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# Per-plant eco-physiological responses of maize to varied nitrogen availability at low and high plant densities

Christopher R. Boomsma * Tony J. Vyn †

*Purdue University
†Purdue University - Main Campus

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## Abstract

Although maize (Zea mays L.) routinely experiences both intra- and inter-specific competition for limited resources, most plant-plant interaction studies have principally focused on maize-weed interactions. Thus very few investigations have considered the impacts of plant crowding and nitrogen (N) availability on maize intra-specific competition. The primary objective of this two-year field study near West Lafayette, IN was to investigate the per-plant eco-physiological responses of modern maize genotypes to varied N availability (0, 165, and 330 kg side-dress N ha-1) at low and high plant densities (54,000 and 104,000 plants ha-1, respectively) by measuring responses among dominated [lowermost 25% per-plant grain yield (GYP)], intermediate, and dominant (uppermost 25% GYP) individual plants in each treatment combination. Parameters measured at the per-plant level included R1 green leaf area (LAP), R1 SPAD, anthesis-silking interval (ASIP), GYP, R6 total aboveground biomass (TBP), and harvest index (HIP). In both years, severe intra-specific competition for soil N in the highly crowded, low-N environment resulted in low R1 LAP and SPAD values, high ASIP values, and reduced GYP, R6 TBP, and HIP values, particularly among dominated plants. Intense competition in this environment also led to (i) high dominant group/dominated group mean ratios for most parameters; (ii) high plant-to-plant variability for R1 SPAD, ASIP, GYP, and HIP; and (iii) high frequencies of barren and low-yielding plants. Insufficient N at high plant densities thus encouraged the formation of plant hierarchies composed of markedly dominated individuals with diminished source capability and severely impaired biomass partitioning to developing grain.

# Introduction

As with many plant species, field crops routinely experience both intra- and inter-specific competition for limited resources during a growing season. Given the well-established negative relationship between inter-specific competition and plant productivity, plant-plant interaction studies in field crops have principally focused on crop-weed interactions. While a limited number of experiments has explored aspects of intra-specific competition in crops such as maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] (e.g., Vega and Sadras, 2003), a majority of studies focusing on intra-specific competition in plant monocultures have been performed by ecologists using species not commonly grown in crop production systems (e.g., Weiner and Thomas, 1986). Those studies which have intensively investigated intra-specific competition in field crops have predominately focused on responses to increased plant density (e.g., Edmeades and Daynard, 1979; Maddonni and Otegui, 2004). Few investigations have considered the impacts of nitrogen (N) availability on intra-specific competition in field crops. This is particularly true in maize, which is strongly dependent upon N application for high productivity. As N fertilizer prices fluctuate upwards, environmental concerns over excessive N application increase, and recommended maize plant densities move progressively higher, it is crucial that both the crowding tolerance and N stress tolerance of current maize germplasm continue to be investigated and improved. Fundamental to these efforts is an understanding of the per-plant eco-physiological responses of modern maize to above- and below-ground intra-specific competition. By describing individual plant behavior within a community context, such an understanding can provide insight into avenues for genetic improvement not offered by more simplistic canopy-level investigations. Thus the objective of this field study was to examine the per-plant eco-physiological responses of modern maize genotypes to varied N availability at both low and high plant densities and, in so doing, to improve the ideotype employed by the maize breeding community for the enhancement of abiotic stress tolerance.

# Materials and Methods

Field research during the 2006 and 2007 growing seasons was conducted at the Purdue University Agronomy Center for Research and Education (ACRE) (40°28′07″ N, 87°00′25″ W) near West Lafayette, Indiana. In each year, maize was grown following no-till soybean. The study was arranged as a split-split-plot design with four blocks. Hybrid (main plot), plant density (subplot), and N rate (sub-subplot) served as the three treatment factors. The Pioneer hybrids 31G68 and 31N28 were planted to achieve final plant densities of 54,000 and 104,000 plants $ha^{-1}$. For all plots, starter fertilizer (10-34-0) was applied at planting 5 cm to the side and 5 cm below the seed at a rate equivalent to 25 kg N $ha^{-1}$. Urea Ammonium Nitrate (UAN) (28-0-0) was applied via side-dressing at a rate equivalent to 165 kg N $ha^{-1}$ once (V3), twice (V3, V5), or not at all, depending upon each plot's prescribed N rate. All other nutrients were kept non-limiting. See Boomsma et al. (2009) for a more complete description of this experiment and mean grain yield responses to treatment combinations.

