weight explained 29% and KN explained 75% of GY variance in 2012, whereas KW explained 44% and KN explained 50% of GY variance in 2013 (Tables 6 and 7). Interestingly, H1 and H2 had higher KN than H3 at both N rates in 2012, but the comparison was reversed in 2013, with H3 demonstrating the largest size projection on the KN vector at both N rates in 2013, while both newer hybrids showed a larger projection on KW than H3 (Fig. 1). This 1975 hybrid (H3) also had a higher SPAD reading at R1 and R3 in 2013 at both N rates, as well as a higher LAI at R3 with the lower N rate (Fig. 1b). The poor GY performance of H4 in 2013 was at least partially due to its large ASI (see large projection on ASI vector) and large SLA at both N rates. In addition, H4 also had a higher LNCC, but not LNCT, in 2013 independent of N rates, indicating a dilution of N concentration but an increased LB in the newer hybrids at both N rates (Fig. 1). The H3 also had longer ASI than both newer hybrids in 2012 and 2013 at both N rates (Fig. 1a and 1c).

The two newer hybrids had the highest KW in both years and the highest KN in 2012 across all three densities (Fig. 2). In 2013, H3 had higher KN at low and medium densities than H1 and H2, whereas it had lower KN at high density (Fig. 2d–2f). Despite poor GY performance in H4, it had high SLA across all densities in both years, and at both N rates in 2013. Overall, ASI was consistently higher in H4 relative to the two 2005 hybrids, and H4 also tended to have higher leaf greenness (SPAD readings) across all conditions (Fig. 2). In 2012, H4 had higher LNCC and LNCT at D2 and D3 densities.

In general, the higher yield of two newer hybrids were due to higher KN and KW in 2012 (across all treatments) and higher KW under both N rates for D1 and D2, but both higher KW and KN at D3 in 2013. In contrast, the lower GY of older hybrids (H3 in 2012 and H4 in 2013) was due to a longer ASI and thinner leaves (higher SLA) across all treatments.

Grain yield always correlated positively with green leaf number during grain filling in both years and at both N rates (Fig. 1, Tables 6 and 7). Green leaf number during grain filling explained 48% of GY's variance in 2012 and 64% in 2013 across all stages and treatments (Tables 6 and 7). Leaf N content and LB accounted for an average of 61 and 48%, respectively, of the GY variance across treatments in 2013. However, leaf N content and LB only explained 14 and 19% of GY variance in 2012 (Tables 6 and 7). Individual year affected correlations between variables. In 2012, the higher N rate enhanced the correlation between GY and LB, LNCT, and SLN but weakened the proportion of GY variation explained by green leaf number during grain filling (except at R5) in 2012 (Table 6). However, in 2013, the higher N rate



Fig. 1. Biplot of first two components for 18 traits and 3 hybrids in 2012, 19 traits and 4 hybrids in 2013, for two N rates: N1, 55 kg N ha<sup>-1</sup>; N2, 220 kg N ha<sup>-1</sup>. Traits are presented by vector and genotypes were shown as dots. PC1 indicates the largest components, and PC2 indicates the second largest components. LB, leaf biomass at silking, g m<sup>-2</sup>; LNCC, leaf N concentration at silking g 100 g<sup>-1</sup>; LNCT, leaf N content at silking, g m<sup>-2</sup>; ASI, anthesis–silking interval, days;  $LAI_{R1}/LAI_{R2}/LAI_{R3}/LAI_{R5}$ , leaf area index at R1/R2/R3/R5, m<sup>2</sup> m<sup>-2</sup>; SLA, specific leaf area, cm g<sup>-1</sup>; SLN, specific leaf nitrogen, g m<sup>-2</sup>; SPAD<sub>R1</sub>/SPAD<sub>R3</sub>, soil plant analysis development at R1/R3; GL<sub>R1</sub>/GL<sub>R2</sub>/GL<sub>R3</sub>/GL<sub>R5</sub>, green leaf number at R1/R2/R3/R5, no. of green leaf plant<sup>-1</sup>; KW, kernel weight, mg kernel<sup>-1</sup>; KN, kernel number, kernels m<sup>-2</sup>; GY, grain yield, g m<sup>-2</sup>; HI, harvest index, g g<sup>-1</sup>.

reduced the proportion of GY variation explained by LB, LNCT, LAI, SLN, and green leaf number at R2 and R3 (Table 7). As for the plant density effect, GY was highly correlated to both LB and LNCT in both years at all densities, except at D1 in 2012 (Tables 6 and 7). Longer ASIs had a negative impact on GY at all densities in both years, except at D1 in 2013. As plant density increased, there was an enhanced negative impact of longer ASI on GY in 2013

(Table 7). Generally, increased density increased the proportion of GY variation that was explained by LAI during the grain-filling period in 2013 (Table 7).

