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Genetic improvement in density and nitrogen stress tolerance traits over 38 years of commercial maize hybrid release

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ABSTRACT

Research attention to improving source and sink strength in maize production is requisite for enhancing yield. Improvement in source strength has been achieved with higher post-silking dry matter accumulation, whereas historical improvement in sink strength has been mostly attributed to increasing kernel number (KN) per unit area, in part because KN is known to be more vulnerable to abiotic stresses compared to kernel weight (KW). However, KW can also vary widely as it is dependent on both genotype and dry matter accumulation during the post-silking period. In order to illustrate the consequences of breeding efforts over a 4-decade period for enhancing source and sink strength at varying nitrogen rates and plant densities, a 2-year and 2-location study was conducted in 2013 and 2014. Eight commercial hybrids from DeKalb released from 1967 to 2005 were compared at 2 nitrogen rates (55 and 220 kg N ha^{-1}) and 3 plant densities (54,000 (D1), 79,000 (D2) and 104,000 (D3) plants ha^{-1}). Breeding progress increased grain yield per hectare (GY) by an average of 66 kg ha^{-1} year^{-1}, and grain yield per plant (GYP) by 0.91 g plant^{-1} year^{-1} across all treatments and environments. This yield increase with hybrid improvement was attributed more to an increase in KW (1.29 mg kernel^{-1} year^{-1} across all treatments and both locations), than to any increase in KN. The overall source-sink ratio (SSR – ratio of post-silking dry matter accumulation to kernel number per plant) also increased by an average of 1.25 mg kernel^{-1} year^{-1} across all treatment and locations. The hybrid improvement in SSR was more pronounced at the high N rate or low plant density. Post-silking dry matter accumulation (PostDM) increased by an average of 54 kg ha^{-1} year^{-1} across all treatments and locations. KW was highly correlated with ear growth rate (EGR) during grain fill. New hybrids had much higher KW gain per unit of EGR. Newer hybrids also had a longer active grain filling period, but the correlation of post-silking dry matter accumulation to the duration of active grain filling period was weak. This study showed that the breeding progress for yield gain in these DeKalb hybrids was achieved by (i) longer duration of the grain filling period plus longer leaf stay green that accompanied a higher PostDM of newer hybrids, (ii) enhanced source to sink strength during grain filling by a higher SSR in newer hybrids, (iii) improved efficiency for transferring source from cob and husk to grain by increasing KW gain per unit of EGR, and (iv) enhanced stress tolerance in newer hybrids to maintain grain yield even under high density.

Introduction

Maize grain yield improvements over the decades have been attributed in rather equal proportions to management and genetic advances (Duvick, 2005). Duvick (2005) observed that there were some traits that breeders intended to change and, on the other hand, there were other traits that improved simultaneously when breeders were narrowly focused on enhancing grain yield. One trait that is of consistent focus is the enhancement in source and sink strength, as well as improving the efficiency of nutrient partitioning from source to sink (Tollenaar and Lee, 2011). Source strength can be quantified using post-silking dry matter accumulation (PostDM). However, PostDM is affected by both pre- and post-silking canopy attributes such as leaf area index (LAI), radiation use efficiency, and specific leaf nitrogen (SLN) (Cirilo et al., 2009). The consequences of breeding improvements on LAI are inconsistent. In one com-

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parison, a 1988 widely used hybrid achieved a higher LAI than a 1959 widely used hybrid in Ontario (Tollenaar and Aguilera, 1992; Tollenaar et al., 1997). Duvick (1984) reported that LAI differences were minimal among hybrids from 1930 to 1980 when a series of 48 Pioneer hybrids were tested in Iowa across 3 densities. Specific leaf nitrogen, representing leaf N per leaf area, was associated with higher N-use efficiency in newer hybrids even at low N supply (McCullough et al., 1994). DeBruin et al. (2013) used SLN at silking for estimating grain yield, KW and KN at maturity; the threshold of SLN for maximum grain yield, KW and KN were 1.5, 1.6 and 1.3 g m^{-2}, respectively.

Kernel number per area (KN), kernel number per plant (KNP) and potential kernel weight are direct variables that contribute to sink strength. Kernel number per area have increased in response to targeted genetic and management (e.g. higher plant density) improvements. Kernel number per plant is well known to be affected by plant growth rate (PGR) during the critical period surrounding silking (Tollenaar et al., 1992; Uhart and Andrade, 1995; Echarte et al., 2004). The association between KNP and PGR was shown to be curvilinear before KNP reaches its maximum and then this association reaches a plateau (Otegui and Andrade, 2000). Andrade et al. (1999, 2002) showed that the incremental rate for KNP of the apical ear decreased to 0 when PGR reached 4 g $plant^{-1}$ d^{-1}. Breeding efforts to increase KNP have been successful when a lower threshold of ear growth rate (EGR) was needed to achieve maximal KNP during the critical period for newer hybrids compared to older hybrids (D'Andrea et al., 2008). Ear growth rate (EGR) during the critical period was proved to be a good estimator of KNP, and KNP reached a maximum when EGR during critical period was over 1.6 g $plant^{-1}$ d^{-1} (D'Andrea et al., 2008). Echarte et al. (2006) indicated that ear demand included KNP and kernel growth rate. Given this, EGR can be treated as a component of ear demand and overall sink strength.

Potential kernel weight is determined about 12–15 days after onset of grain filling period at end of lag phase (Borrás and Gambín, 2010). Whether kernel weight (KW) at maturity achieves its potential kernel weight depends on conditions during grain filling period such as persistence of green leaf area and redistribution of assimilated biomass during grain filling period (Hammer et al., 2010). One lesser-known change in ear traits that potentially coincides with grain yield gain is the increased KW achieved by newer hybrids under well-watered conditions, that trait change was noted in a series of ERA hybrids from 1953 to 2001 tested in Chile (Barker et al., 2005).

It is well known that KW is determined during the grain filling stage, including the lag phase (when KW increases very little) and the active grain filling stage (when KW increases linearly with thermal time) (Maddonni et al., 1998; Echarte and Andrade, 2003). Maddonni et al. (1998) showed that hybrids with a larger potential KW (>300 mg $kernel^{-1}$) had a longer lag phase and a higher kernel growth rate with a longer active grain filling period compared to hybrids with smaller potential KW ($\leq$300 mg $kernel^{-1}$). However, Borrás and Otegui (2001) showed that KW was not correlated with the length of active grain filling period; instead, KW was correlated with the kernel growth rate during grain filling period for both large and small kernel hybrids.

The comparison between source strength and sink strength during grain filling period can be quantified using the source-sink ratio (SSR), which is often known as the ratio of post-silking dry matter accumulation divided by kernel number per plant (Rajcan and Tollenaar, 1999b; Borrás et al., 2003; Borrás and Otegui, 2001; Sala et al., 2007). Modern hybrids exhibited a higher SSR during the post-silking period and these changes were associated with increasing leaf longevity during grain filling period (Rajcan and Tollenaar, 1999b). KW is also affected by the source capacity variation (such as post-silking dry matter accumulation and duration of grain filling period) when ear demand increased due to higher yield potential, especially in newer hybrids compared to older hybrids (Echarte et al., 2006). Breeding efforts to prolong the active grain filling period has been well documented (Ma and Dwyer, 1998; Mi et al., 2003).

Because grain yield gain over time in maize also benefited from steadily increasing plant density, there can be indirect consequences of density on canopy and grain component traits. Cardwell's study on yield gain in Minnesota from 1930 to 1980 demonstrated that increased plant density (30,740–49,780 plants ha^{-1}) over these 50 years had contributed to 21% of the total grain yield gain (Cardwell, 1982). Duvick (2005) also showed newer hybrids perform better under 79,000 plants ha^{-1} than older hybrids for a series of Pioneer hybrids. However, the highest density in that study is now a rather common density in commercial U.S. maize production. Higher density can increase light interception by increasing leaf area index (Tollenaar and Lee, 2002), but higher densities may also increase abiotic stresses that can lead to a reduction in KNP (Poneleit and Egli, 1979; Echarte et al., 2000). Andrade et al. (1999) indicated that the number of kernels set per unit of PGR decreased at high densities and that higher densities therefore contributed to a lower final KNP. High density can also reduce KW due to a reduction in leaf area per plant (Borrás et al., 2003). The performance uncertainty of KNP and KW in newer hybrids both near and well above current plant densities should be investigated.

Maize hybrid evaluations are commonly made under high N conditions due to a large yield loss under N deficient conditions (D'Andrea et al., 2008). However, N deficiency has a large influence on canopy variables, such as green leaf number during the grain filling period, LAI and SLN, which will cause reduction in radiation use efficiency and light interception and eventually lower KN and KW. A series of DeKalb hybrids from 1930s to 1980s showed similar yield increase rates per year under both low fertility and high fertility conditions (Castleberry et al., 1984). However, other previous studies including more recent hybrids (i.e. released after year 2000) showed higher grain yield increases per year under non-stressed conditions. For instance, Barker et al. (2005) observed that a series of Pioneer ERA hybrids (1950–2001) had higher grain yield gain per year under well-watered than in drought stress conditions. Hence, because of the uncertain consequences of abiotic stress factors like N deficiency on hybrids of different eras, the consequence of different N levels on grain yield gain per year warrants further investigation.

Given the risks of yield reduction under both N deficiency and high density stress factors, as well as the opportunities for increasing knowledge to help guide future genetic selection, it is necessary to clarify the traits that have changed over more than three decades of breeding programs under multiple N rates and densities. Therefore, the objectives of this study were to: 1) determine the effects of N rate, plant density and hybrid era on canopy traits, grain yield and its components; 2) evaluate the existence of hybrid interactions with N rate and plant density on these vegetative and reproductive traits; and 3) study whether the correlations between KW and ear growth rate during grain filling period changed with almost 40 years of Dekalb hybrid development.

