

**Friday, December 11
8:00 am**

Corn Session III: Nitrogen

Maize Plant Competition in High Plant Density, High Yield Environments

Christopher R. Boomsma¹, Judith B. Santini², and Tony J. Vyn²

¹Dow AgroSciences, ²Purdue University

Introduction

Improvements in maize (*Zea mays* L.) grain yield over the past 75 to 80 years have been attributed to past genetic gains made by maize breeding programs and to superior agronomic-management practices increasingly adopted by growers, with 50-70% of these yield improvements due to improved genetics and 30-50% attributable to superior agronomic-management practices (Duvick, 2001). Such practices include improved pest control, fertilization practices, and soil tillage programs, in addition to early planting at high plant densities (Duvick, 2001; Tollenaar and Lee, 2002; Kucharik, 2008). Yet the compartmentalization of past maize yield improvements into separate genetic and agronomic-management components fails to adequately acknowledge the pronounced interaction between these two sectors; an interaction that essentially accounts for 100% of prior yield gains (Tollenaar and Lee, 2002; Lee and Tollenaar, 2007). Given that multiple factors account for the greater yields of today's maize hybrids, further understanding the complex impacts and interactions of genetic- and agronomic-related factors is valuable for future advances in maize productivity. If the rate of maize yield advancement is going to improve upon the linear trends observed in the past, advances in maize production must be made at all levels of crop improvement including molecular genetics, eco-physiology, and cropping systems.

Continual examination of maize morpho- and eco-physiology on both a per-plant and canopy-level basis is particularly necessary, especially in high-stress environments [e.g., drought and/or low nitrogen (N) conditions]. As has been previously shown (e.g., Lee and Tollenaar, 2007), such investigations (i) reveal past progress in breeding and cropping systems efforts, (ii) outline areas for future improvement at the genetic and/or crop husbandry level(s), and (iii) help define crop ideotypes for genetic advancement programs. Traditional morpho- and eco-physiological research in maize in low- and high-stress environments has been repeatedly and intensively conducted at the canopy level of organization. Less work to date has been performed at the per-plant level of organization. This is particularly true within the context of plant-to-plant (i.e., intra-specific) competition and by-plant variability. However, a thorough understanding of the functioning and general behavior of the individual plants composing a maize community (i.e., canopy) is essential for future advances in maize productivity. Understanding individual plant behavior within a community context requires intentional manipulation of per-plant resource availability. Two agronomic-management factors which intensely and repeatedly impact resource availability in maize fields are plant density and N fertilization.

High plant density tolerance

Environmental stress tolerance has been recognized as the physiological trait most strongly linked with the genetic improvement of maize hybrids for grain production (Tollenaar and Wu, 1999). Biotic and abiotic stress tolerance can involve tolerance to weed interference, insect feeding, pathogen infection, saturated soils, nutrient deficiency, and/or intense plant crowding (Tollenaar and Wu, 1999; Tokatlidis and Koutroubas, 2004). Tolerance to intense intra-specific competition for available resources (i.e., high plant density tolerance) has improved more than many other environmental stress tolerances over the past 40 to 50 years. This dramatic rate of improvement has been principally driven by maize breeders selecting for yield at high plant densities (Duvick and Cassman, 1999). Consequently, modern hybrids are often grown at greater plant densities than their predecessors to attain maximum grain production.

Nitrogen responsiveness, nitrogen use efficiency, and nitrogen stress tolerance

Besides exhibiting superior tolerance to high plant densities and numerous other stresses, modern hybrids are also more responsive to a variety of inputs, with newer genotypes displaying greater responsiveness than their predecessors in both low- and high-stress environments (Duvick and Cassman, 1999; Tollenaar and Wu, 1999). In particular, modern hybrids are more responsive to N application than their predecessors. While the generally greater responsiveness of modern hybrids has encouraged growers to liberally apply N fertilizer (Duvick, 1992; Duvick, 2001), high N application rates are also the product of increased plant crowding over time (i.e., higher N rates are required to sustain the growth of more plants per unit land area). In addition to improved N responsiveness, some studies suggest greater N use efficiency (NUE) and enhanced N stress tolerance among modern hybrids (e.g., Ding et al., 2005; Coque and Gallais, 2007). However, despite reported advances in NUE and N stress tolerance, recent genetic improvements for these traits have been neither consistently observed nor heavily studied. Furthermore, reductions in yield for modern hybrids are still substantial when soil N is deficient (O'Neill et al., 2004).

Resource availability, intra-specific competition, and plant-to-plant uniformity

Studies to date that have intensively investigated intra-specific competition in maize have often focused on morpho- and eco-physiological responses to increased plant crowding (e.g., Vega and Sadras, 2003; Maddonni and Otegui, 2004). Such studies discovered that as plant density (and concomitantly intra-specific competition) increases in maize, by-plant variability for per-plant grain production, its primary yield components, and other morpho-physiological traits markedly rises (Edmeades and Daynard, 1979; Maddonni and Otegui, 2004; Boomsma et al., 2009) with the end result being reductions in resource use efficiency and total crop productivity (Tollenaar and Wu, 1999). In addition to greater plant crowding, maize plant-to-plant variability can result from cultural practices and/or biological phenomena. Some cultural sources of variability include deviations in planting depth, seed spacing, and crop residue distribution while biological sources of non-uniformity include variations in insect feeding, disease pressure, and soil type (Tokatlidis and Koutroubas, 2004; Andrade and Abbate, 2005). Accordingly, numerous plant-to-plant interaction studies in maize have

focused on the effects of non-uniform intra-row plant spacing and/or uneven seedling emergence on plant performance (Ford and Hicks, 1992; Liu et al., 2004).

Concept justification and study objectives

Despite maize being heavily dependent upon N application for high productivity in crowded environments, few investigations to date have considered the impacts of N availability on maize intra-specific competition (O'Neill et al., 2004; Boomsma et al., 2009). 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 the crowding tolerance, NUE, 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 responses of maize germplasm to above- and below-ground intra-specific competition. This understanding mandates an intense season-long (i.e., seedling emergence through physiological maturity) analysis of the morpho- and eco-physiological dynamics of maize plant hierarchies [i.e., plant populations composed of dominated/unsuccessful and dominant/successful individuals (c.f., Maddonni and Otegui, 2004)] that takes into consideration both temporal (e.g., seedling emergence date) and spatial (e.g., intra-row plant spacing) aspects of above- and below-ground intra-specific competition. Consequently, we recently undertook a three-year study to understand (i) the behavior of maize plant hierarchies in response to varied N availability at multiple plant densities and (ii) the impacts of intra-specific competition on maize high plant density tolerance, NUE, and N stress tolerance. The following sections provide a broad summary of a portion of this work.

Materials and methods

Field research during the 2005, 2006, and 2007 growing seasons was conducted at the Purdue University Agronomy Center for Research and Education (ACRE) near West Lafayette, Indiana. The soil was a Chalmers silty clay loam with approximately 4.0 to 4.5% organic matter content in the top 30 cm. Water stress was generally nonexistent in each growing season due to adequate, timely rainfalls. In each year, maize was grown following no-till soybean [*Glycine max* (L.) Merr.]. Hybrid, plant density, and N rate served as the three treatment factors. The Pioneer hybrids 31G68 (2005, 2006, and 2007), 31N28 (2006 and 2007), and 33N09 (2005) were planted in previously specified years to achieve final plant densities of 54,000, 79,000, and 104,000 plants ha⁻¹ (i.e., ~22,000, 32,000, and 42,000 plants A⁻¹, respectively). For all plots, starter fertilizer (10-34-0) was applied at planting at a rate equivalent to 25 kg N ha⁻¹ (i.e., ~22 lbs. N A⁻¹). Urea Ammonium Nitrate (UAN) (28-0-0) was applied via side-dressing at a rate equivalent to 165 kg N ha⁻¹ (i.e., ~150 lbs. N A⁻¹) once (V3), twice (V3, V5), or not at all, depending upon each plot's prescribed N rate. In a designated sampling area within each plot, extensive, non-destructive, eco-physiological measurements were taken on tagged plants (~4,000 plants year⁻¹) from seedling emergence through physiological maturity. Each plant was classified as dominated, intermediate, or dominant based upon its per-plant grain yield (GY_P). For this manuscript, parameters reported in detail are per-unit-area grain yield (GY_A), NUE, GY_P, per-plant grain yield variability (GY_{CV}), R6 per-plant aboveground total biomass (BP_{R6}), and per-plant harvest index (HI_P). We request readers refer to Boomsma (2009) and Boomsma et al. (2009) for a more in-depth discussion of

this study's cultural practices; treatments; experimental design; statistical analyses; and weather, soil, and plant measurements.