Secondary traits associated with plant N status exerted substantial impacts on both KN and KW GY components. Kernel weight had a strong correlation with SLN in 2013 (Fig. 1), when correlations were significant for H1, H2, and H3, and where the slopes of correlations for these

three hybrids were not different at the  $\alpha = 0.05$ level (Fig. 3). However, the correlation between KW and SLN was poor in 2012 (Fig. 2), when it was only significant for H1 (r = 0.89, P = 0.005; data not shown). Although KW was consistently correlated with green leaf number during grain filling across N rates, higher N rates generally decreased the extent of KW variation explained by green leaf number during all sampling dates during grain filling in 2012 and at R1 and R2 sampling dates in 2013 (Tables 6 and 7). Increased plant density strengthened the proportion of KW variation explained by LNCT and LB in both years, as well as the KW variation explained by green leaf number in 2013 (Tables 6 and 7). However, the variation of KW explained by green leaf number during grain filling was reduced when density increased from D1 to D2 in 2012 and did not change from D2 to D3 (Table 6).

The green leaf number trait itself during grain filling was strongly correlated with leaf N status, and more so in 2013 than in 2012 (Fig. 1 and 2). Approximately 67 and 87% of variance in green leaf number was explained by SLN at R1 for H3 and H4, respectively, and the slopes of correlations for these two hybrids were not different (Fig. 4a). Moreover, at R2 and R3, SLN correlated with green leaf number in a similar manner (the slopes of the correlations were not different among hybrids within each stage) for all four hybrids in 2013 (Fig. 4b and 4c). At R5, SLN explained 79 and 83% of variance in green leaf number for H1 and H3, with similar rates of green leaf number gain per SLN among these two hybrids (Fig. 4d). The correlation between SLN and green leaf number at R1 through R5 was weak in 2012 (averaged  $R^2 = 0.35$  across all stages and hybrids), which could be due to the much smaller difference in green leaf number per plant among hybrids during the grain-filling period in 2012 (Table 2).

Kernel number at maturity appeared to be more strongly correlated with leaf area and LBrelated traits than with plant N status itself. For example, KN had positive correlations with LB and LNCT in both years (Fig. 1, 2). Across two N rates, LB explained 27 and 30% of KN variation, whereas LNCT explained 22 and 32% of KN variation in 2012 and 2013, respectively (Tables 6 and 7). Increased density had enhanced correlations between LNCT and LB with KN in both years (Tables 6 and 7). The poor correlation between KN at maturity and LAI at R1 in 2012 was likely due to a relatively smaller range of LAI (range  $1.5-3.5 \text{ m}^2 \text{ m}^{-2}$  in 2012 vs. 2.2–5.8 m<sup>2</sup> m<sup>-2</sup>

and three plant ha<sup>-1</sup>) z ğ 220 2Z N ha<sup>-1</sup>; I kg N 55 rates (N1, under two N morphophysiological traits 70 000 nlante ha-1. D3 104 000 nlante ha-1) in 2019 components with its and grain yield 5 for ŝ dencities (D1 54 000 plants matrix Correlation 6. Table

