## TRIPLE-STACKS, GENETICS, AND BIOTECHNOLOGY IN IMPROVING NITROGEN USE OF CORN

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### Abstract

Genetic improvement in N use efficiency (NUE) is a clear strategy for enhancing yield and fertilizer N management of corn (Zea mays L.), and has been the subject of our ongoing research. Calculation of the N requirement (N fertilizer usage/grain yield) based on statewide average grain yields and fertilizer sales shows a steady decrease since the mid 1980's, suggestive of a genetic improvement in N use. Direct comparison of the response to N for a 1980's hybrid with its modern counterpart confirmed a 14 bushel improvement in maximum yield with 27 lb/acre less N, for a 25% decrease in the N requirement. An N response curve generated from 37 onfarm sites over 5 states in 2005 and 2006 showed: 1) check plots yields (i.e. no N) of 112 bu/acre; 2) a maximum yield of 173 bu/acre with 130 lbs N/acre; and, 3) a delta-yield of 61 bu/acre. For 55 commercial hybrids grown in our N responsive nursery, the average NUE calculated at the N rate the just optimized yield was 21.6 kg grain/kg N (range of 6-42), with an average uptake efficiency of 0.58 kg plant N/kg N (range 0.26-0.82) and an average utilization efficiency of 38 kg grain/kg plant N (range 18-56). No hybrid was optimized for both N uptake and N utilization suggesting room for improvement in overall NUE. A substantial increase in yield was observed in hybrid isolines containing the rootworm bt trait, which was accompanied by better performance at low N and a higher maximal yield at high N. Based on this research, three difference response models were developed to show the potential ways that yield response to N could be improved in a commercial corn hybrid.

## Introduction

An examination of N fertilizer usage and statewide average yields in Illinois over the last 46 years shows the changing nature of the relationship between yield and fertilizer N (Fig. 1). It must have been nice in the early years (1964-1985) when there was a direct relationship between the amount of N applied and the grain yield, and when each year brought higher N rates and corresponding increases in yield. Recommendation systems devised during this period were yield-based, with the idea that more yield required more N. A yield/N coefficient of 1.2 lbs of N per bushel was typically used, multiplied by the yield the grower wanted to produce. This was later (sometime in the late 1980's) changed to 'proven yield' based on a field's 5 year average.

This N requirement can be roughly shown by dividing the N rate used by the yield achieved (Fig. 1B). The fitted trend line shows it hovers right around 1.2 lbs N/bushel for many years, but it has been decreasing since 1985. This is because fertilizer usage has remained relatively stable (approx 160 lbs N/acre), while the average grain yield ha continued to increase at a rate of about 1.7 bushels per year. As a result, the N fertilizer requirement has steadily decreased since 1985. The N fertilizer usage and the N requirement seem to be relatively independent of fertilizer cost,

which has exhibited large and erratic changes in price, with the overall trend being upward, especially in the last five years (Fig. 1B).



Figure 1. (A) Statewide average grain yield and the average per acre fertilizer N use in Illinois over a 46 year period. The line fitted to the grain yield curve represents the increase in grain yield over time at a rate of 1.7 bushels per acre per year. (B) The N requirement calculated by dividing the N rate by the yield, and the cost reported for fertilizer N. Source USDA-NASS, 2006.

The continued yield increases with a constant N rate, and the lower N requirement, are both indicative of a genetic improvement in N use. Have breeders, by focusing on yield improvement, also indirectly improved the use of N? Genetic variation for numerous aspects related to N use has been reported previously for corn (Bertin and Gallais, 2000, 2001; Hirel et al., 2001; Uribelarrea et al., 2007), with a lot of interest recently toward biotech strategies for improving NUE.

In this report, I use findings from my ongoing research program to address the following five questions related to the current and future use of N by corn: 1) Has there been a genetic improvement in N use of modern hybrids; 2) How do commercial hybrids vary in their use of N;

3) What is the current 'real world' yield N response curve; 4) How might biotechnology improve the N response curve; and 5) Does the rootworm bt trait impact the response to N?

## **Materials and Methods**

Genetic and hybrid evaluations were conducted in our Nitrogen Responsive and Management Evaluation Nurseries at the Crop Sciences Research and Education Center in Champaign, IL between 2001 and 2006. These nurseries have been managed and previously been shown to be responsive to N fertilizer (Gentry et al, 2001). To evaluate genetic improvement we compared the response to N of an older hybrid that was widely grown in the late 70's and early 80's (B73 x Mo17) with its modern counterpart (FR1064 x LH185). To assess how commercial hybrids vary in their use of N a separate array of 55 different commercial hybrids was evaluated. To evaluate the impact of the rootworm bt trait we compared the same hybrid with varying degrees of transgenic traits to include single (herbicide only), double (herbicide and corn borer bt) and triple (herbicide, corn borer, and rootworm bt).