2. Materials and methods

2.1. Experiment design and management

A field study was conducted at ACRE (Agronomy Center for Research and Education, 40∘28′07″N, 87∘00′25″W), West Lafayette, IN, USA and PPAC (Pinney Purdue Agricultural Center, 41∘26′41″N, 86∘56′41″W), Wanatah, IN, USA in 2013 and 2014. The soil type

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was Chalmers silty-clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquolls) in 2013 and Raub-Brenton complex (Fine-silty, mixed, superactive, mesic Aquic Argiudolls) in 2014 at ACRE. The soil type at PPAC was Sebewa loam (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Argiaquolls) in both years. Average soil pH, organic matter, exchangeable P, and available K were 6.9, 3.7 g 100 g^{-1}, 22 mg kg^{-1}, 106 mg kg^{-1} at ACRE in 2013; 6.7, 4.4 g 100 g^{-1}, 17 mg kg^{-1}, 92 mg kg^{-1} at PPAC in 2013; and 6.2, 2.9 g 100 g^{-1}, 75 mg kg^{-1}, 236 mg kg^{-1} at ACRE in 2014, 6.2, 4.8 g 100 g^{-1}, 27 mg kg^{-1}, 129 mg kg^{-1} at PPAC in 2014. Soil N was not measured at sowing in this study; however, it was measured at V14 and R1 stages at both ACRE (2013 and 2014) and PPAC (2013, but not 2014) in immediately adjacent maize hybrid studies planted on the same day in the same field where no N fertilizer was added (De Oliveira Silva, 2015). The soil NH_4^+ ranged from 3.3 to 4.8 mg kg^{-1} and NO_3^- ranged from 1.8 to 3.5 mg kg^{-1} to a 30-cm depth at these locations (De Oliveira Silva, 2015). In both years, the crop rotation was maize after soybean at ACRE, and second year continuous maize at PPAC. ACRE, 2013 was chisel plowed in the fall and field cultivated in the spring. ACRE, 2014 was strip-tilled in both fall and spring with Soil Warrior® (Environmental Tillage Systems Inc.) using coulter-based soil engaging tools. The tillage system was chisel plow in the fall and field cultivated in the spring for PPAC in both years.

Treatments were arranged in a split–split plot design in both years at both locations. Nitrogen rate was the main plot – 55 kg N ha^{-1} (55N) or 220 kg N ha^{-1} (220N). Plant density was the sub-plot – 54,000 (D1), 79,000 (D2), or 104,000 plants ha^{-1} (D3). Hybrid was the sub–sub plot, including 8 commercial DeKalb hybrids, the cultivars used and their decades assigned, as well as their cultivars characteristics, are described in Table 1. Six blocks were planted at ACRE and three blocks were planted at PPAC. All plots were 10 m long and 3.04 m wide with 4 rows and 0.76 m row spacing.

Planting dates were 14 May 2013 and 25 April 2014 at ACRE and 1 June 2013 and 5 May 2014 at PPAC. Nitrogen was side-dressed as urea-ammonium nitrate (UAN, 28% N) applied 30 days after planting (DAP) in 2013 and 33 DAP in 2014 at ACRE and 38 DAP in 2013 and 24 DAP in 2014 at PPAC. All UAN was injected in mid-row positions with a DMI Nutri-Placer 2800.

All grass and broadleaf weeds in the plot areas were controlled with a combination of pre-emerge residual herbicides as well as a single post-emerge application at approximately the V5 stage. All maize seeds were treated in a similar manner with Acceleron™ (Difenoconazole, Fludioxonil, Mefenoxam, and Thiamethoxam). Force 3G (Tefluthrin) was soil-applied at planting to control corn rootworm.

Weather data for ACRE were collected from Purdue University-Indiana State Climate Office at station 'ACRE-West Lafayette' (http://www.iclimate.org/), and for PPAC was collected from station 'Wanatah 2 WNW, IN US' (http://www.ncdc.noaa.gov/cdo-web). Weather recording began with the planting dates at each site-year and continued until biomass harvest at maturity on September 24th, 2013 and September 15th, 2014 at ACRE, and on October 22nd, 2013 and September 29th, 2014 at PPAC.

2.2. Canopy traits, biomass harvest at silking, maturity and grain yield

Leaf area index (LAI) was measured three times – growth stages R1, R3 and R5 in both years and both locations. Five points above the canopy and five points below the canopy were taken for each plot using a Li-Cor 2200 (®2014 LI-COR, Inc.) with a 45° cap to avoid direct sunlight. All plots were 4 rows wide. Hence, the below-canopy points were shaded and followed a diagonal line between row 2 and row 3, and the five points were evenly distributed on this diagonal line. The LAI measurements were conducted in 3 blocks for both ACRE and PPAC each year. Green leaf numbers were recorded from 20 plants per plot at silking (R1) and three times during grain filling (R2, R3 and R5) for 3 blocks for both ACRE and PPAC each year. All leaves retaining at least 50% green area on the leaf surface were counted as "green leaves".

At ACRE, R1 biomass harvest was taken at 7 days (2013) and 0 days (2014) after 50% silking (average of all hybrids). At PPAC, R1 biomass harvest was taken at 2 days (2013) and 4 days (2014) after 50% silking (average of all hybrids). R6 biomass harvest was completed after all treatments reached black layer (representative ears of each hybrid from multiple replications were sampled to insure all treatments reached black layer). For all biomass harvests, the sampling area was 3.04 m^2 for each plot.

All plants in the sampling area were cut at soil level. Five representative plants were then chosen as subsamples from each plot. For the R1 harvest, subsamples were separated into leaf, stem (with husk) and ear for six blocks at ACRE and three blocks at PPAC in both years. For the R6 harvest, subsamples were separated into leaf, stem (with husk), grain and cob for three blocks in ACRE and PPAC in both years. The other three blocks in ACRE were separated into stover (stems, leaves, and husks) and ears (grain and cob) at ACRE in both years. Fresh weight for total plants and all subsample components were recorded before subsample drying at 60 ◦C at ACRE for 5–7 days until a stable dry weight was reached. All subsamples were weighed, ground and sent to A&L Great Lakes Lab (Fort Wayne, Indiana) for determination of plant N composition using combustion analysis (AOAC International 990.03, 1995).

Both grain yield and aboveground biomass were calculated from the R6 sampling areas. After selecting the five subsampled plants, all ears of the remaining plants were collected as "bulk" ears. All "bulk" ears were shelled and weighed, and grain moisture determined with a grain moisture tester. Grain yield was calculated by using all the ears in R6 harvest area, including bulk ears and subsample ears. Grain yield is presented at 0% moisture, as well as grain yield per plant. Number of rows and number of kernels per row was counted for each ear for all subsamples. Kernel number was calculated as the product of number of rows and number of kernels per row. Kernel weights were determined from 200 kernel subsamples for each plot.

Individual plot progression to 50% milkline was determined by sampling at least 3 times from onset of kernel denting to 50% milkline (Butzen, 2014). Sampling began at onset of kernel denting, the second sampling occurred 5 days after the first time sampling, and the third sampling was conducted 7 days after the second sampling. If there were still plots that did not reach 50% milkline, those plots were sampled another 3–5 days later until all the plots reached 50% milkline. For each sampling time, 3 consecutive corn ears were broken in half so that the percentage of milkline of top half of each ear could be recorded. For those plots that were not exactly 50% milkline when sampled, the dates for 50% milkline were calculated based on the fitted linear model of the percentage milkline (y-axis) versus date of sampling (x-axis).

Specific leaf nitrogen (SLN) at silking was calculated as ratio of leaf N content to leaf area index at silking.

$$SLN\left(gm^{-2}\right)=\frac{Leaf\ N\ content\ at\ silking\ \left(kg\ ha^{-1}\right)}{LAI\ at\ silking\ \left(m^{2}m^{-2}\right)}$$

Leaf N content (kg ha^{-1}) is the product of leaf N concentration and leaf biomass at silking.

Source-sink ratio (SSR) was calculated as ratio of post-silking dry matter accumulation per plant to kernel number per plant.

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$$SSR\left(mg\ kernel^{-1}\right)=\frac{Dry\ matter\ at\ maturity\ per\ plant\ \left(mg\ plant^{-1}\right)-Dry\ matter\ at\ silking\ per\ plant\ \left(mg\ plant^{-1}\right)}{Kernel\ number\ per\ plant\ at\ maturity\ \left(kernels\ plant^{-1}\right)}$$

Ear growth rate (EGR) was calculated as the ratio of the gain of ear dry matter from 50% silking to 50% milkline per plant per day (50% milkline was used since it was the last recorded dates to capture the exact thermal time for each treatment).

$$EGR\left(g\ plant^{-1}d^{-1}\right)=\frac{Ear\ dry\ matter\ at 50\%milkline\ \left(g\ plant^{-1}\right)\ -Ear\ dry\ matter\ at\ 50\%silking\ \left(g\ plant^{-1}\right)}{Days\ from\ silking\ to\ 50\%\ milkline\ (d)}$$

Ear dry matter included the dry matter of grain, husk and cob for both 50% milkline and 50% silking. The ear biomass per plant at 50% milkline were estimated as 90% of ear biomass per plant at maturity (Afuakwa and Crookston, 1984).

Harvest index (HI) was calculated as the ratio of grain dry matter (kg ha^{-1}) to total dry matter (kg ha^{-1}) at maturity.

$$HI\ \left(kg\ kg^{-1}\right)=\frac{Grain\ dry\ matter\ at\ maturity\ \left(kg\ ha^{-1}\right)}{Total\ dry\ matter\ at\ maturity\ \left(kg\ ha^{-1}\right)}$$

Thermal time was calculated as an accumulation of average daily temperature − base temperature (8 °C) from sowing (Borrás et al., 2003).

2.3. Statistical analysis

Analysis of variance (ANOVA) was conducted with SAS 9.3 by using "Proc Mixed" (SAS Institute Inc., 2011). Treatment factors of N rate, plant density and hybrid were treated as fixed factors but location was considered as a random factor, and block was considered as a random factor nested within each year. We combined data from two years since Pr ($F > F_0$) of year is larger than 0.05 for most of the measured variables. Neither Error a (year × nitrogen rate × block (year)) or Error b (year × nitrogen rate × density × block (year)) or Error c (year × nitrogen rate × density × hybrid × block (year)) were pooled when the error terms were considered in the split–split plot analyses. Regressions were conducted in SAS 9.3 by "Proc Reg". Slope comparisons were conducted by "Proc GLM" in SAS by setting dummy variables. Plateau quadratic regressions were fitted for ear growth rate vs. era of hybrids by using "Proc nlin" in SAS. Iteration was conducted based on given priors for a, b and c in the quadratic equation: $EGR = a + b \times era + c \times era^2$ when era was less than a certain time point x_0, after x_0 EGR reached a plateau.

3. Results

Average air temperatures were similar in all 4 environments (Table 2). Precipitation accumulated from planting to silking was almost double at PPAC than at ACRE in both years and total growing season precipitation was higher in 2014 than 2013 for both ACRE and PPAC. However, the available water capacity of ACRE was about 2.0 cm available water for each 10 cm zone to a soil depth of 80 cm, and it was about 1.7 cm of available water in each 10 cm zone at PPAC to a soil depth of 90 cm in both years (USDA, 2003); maize rooting depth typically exceeds 60 cm at both locations. Little to no evidence of drought stress was observed in either year at both locations.