		)	<b>Grain yield</b>				ž	srnel weigh	٦t			Ke	rnel numb	er	
Variable†	۶	N2	5	D2	D3	F	N2	Ð	D2	D3	۲ı	N2	Б	D2	B
LB, g m <sup>-2</sup>	0.13ns‡	0.53***	0.14ns	0.38**	0.35*	-0.32**	-0.26*	0.18ns	0.05ns	-0.12ns	0.36**	0.65***	0.03ns	0.40**	0.52***
LNCC, g 100g <sup>-1</sup>	-0.15ns	0.01ns	0.34*	0.23ns	0.32*	-0.05ns	-0.04ns	0.22ns	-0.01ns	0.10ns	-0.15ns	0.06ns	0.20ns	0.24ns	0.31*
LNCT, g m <sup>-2</sup>	0.04ns	0.49***	0.33*	0.40**	0.43**	-0.33**	-0.26*	0.29*	0.04ns	-0.03ns	0.25*	0.62***	0.14ns	0.41**	0.55***
ASI, days	-0.44***	-0.44***	-0.54***	-0.45***	-0.32*	-0.29*	-0.56***	-0.44**	-0.49***	-0.50***	-0.29*	-0.12ns	-0.33*	-0.24ns	-0.08ns
$LAI_{B1}$ , m <sup>2</sup> m <sup>-2</sup>	-0.03ns	0.08ns	-0.29*	0.07ns	0.16ns	-0.06ns	-0.20ns	-0.03ns	0.15ns	-0.08ns	0.00ns	0.15ns	-0.30*	-0.07ns	0.22ns
LAI <sub>B2</sub> , m <sup>2</sup> m <sup>-2</sup>	-0.03ns	-0.01ns	-0.19ns	0.00ns	0.02ns	0.12ns	-0.22ns	0.05ns	0.21ns	-0.08ns	-0.09ns	0.06ns	-0.23ns	-0.12ns	0.01ns
LAl <sub>B3</sub> , m <sup>2</sup> m <sup>-2</sup>	-0.04ns	-0.16ns	0.08ns	-0.03ns	-0.03ns	0.02ns	0.02ns	0.29*	0.37**	0.35**	-0.04ns	-0.20ns	-0.14ns	-0.28*	-0.23ns
SLA, cm <sup>2</sup> g <sup>-1</sup>	-0.14ns	-0.38***	-0.29*	-0.29*	-0.20ns	0.20ns	0.06ns	-0.11ns	0.04ns	-0.01ns	-0.28*	-0.41***	-0.25ns	-0.38**	-0.27*
SLN, g m <sup>-2</sup>	0.08ns	0.36**	0.39**	0.40**	0.43**	-0.14ns	-0.06ns	0.08ns	0.02ns	0.16ns	0.17ns	0.39***	0.39**	0.45***	0.43**
SPAD <sub>B1</sub>	-0.28*	-0.14ns	0.09ns	0.06ns	0.13ns	-0.01ns	-0.06ns	0.00ns	-0.15ns	-0.08ns	-0.31**	-0.10ns	0.07ns	0.11ns	0.14ns
GL <sub>R1</sub> , no. leaves plant⁻¹	0.24*	-0.13ns	0.45***	0.40**	0.29*	0.63***	0.32**	0.64***	0.38**	0.50***	-0.17ns	-0.27*	0.03ns	0.23ns	0.01ns
GL <sub>R2</sub> , no. leaves plant⁻¹	0.40***	0.35**	0.64***	0.73***	0.71***	0.63***	0.36**	0.62***	0.50**	0.56***	0.00ns	0.19ns	0.27*	0.54***	0.49***
$GL_{R3}$ , no. leaves plant <sup>1</sup>	0.40***	0.30**	0.65***	0.72***	0.69***	0.69***	0.39***	0.69***	0.55***	0.56***	-0.03ns	0.16ns	0.24ns	0.49***	0.47***
$GL_{R5}$ , no. leaves plant <sup>1</sup>	0.29*	0.39***	0.56***	0.71***	0.73***	0.44***	0.31***	0.55***	0.42**	0.42**	-0.01ns	0.26*	0.23ns	0.55***	0.60***
KW, mg kernel <sup>-1</sup>	0.38***	0.28*	0.62***	0.53***	0.62***	-		<del>, -</del>	-		-0.21ns	-0.23*	-0.02ns	0.06ns	0.12ns
KN, kernel m <sup>-2</sup>	0.81***	0.85***	0.76***	0.86***	0.83***	-0.21ns	-0.23*	-0.02ns	0.06ns	0.12ns	-	-		-	-
GY, kg ha <sup>-1</sup>	-	-	-	<del>.</del>	-	0.38***	0.28*	0.62***	0.53***	0.62***	0.81***	0.85***	0.76***	0.86***	0.83***
HI, g g <sup>-1</sup>	0.54***	0.62***	0.37**	0.60***	0.60***	0.55***	0.15ns	0.35**	0.29*	0.50***	0.24*	0.55***	0.23ns	0.55***	0.44***
* Significant at the 0.05 probe	ability level; ** ;	significant at t	he 0.01 probs	bility level; **'	* significant a	t the 0.001 pr	obability level				00/00/10 +0	< 10 H			of contraction in the second se
LD, IEAI DIVITIASS AL SIIKITU,		COLICEINIANO	I AL SIINIUG, LI	NOT, TEAL N C	UITETL AL SIIK.	IIU, AOI, aIIIIK		ilerval, LAI <sub>B1</sub> /L	LAIRO/ LAIR3, It	al area illuex		, OLA, SPECIII		NUN, SPECIEC F	aan mirugen,