Key results and conclusions

When grown at optimal and supra-optimal plant densities with 165 kg N ha^{-1} , this study's hybrids exhibited both strong N responsiveness and relatively high NUE (defined: $\Delta \text{GY}_A / \Delta \text{N}$). When N was applied roughly at or above this location's approximate agronomic optimal N rate, GY_A values were very often similar among all plant densities (Figure 1). Such results suggest (i) optimal and supra-optimal levels of crowding generally did not improve overall NUE (defined: GY_A / N) relative to the sub-optimal plant density and (ii) this study's genotypes exhibited a degree of plant density independence in this particular highly productive environment. As discussed by Boomsma et al. (2009), this experiment's hybrids displayed HI_P values in excess of the commonly presumed 0.5 value for modern North American genotypes when N was side-dress applied at any plant density. Such results suggest that the harvest index (HI) of at least a subset of current North American genotypes may now exceed 0.5 when these hybrids are grown in highly productive environments. A similar trend for HI has been observed among Argentine genotypes (Luque et al., 2006).

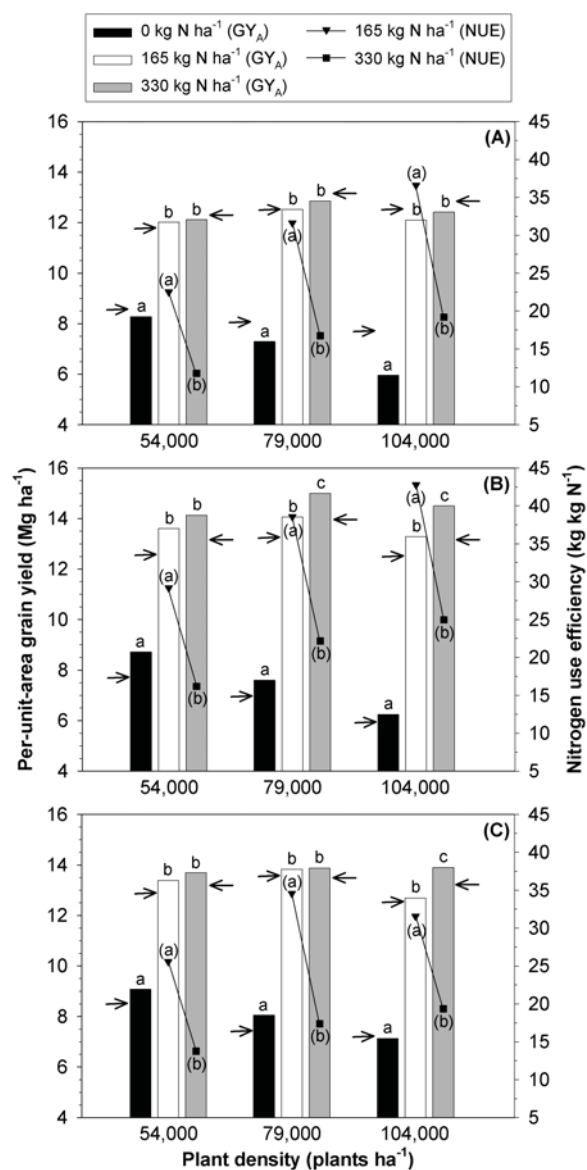


Figure 1. Plant density and nitrogen (N) rate effects on maize per-unit-area grain yield (GY_A) and N use efficiency (NUE) for 2005 (A), 2006 (B), and 2007 (C). Arrows approximately adjacent to each vertical bar indicate the per-unit-area grain yield of each treatment's per-plant sampling area. Values for NUE are expressed as the incremental agronomic efficiency from applied N, which is defined as kilograms of additional grain per kilogram of additional side-dress N ($\Delta GY_A/\Delta N$). For both GY_A and NUE, means with different letters (GY_A : no parentheses, NUE: parentheses) indicate statistically significant differences at the 0.05 probability level within each plant density.

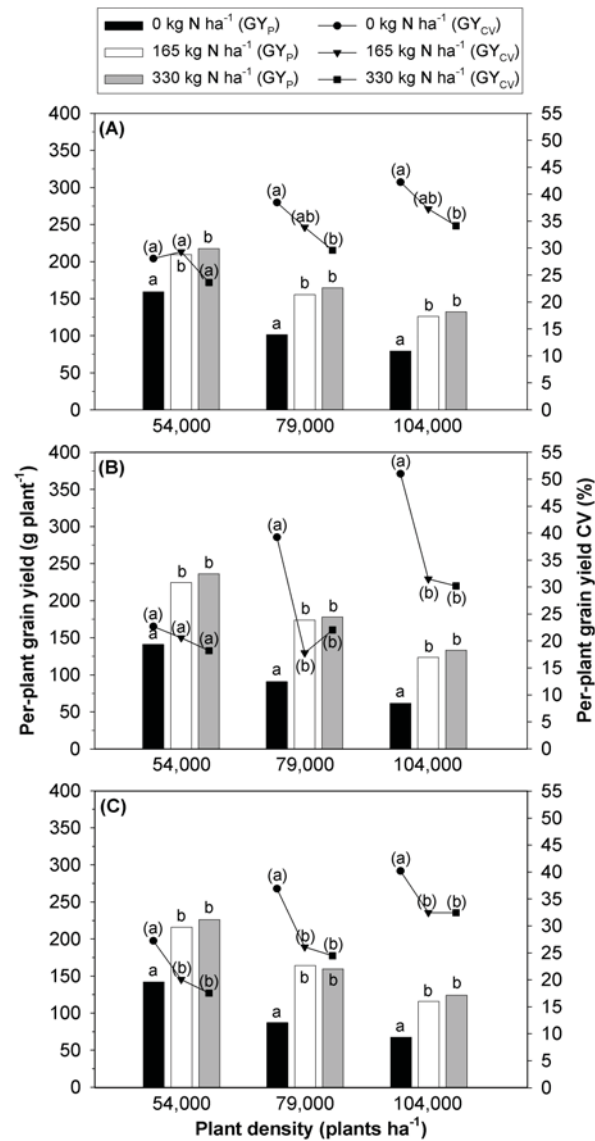


Figure 2. Plant density and nitrogen (N) rate effects on maize per-plant grain yield (GY_p) and per-plant grain yield variability (GY_{cv}) for 2005 (A), 2006 (B), and 2007 (C). Means with different letters (GY_p : no parentheses, GY_{cv} : parentheses) indicate statistically significant differences at the 0.05 probability level within each plant density. The parameter GY_{cv} is expressed using the coefficient of variation (CV).