3.1. Overall nitrogen and density effects

Significant differences in plant parameter responses between the two N rate treatments were unlikely to occur because there were too few degrees of freedom for testing N variance (as N rate was the whole plot in this split–split plot design), and overall LSD values associated with N treatments were large (Tables 3 and 4). Therefore, N rate treatment differences were not significant for grain yield (GY), grain yield per plant (GYP), KN, KW and SSR in both locations (Tables 3 and 4). However, it is interesting to note that the high N rate achieved numerically (1524 and 2136 kg ha^{-1}) higher GY and numerically higher GYP (20 and 27 g plant^{-1}) compared to the low N rate at ACRE and PPAC, respectively (Tables 3 and 4). As for yield components, the high N rate had numerically higher KN – (203 and 536 kernel m^{-2}) and KW – (25 and 27 mg kernel^{-1}) compared to low N rate at ACRE and PPAC, respectively. Source-sink ratio was slightly higher by 27 mg kernel^{-1} with the higher N rate at both ACRE and PPAC. Nitrogen only had minor impacts on thermal time from planting to 50% tassel and from planting to 50% silking, as well as from 50% silking to 50% milkline in both locations (Tables 3 and 4).

The maximum grain yield per area was achieved at D2 at ACRE (Table 3), whereas grain yield was maximized at D1 at PPAC (Table 4). From D1 to D2, GY increased 368 kg ha^{-1} at ACRE and but did not change significantly at PPAC, and it decreased by 432 kg ha^{-1} at ACRE and by 546 kg ha^{-1} at PPAC when density increased from D2 to D3. Grain yield per plant, kernel number per plant (KNP), and KW all decreased as density increased in both locations and the reduction was larger from D1 to D2 than from D2 to D3 (Tables 3 and 4). When density increased from D1 to D2, GYP was reduced 50 and 51 g plant^{-1}, KNP was reduced 131 and 117 kernel plant^{-1}, and KW was reduced 36 and 30 mg kernel^{-1} for ACRE and PPAC, respectively (Tables 3and 4). From D2 to D3,

Table 1
The cultivars used, year of commercial release, cultivar characteristics and relative maturity days.

Cultivars	Commercial Release (yr)	Type of Cultivars	Cultivar Characteristics	Relative Maturity Days (d)
DKC61-69	2005	VT3	Corn rootworm, European corn borer and glyphosate resistant	111
DKC61-72	2005	RR2 (Roundup Ready^{TM})	Glyphosate resistant	111
RX752	2003	VT3	Corn rootworm, European corn borer and glyphosate resistant	112
RX752RR2	2003	RR2 (Roundup Ready^{TM})	Glyphosate resistant	112
RX730	1994	Conventional	Not resistant	111
DK636	1982	Conventional	Not resistant	113
XL72AA	1975	Conventional	Not resistant	115
XL45	1967	Conventional	Not resistant	115

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Table 2
Weather conditions in 2013, 2014 at ACRE and PPAC. The starting points of climate recording for whole growth seasons were: May 14th, 2013 and April 25th, 2014 at ACRE; June 1st, 2013 and May 5th, 2014 at PPAC, which matched with planting dates. The ending points of climate recording were: September 24th, 2013 and September 15th, 2014 at ACRE; October 22nd, 2013 and September 29th, 2014 at PPAC, which matched with harvesting dates. ACRE climate records were collected from Purdue University-Indiana State Climate Office at station 'ACRE-West Lafayette'. PPAC climate records were collected from station 'Wanatah 2 WNW, IN US'. Daily temperature (Daily Temp.) is the mean of averaged daily maximal and minimal temperature. Maximal Temperature (Max. Temp.) averaged daily maximal temperature; Minimal Temperature (Min. Temp.) averaged daily minimal temperature.

	Precipitation (mm)	DailyTemp. °C	Max. Temp. °C	Min. Temp. °C		Precipitation (mm)	DailyTemp. °C	Max. Temp. °C	Min. Temp. °C
	ACRE, 2013					ACRE, 2014			
May	60	19	25	13	April	11	14	20	8
June	106	21	27	16	May	82	17	24	11
July 1st–July 16th	31	22	27	17	June	88	23	29	17
					July 1st – July 9th	26	21	27	15
Total of pre-silking	197				Total of pre-silking	207			
July 17th–July 31st	38	22	28	16	July 9th – July 31st	73	20	27	13
August	44	21	28	15	August	149	22	29	17
September	83	19	27	12	September	80	17	24	12
Total of whole-growing season	362				Total of whole-growing season	509			
	PPAC, 2013					PPAC, 2014			
June	242	20	26	12	May	90	17	23	8
July	63	22	27	16	June	248	21	27	16
August 1st–August 10th	80	20	26	15	July 1st – July 17th	68	20	25	14
Total of pre-silking	385				Total of pre-silking	406			
August 11th–August 31st	32	21	27	14	July 18th – July 31st	18	20	26	12
September	78	18	25	11	August	265	21	27	10
October	80	13	19	7	September	66	19	26	13
Total of whole-growing season	575				Total of whole-growing season	755			

GYP decreased by 37 and 28 g plant^{-1}, KNP decreased by 109 and 103 kernel plant^{-1}, and KW decreased by 19 and 12 mg kernel^{-1} for ACRE and PPAC, respectively (Tables 3 and 4). SSR decreased by 31 and 35 mg kernel^{-1} when density increased from D1 to D2, and it declined further by 15 and 13 mg kernel^{-1} from D2 to D3 for ACRE and PPAC, respectively. D3 delayed tasseling at ACRE compared to D1, whereas D2 and D3 both delayed tasseling at PPAC compared to D1. Increasing density (from D1 to D2, and D2 to D3) delayed 50% silking in both locations. Increasing density from D1 to D2 shortened the interval between 50% silking to 50% milkline at both locations; however, the further increasing density from D2 to D3 shortened this interval in ACRE but not in PPAC (Tables 3 and 4).

3.2. Breeding effort in contributing to canopy traits, yield and yield components

Grain yield per unit area increased linearly from the oldest to the newest hybrids when averaged across all N rates and densities at both locations (Fig. 1a and b). GY increased 62.1 and 86.4 kg ha^{-1} year^{-1} under 55N and 220N, respectively, at ACRE (Fig. 1a) whereas it increased 50.5 and 64.0 kg ha^{-1} year^{-1} under 55N and 220N in PPAC (Fig. 1b). However, the N rate effect on two slopes of grain yield gain per area was not significant for both locations. The relative grain yield gain during 1967–2005 (based on the 2005VT3 hybrid) was 0.5% year^{-1} at ACRE and 0.6% year^{-1} at PPAC across two N rate. Grain yield per plant, KW and SSR also increased linearly in last 40 years (Figs. 2–4). There were no N rate and hybrid interactions, indicating that the rate of improvement for GY, GYP, KW and SSR was not different for these low and high N treatments.

Over time, GY, GYP, KW and SSR also consistently increased across all densities (Fig. 1–4). An interaction of hybrid and density was observed in GY, where GY increased at a slower rate (less steep slope) at D1, than at D2 and D3 at both locations (Fig. 1c and d). The rate of improvement for GY at D1 was 57.5 kg ha^{-1} year^{-1} compared to 82.7 kg ha^{-1} year^{-1} at D2 and 81.7 kg ha^{-1} year^{-1} at D3 at ACRE (Fig. 1c). Similarly, the increasing rate of GY at D1 was 45.7 kg ha^{-1} year^{-1} compared to 59.6 kg ha^{-1} year^{-1} at D2 and 66.4 kg ha^{-1} year^{-1} at D3 at PPAC (Fig. 1d). No interaction between hybrids and density was observed for GYP in either location, suggesting that GYP increased at the same rate under all 3 plant densities (Fig. 2c and 2d). The hybrid x density interaction was significant for KW at ACRE (Fig. 3c), but not at PPAC (Fig. 3d). The increasing rate of KW were much higher at D1 (1.69 mg kernel^{-1} year^{-1}) than D3 (0.81 mg kernel^{-1} year^{-1}), but it did not differ between D1 and D2, or between D2 and D3 at ACRE (Fig. 3c). The hybrid x density interaction also affected the improvement rate for SSR at ACRE (Fig. 4c). Source-sink ratio increased faster under D1 (1.68 mg kernel^{-1} year^{-1}) than D3 (0.70 mg kernel^{-1} year^{-1}), while rate of SSR increase was similar between D1 and D2, and between D2 and D3 at ACRE (Fig. 4c). However, rates of SSR gain did not differ among the three densities at PPAC (Fig. 4d).

The effect of hybrid era on EGR reached a plateau at alternate decades under different environments (Fig. 5). At ACRE, the plateau of EGR was reached at the end of 1980s, and N rate had minor impact on the time of plateau occurrence (Fig. 5a). At PPAC, the EGR plateau was achieved earlier at high N rate compared to low N rate (Fig. 5b). The density effect was consistent in both locations; low density reached a plateau earlier than both medium and high density (Fig. 5c and d). For D3 in PPAC, the estimated plateau year is beyond the most recent hybrid year in this experiment (Fig. 5d). Lastly, post-silking dry matter accumulation (PostDM) was higher with more recent hybrids (Fig. 6). There were no N treatment and hybrid era interactions in PostDM rate gains, suggesting that the increasing rate of PostDM were consistent at different N rates (Fig. 6a and b). Furthermore, there was no density and era interaction in PostDM rate gains at ACRE. However, there was a density and era interaction at PPAC with low density having a much higher PostDM increase rate compared to medium density, even though there was no such difference between medium density and high density (Fig. 6d).

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Table 3
Nitrogen rate, plant density and hybrid era impacts on treatment means for grain yield per area (at 0% moisture), grain yield per plant (at 0% moisture), kernel number per plant, kernel number, kernel weight, source-sink ratio, green leaf number at R1, R2, R3 and R5, thermal time from planting to 50% tassel, from planting to 50% silking and from silking to 50% milkline, specific leaf nitrogen, leaf area index at R1, R3 and R5 at ACRE.

