

PLANT POPULATION INFLUENCES ON MAIZE PHYSIOLOGICAL RESPONSES TO NITROGEN APPLICATION

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Abstract

Past genetic improvements in maize (*Zea mays* L.) have led to the creation of modern hybrids that are increasingly tolerant to a variety of stresses and, in particular, to high plant populations. However, high plant densities can adversely affect overall grain yield due to greater intra-specific (within species) competition for limiting resources. This competition increases barrenness and plant-to-plant variability for grain yield (GY_{CV}) and other morpho-physiological traits. In this study, we hypothesize that nitrogen (N) application will reduce intra-specific competition for N, thus decreasing GY_{CV} while increasing per-unit-area grain yield (GY_A). We further suggest that reductions in GY_{CV} will be more pronounced at higher than at lower plant populations. In 2005 and 2006, hybrid Pioneer 31G68 was grown at densities of 54,000, 79,000, and 104,000 plants ha^{-1} . Urea Ammonium Nitrate (UAN) (28-0-0) was applied via side-dressing at a rate of 170 kg N ha^{-1} once (V3), twice (V3, V5), or not at all. Individual plants were measured for numerous morpho-physiological traits including leaf N content (SPAD) (2005, 2006), R6 total biomass (TB_P) (2006), harvest index (HI) (2006), and grain yield (2005, 2006), and a number of canopy-level measurements were taken. The application of 170 kg N ha^{-1} generally increased GY_A , per-plant grain yield (GY_P), HI, TB_P , and leaf N content relative to the zero N rate regardless of plant population. Corresponding with the intensity of intra-specific competition during vegetative and reproductive stages, GY_{CV} was greatest at the highest plant density when no N was applied and smallest at the lowest plant population when 340 kg N ha^{-1} was applied. The positive GY_A response to N application was much bigger at higher plant populations. Without sufficient N at high plant densities, intra-specific competition for soil mineral N was severe. Limitations in available N lead to reduced foliar N concentrations and earlier leaf senescence that limited TB_P and GY_P . Overall, this study demonstrates that N application plays a critical role in decreasing plant-to-plant variability which was more pronounced when maize was grown at high plant densities..

Introduction

Genetic improvements in maize (*Zea mays* L.) have led to modern hybrids that are increasingly tolerant to a variety of stresses and, in particular, to high plant populations (Tollenaar and Wu, 1999; Duvick, 2001). One result of higher plant density tolerance is that the optimum plant population for maximum grain yield has increased steadily over the past 70 years (Duvick and Cassman, 1999). However, past yield gains cannot be attributed to genetic improvements alone. Instead, they are the product of an improved genetic by agronomic-management interaction (Tollenaar and Lee, 2002).

The adoption of superior agronomic-management practices has included the application of economically and environmentally appropriate rates of inorganic nitrogen (N) fertilizers. All

maize hybrids rely on adequate N inputs to reach maximum yield potential, and modern hybrids are generally more N efficient (i.e. grain yield per unit N applied) than their older counterparts (Tollenaar and Wu, 1999). However, even when density-tolerant hybrids and N applications are employed, high plant populations can adversely affect overall grain yield due to increased plant-to-plant variability for grain yield (GY_{CV}) and higher incidence of barrenness (Tokatlidis and Koutroubas, 2004).

Both management practices and environmental conditions typically cause increased GY_{CV} and decreased uniformity for other morpho-physiological traits. Some management-related causes of plant-to-plant variability include deviations in planting depth and seed spacing, uneven nutrient application and crop residue distribution, wheel-track compaction, and tillage/rotation practices (Boomsma and Vyn, 2007). Environmental-related causes include variations in insect feeding and disease pressure along with inherent soil spatial variability (Andrade and Abbate, 2005). In modern maize production systems, enhanced plant-to-plant variability often results from increased competition among individual plants at progressively higher plant densities for limiting resources such as N, incident photosynthetically active radiation (IPAR), and soil moisture. Past studies have often emphasized that stand uniformity is essential for high productivity levels, and that increased plant-to-plant variability (determined and expressed using a variety of maize growth and developmental parameters) reduces per-unit-area maize grain yields (GY_A) through reduced stress tolerance (Tollenaar and Wu, 1999). Therefore, at higher plant populations, resource availability must be adequate to help maintain uniform growth, development, and grain yield of adjacent plants in a maize canopy.