While this experiment's hybrids exhibited pronounced tolerance to high plant densities, this tolerance was heavily dependent upon the application of side-dress N. In the absence of side-dress N, these hybrids displayed low crowding tolerance and poor N stress tolerance (Figure 1). As similarly discussed by Boomsma et al. (2009), markedly low grain production in the high stress environment (i.e., 104,000 plants ha^{-1} , 0 kg N ha^{-1}) was primarily the product of (i) reduced production and activity of source tissues during the pre-silking period; (ii) decreased plant growth and poor biomass partitioning to the developing ear around silking; (iii) early remobilization of leaf N and subsequent reduction in photosynthesis during the grain-filling period (i.e., loss of functional stay-green); and (iv) unequal resource sharing between plants, the formation of pronounced

plant hierarchies composed of dominated and dominant plants, and enhanced plant-to-plant variability for key morpho-physiological traits (Figure 2). As evident by trends and Gini coefficients (G) for GY_P in Figure 3, a relatively large percentage of individuals accounted for a pronouncedly small percentage of total grain production when intra-specific competition for available N was intense. When considered in conjunction with GY_{CV} trends in Figure 2, such results confirm the existence of pronounced plant hierarchies in this high stress environment, with these hierarchies composed of numerous individuals with severely limited reproductive output (i.e., a large number of dominated plants).

As detailed by Boomsma (2009), relatively successful plants (i.e., intermediate and dominant plants) within the highly competitive environment (i.e., 104,000 plants ha^{-1} , 0 kg N ha^{-1}) were characterized by their ability to:

- (i) Effectively compete for solar radiation through pre-silking stem elongation;
- (ii) Maintain relatively high rates of pre-silking biomass accumulation (i.e., sustain high pre-flowering plant growth rates);
- (iii) Sustain ear biomass accumulation during the critical period bracketing silking (i.e., limit silking delays and early kernel abortion);
- (iv) Produce a relatively large leaf area with high leaf N/chlorophyll levels for sustaining plant and ear growth;
- (v) Maintain leaf N/chlorophyll levels during the grain-filling period (i.e., sustain post-silking photosynthesis) to ensure assimilate availability for kernel growth;
- (vi) Limit the premature remobilization of lower stem assimilates to root tissues; and
- (vii) Markedly remobilize vegetative assimilates for ear growth and development.

As also described by Boomsma (2009), the failure of dominated plants in this study's high stress environment principally resulted from:

- (i) Markedly delayed vegetative and reproductive development;
- (ii) Lower pre-silking source tissue production (i.e., markedly reduced leaf production);
- (iii) Preferential pre-silking biomass partitioning to leaf versus stem tissue;
- (iv) Reduced ability to exploit available space (i.e., low GY_P per unit available space);
- (v) Lower pre- and post-silking leaf N levels;
- (vi) Premature leaf chlorophyll losses (i.e., loss of functional stay-green);
- (vii) Reduced post-silking vegetative assimilate remobilization; and
- (viii) Decreased biomass partitioning to the developing ear.

Interestingly, a plant's success or failure was not strongly contingent upon relatively early seedling emergence among neighboring plants or on relative intra-row plant space at a given plant density (Boomsma, 2009). Overall, such findings (i) affirm that

increased plant-to-plant variability (the majority of which is only evident after seedling emergence) is a key restraint in achieving high grain production at high maize plant densities, (ii) give insight into the morpho- and eco-physiological dynamics of maize intra-specific competition, and (iii) help transform the current view of the maize ideotype from a mainly canopy-level-based model (Mock and Pearce, 1975; Lee and Tollenaar, 2007) to an increasingly individual-plant-based model (Boomsma, 2009).

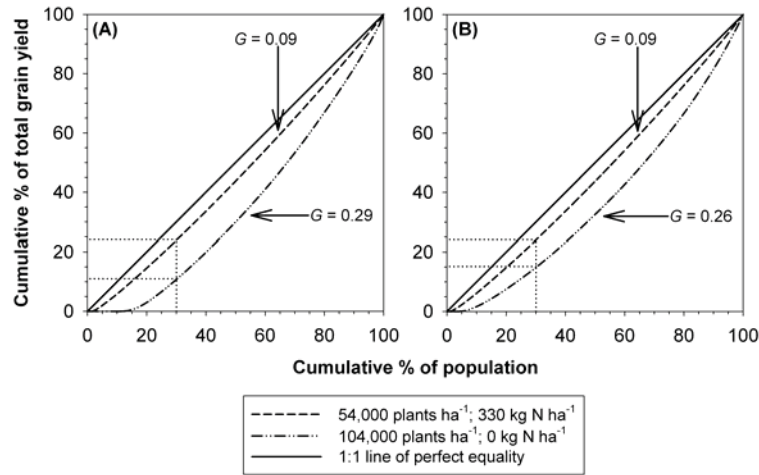


Figure 3. Lorenz curves and accompanying Gini coefficients (G) for per-plant grain yield (GY_p) for the 54,000 plants ha^{-1} , 330 kg $N\ ha^{-1}$ and 104,000 plants ha^{-1} , 0 kg $N\ ha^{-1}$ treatment combinations in 2006 (A) and 2007 (B). For each treatment combination in both (A) and (B), dotted lines (lower left corner of each figure) indicate the cumulative percentage of a treatment combination's total grain yield accounted for by 30% of that treatment combination's population of plants.

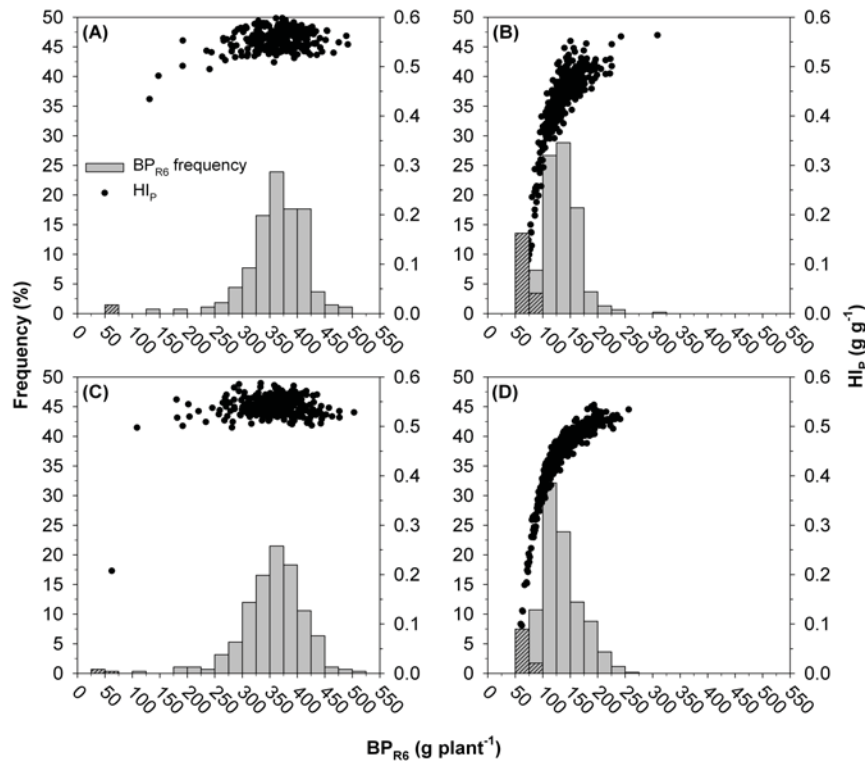


Figure 4. Frequency distributions for R6 per-plant aboveground total biomass (BP_{R6}) and superimposed scatter plots of per-plant harvest index (HI_P) values for the 54,000 plants ha^{-1} , 330 kg N ha^{-1} (A,C) and 104,000 plants ha^{-1} , 0 kg N ha^{-1} (B,D) treatment combinations in 2006 (A,B) and 2007 (C,D). For a given year and treatment combination, bars with diagonal lines indicate the frequency of ultimately barren plants by BP_{R6} increment.