		Nitrogen (kg ha^{-1})		*LSD(N)*	Density (plants ha^{-1})			*LSD(D)*	Era of hybrids								*LSD(H)*
	Unit	55N	220N		D1	D2	D3		1967	1975	1982	1994	2003RR2	2003VT3	2005RR2	2005VT3	
Grain Yield (GY)	kg ha^{-1}	9934	11458	***6640***	10600	10968	10536	***348***	8385	9985	10160	10848	11527	11451	11755	11522	***561***
Grain Yield per plant (GYP)	g plant^{-1}	136	156	***81***	192	142	105	***4.4***	112	138	138	149	157	156	159	160	***7***
Kernel Number per Plant (KNP)	kernels plant^{-1}	506	532	***55***	633	516	407	***14***	517	522	534	548	550	493	538	450	***23***
Kernel Number (KN)	kernels m^{-2}	3772	3975	***496***	3510	4004	4110	***104***	3372	3955	3694	4092	4139	4003	3915	3815	***170***
Kernel Weight (KW)	mg kernel^{-1}	263	288	***69***	302	272	253	***6***	245	249	273	266	281	287	298	303	***9***
Post-silking Dry Matter Gain	kg ha^{-1}	10445	12644	***2394***	11793	11711	11152	***524***	9423	11253	11989	10936	11832	11688	12620	12670	***764***
Harvest Index (HI)	kg kg^{-1}	0.53	0.55	***0.03***	0.54	0.54	0.53	***0.01***	0.53	0.52	0.55	0.55	0.56	0.56	0.54	0.55	***0.01***
Source Sink Ratio (SSR)	mg kernel^{-1}	264	291	***63***	303	272	257	***6***	249	254	275	268	283	289	297	305	***9***
Ear Growth rate (EGR)	g plant^{-1} d^{-1}	2.8	3.1	***0.6***	3.9	2.9	2.2	***0.1***	2.3	3.0	3.0	2.9	3.1	3.2	3.1	3.1	***0.2***
Green leaf number at R1	# green leaf pl^{-1}	12.3	13.2	***1.6***	13.4	12.8	12.2	***0.2***	12.3	12.6	13.1	13.0	12.8	12.8	12.7	13.0	***0.2***
Green leaf number at R2	# green leaf pl^{-1}	11.9	12.9	***1.9***	13.0	12.3	11.8	***0.2***	11.9	12.1	12.6	12.6	12.4	12.5	12.4	12.6	***0.3***
Green leaf number at R3	# green leaf pl^{-1}	10.8	12.2	***2.1***	12.2	11.5	10.8	***0.2***	11.1	11.3	11.5	11.7	11.5	11.6	11.5	11.8	***0.3***
Green leaf number at R5	# green leaf pl^{-1}	9.9	11.2	***1.9***	11.3	10.4	9.9	***0.3***	10.1	10.3	10.6	10.9	10.6	10.7	10.5	10.8	***0.3***
Thermal time (planting to 50% tassel)	°Cd	876	882	***6***	872	879	886	***9***	849	903	904	883	872	875	873	873	***10***
Thermal time (planting to 50% silking)	°Cd	869	872	***8***	856	869	886	***9***	855	915	899	869	854	862	856	852	***13***
Thermal time(50% silking to 50% milkline)	°Cd	634	639	***6***	649	638	622	***6***	639	605	590	643	653	633	666	665	***15***
Specific Leaf Nitrogen (SLN)	g m^{-2}	1.94	2.35	***0.71***	2.24	2.19	2.01	***6.37***	1.83	2.14	2.18	2.03	2.35	2.29	2.20	2.15	***0.19***
Leaf Area Index (LAI) at R1	m^2 m^{-2}	3.7	3.7	***0.2***	3.1	3.7	4.3	***0.3***	3.5	3.8	3.8	3.8	3.6	3.5	3.8	3.8	***0.2***
Leaf Area Index (LAI) at R3	m^2 m^{-2}	3.3	3.5	***1.9***	2.8	3.4	4.0	***0.2***	3.5	3.4	3.6	3.5	3.2	3.3	3.5	3.5	***0.2***
Leaf Area Index (LAI) at R5	m^2 m^{-2}	1.7	2.2	***2.4***	1.7	1.9	2.2	***0.2***	2.2	1.8	2.1	1.8	1.9	1.9	2.0	1.9	***0.2***

Table 4
Nitrogen rate, plant density and hybrid era impacts on treatment means for grain yield per area (at 0% moisture), grain yield per plant (at 0% moisture), kernel number per plant, kernel number, kernel weight, source-sink ratio, green leaf number at R1, R2, R3 and R5, thermal time from planting to 50% tassel, from planting to 50% silking and from silking to 50% milkline, specific leaf nitrogen, leaf area index at R1, R3 and R5 at PPAC.

		Nitrogen (kg ha^{-1})		*LSD(N)*	Density (plants ha^{-1})			*LSD(D)*	Era of hybrids								*LSD(H)*
	Unit	55N	220N		D1	D2	D3		1967	1975	1982	1994	2003RR2	2003VT3	2005RR2	2005VT3	
Grain Yield (GY)	kg ha^{-1}	6955	9091	***2187***	8369	8123	7577	***317***	6556	7085	7525	8188	8487	8610	8595	9140	***402***
Grain Yield per plant (GYP)	g plant^{-1}	96	123	***31***	153	102	74	***5***	88	98	103	111	116	117	117	125	***6***
Kernel Number per Plant (KNP)	kernels plant^{-1}	410	477	***77***	565	434	331	***19***	454	433	457	455	465	422	446	414	***29***
Kernel Number (KN)	kernels m^{-2}	3037	3573	***612***	3098	3453	3363	***155***	3082	3295	3142	3515	3398	3420	3229	3351	***200***
Kernel Weight (KW)	mg kernel^{-1}	228	255	***36***	270	234	222	***7***	210	213	238	233	249	252	266	273	***9***
Post-silking Dry Matter Gain	kg ha^{-1}	6511	9188	***8456***	8346	8041	7161	***569***	5383	7346	8183	7578	7760	8503	8616	8971	***809***
Harvest Index (HI)	kg kg^{-1}	0.48	0.52	***0.15***	0.52	0.50	0.49	***0.01***	0.51	0.47	0.47	0.52	0.51	0.52	0.51	0.52	***0.01***
Source Sink Ratio (SSR)	mg kernel^{-1}	229	256	***34***	270	235	222	***7***	212	215	241	234	249	250	267	272	***11***
Ear Growth rate (EGR)	g plant^{-1} d^{-1}	2.0	2.5	***0.7***	3.0	2.1	1.5	***0.1***	1.8	2.2	2.2	2.2	2.3	2.3	2.3	2.4	***0.1***
Green leaf number at R1	# green leaf pl^{-1}	11.2	12.7	***2.2***	12.6	11.9	11.4	***0.3***	11.4	12.0	12.2	12.0	12.1	12.2	11.9	12.0	***0.3***
Green leaf number at R2	# green leaf pl^{-1}	10.3	12.2	***1.7***	11.9	11.2	10.6	***0.2***	10.9	11.0	11.4	11.2	11.2	11.4	11.3	11.4	***0.2***
Green leaf number at R3	# green leaf pl^{-1}	9.4	11.0	***4.1***	10.9	10.1	9.6	***0.3***	10.0	9.9	10.3	10.2	10.3	10.4	10.3	10.4	***0.3***
Green leaf number at R5	# green leaf pl^{-1}	8.2	9.9	***1.3***	9.7	9.0	8.4	***0.3***	8.1	8.8	9.2	9.0	9.3	9.3	9.3	9.3	***0.3***
Thermal time (planting to 50% tassel)	°Cd	886	886	***5***	877	887	893	***8***	856	909	907	881	878	881	890	883	***7***
Thermal time (planting to 50% silking)	°Cd	886	883	***7***	872	886	896	***8***	862	927	913	870	877	875	881	873	***8***
Thermal time(50% silking to 50% milkline)	°Cd	594	603	***6***	607	597	591	***9***	586	574	571	605	612	604	616	619	***15***
Specific Leaf Nitrogen (SLN)	g m^{-2}	1.54	1.89	***0.7***	1.97	1.70	1.49	***0.09***	1.48	1.83	1.65	1.58	1.89	1.86	1.70	1.74	***0.13***
Leaf area index (LAI) at R1	m^2 m^{-2}	3.3	3.4	***0.1***	2.7	3.4	3.9	***0.2***	3.3	3.2	3.5	3.4	3.2	3.3	3.4	3.5	***0.2***
Leaf area index (LAI) at R3	m^2 m^{-2}	2.2	2.5	***0.1***	2.1	2.3	2.6	***0.1***	2.4	2.2	2.4	2.3	2.2	2.2	2.5	2.4	***0.2***
Leaf area index (LAI) at R5	m^2 m^{-2}	1.6	2.1	***2.3***	1.6	1.8	2.0	***0.2***	1.7	1.6	2.0	1.7	1.8	1.9	1.9	1.9	***0.2***

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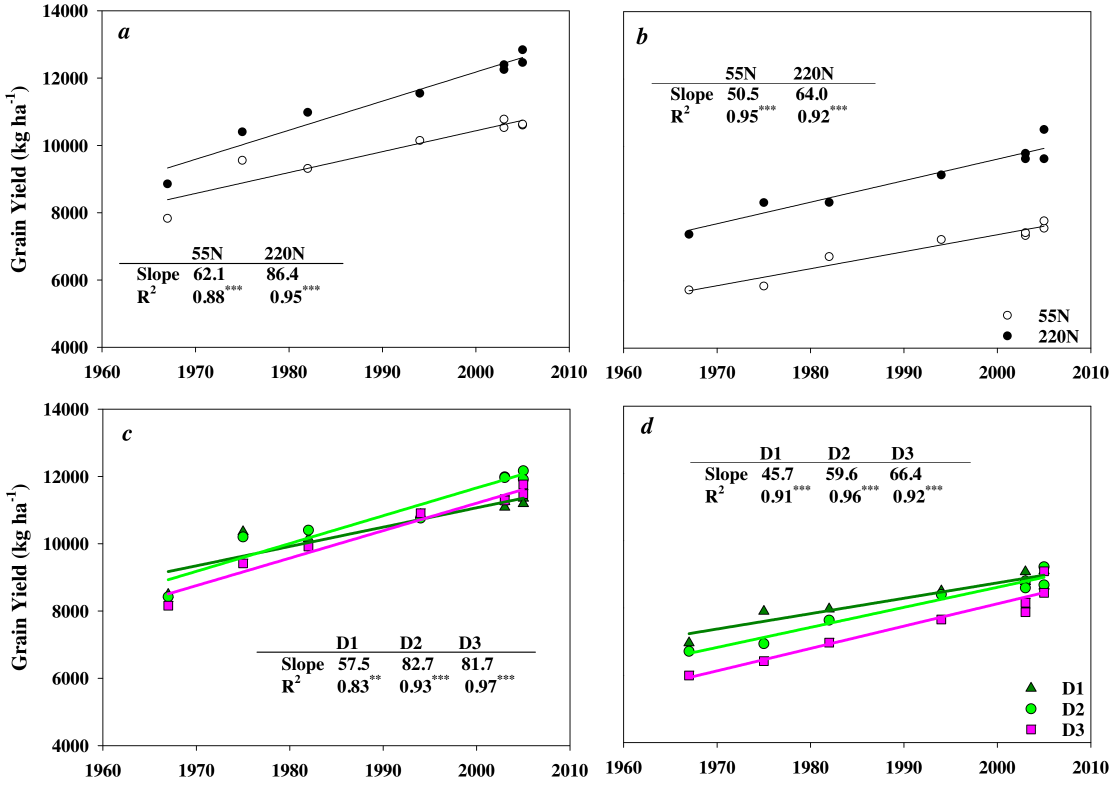

Fig. 1. Nitrogen by hybrid era interaction effects on grain yield (at 0% moisture) at ACRE (a) and PPAC (b). Means are averaged over two years and plant density of 54,000, 79,000, and 104,000 plants ha^{-1}. The slope difference in (a) is 24.3^{ns} and in (b) is 13.5^{ns}. Plant density by hybrid era interaction effects on grain yield at ACRE (c) and PPAC (d). Means are averaged over two years and N rates of 55 and 220 kg N ha^{-1}. The slope difference between D1 and D2 is 25.2*, between D2 and D3 is 1.0^{ns}, between D1 and D3 is 24.2^{*} in (c) and is 13.9^{ns}, 6.8^{ns} and 20.7^{ns} in (d). Legends for treatment variables are shown in (b) and (d). *, **, *** indicates slope significance at p-value <0.05, <0.01, and <0.001, respectively.