SPAD<sub>R1</sub>, soil plant analysis development at R1; GL<sub>R1</sub>/GL<sub>R2</sub>/GL<sub>R2</sub>/GL<sub>R2</sub>, green leaf number at R1/R2/R3/R5; KW, kernel weight; KN, kernel number; ĜY, grain yield; HI, harvest index.

t ns, not significant at the 0.05 probability level

55 kg N ha <sup>-1</sup> ; N2 220 kg N ha <sup>-1</sup> ) and three plant	
aits under two N rates (N1,	
vith morphophysiological tr	$(000 \text{ plants ha}^{-1})$ in 2013.
ו yield and its components w	2, 79,000 plants ha <sup>-1</sup> ; D3, 104,
7. Correlation matrix for grain	ies (D1, 54,000 plants ha <sup>-1</sup> ; D
Table 7	densiti

												2	_		
			arain yieid				¥٩	ernel weigr				Ve	ernei numb	er	
Variable†	Ę	N2	5	D2	D3	£	N2	Б	D2	D3	Ę	N2	5	D2	D3
LB, g m <sup>-2</sup>	0.73***	0.65***	0.78***	0.81***	0.77***	0.41***	0.39***	0.76***	0.68***	0.63***	0.63***	0.46***	0.32**	0.56***	0.57***
LNCC, g 100g <sup>-1</sup>	0.35**	0.08 ns	0.22 ns	0.29*	0.36*	0.36**	0.14ns	-0.02ns	0.26ns	0.35*	0.21ns	-0.07ns	0.28ns	0.25ns	0.24ns
LNCT, g m <sup>-2</sup>	0.83***	0.72***	0.84***	0.81***	0.81***	0.57***	0.45***	0.70***	0.71***	0.64***	0.66***	0.45***	0.40***	0.49***	0.65***
ASI, days	-0.30*	-0.31**	-0.11 ns	-0.30*	-0.45**	-0.48***	-0.33**	-0.24ns	-0.37*	-0.49***	-0.08ns	-0.08ns	0.09ns	-0.09ns	-0.34*
LAI <sub>B1</sub> , m <sup>2</sup> m <sup>-2</sup>	0.53***	0.41***	0.60***	0.50***	0.73***	0.24*	0.12ns	0.46***	0.48***	0.63***	0.47***	0.38**	0.32*	0.23ns	0.50***
LAI <sub>B2</sub> , m <sup>2</sup> m <sup>-2</sup>	0.64***	0.54***	0.54***	0.67***	0.78***	0.31**	0.20ns	0.38**	0.50***	0.64***	0.59***	0.47***	0.30*	0.49***	0.61***
LAI <sub>B3</sub> , m <sup>2</sup> m <sup>-2</sup>	0.34**	0.49***	0.42**	0.47***	0.48***	0.30*	0.37*	0.48***	0.59***	0.42**	0.18ns	0.25*	0.08ns	0.09ns	0.34*
SLA, cm <sup>2</sup> g <sup>-1</sup>	-0.29*	-0.26*	-0.44**	-0.34*	-0.17ns	-0.35**	-0.32**	-0.54***	-0.28ns	-0.07ns	-0.16ns	-0.04ns	-0.06ns	-0.26ns	-0.23ns
SLN, g m <sup>-2</sup>	0.46***	0.33**	0.53***	0.45**	0.33*	0.49***	0.38**	0.51***	0.36*	0.20ns	0.27*	0.05ns	0.19ns	0.35*	0.36*
SPAD <sub>R1</sub>	0.55***	0.39***	0.61***	0.58***	0.49***	0.53***	0.51***	0.34*	0.48***	0.45**	0.33**	-0.07ns	0.44**	0.41**	0.35*
SPAD <sub>B3</sub>	0.50***	0.13 ns	0.51***	0.43**	0.42**	0.52***	0.21ns	0.17ns	0.37*	0.34*	0.27*	0.00ns	0.51***	0.33*	0.35*
GL <sub>B1</sub> , no. leaves plant <sup>-1</sup>	0.56***	0.58***	0.76***	0.65***	0.67***	0.80***	0.58***	0.56***	0.68***	0.68***	0.13ns	0.18ns	0.46**	0.31*	0.46***
GL <sub>R2</sub> , no. leaves plant <sup>-1</sup>	0.67***	0.59***	0.76***	0.68***	0.73***	0.79***	0.69***	0.63***	0.66***	0.70***	0.30*	0.07ns	0.39**	0.41**	0.51***
GL <sub>R3</sub> , no. leaves plant <sup>1</sup>	0.56***	0.54***	0.68***	0.60***	0.63***	0.67***	0.74***	0.57***	0.65***	0.64***	0.26*	-0.05ns	0.34*	0.32*	0.41**
GL <sub>R5</sub> , no. leaves plant <sup>1</sup>	0.54***	0.63***	0.69***	0.62***	0.70***	0.72***	0.80***	0.74***	0.72***	0.70***	0.17ns	0.01ns	0.17ns	0.21ns	0.48**
KW, mg kernel <sup>-1</sup>	0.68***	0.67***	0.63***	0.78***	0.77***	-		-	-	<del>.</del>	0.13ns	-0.08ns	-0.08ns	0.19ns	0.26*
KN, kernel m <sup>-2</sup>	0.80***	0.65***	0.70***	0.75***	0.79***	0.13ns	-0.08ns	-0.08ns	0.19ns	0.26*	-	-	-	-	÷
GY, kg ha⁻¹	-	-	-	-	-	0.68***	0.67***	0.63***	0.78***	0.77***	0.80***	0.65***	0.70***	0.75***	0.79***
HI, g g <sup>-1</sup>	0.10ns‡	0.12ns	-0.21ns	0.24*	0.22ns	0.12ns	0.02ns	-0.20ns	0.21ns	0.29*	0.10ns	0.08ns	-0.11ns	0.24*	0.05ns
* Significant at the 0.05 prob	ability level; **	significant at	the 0.01 prob	ability level; *"	** significant a	it the 0.001 p	robability leve								
t LB, leaf biomass at silking	; LNCC, leaf N	I concentratic	n at silking; L	NCT, leaf N c	content at silk	ing; ASI, anth	nesis–silking ir	nterval; LAI <sub>B1</sub> /	/LAI <sub>Bo</sub> /LAI <sub>B3</sub> , I	leaf area inde	x at R1/R2/R:	3; SLA, speci	ific leaf area;	SLN, specific	leaf nitrogen;
SPAD <sub>R1</sub> /SPAD <sub>R3</sub> , soil plant a	analysis develc	pment at R1/	'R3; GL <sub>R1</sub> /GL <sub>R</sub>	2/GL <sub>R3</sub> /GL <sub>R5</sub> ,	green leaf nui	nber at R1/R	2/R3/R5; KW	, kernel weigh	nt; KŇ, kernel	number; GY,	grain yield; HI	, harvest inde	ex.	•	1