Historically, numerous growth and developmental parameters such as plant height and V-stage have been used to investigate the morpho-physiological progression of maize hybrids throughout the growing season. Plant height has also been used to measure plant-to-plant variability, although correlations between the height of individual plants at particular growth stages and their respective grain yields have often been weak (Muldoon and Daynard, 1981; Daynard and Muldoon, 1983). Other per-plant morpho-physiological parameters that have enabled an enhanced understanding of intra-specific competition, per-plant variability, and resulting effects on grain yield include leaf N concentration, total biomass, and harvest index (HI). Previous studies have examined a number of these per-plant morpho-physiological traits with respect to intra-specific competition and plant-to-plant variability at multiple plant densities (e.g. Edmeades and Daynard, 1979; Vega et al., 2000; Vega and Sadras, 2003; Pagano and Maddonni, 2007). However, no study to date has investigated the morpho-physiological effects of N availability on intra-specific competition in maize at multiple plant densities.

Overall, this study seeks to understand the effects of plant population and N rate on maize intra-specific competition and growth and developmental uniformity at both the per-plant and canopy levels of organization through the measurement of numerous morpho-physiological traits at multiple stages of vegetative and reproductive development. We hypothesize that (a) mineral N availability may be more essential for proper maize growth and development at high plant populations than at low plant populations and (b) grain yield loss due to increased plant-to-plant variability at higher plant populations will be reduced when N rates are increased. We also suggest that with increased N rates at higher plant densities, GY_A may be increased due to decreased GY_{CV} , improved per-plant total biomass production (TB_P) (i.e. enhanced photo-

assimilate production), greater partitioning of biomass to the grain (i.e. improved HI), and both delayed and reduced leaf senescence during the grain-filling period.

Materials and Methods

Experimental Design and Management Practices

Research in 2005 and 2006 was conducted at Purdue University's Agronomy Center for Research and Education (ACRE) near West Lafayette, Indiana (40° 28' N Lat.). The soil, which had developed under prairie vegetation, was a Chalmers (fine-silty, mixed, mesic Typic Endoaquoll) silty clay loam with approximately 4.0% organic matter content in the top 30 cm of the soil profile. The experimental area had less than 2% slope and was systematically tile drained. In both years, maize was grown following soybeans (*Glycine max* L. Merrill). Strip-tillage was performed in the fall. The study was analyzed as a split-plot design with four blocks. Plant population (whole plot) and N application rate (subplot) served as the two treatment factors. Hybrid Pioneer 31G68 was planted at rates of 58,000, 83,000, and 108,000 seeds ha⁻¹ to achieve the desired final plant populations of 54,000, 79,000, and 104,000 plants ha⁻¹. Starter fertilizer (10-34-0) was applied at a rate of 185 L ha⁻¹ 5 cm to the side and 5 cm below the seed. Urea Ammonium Nitrate (UAN) (28-0-0) was applied via side-dressing at a rate of 170 kg N ha⁻¹ once (V3), twice (V3, V5), or not at all, depending upon each plots' prescribed N application rate. Soil samples (0-30 cm) were taken at V4 and V8 in plots receiving only starter fertilizer to determine soil nitrate (NO₃⁻) and ammonium (NH₄⁺) concentrations. Each plot consisted of 6 rows (76-cm row spacing) and was 4.6 m in width and 27.5 m or 30.5 m in length in 2006 and 2005, respectively.

Individual Plant Non-destructive and Destructive Measurements

In both years, sampling areas (4 m in length) were established in rows 3 and 4 of each 6-row plot. Within each of the designated sampling areas, individual plants (≈ 36-70 per sampling area depending upon plant population, ≈ 1,850 in total yr⁻¹) were tagged with barcodes that were individually numbered. The growth and development of each of the tagged plants was measured throughout the growing season using the following parameters: emergence date; pre- (V15) and post-silking (R3 and R6) stalk diameter (6th internode); pre-silking (V5 and V15) plant height and V-Stage; final plant height (R6); pre- (V15; 12th leaf) and post-silking (R1, R3, and R5; earleaf) SPAD (i.e. leaf greenness measured with chlorophyll meters); earleaf position; total leaf number; plant spacing; and anthesis (2006) and silking (2005, 2006) date. In 2006, the LAI of each tagged plant was determined at R1 using a modified procedure from Valentinuz and Tollenaar (2006). In both years, individual ears of each tagged plant were hand-harvested, shelled, and measured for moisture content, total kernel weight, and total kernel number. In 2006, the R6 TB_p and resulting HI of 6 consecutive, representative, tagged plants from each sampling area was determined. All plots were machine harvested after hand-harvesting of the sampling areas. Yields were corrected to 15.5% moisture content.