Table 5
Progressive hybrid era correlation relationships for green leaf number at R1, R2, R3, and R5 in response to N rate and plant density treatments at ACRE and PPAC.

	ACRE		PPAC	
	Slope	R^2	Slope	R^2
	Green leaf at R1 (# of green leaf pl^{-1})		Green leaf at R1 (# of green leaf pl^{-1})	
55N	9.5×10^{-3}	0.34 NS	1.1×10^{-2}	0.42 NS
220N	6.1×10^{-3}	0.36 NS	1.0×10^{-2}	0.34 NS
54,000 pls ha^{-1}	8.8×10^{-3}	0.24 NS	7.0×10^{-3}	0.15 NS
79,000 pls ha^{-1}	8.7×10^{-3}	0.27 NS	9.9×10^{-3}	0.31 NS
104,000 pls ha^{-1}	6.0×10^{-3}	0.15 NS	1.6×10^{-2}	0.60^{*}
	Green leaf at R2 (# of green leaf pl^{-1})		Green leaf at R2 (# of green leaf pl^{-1})	
55N	1.3×10^{-2}	0.54^{*}	8.3×10^{-3}	0.37 NS
220N	1.0×10^{-2}	0.39 NS	1.2×10^{-2}	0.55^{*}
54,000 pls ha^{-1}	1.4×10^{-2}	0.59^{*}	4.9×10^{-3}	0.14 NS
79,000 pls ha^{-1}	1.1×10^{-2}	0.34 NS	1.5×10^{-2}	0.75^{*}
104,000 pls ha^{-1}	1.0×10^{-2}	0.42 NS	1.1×10^{-2}	0.61^{*}
	Green leaf at R3 (# of green leaf pl^{-1})		Green leaf at R3 (# of green leaf pl^{-1})	
55N	1.3×10^{-2}	0.74^{**}	1.0×10^{-2}	0.45 NS
220N	1.2×10^{-2}	0.41 NS	1.1×10^{-2}	0.85^{***}
54,000 pls ha^{-1}	1.4×10^{-2}	0.69^{*}	5.5×10^{-3}	0.18 NS
79,000 pls ha^{-1}	1.6×10^{-2}	0.70^{*}	1.5×10^{-2}	0.86^{***}
104,000 pls ha^{-1}	7.0×10^{-3}	0.24 NS	1.1×10^{-2}	0.66*
	Green leaf at R5 (# of green leaf pl^{-1})		Green leaf at R5 (# of green leaf pl^{-1})	
55N	1.2×10^{-2}	0.59^{*}	2.1×10^{-2}	0.69^{*}
220N	1.4×10^{-2}	0.48^{*}	2.6×10^{-2}	0.74^{*}
54,000 pls ha^{-1}	2.1×10^{-2}	0.69^{*}	2.8×10^{-2}	0.64^{*}
79,000 pls ha^{-1}	1.3×10^{-2}	0.35 NS	2.1×10^{-2}	0.64^{*}
104,000 pls ha^{-1}	6.2×10^{-3}	0.24 NS	2.1×10^{-2}	0.82^{***}

The era effects on green leaf number (GL) were weak at onset of the grain filling period (R1) in both locations (Table 5). However, the era effects on GL were greater at later stages of grain filling period (R3-R5) for both locations. For instance, GL per plant increased 0.012 and 0.014 leaves $\text{plant}^{-1}\,\text{year}^{-1}$ with 55N and 220N at ACRE at R5, and GL per plant increased 0.021 and 0.026 leaves $\text{plant}^{-1}\,\text{year}^{-1}$ at PPAC at R5 (Table 5). D1 showed a higher increasing rate for GL at R5 for both locations with 0.021 leaves

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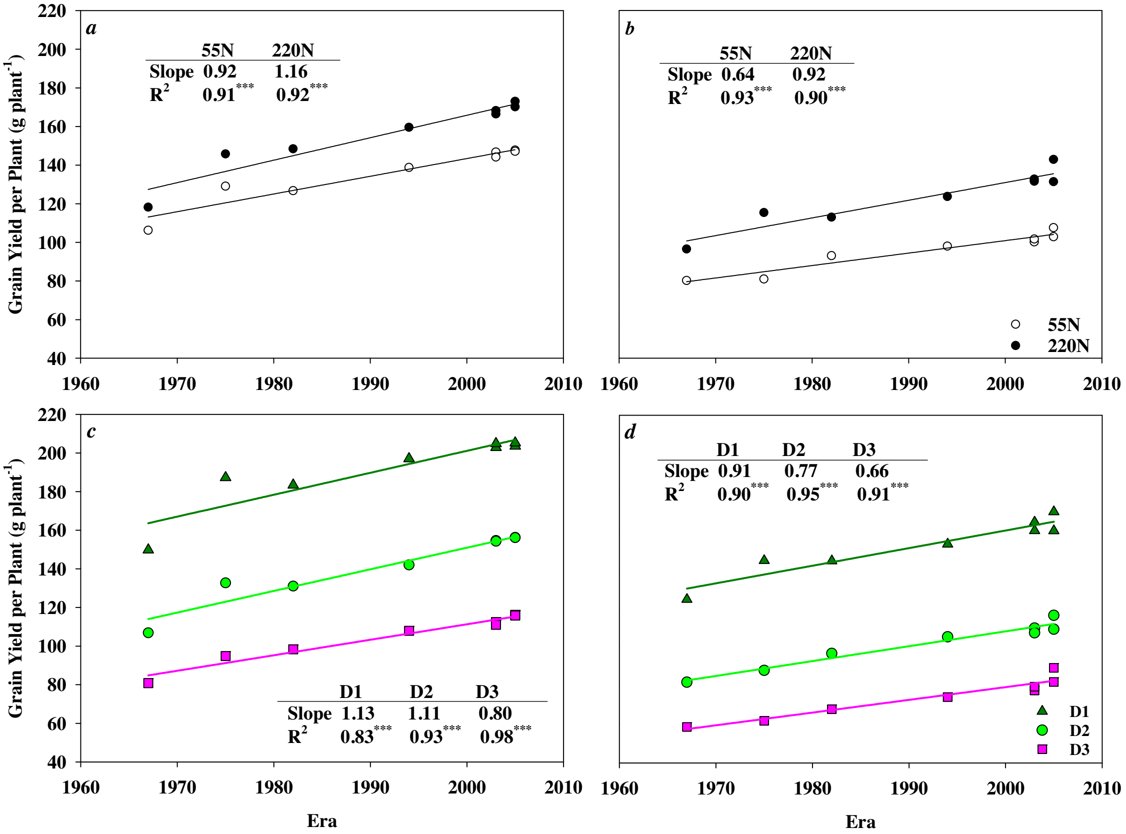

Fig. 2. Nitrogen by hybrid era interaction effects on grain yield per plant (at 0% moisture) at ACRE (a) and PPAC (b). Means are averaged over two years and plant density of 54,000, 79,000, and 104,000 plants ha^{-1}. The slope difference in (a) is 0.24^{ns} and in (b) is 0.28^{ns}. The plant density by hybrid era interaction effects on grain yield at ACRE (c) and PPAC (d). Means are averaged over two years and N rates of 55 and 220 kg N ha^{-1}. The slope difference between D1 and D2 is 0.02^{ns}, between D2 and D3 is 0.31^{ns}, between D1 and D3 is 0.33^{ns} in (c) and is 0.14^{ns}, 0.11^{ns} and 0.25^{ns} in (d). Legends for treatment variables are shown in (b) and (d). *, **, *** indicates slope significance at *p*-value <0.05, <0.01, and <0.001, respectively.

plant^{-1} year^{-1} at ACRE and 0.028 leaves plant^{-1} year^{-1} at PPAC. The slopes of linear regression lines for SLN at silking versus era were not significant between the two N rates or among the three densities at both locations (data not shown). Similar to SLN, the slopes of linear regression lines for LAI at R1, R3 and R5 versus era were not different between N rates and densities in both locations (data not shown).

Kernel weight was positively correlated with EGR during grain filling period (Fig. 7). At low N rate, the KW increment per unit of EGR (g plant^{-1} d^{-1}) ranged from 182 to 226 mg kernel^{-1} for hybrids from 1982 to 2005, which were all significantly higher than the rate of KW gain per unit EGR for the 1967 hybrid, with *p*-value = 0.003 when 2003RR2 (which had lowest rate of KW gain among hybrids from 1982 to 2005) compared with 1967 hybrid (Fig. 7). Hybrids from 1982 to 2005 also had higher rates of KW gain per unit EGR than 1975 hybrid, although hybrids 2005RR2 (*p*-value = 0.06) and 2003RR2 (*p*-value = 0.09) hybrids were just marginally significantly different than the 1975 hybrid. At high N rate, the KW incremental gain per unit EGR ranged from 167 to 244 mg kernel^{-1} for hybrids from 1994 to 2005 with no significant difference among these hybrids. However, these hybrids had a much higher rate of KW gain per unit EGR than the 1967 hybrid, with *p*-value = 0.01 when 2003RR2 (which had lowest rate of KW gain among hybrids from 1975 to 2005) was compared with the 1967 hybrid (Fig. 7).