in 2013). Higher density also increased the variation of KN at maturity explained by LAI at R1 and R3 in 2013 (Table 7). Overall, the correlation between KN at maturity and LAI at silking could be described as a bilinear function for H1, H2, and H3 and as a quadratic function for H4 (Fig. 5). Across two growing seasons, H1 and H2 reached maximum KN per area at a higher LAI at silking (3.85 m<sup>2</sup> m<sup>-2</sup> for H1 and 4.04 m<sup>2</sup> m<sup>-2</sup> for H2) compared with H3, which reached a plateau at 3.28 m<sup>2</sup> m<sup>-2</sup>. However, the quadratic correlation for H4 indicated a decline in KN at D3 for this hybrid (Fig. 5). It is interesting to note that the thresholds for the two newer hybrids to reach maximum KN (3940 kernels  $m^{-2}$  for H1 and 3893 kernels  $m^{-2}$ for H2) were between D2 and D3, whereas the maximum KN threshold for H3 (3838 kernels  $m^{-2}$ ) occurred between D1 and D2 (Fig. 5).

## DISCUSSION Management Impacts on Leaf Area Index and Specific Leaf N

Among the two components that affect RUE (LAI and SLN) at flowering, LAI at silking did not differ between low and high N rates in our research, whereas SLN at silking decreased 13 to 16% from the high N rate to the low N rate across years (Tables 4 and 5). The lack of interaction between N rate and hybrids in LAI and SLN at silking (Tables 2 and 3) indicated that hybrids responded in a similar manner for these two components. Therefore, despite inadequate available N and the resultant lower leaf N storage per unit leaf area at the low N rate, all hybrids were stable in radiation interception through the silking stage in this study. Similar to our findings, previous studies have shown that, under N-deficit conditions, maize is more susceptible to loss in RUE than loss in radiation interception (Muchow and Davis, 1988; Lemaire et al., 2007; Massignam et al., 2009). Lemaire et al. (2007) indicated that the critical SLN for radiation interception was lower than that for RUE (1.0 vs. ~1.5 g m<sup>-2</sup>, respectively). Munaro et al. (2011) also observed a maize canopy preference to attain maximum LAI ahead of RUE when N and water stress occur before silking.