Agronomy- and eco-physiology-based genetic improvement

This study, in addition to a number of its predecessors (e.g., Vega and Sadras, 2003; Maddonni and Otegui, 2004, 2006), provides a call for future research and discussion on the morpho- and eco-physiological dynamics of maize intra-specific competition. Such research could further unravel the morpho- and eco-physiological complexities of maize growth, development, resource use efficiency, and abiotic stress tolerance and, in so doing, provide guidance for the future advancement of maize genetics and agronomic-management practices. The following areas of research are therefore recommended for further understanding maize intra-specific competition and its impact on future yield advances.

First, the maize improvement community needs to further investigate the causal agents of stand non-uniformity in maize. In so doing, future investigations should thoroughly examine the above- and below-ground morpho- and eco-physiological responses of maize to (i) single (e.g., low N availability) and multiple (e.g., low water availability and high plant density) abiotic stresses and (ii) various crop management practices (e.g., tillage system). Such experiments, when performed across multiple environments, could help identify agronomic-management practices that improve plant-to-plant uniformity and may lead to advances in site-specific crop management. For maximum effectiveness, these studies should be performed within a genetic ($\pm$ trait) $\times$ environment $\times$ management [i.e., $G (\pm T) \times E \times M$] framework so that all major factors impacting maize intra-specific competition (and by extension yield) can be explored.

Second, the maize improvement community needs to examine and refine the maize ideotype so that it more fully incorporates a community-based perspective in which plant-to-plant uniformity and/or morpho- and eco-physiological plasticity are viewed as requisite for improving yield potential, resource use efficiency, and abiotic stress tolerance. Key areas of investigation for future studies include:

- (i) The possibility of improved ear growth rates (i.e., altered reproductive allometry) in stress conditions (c.f., Lee and Tollenaar, 2007);
- (ii) The benefits and drawbacks of per-plant and canopy-level plasticity (i.e., efficient versus non-efficient *in planta* resource allocation) (c.f., Bradshaw, 2006);
- (iii) The potential for marked compensatory growth among dominant plants whereby reduced grain production among dominated plants is fully compensated by greater grain production among dominant plants;
- (iv) The worth of Donald's proposed ideotype (i.e., a "communal" ideotype that minimizes canopy-level growth redundancy by partitioning less energy to resource-acquiring organs and more to reproductive output) for future maize genetic improvement (c.f., Zhang et al., 1999); and

- (v) The definition of belowground ideotypes for improved yield potential, resource use efficiency, and abiotic stress tolerance.

Examinations in some of these areas would likely benefit from being tightly linked with complex genetic analyses of quantitative traits, intensive investigations of metabolic processes at the molecular and genomic levels, and extensive application of crop growth models (e.g., Hammer et al., 2009).

Acknowledgements

Funding for this research was provided by a Pioneer Fellowship in Plant Sciences (2006-2009), a Purdue University Andrews Foundation Fellowship (2004-2006), and the Purdue Research Foundation. Pioneer Hi-Bred Intl., Inc. provided seed and Deere & Company loaned field and automatic guidance equipment for this study. We express our thanks to numerous field technicians, undergraduate and graduate students, and volunteers for their extensive and indispensable help in both the field and laboratory. Special thanks to research agronomist T.D. West and the staff at the Purdue University Agronomy Center for Research and Education (ACRE) for help in establishing and managing this study's research plots. Thanks also to Drs. M Tollenaar, A. Dobermann, J.J. Camberato, and R.L. Nielsen for their contributions to this study as members of the first author's Ph.D. advisory committee.

References

- Andrade, F.H., Abbate, P.E., 2005. Response of maize and soybean to variability in stand uniformity. *Agron. J.* 97, 1263-1269.
- Boomsma, C.R., 2009. Resource availability and intra-specific competition in maize: A per-plant and canopy-level morpho- and eco-physiological analysis. Ph.D. Dissertation, Purdue University.
- Boomsma, C.R., Santini, J.B., Tollenaar, M., Vyn, T.J., 2009. Maize morphophysiological responses to intense crowding and low nitrogen availability: An analysis and review. *Agron. J.* 101, 1426-1452.
- Bradshaw, A.D., 2006. Unraveling phenotypic plasticity—why should we bother? *New Phytol.* 170, 644-648.
- Coque, M., Gallais, A., 2007. Genetic variation among European maize varieties for nitrogen use efficiency under low and high nitrogen fertilization. *Maydica* 52, 383-397.
- Ding, L., Wang, K.J., Jiang, G.M., Biswas, D.K., Xu, H., Li, L.F., Li, Y.H., 2005. Effects of nitrogen deficiency on photosynthetic traits of maize hybrids released in different years. *Ann. Bot.* 96, 925-930.

- Duvick, D.N., 1992. Genetic contributions to advances in yield of U.S. maize. *Maydica* 37, 69-79.
- Duvick, D.N., 2001. Biotechnology in the 1930s: the development of hybrid maize. *Nature Rev.—Genet.* 2, 69-74.
- Duvick, D.N., Cassman, K.G., 1999. Post-green revolution trends in yield potential of temperate maize in the north-central United States. *Crop Sci.* 39, 1622-1630.
- Edmeades, G.O., Daynard, T.B., 1979. The development of plant-to-plant variability in maize at different planting densities. *Can. J. Plant Sci.* 59, 561-576.
- Ford, J.H., Hicks, D.R., 1992. Corn growth and yield in uneven emerging stands. *J. Prod. Agric.* 5, 185-188.
- Hammer, G.L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Paskiewicz, S., Cooper, M., 2009. Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. Corn Belt? *Crop Sci.* 49, 299-312.
- Kucharik, C.J., 2008. Contribution of planting date trends to increased maize yields in the central United States. *Agron. J.* 100, 328-336.
- Lee, E.A., Tollenaar, M., 2007. Physiological basis of successful breeding strategies for maize grain yield. *Crop Sci.* 47(S3), S202-S215.
- Liu, W., Tollenaar, M., Stewart, G., Deen, W., 2004. Response of corn grain yield to spatial and temporal variability in emergence. *Crop Sci.* 44, 847-854.
- Luque, S.F., Cirilo, A.G., Otegui, M.E., 2006. Genetic gains in grain yield and related physiological attributes in Argentine maize hybrids. *Field Crops Res.* 95, 383-397.
- Maddonni, G.A., Otegui, M.E., 2004. Intra-specific competition in maize: early establishment of hierarchies among plants affects final kernel set. *Field Crops Res.* 85, 1-13.
- Maddonni, G.A., Otegui, M.E., 2006. Intra-specific competition in maize: Contribution of extreme plant hierarchies to grain yield, grain yield components and kernel composition. *Field Crops Res.* 97, 155–166.
- Mock, J.J., Pearce, R.B., 1975. An ideotype of maize. *Euphytica* 24, 613-623.
- O'Neill, P.M., Shanahan, J.F., Schepers, J.S., Caldwell, B., 2004. Agronomic responses of corn hybrids from different eras to deficit and adequate levels of water and nitrogen. *Agron. J.* 96, 1660-1667.

- Tokatlidis, I.S., Koutroubas, S.D., 2004. A review of maize hybrids' dependence on high plant populations and its implications for crop yield stability. *Field Crops Res.* 88, 103-114.
- Tollenaar, M., Lee, E.A., 2002. Yield potential, yield stability and stress tolerance in maize. *Field Crops Res.* 75, 161-169.
- Tollenaar, M., Wu, J., 1999. Yield improvement in temperate maize is attributable to greater stress tolerance. *Crop Sci.* 39, 1597-1604.
- Vega, C.R.C., Sadras, V.O., 2003. Size-dependent growth and the development of inequality in maize, sunflower and soybean. *Ann. Bot.* 91, 795-805.
- Zhang, D., Sun, G., Jiang, X., 1999. Donald's ideotype and growth redundancy: a game theoretical analysis. *Field Crops Res.* 61, 179-187.