4. Discussion

4.1. Genetic improvement contribution to grain yield under nitrogen and density stress

The average annual rate of grain yield improvement was 66 kg ha^{-1} year^{-1} in this study across all treatments and locations. The rate of yield gain averaged 56 kg ha^{-1} at low N rate and 75 kg ha^{-1} year^{-1} at high N rate when averaged across the three densities and two locations (Fig. 1). The higher rate of grain yield increase at the higher N rate (Fig. 1) was due to a higher GYP gain at high N than at low N rate (Fig. 2). Duvick (2005) reported grain yield increases of 109 kg ha^{-1} year^{-1} in US maize production from 1961 to 2002. However, our yield gain rate was almost identical to the results reported in Castleberry et al. (1984) with a series of DeKalb hybrids from 1930′s to 1980′s (which was 51 for low fertility and 86 kg ha^{-1} year^{-1} for high fertility across two years and two locations). Castleberry et al. (1984) discussed that their yield gain rate was lower than US national rate during 1930–1980 (110 kg ha^{-1} ha^{-1}), and they attributed the discrepancy to over estimation of yield increases over time by planting older hybrids at then-current densities in hybrid comparison trials. Additionally, many trials are machine harvested which could cause greater loss for older hybrids because of more stem lodging. However, in our study, the low density (54,000 plants ha^{-1}) was a common plant density for 1967–1975 hybrids when these were grown commercially. In addition, all plots in our experiment were hand harvested

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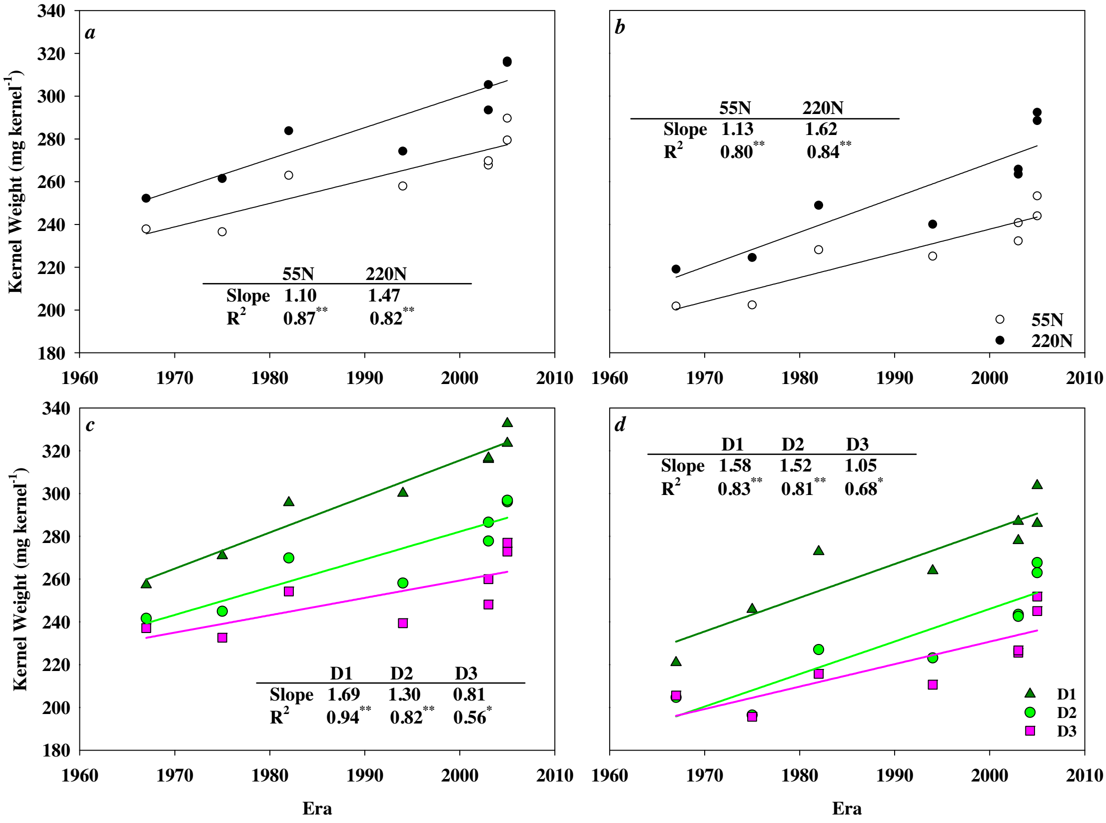

Fig. 3. Nitrogen by hybrid era interaction effects on kernel weight at ACRE (a) and PPAC (b). Means are averaged over two years and plant density of 54,000, 79,000, and 104,000 plants ha^{-1}. The slope difference in (a) is 0.37^{ns} and in (b) is 0.49^{ns}. Plant density by hybrid era interaction effects on grain yield at ACRE (c) and PPAC (d). Means are averaged over two years and N rates of 55 and 220 kg N ha^{-1}. The slope difference between D1 and D2 is 0.39^{ns}, between D2 and D3 is 0.49^{ns}, between D1 and D3 is 0.88^{*} in (c) and is 0.06^{ns}, 0.47^{ns} and 0.53^{ns} in (d). Legends for treatment variables are shown in (b) and (d). *, **, *** indicates slope significance at *p*-value <0.05, <0.01, and <0.001, respectively.

which would eliminate the grain yield loss problem during harvest from any extra lodging in older hybrids.

Grain yield increased consistently with advancing hybrid era under all three densities in ACRE (Fig. 1c), but the two yield intersections at 1970–1980 and at 1990–2000 for yields at the different densities indicate that grain yields were higher at 79,000 versus 54,000 plants ha^{-1} after around 1975, and that grain yields were higher at 104,000 versus 54,000 plants ha^{-1} after around 1995 (Fig. 1c). Duvick (2005) studied grain yield under three densities for a series of Pioneer hybrids from 1930 to 2000. The intersection of 30,000 versus 79,000 plants ha^{-1} occurred during 1950–1960 in that study (Duvick, 2005). Even though there was an intersection between 104,000 and 54,000 plants ha^{-1} around 1995 in ACRE in our study, we did not observe an intersection between 104,000 and 79,000 plants ha^{-1}. The failure to achieve higher yields at 104,000 for 2003 or 2005 hybrids in our study is due to low grain yield per plant at 104,000 plants ha^{-1} in ACRE (Fig. 2c). In comparison, average final plant population in US grain maize production in 2015 was estimated at ~73,000 plants ha^{-1} by USDA Crop Production Summary (USDA, 2016). The limitations for further yield gains at the highest population in this study included dramatic reductions in PostDM, KW and KN per plant across all N rates and environments (Tables 3 and 4). There were no grain yield intersections among the three plant densities among the hybrid era yield means at PPAC (Fig. 1c). The lack of intersection occurred in the context of lower overall yields at PPAC resulting from corn being grown after corn, at least 10 day later planting dates, and by above normal precipitation levels before silking in both 2013 and 2014 (Table 2).

Tollenaar and Lee (2011) addressed the importance of enhanced grain yield stability in modern hybrids that is achieved by 1) increasing stress tolerance, 2) maintaining yield potential, and 3) minimizing the genotype × environment interaction. In our study, grain yield improvement over decades was consistent over all density levels. Even though the high density (D3) did not lead to the highest grain yield, the superior performance of newer hybrids at high density illustrated a better tolerance to stress in these hybrids. Secondly, GYP of newer hybrids increased consistently across all locations, densities and N rates (Fig. 2). Although our density level was not low enough to measure yield potential, more recent hybrids still have a better GYP in comparison with older hybrids in the same environments. Lastly, incremental grain yield improvements were consistent over two locations (Fig. 1). Grain yield of 1967 hybrid was 70% of 2005VT3 hybrid, 1975 and 1982 hybrids achieved about 80–88% of grain yield of 2005VT3 hybrid, and the two 2003 hybrids plus 2005RR2 hybrid had about 90%–100% grain yield of 2005VT3 hybrid at both locations. Although the increasing rate of grain yield is always higher in ACRE than PPAC at same treatment management combination, the consistent increase at both locations indicates lack of genotype × environment interaction for grain yield.

4.2. Genetic justification in source versus sink strength

Source-sink ratio (SSR) increased by 1.2 mg kernel^{-1} year^{-1} in ACRE and 1.3 mg kernel^{-1} year^{-1} in PPAC, across all plant density and N treatments (Fig. 4). Previously, SSR has more often

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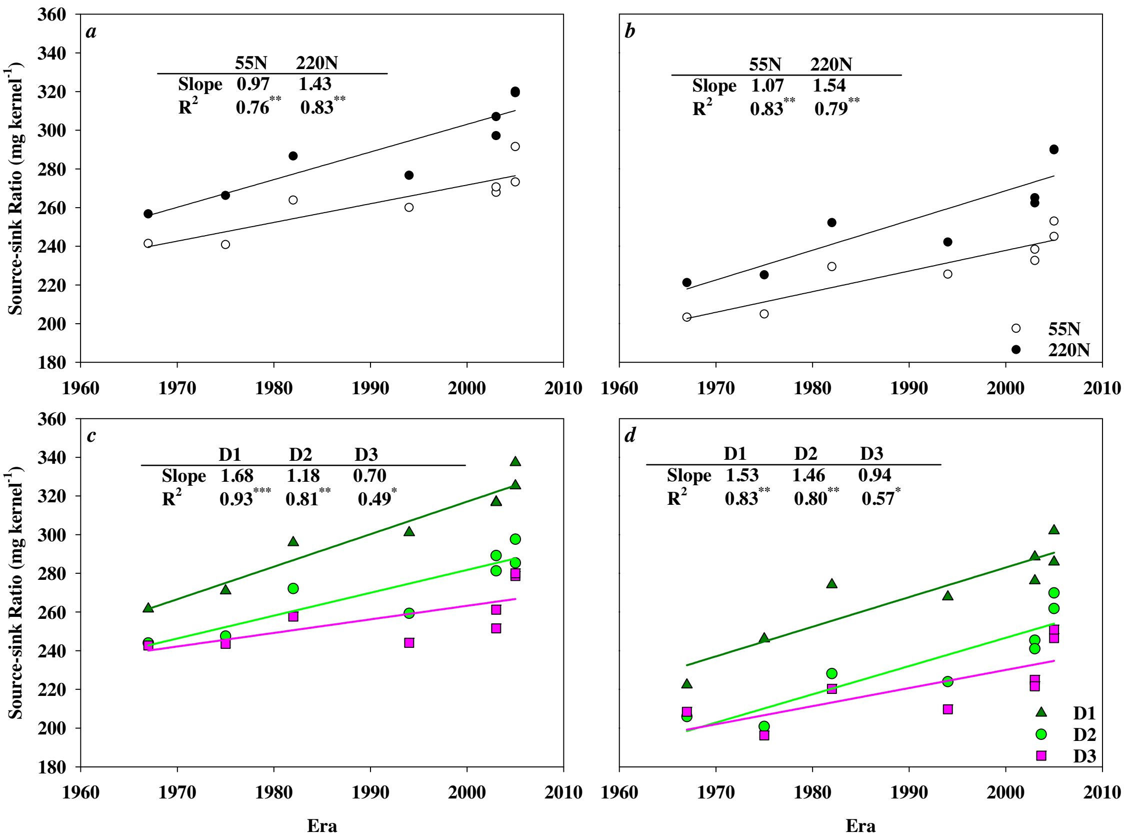