Plant density effects on SLN at silking were not consistent across years, as a decrease in SLN at higher densities was observed in 2013, but no density effects on SLN were apparent in 2012 (Tables 4 and 5). This difference was due to similar trends of LAI and LNCT gains in response to density in 2012 (Table 4), but a proportionately

not significant at the 0.05 probability level.

‡ ns, r



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GL<sub>Rs</sub>, green leaf number at R1/R2/R3/R5, no. of green leaf plant<sup>-1</sup>; KW, kernel weight, mg kernel<sup>-1</sup>; KN, kernel number, kernels m<sup>-2</sup>; GY, grain yield, g m<sup>-2</sup>; HI, harvest index, g g<sup>-1</sup>

eaf area index at R1/R2/R3/R5, m<sup>2</sup> m<sup>-2</sup>; SLA, specific leaf area, cm g<sup>-1</sup>; SLN, specific leaf nitrogen, g m<sup>-2</sup>; SPAD<sub>R1</sub>/SPAD<sub>R3</sub>, soil plant analysis development at R1/R3; GL<sub>R1</sub>/GL<sub>R2</sub>



Fig. 3. Regression of specific leaf nitrogen at silking (SLN at R1) with kernel weight (KW) at maturity in 2013 for four hybrids. Each point was averaged across N rates and densities. \* Slope of linear regression is significant at the 0.05 probability level; \*\* slope of linear regression is significant at the 0.01 probability level; ns = not significant at the 0.05 probability level.

higher gain in LAI compared with LNCT at higher densities during silking in 2013 (Table 5). The lack of density and hybrid interaction in 2013 indicated a similar pattern of reductions in SLN at higher densities for all tested hybrids. Across all hybrids and N rates, LAI at silking increased 17% from D1 to D2 and 20% from D2 to D3, whereas LNCT increased 14% from D1 to D2 and only 3% from D2 to D3 in 2013 (Table 5).

Although one of the typical explanations for higher GY at optimum density is higher LAI and higher radiation interception (Barbieri et al., 2000; Luque et al., 2006; Amanullah et al., 2007), multiple plant trait factors exert influence on KN and KW. The tradeoff between LAI and SLN at silking at various densities can be critical in a specific hybrid's grain production due to their impact on RUE and light interception. As a result, simply increasing LAI by increasing planting rate could negatively affect SLN and reduce KW. On the other hand, KN per area achieved its maximum when LAI reached  $3.85 \text{ m}^2 \text{ m}^{-2}$  for H1,  $4.04 \text{ m}^2 \text{ m}^{-2}$  for H2,  $3.28 \text{ m}^2 \text{ m}^{-2}$  for H3, and 3.43



Fig. 4. Regression of specific leaf nitrogen at silking (SLN) with green leaf number at silking (a) at R1, (b) at R2, (c) at R3, and (d) at R5 in 2013 for four hybrids. Each point was averaged across N rates and densities. \* Slope of linear regression is significant at the 0.05 probability level; \*\* slope of linear regression is significant at the 0.01 probability level; ns = not significant at the 0.05 probability level.



Fig. 5. Correlation between kernel number and leaf area index (LAI) at silking for four hybrids. Two growth seasons-2012 and 2013-are combined, which leads to total of 12 treatment mean points for DKC61-69, DKC61-72, and XL72AA and six treatment mean points for XL45. Bilinear model for DKC61-69: kernel number (KN) = 2207 + 450(LAI) at LAI < 3.85,  $KN = 3940 \text{ at } LAI > 3.85 (R^2 = 0.44, P < 0.001).$ Bilinear model for DKC61-72: KN = 2343 + 384(LAI) at LAI < 4.04, KN = 3893 at LAI >  $4.04 (R^2 = 0.41, P < 0.001)$ . Bilinear model for XL72AA: KN = 178 + 1113(LAI) at LAI < 3.28,  $KN = 3838 \text{ at } LAI > 3.28 (R^2 = 0.72, P < 0.001).$ Quadratic regression for XL45: KN = -5966 + $5498(LAI) - 801(LAI)^2 (R^2 = 0.47, P = 0.3679).$ 