For this publication, only a subset of the previously mentioned individual plant measurements was analyzed. Although results presented here are for only two years and a single hybrid, this

experiment involves four years of data (2004-2007) with two hybrids each year; hybrids were the first split, plant population the second split, and N rate the third split in all these tests.

Statistical Analyses

Analysis of variance (ANOVA) was performed using SAS[®] (SAS Institute, 2004) PROC GLM or PROC MIXED for balanced or unbalanced data, respectively. The whole-unit error was pooled with the sub-unit error. A combined year analysis (2005 and 2006) was performed, with all year \times treatment interactions pooled. When treatment effects were significant, least significant difference (LSD) mean separation tests and least-squares mean (LS-mean) separation tests (t-test) were performed for balanced and unbalanced data, respectively.

Results

As shown in Figure 1, GY_A increased for each plant population with an initial application of 170 kg N ha⁻¹, however, no increase in GY_A resulted from a second 170 kg N ha⁻¹ application at each plant density. The particularly low GY_A for the 104,000 plants ha⁻¹, 0 kg N ha⁻¹ treatment combination likely resulted from intense intra-specific competition for soil mineral N. Slightly greater GY_A at 79,000 plants ha⁻¹ than at 104,000 plants ha⁻¹ for the mid and high N rates likely resulted from the highest plant density being above that for maximum GY_A for Pioneer 31G68 (Pioneer Hi-Bred Intl., Inc., 2005). Although GY_A was relatively similar across plant densities for the 170 and 340 kg N ha⁻¹ rates, GY_A decreased dramatically with increasing plant population for the 0 kg N ha⁻¹ rate. This potentially suggests that N application was more important for sustaining GY_A at higher plant densities than at lower plant densities.

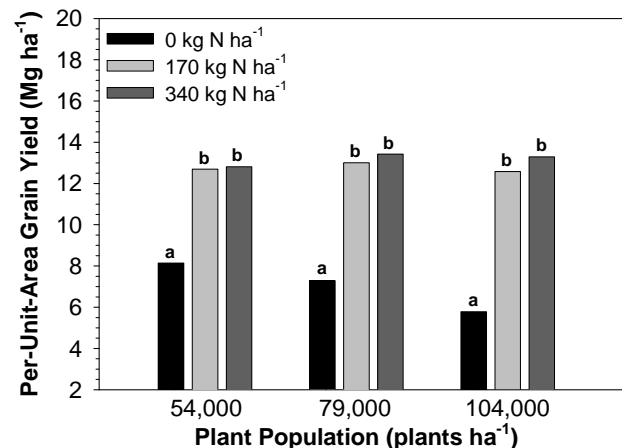


Figure 1. Effect of plant population and N rate on per-unit-area grain yield (GY_A). Means with different letters indicate statistically significant differences at $P \leq 0.05$ within each plant population. The plant population \times N-rate interaction was significant at $P \leq 0.05$.

As shown in Figure 2A, increases in plant population resulted in decreases in mean per-plant grain yield (GY_P) as a result of intensifying intra-specific competition. While the first application of 170 kg N ha⁻¹ significantly increased GY_P , a second application of N at the same rate had no significant effect on GY_P regardless of plant population (Figure 2B). A lack of increase in GY_A (Figure 1) and GY_P from a second application of 170 kg N ha⁻¹ (Figure 2B) suggests that other resources (e.g. IPAR and soil moisture) may have been more limiting than mineral N after the initial side-dress application. The greater GY_A at 79,000 plants ha⁻¹ than at 104,000 plants ha⁻¹ for the mid and high N rates (Figure 1) likely resulted from limited per-plant

resource availability during the period bracketing silking at the highest plant density (Pagano and Maddonni, 2007).