Technology and Biotechnology of Nitrogen Use for High Yield Corn

Fred E. Below¹, Jason W. Haegele¹ and Matias L. Ruffo²

¹Department of Crop Sciences, University of Illinois, Urbana, IL

²Research and Development, Mosaic Fertilizer, Buenos Aires, Argentina

ABSTRACT

A better understanding of the N requirement, the different strategies for N use, and the degree of field spatial variability for N use is needed to devise technology and biotechnology strategies for high yield corn (*Zea mays* L) production. A series of 78 on-farm N titrations conducted across the U.S. corn belt during the last four years showed: 1) average check plots yields (i.e. no N) of 115 bu/acre; 2) a maximum yield of 180 bu/acre with 150 lbs N/acre for an average N requirement of 0.83 lbs N/bu; and 3) an average delta yield of 65 bu/acre. This research also showed that field spatial variability for N use was primarily a function of variation in the check plot yield, suggesting that variable rate application algorithms should target the low yield areas, and showing the value of the biotechnology product concept of lower N rates (25-30% less N) to achieve the same yield. Evaluation of an array of commercial hybrids, as well as hybrids with the intermated B73 x Mo17 recombinant inbred line population, showed the existence of two major N use strategies: 1) a high yield at low N accompanied by a small delta yield response to fertilizer N; and 2) a low yield at low N accompanied by a large delta yield response to fertilizer N application. The check plot yield and delta yield were always negatively related among hybrids. Comparison of rootworm Bt hybrids with their non-rootworm counterparts showed that rootworm hybrids have a marked improvement in NUE and grain yield that is primarily the result of higher uptake efficiency (average of 54% in non-rootworm vs. 71% in rootworm hybrids), and a greater delta yield in response to N. Combining biotechnology traits for better N use with technology for better N application should result in higher corn yields.

INTRODUCTION

Because the application of fertilizer N is one of the most important factors governing corn yields, understanding processes associated with the efficient use of N is paramount to developing management strategies for high yield corn production. Nitrogen plays many key roles in corn yield determination including the development and maintenance of the photosynthetic machinery, and the establishment and fulfillment of kernel sink capacity (Below, 2002).

From an agronomic perspective, the optimum use of fertilizer N would be to apply the exact N rated needed to maximize productivity, while accounting for the cost of the fertilizer and the value of the grain (often called the economic optimum N rate). In practice, however, uncertainties related to weather make it difficult to know the optimum N rate, because weather affects the ability to apply N, as well as its availability and susceptibility to loss. Also interacting with weather is field spatial variability in the response to fertilizer N, which occurs both between (Kyveryga et al., 2007; Williams et

al., 2007) and within (Anselin et al., 2004; Mamo et al., 2003) fields. Spatial variability is one of the more important factors impacting an individual field's response to fertilizer N, and most farmers tend to apply a uniform N rate to whole fields that they believe will serve the needs of the most demanding areas within that field.

Adding to the variability due to spatial field differences is hybrid variation in N use. Genetic variation for numerous aspects related to N use has been reported previously, with considerable interest toward identifying biotech strategies and genes for improving N use efficiency (Gallais and Hirel, 2004; Hirel et al., 2007; Moose and Below, 2009). Comparing the yield response to N of an older hybrid (1980) with its modern counterpart (2005) demonstrates the ability to improve N use with genetic selection as the newer hybrid produced higher grain yields at all rates of N, and importantly, even in the absence of added N (Below et al., 2007). This is indicative of greater tolerance to N deficiency stress, which should be of comparative value in production systems with low N inputs or limited N availability.

This paper describes some of our ongoing research efforts to combine better technologies for N application with improved genetics to improve the overall use of N by corn. We use the check plot yield (no added N) and the delta yield (maximum response to N) of the N response curve to characterize differences in N response due to field spatial variability and due to hybrid, and then we use this information to make site specific N rate and hybrid selection recommendations.

MATERIALS AND METHODS

A series of on-farm trials was used to determine the yield response function of current corn hybrids to fertilizer N, and the degree of spatial variation within and between fields. Seventy-eight commercial fields in six states (Indiana, Illinois, Iowa, Minnesota, Nebraska, and North Dakota) were evaluated over a four year period (2005-2008). Crop and soil management practices varied between fields and followed each cooperator's typical management for high yield. Forty-five fields were on continuous corn rotation and the remaining had soybean as the previous crop. Nitrogen was side-dress applied on fields in Illinois, Indiana, Iowa, and Nebraska, and pre-plant applied in Minnesota and North Dakota. Nitrogen sources were anhydrous ammonia (55 fields), urea-ammonium nitrate solutions (19 fields), and urea (4 fields).

Six N rates ranging from 0 to 250 lbs N/acre in 50 lb increments were applied in six to nine stamps that were strategically situated within different management zones in each field. Management zones were determined using the layers of information that were available for each field which could include: yield maps, bare soil images, topography, satellite images, and soil types. Nitrogen plot dimensions varied slightly between fields but were at least 300 feet long and wide enough to accommodate two passes of the commercial variable rate N applicator. As-applied data for each field was collected from each producer at 2-second intervals and the absolute and relative differences between the target and applied N rate were calculated for each point. Grain yield was measured and recorded using calibrated commercial yield monitors mounted on combines equipped with DGPS. Corn yield was recorded every second and corrected to 15.5% grain moisture. Yield monitor data was cleaned using YieldEditor (Sudduth and Dummond,

2007) considering flow, delay, minimum and maximum speed, speed change from point to point, and swath width.

Yield monitor and applied N data were imported into ArcView GIS and the as-applied point data converted to polygons. The yield data points at a distance shorter than 25 feet from the start or end of a plot were discarded. Yield data points located in plot areas where the actual N rate applied was higher than 20 lbs/acre (for the check plots), or where the relative difference between the target N and actual N was larger than 15% were also discarded. Typically, more than 25 points were used to calculate mean corn yield for each plot. Linear and linear-plateau response models were used to analyze the effect of N rate on grain yield, with significance tested at the $\alpha = 0.1$ level. The inflection point of the linear-plateau response model for grain yield was considered the optimal N rate when the slope of the linear term was larger than 0.1 bu/lb N.

Hybrid evaluations were conducted in our Nitrogen Responsive Nurseries at the Crop Sciences Research and Education Center in Champaign, IL between 2006 and 2008. These nurseries have been managed and previously shown to be responsive to N fertilizer (Gentry et al., 2001). To assess how commercial hybrids vary in their use of N, an array of 47 different commercial hybrids was evaluated for their check plot yields, the N rate that gave maximum yield, and the delta yield. All hybrids were grown under various N levels ranging from deficient (0 lb N/acre) to excessive (250 lb N/acre), with N as the main plot and hybrid as the subplot of a split-plot experimental design with 4 replications. An experimental unit consisted of a two-row plot, with rows 17.5 foot long spaced 30 inches apart. Plots were over-planted and then thinned to a plant population of 32,000 plants/acre. The hybrids had a relative maturity range of 108 to 118 days. For all hybrid trials, the fertilizer N was hand applied in a diffuse band down the center of the row as ammonium sulfate and incorporated when the crop was between the V2 and V3 growth stages.

To evaluate the impact of the rootworm Bt trait on N use two commercial RW hybrids DKC61-69 VT3 (111 RM) and DKC63-42 VT3 (113 RM) were grown with five N rates (0 to 240 lbs N/acre in 60 lb increments) along with their near-isogenic non-RW counterparts (DKC61-72 RR2 and DKC63-46 RR/YGCB) at Champaign, IL in 2008. Treatments were arranged in a split-plot experimental design with four replications where hybrid was the main plot and N rate the subplot. An experimental unit consisted of a six row plot with rows 35 feet in length and spaced 30 inches apart. The non-RW hybrids received an in-furrow application of tefluthrin at planting. All hybrids were overplanted and thinned to a final population of 31,000 plants/acre and N was applied as ammonium sulfate as described above.