In a designated sampling area ($\approx$ 6 $m^2$) within each plot, extensive, non-destructive, eco-physiological measurements were taken on tagged plants from seedling emergence through physiological maturity. Discussion here is limited to the following per-plant parameters: R1 green leaf area ($LA_P$), R1 SPAD, anthesis-silking interval ($ASI_P$), grain yield ($GY_P$), R6 aboveground total biomass ($TB_P$), and harvest index ($HI_P$). At R1 in both years, $LA_P$ was determined using a modified procedure from Valentinuz and Tollenaar (2006). Per-plant SPAD measurements at R1 were taken on each plant's uppermost earleaf. For determination of $ASI_P$, anthesis and silking were defined according to Borrás et al. (2007). In each year, $GY_P$ was corrected to 0% moisture content. For each plant, R6 $TB_P$ was calculated as the sum of each plant's $GY_P$ and R6 vegetative biomass ($VB_P$). In both years, R6 $VB_P$ was determined using a modification of the allometric model-based procedure employed by Maddonni and Otegui (2004). For each plant, $HI_P$ was calculated as the quotient of $GY_P$ and R6 $TB_P$. Plant-to-plant variability for each of these six parameters was calculated on a plot-level basis using the coefficient of variation (CV) or standard deviation (SD). To examine plant hierarchy responses, plants were ranked in ascending order by $GY_P$ within each plot. The cumulative frequency was calculated for each plant based upon its respective rank. A plant was classified as dominated, intermediate, or dominant when its $GY_P$ rank position was in the lowermost 25%, middle 50%, or uppermost 25% of the plot-level population of plants, respectively. Each plant was thus assigned to a single plant group based solely upon its $GY_P$.

For all analyses of variance (ANOVA), hybrid was treated as a random effect. For ANOVA on plant hierarchy responses, the study was analyzed as a split-split-plot design with plant density (main plot), side-dress N rate (subplot), and plant group (sub-subplot) serving as the three treatment factors. For ANOVA on plant-to-plant variability responses, the study was analyzed as a split-plot design with plant density (main plot) and side-dress N rate (subplot) serving as the two treatment factors. In all instances, ANOVA was performed using SAS PROC MIXED. When treatment effects were significant at $P$ = 0.05, least-squares mean (LS-mean) separation tests were performed for appropriate fixed effects.

## Results

As indicated in Tables 1 and 2, the application of 165 kg ha$^{-1}$ of side-dress N often increased each set of mean values (i.e., overall, dominated, intermediate, and dominant) for R1 $LA_P$, R1 SPAD, $GY_P$, R6 $TB_P$, and $HI_P$ and additionally decreased these mean values for $ASI_P$ at both the low and high plant densities for each year. For either plant density in both years, a second side-dress N application often had a minimal effect on each set of means. Depending upon the parameter, the 104,000 plants ha$^{-1}$; 0 kg N ha$^{-1}$ treatment combination often exhibited either the lowest or highest values for the overall, dominated, intermediate, and dominant means in both years. Regardless of year or treatment combination, values for each parameter (except $ASI_P$) were nearly always lower for the dominated relative to intermediate and dominant plant group(s). For all parameters but $ASI_P$, the application of either 165 or 330 kg N ha$^{-1}$ often decreased the dominant group/dominated group mean ratio in both years for either plant density. In both years, increasing plant density produced the opposite effect for these parameters regardless of N rate. For these same parameters, the highly crowded, low-N treatment combination often exhibited the numerically greatest dominant group/dominated group mean ratio in both years.

As evident in Table 3, an initial application of 165 kg N ha$^{-1}$ at either plant density resulted in a decrease in plant-to-plant variability for some parameters in 2006 and/or 2007. Regardless of year or plant density, a second side-dress N application rarely impacted plant-to-plant variability for all parameters. The highly crowded, low-N environment exhibited the highest plant-to-plant variability for R1 SPAD and $ASI_P$ in 2006 and for $GY_P$ and $HI_P$ in both 2006 and 2007.