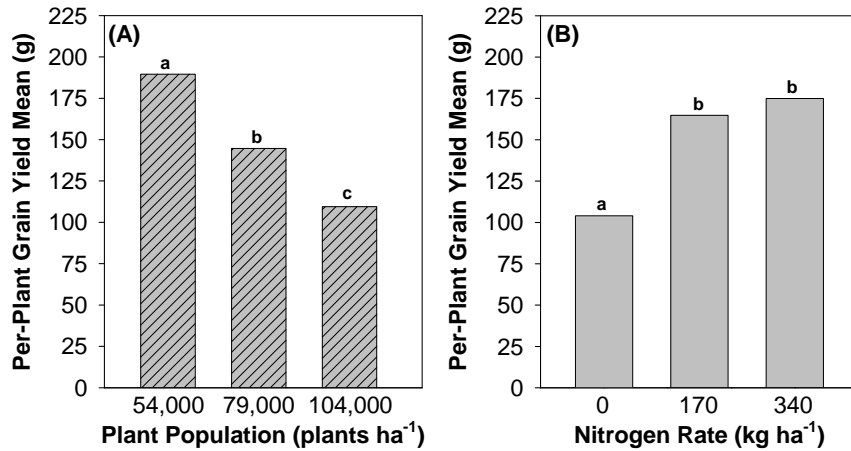


Figure 2. Effect of plant population (A) and N rate (B) on mean per-plant grain yield (GY_P). Means with different letters indicate statistically significant differences at $P \leq 0.05$. The plant population \times N-rate interaction was not significant at $P \leq 0.10$.

Figure 3 shows that per-plant grain yield variability (GY_{CV}) generally increased with increases in plant population regardless of N rate (with exception to the 170 kg N ha⁻¹ rate at 54,000 plants ha⁻¹ and 79,000 plants ha⁻¹). An initial N application of 170 kg ha⁻¹ decreased GY_{CV} at 54,000 plants ha⁻¹ and 79,000 plants ha⁻¹ but not at 104,000 plants ha⁻¹. Although a second N application of 170 kg ha⁻¹ did not increase GY_A (Figure 1) and GY_P (Figure 2) at the highest plant density, it did result in a significant decrease in GY_{CV} . The effect of N application was smallest at 54,000 plants ha⁻¹ due to reduced intra-specific competition for applied and residual soil N. In accordance with the degree of intra-specific competition, the treatment combination of 104,000 plants ha⁻¹, 0 kg N ha⁻¹ had the highest GY_{CV} , while the treatment combination of 54,000 plants ha⁻¹, 340 kg N ha⁻¹ had the lowest GY_{CV} .

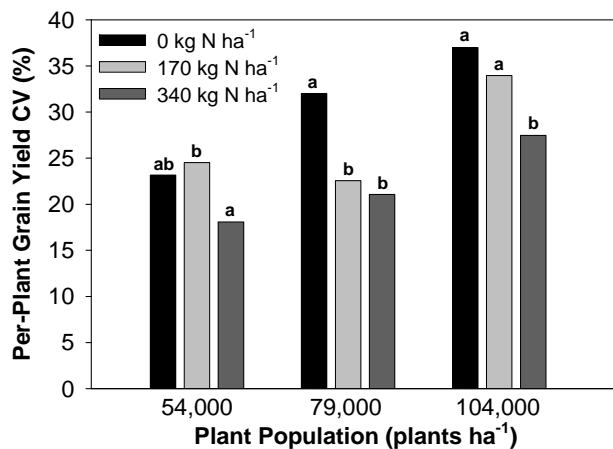


Figure 3. Effect of plant population and N rate on per-plant grain yield variability (GY_{CV}). GY_{CV} is expressed as the coefficient of variation (CV) of per-plant grain yield. Means with different letters indicate statistically significant differences at $P \leq 0.05$ within each plant population. The plant population \times N-rate interaction was significant at $P \leq 0.10$.

Figure 4A shows that HI was significantly less at 104,000 plants ha⁻¹ than at 54,000 plants ha⁻¹ and 79,000 plants ha⁻¹. It was also significantly less for the 0 kg ha⁻¹ N rate than the 170 kg ha⁻¹ and 340 kg ha⁻¹ N rates (Figure 4B). Maize HI typically decreases at above-optimum plant densities as intra-specific competition reduces biomass partitioning to the ear (Pagano and Maddonni, 2007). At the highest plant population in this study, intense intra-specific competition led to greater proportional reductions in grain yield than total above-ground dry

matter accumulation, incidentally resulting in a reduced HI. A lack of N application similarly resulted in a disproportional allocation of photoassimilates to non-reproductive tissues.