For NUE measurements, four representative whole plants were harvested at physiological maturity and separated into stover, and grain fractions, and each fraction analyzed for N by either a combustion technique (NA2000 N-Protein, Fisons Instruments), or by near infra-red transmittance (Foss 1241 Grain Analyzer). NUE (kg grain/kg N) and its components, N uptake (kg plant N/kg N) and N utilization (grain/kg plant N), were calculated as shown in Uribe Larrea et al., (2007). For yield determination, all ears in two rows were combine- or hand-harvested and mechanically shelled, and weight and moisture level determined. All grain yields expressed throughout this report are as bushels/acre at 15.5% moisture. Data were analyzed using the MIXED procedure of SAS. Hybrid and N rate were considered as fixed effects while replication was

considered random. Segmented regression of grain yield versus N rate was performed using the NLIN procedure of SAS.

RESULTS AND DISCUSSION

The average response of grain yield to increasing fertilizer N rate is shown in Figure 1. Three important aspects can be determined from this curve including: 1) the check plot yield, or the yield without added fertilizer N; 2) the delta yield, or the maximum magnitude of increase in yield from applying the optimal N rate; and 3) the minimum N level required for maximum yield that allows for calculation of the N requirement.

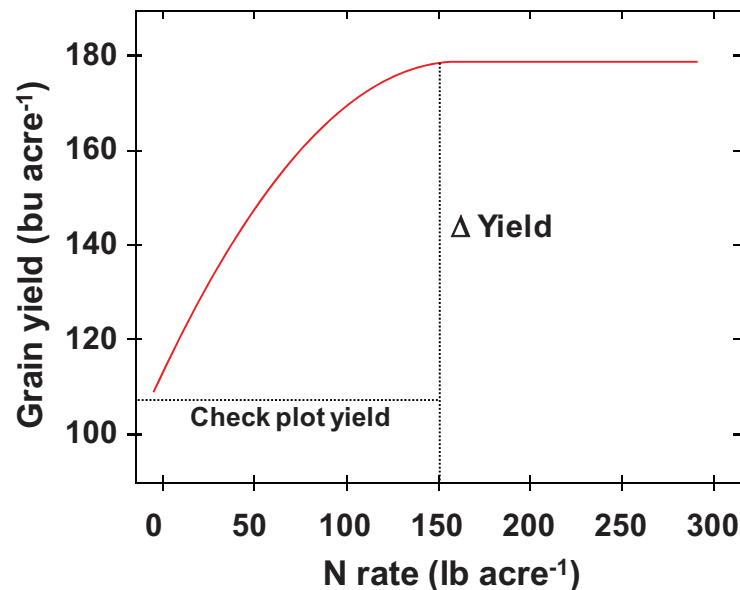


Figure 1. The grain yield response of corn to increasing rates of fertilizer applied N. Data are the field averages of 78 on-farm N response trails conducted in six states in the corn-belt over a four year period (2005-2008). The dashed line at the inflection point indicates the N rate associated with maximum biological yield.

Especially surprising to our farmer cooperators were the check plot yields, averaging 115 bushels/acre without any fertilizer N. Based on this response function, 150 lbs of N produced a maximum yield of 180 bushels/acre. This represents a biological N requirement of 0.83 lbs N/bushel, which is considerably less than the 1.0 to 1.2 lbs of N/bushel typically used by most of these growers. We believe this is why yields have continued to increase even though N rates have remained constant, and why University extension is promoting new recommendation systems for fertilizer N, like the Corn N Rate Calculator (Sawyer et al., 2006). Based on the difference between maximum yield and check plot yield an average delta yield of 65 bushels was observed, which is lower than most of the farmer cooperators thought would be achieved from optimal N fertilization. Because this function was generated on-farm using commercial variable rate applicators, and grain yields obtained using as-applied N maps and yield monitors, we

believe this curve represents a reasonable ‘real world’ picture of fertilizer N use in commercial corn production.

As expected, considerable variation in the yield response to N was observed between years, between fields, and within the same field in a given year. An example of the degree of spatial variability for response to N within an individual field is shown in Figure 2. Within this same field are areas of linear response to N, which are generally indicative of large losses, as well as areas of no response which typically signal high residual availability. Other areas of this field exhibit distinct differences in the N rate that was required to obtain maximum yields. Despite this considerable spatial variation in N response functions, and contrary to our expectations, the maximum yield obtained in each stamp was relatively similar throughout the field at a little over 200 bushels/acre (Figure 2). Rather, it was the check plot yield that varied spatially in this field ranging from 120 to 192 bushel/acre, and with the high check plot areas roughly associated with the high management zones and the low check plot areas the low management zones.

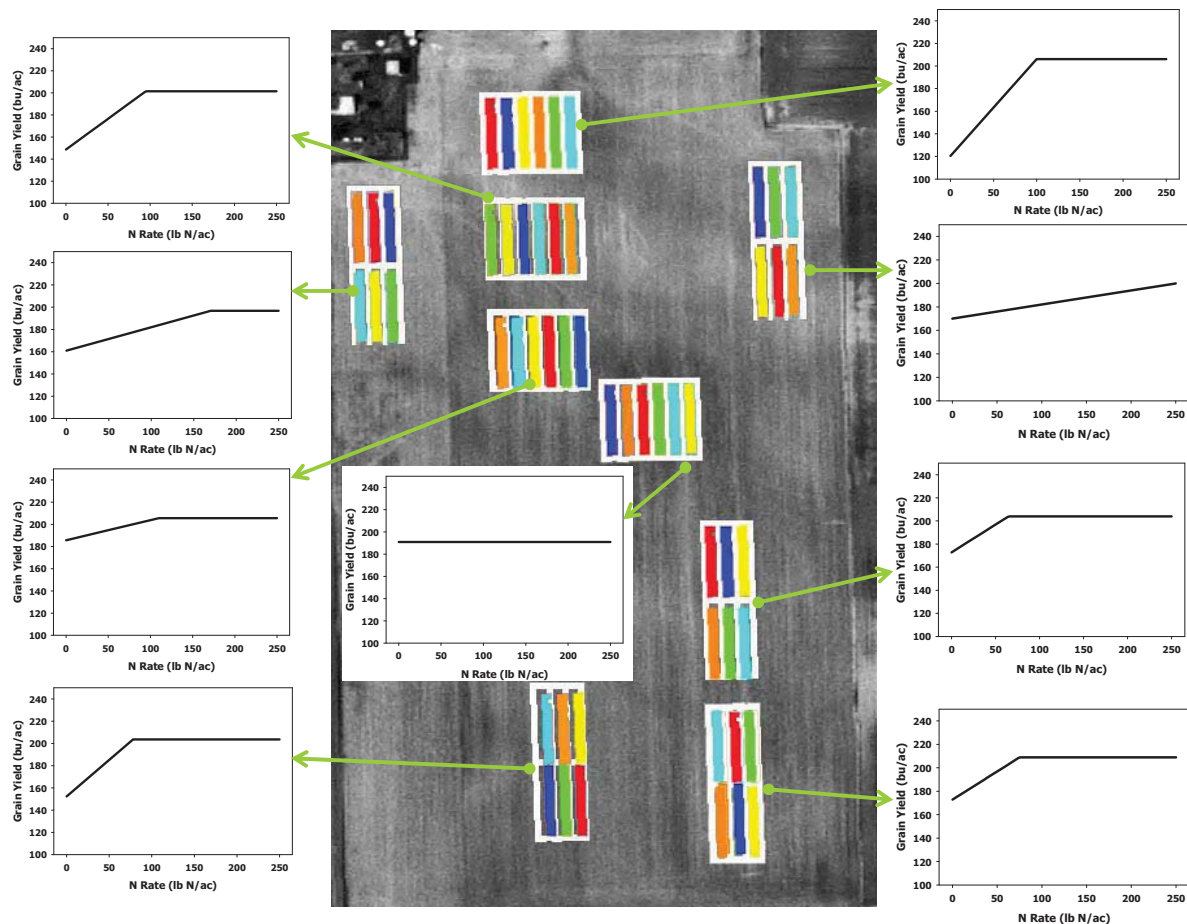


Figure 2. The degree of variation in yield response to fertilizer N that can occur within an individual farm field in a given year. Treatment stamps containing varying N rates (0 to 250 lbs N/acre in 50 lb increments) were situated in areas of different management potential based on soil and topographic factors and/or on previous crop growth and yield information. The location of each stamp is shown on the bare soil image of a 125 acre field located near Beardstown, IL in 2006.