As shown in Figure 1A-D, the high plant density, low-N environment displayed a greater frequency of barrenness and a higher frequency of low-yielding plants than the low plant density, high-N environment in both years. For the highly crowded, low-N environment, low-yielding plants displayed markedly high $ASI_P$ values in both years (Figure 1B,D).

As displayed in Figure 2A-D, the high plant density, low-N environment displayed a higher frequency of poorly productive plants than the low plant density, high-N environment in both years. In the high plant density, low-N environment, $HI_P$ pronouncedly declined with decreasing R6 $TB_P$ in both years (Figure 2B,D). Contrarily, in the low plant density, high-N environment, $HI_P$ was relatively stable across R6 $TB_P$ values in both years (Figure 2A,C).

Table 1. Plant density and nitrogen (N) rate effects on maize R1 per-plant green leaf area ($LA_P$), R1 per-plant SPAD, and per-plant anthesis-silking interval ($ASI_P$) for 2006 and 2007. Within each data cell for each parameter, the top number indicates the overall mean for all plants for that treatment combination while the lower set of numbers indicates the means (from left to right) for the dominated, intermediate, and dominant plant groups for that treatment combination.

| Year | Plant density | N rate | R1 $LA_P$ | R1 SPAD | $ASI_P$ |
|---|---|---|---|---|---|
| | plants $ha^{-1}$ | kg N $ha^{-1}$ | $cm^2$ $plant^{-1}$ | | days |
| 2006 | 54,000 | 0 | 5176a[1] | 44a | 0.5a |
| | | | 4763aA[2]/5231aB/5537aC | 41aA/45aB/47aC | 1.0aA/0.4aB/0.0aB |
| | | 165 | 7063b | 58b | 0.2a |
| | | | 6495bA/7222bB/7472bC | 55bA/59bB/61bC | 0.5aA/0.1aAB/0.0aB |
| | | 330 | 7186b | 59b | 0.2a |
| | | | 6664bA/7313bB/7581bC | 57bA/60bB/61bB | 0.7aA/0.1aB/0.0aB |
| | 104,000 | 0 | **3522a**[3] | **36a** | **2.8a** |
| | | | **3047aA/3547aB/3974aC** | **32aA/36aB/39aC** | **4.6aA/2.7aB/1.3aC** |
| | | 165 | 5668b | 53b | 1.2b |
| | | | 5006bA/5788bB/6212bC | 49bA/54bB/55bB | 2.1bA/0.8bB/0.6bB |
| | | 330 | 5893b | 54b | 1.2b |
| | | | 5219bA/6018bB/6441bC | 52cA/55bB/56bB | 2.1bA/0.9bB/0.7bB |
| 2007 | 54,000 | 0 | 5166a | 44a | 0.2a |
| | | | 4555aA/5227aB/5715aC | 39aA/44aB/48aC | 1.0aA/-0.0aB/-0.4aB |
| | | 165 | 6753b | 57b | -0.3a |
| | | | 6184bA/6829bB/7245bC | 55bA/57bB/60bC | 0.1bA/-0.4aAB/-0.6aB |
| | | 330 | 6873b | 57b | -0.3a |
| | | | 6123bA/7151bB/7345bB | 54bA/58bB/59bB | 0.2abA/-0.5aB/-0.5aB |
| | 104,000 | 0 | **2891a** | **36a** | **3.0a** |
| | | | **2470aA/2941aB/3262aC** | **33aA/36aB/39aC** | **4.7aA/3.0aB/1.4aC** |
| | | 165 | 4957b | 50b | 1.2b |
| | | | 4337bA/5022bB/5513bC | 45bA/51bB/54bC | 2.3bA/1.1bB/0.1bC |
| | | 330 | 5095b | 51b | 1.3b |
| | | | 4444bA/5153bB/5687bC | 47bA/52bB/55bC | 2.8bA/1.0bB/0.2bC |

[1] For a given type of mean within column, year, and plant density, means with different lowercase letters indicate statistically significant differences at $P \leq 0.05$.
[2] Within column, year, plant density, and N rate, means with different uppercase letters indicate statistically significant differences between plant groups at $P \leq 0.05$.
[3] For a given type of mean, a bolded value indicates that the 104,000 plants $ha^{-1}$; 0 kg N $ha^{-1}$ treatment combination exhibits the lowest or highest value for that parameter in that year.