m<sup>2</sup> m<sup>-2</sup> for H4 at silking across all treatments and years (Fig. 5). The reduction of KN at D3 for H4 was clear in Fig. 5, even though the P-value for this quadratic function was >0.05. This indicated that above-optimum plant densities will lead to a potential plateau in KN per unit area for more recent hybrids, plus an actual reduction in KW. We did not observe a significant yield loss at high density (104,000 plants ha<sup>-1</sup>) in either year of this study (Fig. 4 and 5), but this implies that even higher densities must be tested to identify the threshold density for significant yield loss in these hybrids and environments. Plant-toplant variability in GY also increases at high density, as observed previously for GY per plant and KN per plant when densities ranged from 30,000 to 150,000 plants ha<sup>-1</sup> (Maddonni and Otegui, 2006) or from 54,000 to 104,000 plants ha<sup>-1</sup> (Boomsma et al., 2009).

## Canopy Traits as Simple Markers for Yield and Yield Components

Grain yield correlated strongly with green leaf number at both N rates in both years, albeit with lower coefficients at the higher N rate (Fig. 1 and 2). The varying dependency of per-plant yield on light capture traits versus actual growth rates at N rates from 0 to 400 kg N ha<sup>-1</sup> was also observed by Munaro et al. (2011). Green leaf number at grain filling was 3 to 5% higher in 2012 and 3 to 15% higher in 2013 in newer hybrids compared with older hybrids from R2 to R5 during grain filling (Tables 4 and 5). High correlations between green leaf number and KW were also observed in our study (Tables 6 and 7), and 10 to 48% (2012) and 31 to 64% (2013) of KW variation was explained by green leaf number across all treatments (Table 7). It is well known that KW is determined both in the lag phase at the onset of grain filling (potential KW) and in the active grain-filling period (Maddonni et al., 1998). Barker et al. (2005) also indicated a higher KW and visual stay green in newer hybrids under well-watered conditions when a series of ERA hybrids from 1953 to 2003 was evaluated in Chile.

The actual leaf N concentrations during grain fill were also important to kernel attributes. In our study, KW was strongly correlated with SLN at silking for H1, H2, and H3 in 2013 (Fig. 3). DeBruin et al. (2013) also observed a positive correlation of SLN with KW, and the critical SLN at silking for maximum KW and GY was  $1.5 \text{ g m}^{-2}$  across two hybrids and five N rates. The strong correlations among KW with SLN in 2013 (Fig. 3), and SLN in turn with green leaf number during grain filling, indicated a possibly longer active grain filling in newer hybrids (H1 and H2) that contributed to higher KW in 2013. Similar to our study, Cirilo et al. (2009) showed that analyzing morphophysiological traits can help in hybrid selection for higher GY under varied environments. In that study, the hybrid that yielded most under low-N conditions had the combined traits of a high green leaf number during grain filling, high SLN at silking, and a low N harvest index.

Grain yield was also correlated with LAI at R1, R3, and R5 stages in 2013, and the variance of GY explained by LAI ranged from 11 to 61% across these stages (Table 7). Obviously, the latter correlations can be partially attributed to the correlation between KN and LAI at silking (Fig. 5). D'Andrea et al. (2009) documented that higher leaf area resulted in 31% greater KN at 0 kg N ha<sup>-1</sup> when hybrids were compared with inbred lines.

Higher density always increased LAI, and LAI reached  $4 \text{ m}^2 \text{ m}^{-2}$  at maximum density for all tested hybrids in 2013 (Table 5, Fig. 5). Maddonni and Otegui (1996) showed