Another representation of the spatial variability in check plot and maximum yield is shown in Figure 3. Data in this figure shows the check plot and the maximum yields (at the N rate that just optimized yield) of each individual stamp from nine other fields that were examined in this study. The coefficient of variation (CV) is also shown above each N treatment for each field to give an indication of the degree of variation among the stamps at that location. In all cases, the range in the yields and the CV's was considerably greater for the check plot yields than the maximum yields, in agreement with the idea that it is the check plot yield that varies spatially in most fields. A similar response was observed at virtually all the other fields (data not shown), suggesting that the maximum yield at a given site is determined by the weather experienced during the season at that site, and to a lesser extent the hybrid used. This means that spatial variation in yield level is mainly a function of the availability of N, which we believe can be managed by variable rate N applications and/or by hybrid selection. For variable rate N applications our data would suggest applying a higher N rate on the areas of low check plot yield (i.e. the low management zones), and a lower N rate on the areas of high check plot yields (i.e. the high management zones). We believe that most proprietary models currently being used for commercial variable rate N applications do not use this approach. Similarly, we believe that future biotech-improved hybrids exhibiting the strategy of a reduction in N level required for maximum yield (i.e. same yield with 25% less applied N) would have utility for helping to control field spatial variation in check plot yields.

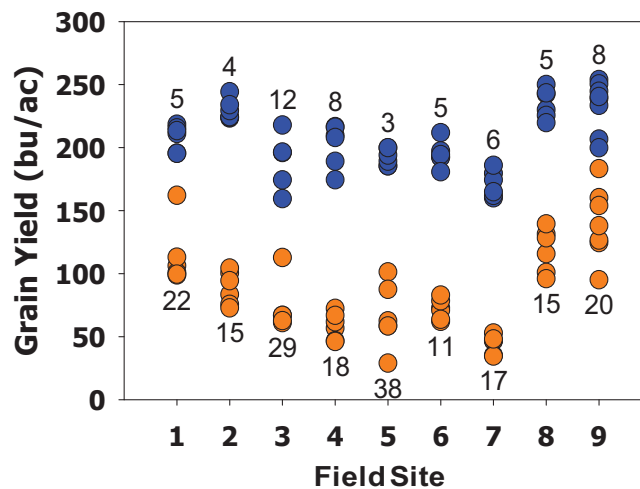


Figure 3. The degree of variation in check plot yields (orange symbols) and in the maximum yields obtained with the optimal N rate (blue symbols) for nine representative fields from 2008. The coefficient of variation for each N level is shown above or below the symbols for that location as an indication of variation in yield in the individual treatment stamps.

Determining which hybrid is best for controlling field spatial variability necessitates a thorough understanding of how modern corn hybrids use N and the degree of variation

that exists among commercial hybrid for variation in N use. To characterize a hybrid's response to N one must know how well it accumulates N under deficient conditions (i.e. with no added fertilizer N), and then how well it uses this N to produce yield. This is known as genetic utilization. Although there are many definitions of NUE, we take the agronomic approach where NUE is defined as the yield increase per unit of applied N for a specific portion of the yield N response curve. It is adjusted for yield and N uptake without added N, and so it represents the additional yield derived from the fertilizer. This NUE or 'yield efficiency' is a product function of uptake efficiency (plant N accumulation per unit of applied N) and utilization efficiency (grain produced per unit of plant accumulated N), and can be improved by increasing the uptake or the utilization, or both. We measured these NUE parameters for each of the 47 commercial hybrids, and present the average and range among hybrids, along with check plot, maximum, and delta yields in Table 1.

Table 1. The average and the range in grain yield and N use traits for 47 commercial hybrids grown at Champaign IL between 2006 and 2008.

Trait	Average	Range
Check plot yield (bu/acre)	130	92-172
Maximum yield (bu/acre)	195	144-239
Delta Yield (bu/acre)	65	25-97
Genetic N utilization (kg grain/kg plant N)	78	63-95
NUE (kg grain/kg N)	23	10-38
N uptake (kg plant N/kg N)	0.54	0.36-0.78
N utilization (kg grain/kg plant N)	41	27-97

There was a wide range among the commercial hybrids in both check plot and maximum yields, and the average delta yield for the hybrids (65 bu/acre) was the same as the average delta yield obtained in our on-farm studies (Fig. 1). Associated with a hybrid's check plot yields was its genetic N utilization which accounts for the plants ability to acquire N from the soil when it is limiting, and/or to make better physiological use of limiting tissue N for growth and yield production. Differences in N acquisition could be due to variation in root architecture or root metabolism, while differences in N utilization at low N are almost always related to an increase in kernel number due to less kernel abortion (Moose and Below, 2009; Uhart and Andrade, 1995a,b).

Considerable hybrid variation was observed for all NUE parameters (Table 1). The NUE averaged 23 kg grain/kg N, (range of 10-38), with an average uptake efficiency of 0.54 kg plant N/kg N (range 0.36-0.78) and an average utilization efficiency of 41 kg grain/kg plant N (range 27-97). No hybrid was optimized for both N uptake and N utilization suggesting room for improvement in overall NUE.

When each hybrid's check plot yield is plotted against its delta yield the relationship is negative ($r = -0.45$), and it is apparent that hybrids exhibit two major strategies for their response to fertilizer N (Fig. 4). One strategy involves producing a high check plot yield (or a high yield at low N) accompanied by a low delta yield, while another involves a low check plot yield and a high delta yield. Hybrids with high check plots yields could presumably be used to help control field spatial variability, since it is check plot field that varies spatially within fields (Figs. 2 & 3). Conversely, hybrids with

high delta yields should be more suited to situations where N can be intensely managed. Few hybrids exhibited relatively high check plot and high delta yields, which is the most desired strategy, as these hybrids would produce good yields when N was limiting, but even higher yields with adequate N. Because the absolute values for check plot yields (average of 130 bushels/acre) are twice as high as delta yields (average of 65 bushels), we believe that research efforts targeting N use for 300 bushels/acre will have to focus on increasing the magnitude of response to fertilizer N, while maintaining high check plot yields. A big challenge to this approach is the negative relationship between check plot and delta yield (Fig. 4), which may require the introgression of novel genes from exotic germplasm, or new gene combinations from biotechnology.

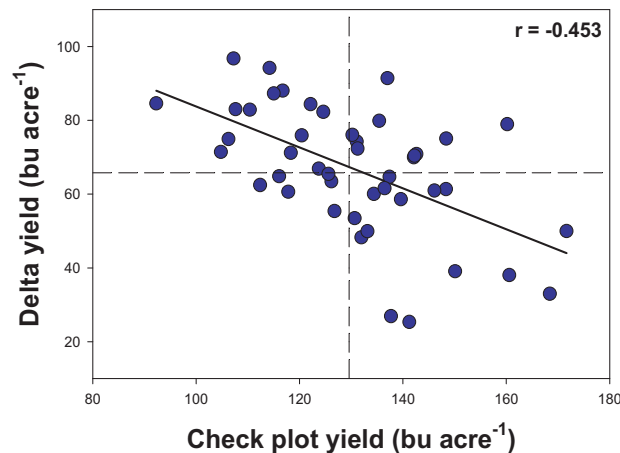


Figure 4. Relationship between check plot yield and delta yield for 47 commercial hybrids grown at Champaign, IL over a three-year period (2006-2008). The dashed lines represent the average check plot and delta yields of these hybrids. The negative relationship between check plot and delta yield among the hybrids is indicated by the solid line.