# Discussion

As evidenced by pronouncedly low $LA_P$ and SPAD values at R1, severe intra-specific competition for N in the highly crowded, low-N environment before and during flowering resulted in reductions in per-plant source activity around silking. As indicated by high $ASI_P$ values for this treatment combination, such decreases in C assimilation severely reduced C partitioning to the developing ear at silking, particularly among dominated plants (Borrás et al., 2007). Exceptionally low $GY_P$, R6 $TB_P$, and $HI_P$ values among plants in this treatment combination suggest that C partitioning to the developing grain was further limited during the grain-filling period, especially among dominated plants. Thus, as suggested by (i) high dominant group/dominated group mean ratios for most parameters; (ii) high CV values for R1 SPAD, $ASI_P$, $GY_P$, and $HI_P$; and (iii) high frequencies of barren, low-yielding, poorly productive plants; severe intra-specific competition in the highly crowded, low-N environment led to the formation of plant hierarchies composed of dominated individuals with a diminished amount of source activity and a drastically decreased ability to partition C to developing grain (Maddonni and Otegui, 2004; Pagano and Maddonni, 2007). Overall, such results suggest that adequate N availability is critical for high grain production in crowded maize stands (Boomsma et al., 2009) since it reduces plant-to-plant variability for key eco-physiological traits and limits the formation of plant hierarchies. Maize genetic improvement efforts in abiotic stress tolerance should focus on (and potentially select for) enhanced by-plant uniformity under stress conditions. Improved uniformity requires (i) greater C partitioning to the developing grain among dominated plants and/or (ii) improved compensatory grain production among dominant individuals.

Table 2. Plant density and nitrogen (N) rate effects on maize per-plant grain yield ($GY_P$), R6 per-plant aboveground total biomass ($TB_P$), and per-plant harvest index ($HI_P$) for 2006 and 2007. Within each data cell for each parameter, the top number indicates the overall mean for all plants for that treatment combination while the lower set of numbers indicates the means (from left to right) for the dominated, intermediate, and dominant plant groups for that treatment combination.

| Year | Plant density | N rate | $GY_P$ | R6 $TB_P$ | $HI_P$ |
|---|---|---|---|---|---|
| | plants $ha^{-1}$ | kg N $ha^{-1}$ | g $plant^{-1}$ | g $plant^{-1}$ | g $g^{-1}$ |
| 2006 | 54,000 | 0 | 118a[1] | 234a | 0.49a |
| | | | 85aA[2]/120aB/148aC | 186aA/236aB/280aC | 0.44aA/0.51aB/0.53aB |
| | | 165 | 186b | 337b | 0.55b |
| | | | 140bA/195bB/225bC | 265bA/347bB/399bC | 0.51bA/0.56bB/0.56aB |
| | | 330 | 196b | 355b | 0.54b |
| | | | 155cA/204bB/230bC | 285cA/366bB/413bC | 0.51bA/0.56bB/0.56aB |
| | 104,000 | 0 | **52a**[3] | **127a** | **0.36a** |
| | | | **19aA/54aB/82aC** | **85aA/127aB/168aC** | **0.19aA/0.42aB**/0.48aC |
| | | 165 | 102b | 190b | 0.51b |
| | | | 61bA/109bB/136bC | 131bA/199bB/241bC | 0.42bA/0.55bB/0.56bB |
| | | 330 | 110b | 209b | 0.50b |
| | | | 67bA/116bB/148bC | 147bA/215bB/264cC | 0.41bA/0.54bB/0.56bB |
| 2007 | 54,000 | 0 | 119a | 244a | 0.47a |
| | | | 80aA/120aB/157aC | 176aA/244aB/311aC | 0.42aA/0.49aB/0.50aB |
| | | 165 | 180b | 337b | 0.53b |
| | | | 137bA/186bB/218bC | 259bA/346bB/407bC | 0.51bA/0.54bB/0.54bB |
| | | 330 | 189b | 350b | 0.53b |
| | | | 148bA/194bB/224bC | 277bA/358bB/414bC | 0.52bA/0.54bA/0.54bA |
| | 104,000 | 0 | **56a** | **129a** | **0.40a** |
| | | | **28aA/58aB/83aC** | **89aA/130aB/168aC** | **0.28aA/0.44aB**/0.49aC |
| | | 165 | 96b | 187b | 0.49b |
| | | | 56bA/101bB/132bC | 122bA/193bB/248bC | 0.41bA/0.52bB/0.53bB |
| | | 330 | 103b | 197b | 0.50b |
| | | | 59bA/108bB/141bC | 127bA/204bB/260bC | 0.42bA/0.53bB/0.54bB |

[1] For a given type of mean within column, year, and plant density, means with different lowercase letters indicate statistically significant differences at $P \leq 0.05$.