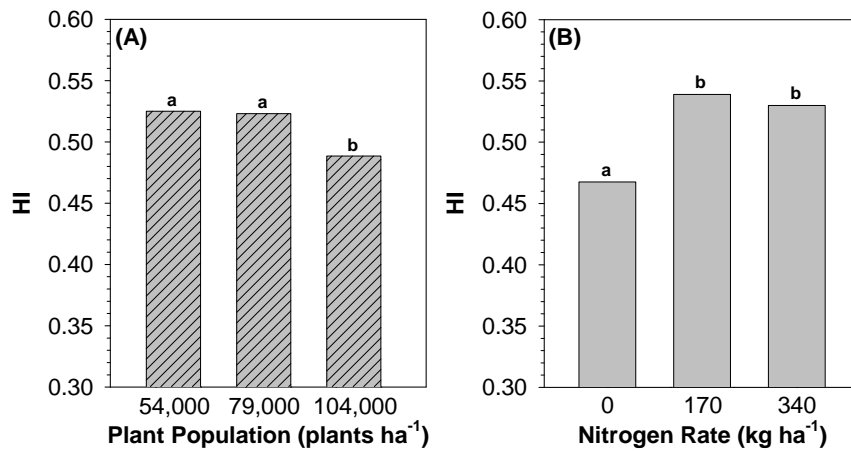


Figure 4. Effect of plant population (A) and N rate (B) on harvest index (HI). Means with different letters indicate statistically significant differences at $P \leq 0.05$. The plant population \times N-rate interaction was not significant at $P \leq 0.10$. Data is from 2006 only.

Figure 5 indicates that at each N rate, increases in plant population resulted in decreases in R6 TB_P. A single application of 170 kg N ha⁻¹ significantly increased TB_P at each plant density. A second application of 170 kg N ha⁻¹ increased TB_P at only the lowest plant population. This suggests that after an initial application of 170 kg N ha⁻¹, N availability limited TB_P at the low but not at the mid and high plant densities. The highest TB_P occurred for the 54,000 plants ha⁻¹, 340 kg N ha⁻¹ treatment combination, while the lowest TB_P was present for the 104,000 plants ha⁻¹, 0 kg N ha⁻¹ treatment combination. This suggests plasticity for TB_P in response to resource availability for Pioneer 31G68.

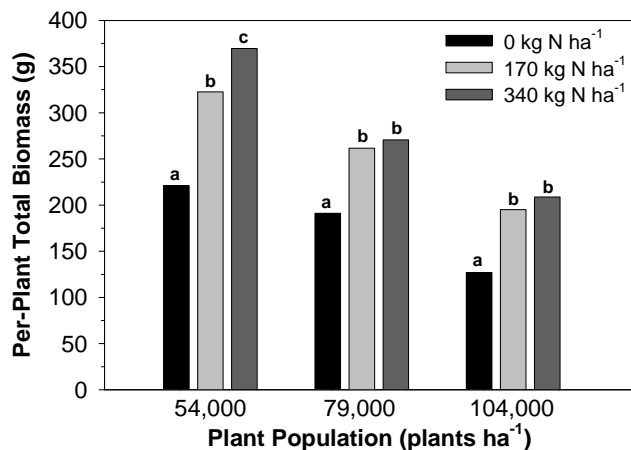


Figure 5. Effect of plant population and N rate on R6 per-plant total biomass (TB_P). Means with different letters indicate statistically significant differences at $P \leq 0.05$ within each plant population. The plant population \times N-rate interaction was significant at $P \leq 0.10$. Data is from 2006 only.

Enhanced N uptake as a result of greater N availability can result in lower N mobilization from plant leaves, delays in leaf senescence (i.e. “stay-green”), and, resultantly, prolonged accumulation of dry matter (Maddonni and Otegui, 2004). Chlorophyll meters have been routinely used to estimate leaf N content and examine patterns of leaf senescence in maize through the measurement of relative leaf greenness (i.e. SPAD) (e.g. Ma et al., 1996; Ma and Dwyer, 1997). When used in this study on a per-plant basis, SPAD values declined with increasing plant population (Figure 6A) and decreasing N rate (Figure 6B) at each growth stage. For all three populations, SPAD increased from V15 to R1, peaked between R1 and R3, and