There is some indication that biotechnology has already altered N use through the introduction of the rootworm Bt trait that confers resistance to larval feeding of the corn rootworm. The hypothesis is that limited root damage could lead to greater access to N (and potentially other mineral nutrients), and as a result higher yield compared to the non-rootworm corn with similar genetics. Upon their widespread commercial release in 2006, we immediately observed large increases in grain yield conferred by the rootworm trait that were hard to attribute entirely to rootworm control (Below et al. 2007). Studies in subsequent years have confirmed this yield advantage, and helped to demonstrate that an improvement in N use is involved (Fig. 5, Table 2). The yield response to N for two pairs of near-isogenic hybrids with and without the rootworm trait is shown in Figure 5. Although the overall yield response to N was different for the two hybrid pairs, both rootworm resistant hybrids exhibited larger delta yields in response to N and produced higher yields. For both hybrid pairs, the rootworm resistant hybrid had higher overall fertilizer NUE compared to its non-rootworm counterpart, which was related to an improvement in uptake efficiency (Table 2).

Uptake efficiency was notably higher (71 compared to an average of 54%) for the hybrids with rootworm protection, showing that a better ability to take up N from the soil is a key factor contributing to higher N use efficiency. Genetic technologies like rootworm protection, which enable the plant to recover more soil N, might also be important tools to help minimize spatial variability and promote consistent yield potential across the entire field. While the future promises to bring a suite of traits that will enable more efficient use of N, corn rootworm protection appears to be the first generation of N management traits.

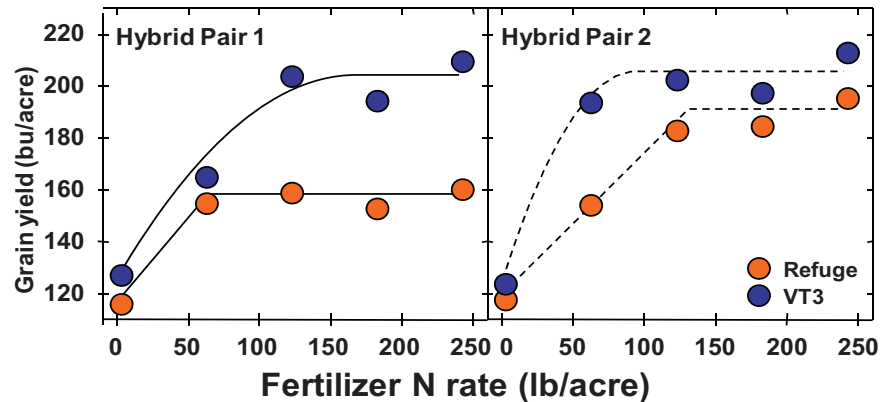


Figure 5. Comparative effect of the rootworm Bt trait (as indicated by VT3) on the yield response to fertilizer N for two pairs of near-isogenic hybrids with and with the rootworm trait grown at Champaign, IL in 2008. Hybrids without the rootworm trait (indicated by refuge) received a soil applied insecticide (tefluthrin) at planting.

Table 2. The average values for fertilizer NUE and its components uptake and utilization for two pairs of near-isogenic hybrids with and with the rootworm trait (VT3) grown at Champaign, IL in 2008. Hybrids without the rootworm trait received a soil applied insecticide (tefluthrin) at planting.

Hybrid pair	NUE	Uptake	Utilization
	kg kg _N ⁻¹	%	kg kg _N ⁻¹
DKC61-72 RR2	17.0	52	33.1
DKC61-69 VT3	25.9*	71*	36.4
DKC63-46 RR2/YGCB	22.4	56	40.1
DKC63-42 VT3	31.7*	71*	44.6

*Different from non-RW counterpart (P < 0.05).

ACKNOWLEDGEMENTS

We thank the Illinois Fertilizer Research Council, Mosaic, Honeywell, Monsanto, and Dow AgroSciences for financial support of this research. We also express our gratitude to Matt Wiebers, Mark Harrison, Juliann Seebauer, Jim Kleiss, Brad Brandy, Adam Henninger, and Martin Uribe Larrea for technical assistance.

REFERENCES

- Anselin, L., R. Bongiovanni, and J. Lowenberg-DeBoer. 2004. A spatial econometric approach to the economics of site-specific nitrogen management in corn production. *Am. J. Agric. Econ.* 86:675-687.
- Below, F.E. 2002. Nitrogen metabolism and crop productivity. p. 385-406, *In*: M. Pessaraki, ed. *Handbook of plant and crop physiology*, Second Edition ed. Marcel Dekker, Inc., New York.
- Below, F.E., M. Uribeharrea, M. Ruffo, S.P. Moose, and A.W. Becker. 2007. Triple stacks, genetics, and biotechnology in improving nitrogen use of corn. *In*: Proc. N.C. Ext.-Ind. Soil Fertility Conf., Des Moines, IA, 14-15 Nov. 2007. International Plant Nutrition, Inst., Brookings, SD.
- Gentry, L.E., F.E. Below, M.B. David, and J.A. Bergerou. 2001. Source of the soybean N credit in maize production. *Plant Soil* 236:175-184.
- Gallais, A., and B. Hirel. 2004. An approach to the genetics of nitrogen use efficiency in maize. *J. Exp. Bot.* 55:295-306.
- Hirel, B., J. LeGousi, B. Ney, and A. Gallais. 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot.* 58:2368-2387.
- Kyverga, P.M., A.M. Blackmer, and T.F. Morris. 2007. Disaggregating model bias and variability when calculating economic optimum rates of nitrogen fertilization for corn. *Agron. J.* 99:1048-1056.
- Mamo, M., G.L. Malzer, D.J. Mulla, D.R. Huggins, and J. Strock. 2003. Spatial and temporal variation in economically optimum nitrogen rate for corn. *Agron. J.* 95:958-964.
- Moose, S.P., and F.E. Below. 2009. Biotechnology approaches to improving maize nitrogen use efficiency. p. 65-77. *In*: A.L. Kriz and A.B. Larkins (eds.). *Molecular Genetic Approaches to Maize Improvement*. Biotechnology in Agriculture and Forestry, Vol. 63. Springer-Verlag, Berlin Heidelberg.
- Sawyer, J., E. Nafziger, G. Randall, L. Bundy, G. Rehm, and G. Joern. 2006. Concepts and rationale for regional nitrogen rate guidelines for corn. PM 2015 April 2006. Cooperative Ext. Serv., Iowa State Univ. Ames, IA.
- Sudduth, K.A., and S.T. Drummond. 2007. Yield editor: Software for removing errors from crop yield maps. *Agron. J.* 99:1471-1482.
- Williams, J.D., C.R. Crozier, J.G. White, and D.A. Crouse. 2007. Comparison of soil nitrogen tests for corn fertilizer recommendations in the humid southeast USA. *Soil Sci. Soc. Am. J.* 71:171-180.
- Uhart, S.A., and F.H. Andrade. 1995a. Nitrogen deficiency in maize. I. Effects on crop growth, development, dry matter partitioning, and kernel set. *Crop Sci.* 35:1376-1383.
- Uhart, S.A., and F.H. Andrade. 1995b. Nitrogen deficiency in maize. II. Carbon-nitrogen interactions effects on kernel number and grain yield. *Crop Sci.* 35:1384-1389.
- Uribeharrea, M., S.P. Moose, and F.E. Below. 2007. Divergent selection for grain protein affects nitrogen use efficiency in maize hybrids. *Field Crops Res.* 100:82-90.