[2] Within column, year, plant density, and N rate, means with different uppercase letters indicate statistically significant differences between plant groups at $P \leq 0.05$.

[3] For a given type of mean, a bolded value indicates that the 104,000 plants $ha^{-1}$; 0 kg N $ha^{-1}$ treatment combination exhibits the lowest or highest value for that parameter in that year.

Table 3. Plant density and nitrogen (N) rate effects on maize plant-to-plant variability for R1 green leaf area (R1 $LA_{CV}$), R1 SPAD (R1 $SPAD_{CV}$), anthesis-silking interval ($ASI_{SD}$), grain yield ($GY_{CV}$), R6 aboveground total biomass (R6 $TB_P$), and harvest index ($HI_P$) for 2006 and 2007.

| Year | Plant density | N rate | R1 $LA_{CV}$[1] | R1 $SPAD_{CV}$ | $ASI_{SD}$[2] | $GY_{CV}$ | R6 $TB_{CV}$ | $HI_{CV}$ |
|---|---|---|---|---|---|---|---|---|
| | plants $ha^{-1}$ | kg N $ha^{-1}$ | % | | Days | % | | |
| 2006 | 54,000 | 0 | 9.2a[3] | 9.7a | 1.0a | 22.3a | 16.4a | 9.5a |
| | | 165 | 8.9a | 8.3a | 0.8a | 20.6a | 17.1a | 9.1a |
| | | 330 | 10.4a | 9.2a | 0.9a | 18.2a | 16.5a | 9.3a |
| | 104,000 | 0 | 15.3a | **13.9a**[4] | **1.9a** | **51.5a** | 27.2a | **39.7a** |
| | | 165 | 12.8ab | 9.9b | 1.2b | 31.3b | 23.7ab | 20.7b |
| | | 330 | 11.5b | 8.5b | 1.2b | 30.2b | 22.9b | 19.1b |
| 2007 | 54,000 | 0 | 12.6a | 12.2a | 1.3a | 27.2a | 23.1a | 13.5a |
| | | 165 | 10.4a | 8.0b | 1.0a | 20.1b | 19.2ab | 7.8a |
| | | 330 | 13.5a | 9.0b | 1.1a | 17.6b | 17.0b | 7.0a |
| | 104,000 | 0 | 15.9a | 11.3a | 2.0a | **40.2a** | 24.5a | **26.3a** |
| | | 165 | 14.5a | 10.7a | 1.7a | 32.5b | 27.7a | 19.0b |
| | | 330 | 13.8a | 10.2a | 1.7a | 32.5b | 27.5a | 18.6b |

[1] CV, coefficient of variation.
[2] SD, standard deviation.
[3] Within column, year, and plant density, means with different lowercase letters indicate statistically significant differences at $P \leq 0.05$.
[4] A bolded value indicates that the 104,000 plants $ha^{-1}$; 0 kg N $ha^{-1}$ treatment combination exhibits the lowest or highest value for that parameter in that year.

Figure 1. Frequency distributions for per-plant grain yield ($GY_P$) and scatter plots for per-plant anthesis-silking interval ($ASI_P$) values for the 54,000 plants $ha^{-1}$; 330 kg N $ha^{-1}$ (A,C) and 104,000 plants $ha^{-1}$; 0 kg N $ha^{-1}$ (B,D) treatment combinations in 2006 (A,B) and 2007 (C,D). Bars with diagonal lines indicate the frequency of barren ($GY_P \leq 25$ g) plants.