decreased from R3 to R5 (Figure 6A). Values for SPAD at V15, R1, R3, and R5 did not differ for the 170 kg N ha⁻¹ and 340 kg N ha⁻¹ application rates (Figure 6B), indicating that the second N application did not improve leaf N content or decrease the rate of leaf senescence for these treatments. However, the contribution of an initial N application of 170 kg ha⁻¹ did significantly improve both leaf N content and “stay-green” for all three plant populations in a similar fashion. While plants receiving 170 kg ha⁻¹ or 340 kg ha⁻¹ of N showed increases in leaf N from V15 to R1 and from R1 to R3 and decreases in leaf N from R3 to R5, plants receiving no N application exhibited decreasing leaf N content from V15 to R5. The rate of leaf senescence was also markedly greater at 0 kg N ha⁻¹ than at 170 kg N ha⁻¹ and 340 kg N ha⁻¹ (Figure 6B).

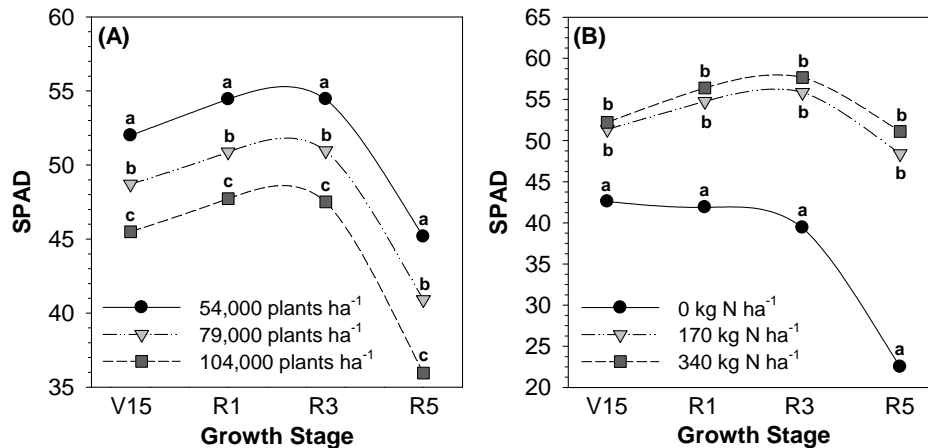


Figure 6. Effect of plant population (A) and N rate (B) on leaf greenness at V15, R1, R3, and R5 growth stages. Means with different letters indicate statistically significant differences at $P \leq 0.05$. The plant population \times N-rate interaction was not significant at $P \leq 0.10$.

Summary

Plants that experience high levels of intra-specific competition due to poor resource availability have been shown to have a reduced ability for resource capture that results in reduced vegetative and reproductive biomass production. Decreased TB_P and increased GY_{CV} are therefore expected responses to reduced resource availability in maize (Maddonni and Otegui, 2004). In this study, GY_{CV} was greatest and GY_A was lowest when N availability was most limiting (104,000 plants ha⁻¹, 0 kg N ha⁻¹) (Figures 3 and 1, respectively). At the highest plant population in this study (104,000 plants ha⁻¹), a lack of N application also limited TB_P accumulation (Figure 5). Since improved TB_P accumulation is associated with the interception and utilization of IPAR (Tollenaar and Lee, 2006), lower TB_P values likely resulted from reduced leaf N content and increased foliar senescence (Figures 6A and 6B). These foliar responses to low N availability likely limited per-plant photosynthetic rates and induced source limitations during the grain-filling period that resultantly restricted both GY_A and GY_P (Figures 1 and 2, respectively).

Intra-specific competition in maize for limited resources leads to the formation of plant hierarchies that consist of “dominated” and “dominating” plants (Maddonni and Otegui, 2004). Low canopy-level HI values at high plant densities have been shown to be related to reductions in the ear biomass of “dominated” plants at physiological maturity (Maddonni and Otegui, 2006). Increased competition at the highest plant density and lowest N rate in this study also

reduced HI values (Figures 4A and 4B), likely indicating a limitation among “dominated” plants exposed to severe N stress to partition photoassimilates to the developing ear.

In conclusion, the availability of N is more critical for maintaining GY_A at higher plant populations than at lower plant populations. Without sufficient N at high plant densities, intra-specific competition for soil N is severe. Limitations in available N lead to reduced foliar N concentrations and earlier leaf senescence that limit TB_P and GY_P . These reductions in GY_P cannot be compensated for by increases in plant density, thus resulting in decreased GY_A .

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