Fig. 4. Nitrogen by hybrid era interaction effects on Source-sink ratio at ACRE (a) and PPAC (b). Means are averaged over two years and plant densities of 54,000, 79,000, and 104,000 plants ha^{-1}. The slope difference in (a) is 0.46^{ns} and in (b) is 0.47^{ns}. Plant density by hybrid era interaction effects on grain yield at ACRE (c) and PPAC (d). Means are averaged over two years and N rates of 55 and 220 kg N ha^{-1}. The slope difference between D1 and D2 is 0.50^{ns}, between D2 and D3 is 0.48^{ns}, between D1 and D3 is 0.98* in (c) and is 0.07^{ns}, 0.52^{ns} and 0.59^{ns} in (d). Legends for treatment variables are shown in (b) and (d). *, **, *** indicates slope significance at p-value <0.05, <0.01, and <0.001, respectively.

been discussed as an intentional treatment by controlling pollination or imposing leaf defoliation (Rajcan and Tollenaar, 1999b; Borrás and Otegui, 2001; Jones and Simmons, 1983; Tollenaar and Daynard, 1982). Discussion of how SSR is impacted by hybrid development has not been well-documented (Luque et al., 2006). Source strength during the grain filling period can be inferred from post-silking dry matter accumulation (PostDM). Tollenaar and Lee (2011) illustrated two ways to improve source strength: 1) increase dry matter accumulation rate during grain filling period; 2) increase the duration of grain filling period by advancing silking but keeping physiological maturity constant. Both mechanisms were evident in our research. With respect to the first approach, PostDM increased 54.2 and 53.6 kg ha^{-1} year^{-1} at ACRE and PPAC, respectively, across all N rate and density treatments (Fig. 6). With respect to the second approach, our study confirmed longer duration of grain filling in newer hybrids. For example, the newest hybrid – 2005VT3 – had reached 50% silk emergence at the same time as other 2000s hybrids, but it silked 47 ~ 63 °Cd and 40 ~ 54 °Cd earlier than 1970–1980 hybrids in ACRE and PPAC, respectively (Tables 3 and 4). The same 2005VT3 hybrid had active grain filling periods (i.e. from 50% silking to 50% milkline) that averaged from 14 to 75 °Cd longer than those with hybrids released from 1967 to 1994 (Tables 3 and 4).

Overall sink strength was estimated in our study by EGR, which includes kernel number and kernel growth rate, as well as cob and husk growth rates. EGR increased with hybrid era across N rate and density treatments, but it reached a maximum around 1990 in ACRE, and 2000 in PPAC except under high density (Fig. 5). It is well known that sink strength can be improved by reducing plant-to-plant variation in HI and by achieving higher absolute value of HI (via proportionately more transfer of dry matter from vegetative organs to grain), higher KNP or KN, or higher potential kernel weight (Tollenaar and Lee, 2011). In our study, HI was similar across hybrids except for low values with 1967 and 1975 hybrids at ACRE and with the 1975 and 1982 hybrids at PPAC (Tables 3 and 4). Neither KNP nor KN was higher in more recent hybrids in this study (Table 3, 4). Although potential kernel weight, a function of kernel growth rates during silking, was not determined in this research, substantial gains in final KW were detected across both locations. KW increased 1.3 and 1.4 mg kernel^{-1} year^{-1} at ACRE and PPAC across all N rates and densities, respectively (Fig. 3). This substantial KW gain contrasts with previous expectations. For example, Luque et al. (2006) discussed the lack of breeding focus on KW since it is normally considered to be a more stable parameter in comparison with KN or KNP.

Overall, SSR explained 98% (R^2 = 0.98) of total variance of KW at ACRE and 99% (R^2 = 0.99) at PPAC (data not shown) in this study. Borrás and Otegui (2001) also showed a high correlation between SSR and KW, and that maximum KW was achieved only if SSR: KW was over 1:1 due to a saturation in post-silking dry matter accumulation per kernel. However, other research on hybrid eras has not found that maize breeding progress necessarily leads to higher SSR (Luque et al., 2006). We also noticed a high correlation between KW and EGR (Fig. 7), which was similar with the results from Borrás and Otegui (2001), who also reported a poor correlation between KW with duration of the active grain filling period. In our study, considering the fact that EGR also included husk and cob growth rates, the correlation between KW and EGR demon-

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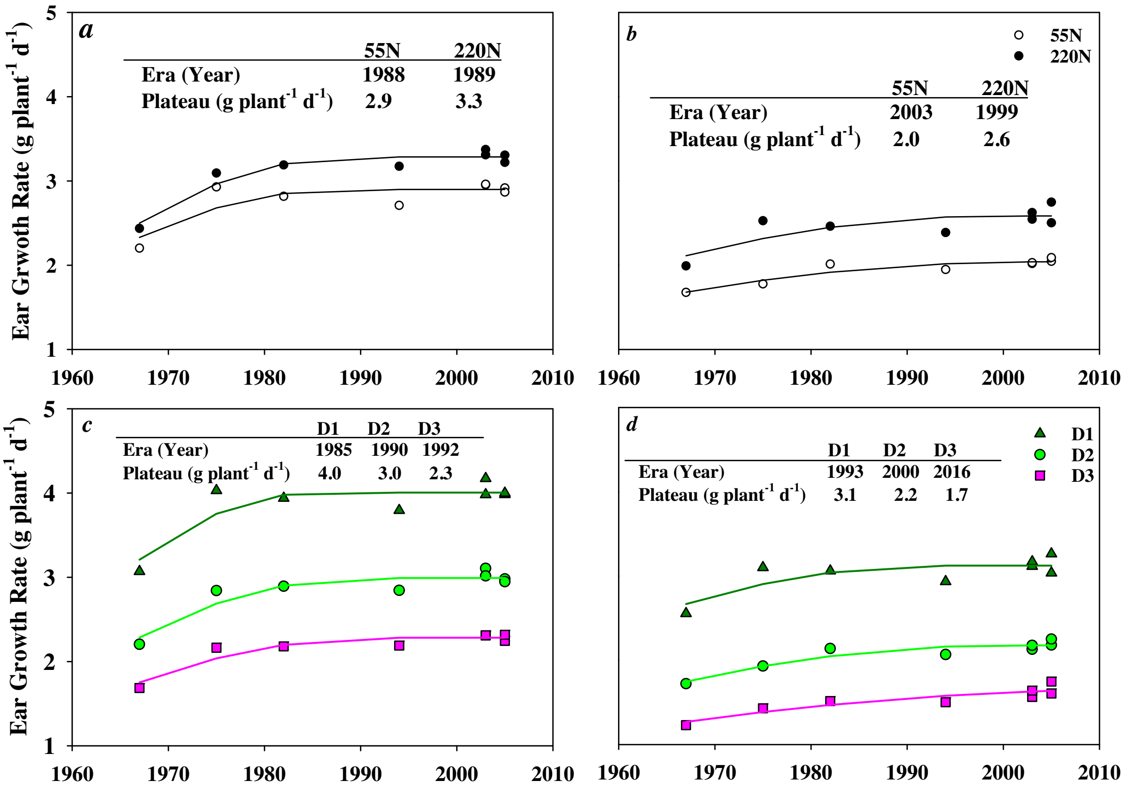

Fig. 5. Nitrogen by hybrid era interaction effects on ear growth rate at ACRE (a) and PPAC (b). Means are averaged over two years and plant density of 54,000, 79,000, and 104,000 plants ha^{-1}. Plant density by hybrid era interaction effects on grain yield at ACRE (c) and PPAC (d). Means are averaged over two years and N rates of 55 and 220 kg N ha^{-1}. Quadratic with plateau was fitted. For each condition, era (year) to reach the plateau, and ear growth rate at plateau, were calculated.

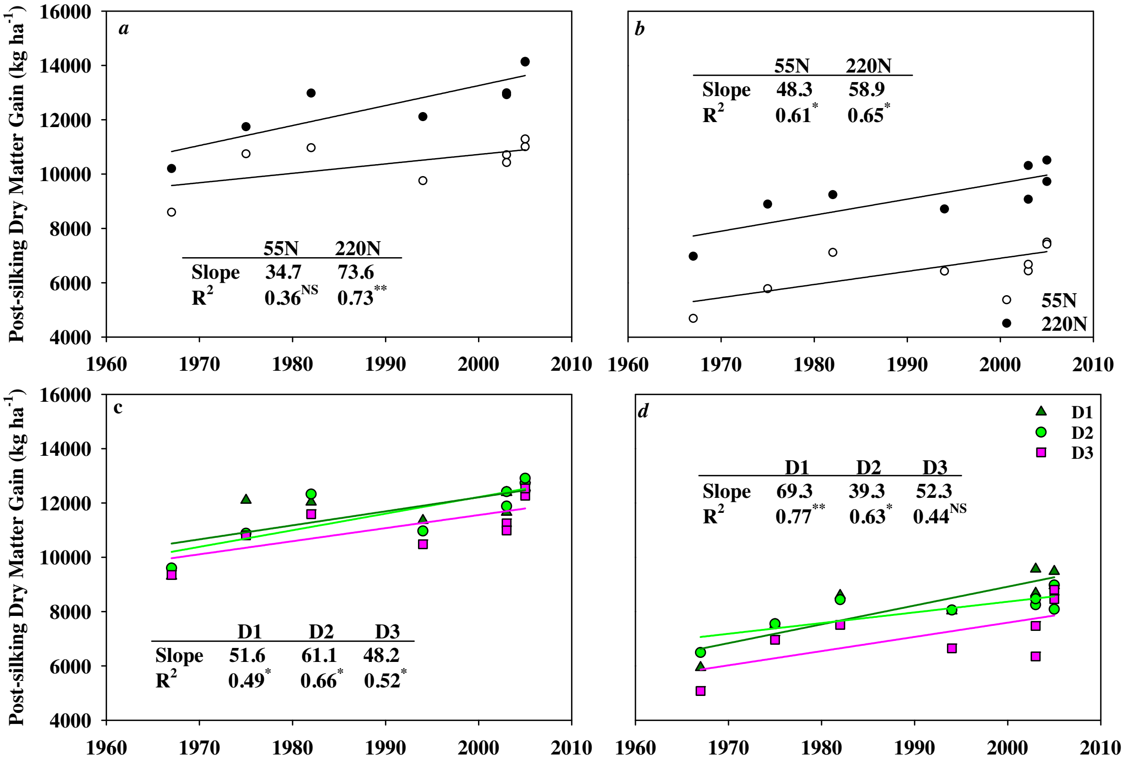

Fig. 6. Nitrogen by hybrid era interaction effects on Post-silking dry matter gain at ACRE (a) and PPAC (b). Means are averaged over two years and plant density of 54,000, 79,000, and 104,000 plants ha^{-1}. The slope difference in (a) is 38.9^{ns} and in (b) is 10.6^{ns}. Plant density by hybrid era interaction effects on grain yield at ACRE (c) and PPAC (d). Means are averaged over two years and N rates of 55 and 220 kg N ha^{-1}. The slope difference between D1 and D2 is 9.5^{ns}, between D2 and D3 is 12.9^{ns}, between D1 and D3 is 3.4^{ns} in (c) and is 29.9*, 13.0^{ns} and 16.9^{ns} in (d). Legends for treatment variables are shown in (b) and (d). *, **, *** indicates slope significance at *p*-value <0.05, <0.01, <0.001, respectively.