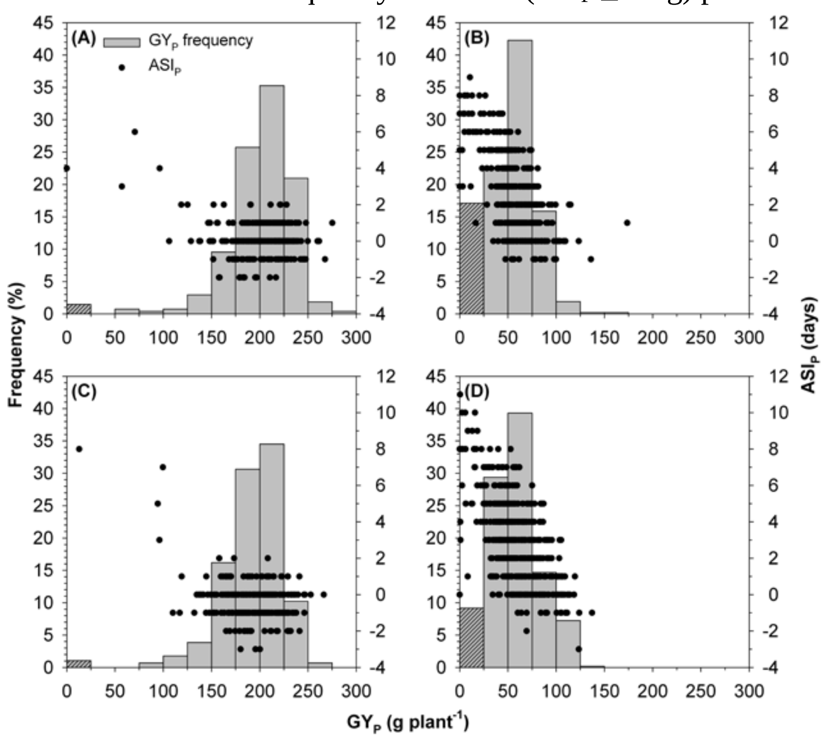
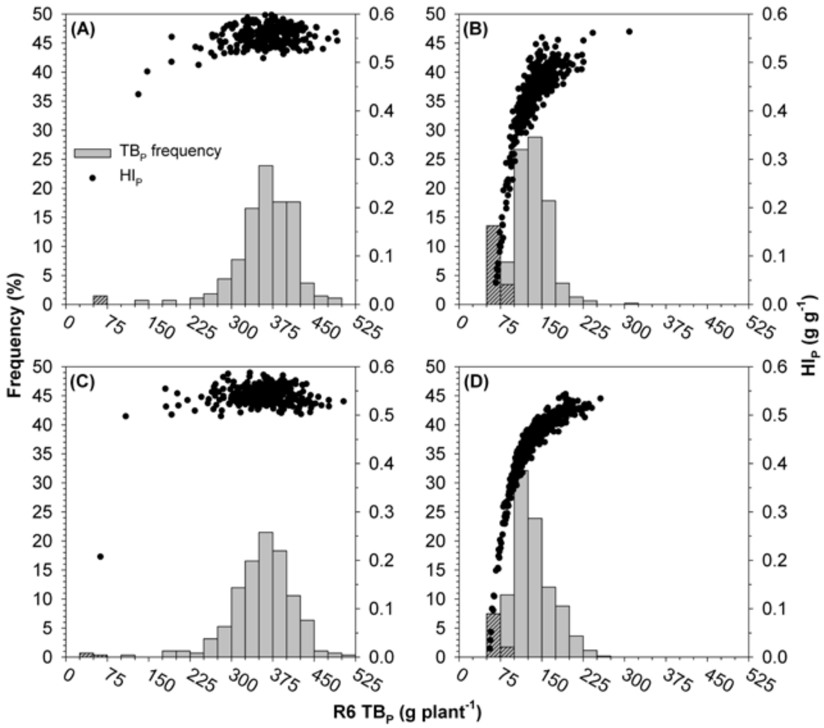


Figure 2. Frequency distributions for R6 per-plant aboveground total biomass ($TB_P$) and scatter plots for per-plant harvest index ($HI_P$) (non-zero) values for the 54,000 plants ha$^{-1}$; 330 kg N ha$^{-1}$ (A,C) and 104,000 plants ha$^{-1}$; 0 kg N ha$^{-1}$ (B,D) treatment combinations in 2006 (A,B) and 2007 (C,D). Bars with diagonal lines indicate the $TB_P$ and frequency of barren ($GY_P \leq 25$ g) plants.

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