that maximum light interception was reached when incident solar radiation exceeded 90%, which occurred at LAI levels above 4 m<sup>2</sup> m<sup>-2</sup>. In the present study, a plateau in KN was reached at a R1-stage LAI of ~4  $m^2 m^{-2}$  in two newer hybrids and at a LAI of about 3 m<sup>2</sup> m<sup>-2</sup> in H3, whereas the oldest hybrid (H4) had a large decline in KN at the highest density (Fig. 5). Nunez and Kamprath (1969) showed that GY of their then-current hybrids (commercially released in the same decade as our oldest hybrid, H4) reached a plateau when LAI was at  $3.5 \text{ m}^2 \text{ m}^{-2}$  under both 168 and 280 kg N ha<sup>-1</sup>. Hybrids with higher LAI had lower proportions of incident solar radiation transmitted in the Maddonni et al. (2006) study, where hybrids with small versus large kernels were compared at densities ranging from 30,000 to 120,000 plants ha<sup>-1</sup>. They also showed that relative KN was much higher at densities  $\geq 90,000$ plants ha<sup>-1</sup> than at lower densities when intercepted photosynthesis active radiation was ~90% (Maddonni et al., 2006). In our study, the observation that newer hybrids maintained greater KN per unit area at high density compared with older hybrids confirmed that newer hybrids maintained a higher capability of light interception at high density.

Grain yield was also correlated with LB and LNCT at silking in both years (Tables 6 and 7). These correlations were reflected in the LB and LNCT associations with KN in 2012 (Table 6) and in their association with both KN and KW in 2013 (Tables 6 and 7). Leaf biomass and LNCT at silking was higher in H1, H2, and H3 compared with H4 in 2013 (Table 5). The higher LB and LNCT raised SLN and lowered SLA at silking, even when average LAI values at silking were the same for H1, H2, and H3 (Table 5). Hammer et al. (2010) documented the influence of SLA on biomass partitioning to leaf at silking, as well as the importance of SLN to photosynthesis in their development of the APSIM model.

Lastly, we noticed negative associations between GY and ASI but a lack of association between ASI and KN in both years (Tables 6 and 7). Older hybrids (H3 and H4) always had poorer (i.e., longer) ASI performance under varied management conditions (Fig. 1 and 2). Munaro et al. (2011) also noticed a poor correlation of ASI and GY per plant at 0 kg N ha<sup>-1</sup>, but a significant negative correlation between these two variables when 200 or  $400 \text{ kg N} \text{ ha}^{-1}$  was applied. Anthesis-silking interval was also negatively correlated with KN per plant under various N supply treatments in that study (Munaro et al., 2011). In the present study, ASI showed a stronger negative effect on KW in both years than on KN (Tables 6 and 7). This contrast with the previous literature documenting the expected stronger ASI influence on KN than on KW could be related to greater variation of KW in this series of hybrids. The latter impact of ASI could be related to potential KW achieved during the critical period. Echarte et al. (2004) observed that the shorter ASI in a modern hybrid was related to a higher partitioning of dry matter into kernels during the period bracketing silking. Our hybrid era investigation is unique in pointing to a strong ASI influence on KW, and not just on KN alone.

### CONCLUSIONS

Our central findings with respect to the secondary trait responses of maize to the experiment's main N rate, density, and hybrid treatments were that (i) the higher N rate had positive effects on LNCC, LB, LNCT, SLN, leaf greenness, and green leaf number, (ii) higher plant densities increased LAI and decreased SLN for all hybrids, (iii) newer hybrids had higher SLN and green leaf number, but lower SLA, than older hybrids, (iv) the higher N rate lowered the correlations between GY with canopy attributes, including green leaf number in both years, as well as LB, LNCT, SLN, and LAI in the more favorable production year, and (v) increasing density enhanced light interception by increasing canopy LAI, which also enhanced its correlation with GY.

The higher LB and LNCT in more recent hybrids contributed to higher SLN and lower SLA. Enhanced SLN in newer hybrids contributed to higher KW and green leaf number during grain filling, which was a positive factor in the higher GY achieved by newer hybrids in the more favorable year (2013). The high correlations between GY and green leaf number during grain filling, as well as the coinciding positive correlation between green leaf number and KW, showed that newer hybrids retained leaf greenness later in grain filling and that the latter led to higher KW. On the other hand, the bilinear function between KN and LAI at silking showed that newer hybrids reached their KN plateau at higher LAI levels than was the case for older hybrids. The latter confirmed one of the mechanisms of enhanced plant density tolerance that has been achieved during these decades of hybrid selection.

Attributing GY (and KN or KW component) increase with modern hybrids to specific secondary traits is complicated by the environmental and management regimes under which hybrids are compared. Changes in either plant densities or N rates in maize hybrid era studies can strengthen or weaken the correlations between GY or kernel component and secondary traits but do not change the type of correlation if the correlations were significant (i.e., negative or positive). Because year had such a major impact on the strength of the correlations in this study, using even more site-years in such hybrid era studies would enable more precision in attributing GY, KN, or KW gains to specific secondary trait(s).

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