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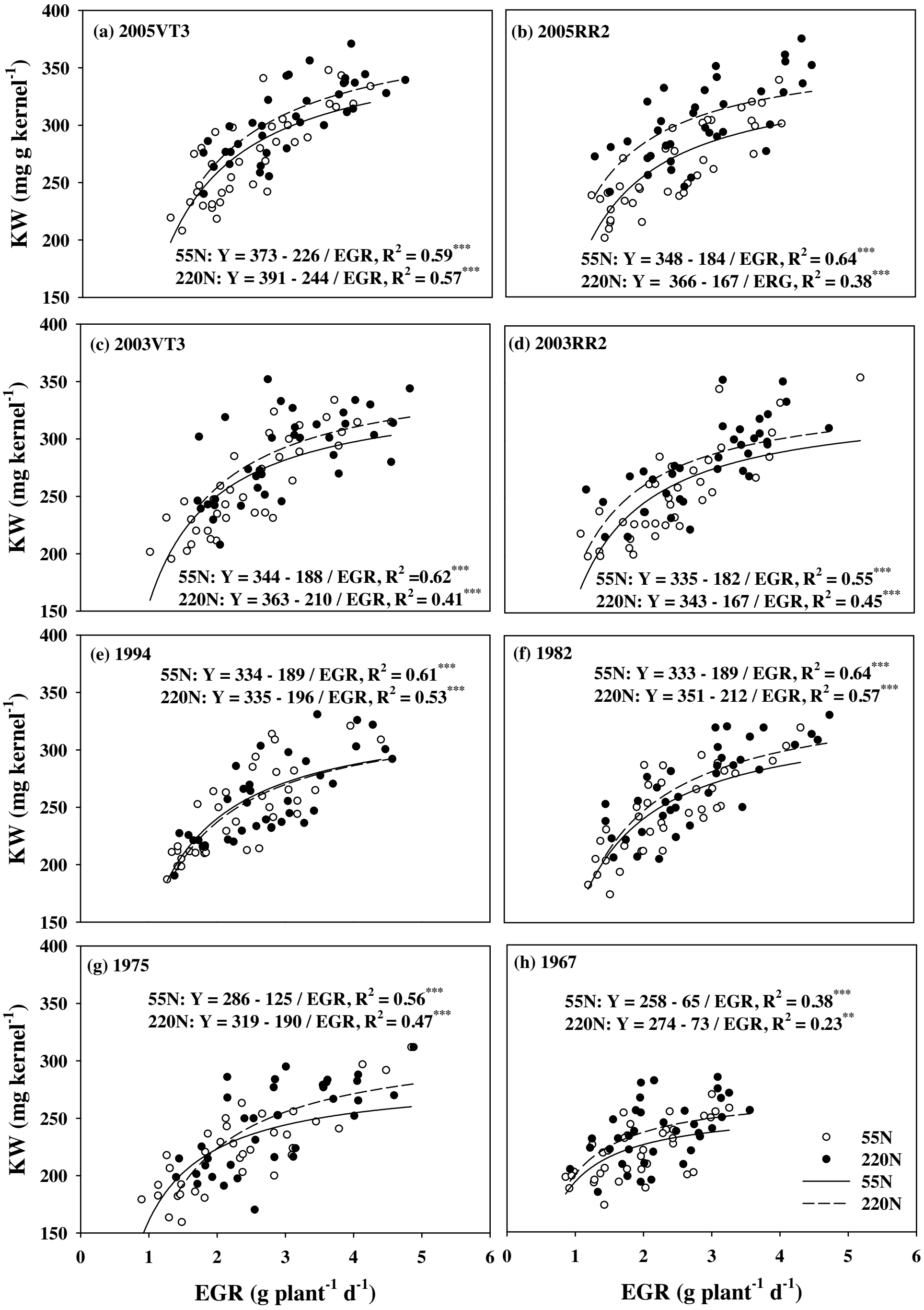

Fig. 7. Differential hybrid era responses to correlation between ear growth rate per plant per thermal time (EGR) and kernel weight per kernel (KW) when ACRE and PPAC data are combined. Closed circle represents 220N and open circle represents 55N *, **, *** indicates slope significant at *p*-value < 0.05, < 0.01, and <0.001, respectively.

strated the efficiency of transferring dry matter from husk and cob to kernel. Recent hybrids showed a greater KW increase per unit of EGR compared to 1967 and 1975 hybrids at the lower N rate, and relative to the 1967 hybrid at higher N rate. This implies a higher efficiency in transferring dry matter to kernel from husk and cob.

Breeding efforts over time in these DeKalb hybrids contributed to an improvement of SSR which was associated with incremental retention gains of green leaf number in newer hybrids during grain filling period. Green leaf number retained at the R3 and R5 stages increased over time at both ACRE and PPAC (Tables 5 and 6). The green leaf number of the 2005 VT3 hybrid, compared to the 1967 hybrid, increased about 0.8 green leaves (when averaged over R3 and R5 stages) at ACRE, and 0.7 green leaves for the same 2 stages at PPAC. These strong era effects on GL at the later stages (R5) of grain filling period indicate a breeding effort which promoted hybrids with longer visual stay green during grain filling (Table 5). Previous studies also documented that stay green enhanced post-silking dry matter and nutrient accumulation (Rajcan and Tollenaar, 1999a; Tollenaar et al., 2004). In our study, the correlation coefficients between green leaf number and PostDM were 0.63–0.70 for green leaf number at R1 to R5 across all treatments and environments (data not shown), and very similar correlation coefficients were found between green leaf number and KW, as well as with SSR (data not shown).

Green leaf number during grain filling period did not show correlations with LAI during grain filling period, with correlation

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coefficients ranging from 0 to 0.3 across both locations, N rates and all densities. In a study with a series of 47 commercial hybrids from 1934 to 1978 tested in Iowa, LAI at silking did not change, although KW and stay green scores increased in more recent hybrids (Duvick, 1984). The lack of correlation of LAI with green leaf number during grain filling in our study could be because LAI was measured for the whole canopy instead of only for green leaves. Cirilo et al. (2009) used green leaf area during grain filling period as a variable for canopy traits, and discovered that the hybrids with higher green leaf area during grain filling had higher PostDM. Borrás et al. (2003) used the ratio of green leaf area during grain filling with kernel number as source-sink ratio, and concluded that leaf senescence is more related to local light source during grain filling period than photosynthetic activity at onset of grain filling period. Hence, leaf area index for canopy did not reflect green leaf changes during grain filling period, whereas green leaf area could be a better measurement for tracking stay green function during the grain filling period.

The causes of yield gains over time in this series of DeKalb hybrids can be summarized as: 1. An enhanced grain filling period with longer duration and more persistent leaf stay green, which leads to higher PostDM (source) in newer hybrids; 2. A higher sink demand in newer hybrids due more to a higher KW than to a higher KN at maturity; 3. A higher source-sink ratio indicating an enhanced source strength in comparison to sink in newer hybrids during the grain filling period; and 4. A higher KW gain per unit of EGR indicating a higher efficiency of transferring dry matter from husk and cob to kernel during grain filling.

5. Conclusion

We studied the physiological basis for yield gains for a 38-year period of commercial DeKalb hybrid release with respect to canopy, yield and yield component traits. Eight hybrids were compared side by side under both limiting and optimal N and with plant densities ranging from 54,000 to 104,000 plants ha^{-1} for a 2-year period at 2 locations. We concluded that: 1) no gain in maximum mid-season LAI or SLN was observed in hybrids spanning 38 years of development, whereas green leaf number during mid or late grain filling period increased in newer hybrids; 2) GY, GYP, KW and SSR, but not KN or KNP, were all increased with more recent hybrids at both N rates and at three plant densities.; 3) EGR during active grain filling period (50% silking to 50% milkline) reached a plateau around 1990 (year) at ACRE across N rate and density treatments, whereas it reached plateau around 2000 (year) at PPAC except at high density where the stress was most severe; and 4) newer hybrids had a longer grain filling period with low correlation between thermal time of grain filling and PostDM, whereas KW showed a high correlation with EGR as a higher rate of KW gain in newer hybrids was apparent per unit of EGR. Increases with hybrid development over time were GY – 65.8 kg ha^{-1} year^{-1}, GYP – 0.91 g plant^{-1} year^{-1}, KW – 1.29 mg kernel^{-1} year^{-1}, and SSR – 1.25 mg kernel^{-1} year^{-1} across all treatments and locations.

Analysis of DeKalb hybrids developed over this 38-year period revealed 1) enhanced grain filling period with both longer duration, as well as retention of source strength capacity (leaves staying green longer); 2) improved source strength with higher PostDM, as well as sink strength with greater KW in newer hybrids; 3) increased efficiency of transferring source from cob and husk to grain by increasing KW gain per unit of EGR; 4) enhanced source to sink strength during grain filling period by increasing SSR; 5) enhanced stability of grain yield, as well as reduced genotype × environment interaction impacts on grain yield. These results are distinct from other maize hybrid era studies that have more frequently reported that KNP or KN are the primary yield component factors that changed over time. However, the precipitation during these two testing years was above normal at both locations and there was no moisture deficit during the critical period at any location-year. Hence, these experiments may need to be repeated under water limited conditions to test the consistency of these efforts in water limited environments. In addition, direct measurements of "functional stay green" – photosynthesis rate or respiration rates – are recommended for future studies to complement our findings that newer hybrids were more "visually stay green".

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