All hybrids were grown under various N levels ranging from deficient (0 lb N/acre) to excessive (300 lb N/acre). Experiments were arranged in the field as randomized complete block designs with 4 to 8 N rates and three or four replications. Plots were over planted and then thinned to 32,000 plants/acre for commercial hybrids and 28,000 for the older hybrid. In all cases, 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.

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 reflectance (DickeyJohn Instalab-6000). 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 Uribelarrea et al, (2007). For yield determination, all ears in the row, were harvested and mechanically shelled, and weight and moisture level determined. All grain yields expressed throughout this report are as bushels/acre at 15% moisture.

Our estimate of the current yield response function to fertilizer N was obtained on-farm as part of a larger study evaluating field spatial variability in N response. Seventeen sites were located in three geographic regions near Beardstown and Paris, IL, and Lewisville, IN in 2005, and 20 sites spanned 5 states (IN, IL, IA, MN, ND) in 2006. All sites were large-scale production fields with 6-9 'treatment stamps' of six N rates (0 to 250 lb N/acre) strategically situated through the field. The N was applied with commercial variable rate N applicators, and yields obtained using asapplied N maps and yield monitors. Yield monitor points were only taken when the as-applied N rate was within 10% of the target rate, and yield data was cleaned with YieldEditor considering combine speed, speed change, and the minimum and maximum yields. The N response curve presented in this report is derived from the field-averages of all 37 sites. Nearly identical functions were obtained for the 2005 and the 2006 data analyzed separately.

## **Results and Discussion**

## **Genetic Improvement**

Evidence for a genetic improvement in N fertilizer use is shown in the comparison of the yield response to N for an older hybrid with its modern counterpart (Fig. 2). Based on the fitted inflection point, the modern hybrid was more efficient in using N requiring 27 lbs less N to produce 14 bushels more grain. The modern hybrid also has a lower N requirement 0.9 lb N/bushel compared to 1.2 lb N/bushel for the older hybrid. This improvement, however, is not the result of direct selection for better N use, but rather has occurred indirectly as plant breeders have selected for higher yields. Much of the yield improvement of modern hybrids is due to better stress tolerance (Duvick, 2005), and plants less challenged by stress might be able to make better use of fertilizer N.



Figure 2. Comparison of the yield response to N for an older hybrid (B73 x Mo17), that would have been widely grown in the early 1980s, with its modern-day counterpart (FR1064 x LH185). The dotted line at the inflection points indicate the N rate associated with maximum biological yield. Data are the average of four years at Champaign, IL.

## Hybrid Variation in NUE

The overall response to fertilizer N depends on both the grain yield when no N is applied and the magnitude of yield increase with a particular N rate. Thus, to characterize a hybrid's response to N one must how well it accumulates N under deficient conditions (i.e with no added fertilizer N), and then 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.

Since NUE and its components are efficiency indicators they always decrease with N supply. For this reason we calculate an individual hybrid's optimal NUE (and components) at the N rate that just maximizes its grain yield. The average yield, genetic utilization, and NUE components, and their ranges, for the 55 hybrids are shown in Table 1. Considerable hybrid variation was observed for all parameters. The NUE averaged 21.6 kg grain/kg N, (range of 6-42), with an average uptake efficiency of 0.58 kg plant N/kg N (range 0.26-0.82) and an average utilization efficiency of 38 kg grain/kg plant N (range 18-56). No hybrid was optimized for both N uptake and N utilization suggesting room for improvement in overall NUE.

Table 1. Grain yield and N use traits for 55 commercial hybrids grown with no fertilizer N, and at the optimum N rate for grain yield. Values in parenthesis are the hybrid ranges. Trials were conducted in Champaign IL between 2001 and 2006.

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Trait	No Applied N	Optimum N rate
Grain yield (bu/acre)	128 (62 - 174)	174 (137 - 224)
Genetic utilization (kg grain/kg plant N)	73 (44 - 97)	N/A
NUE (kg grain/kg N)	N/A	21.6 (5.9 - 42.4)
N uptake (kg plant N/kg N)	N/A	0.58 (0.26 - 0.82)
N utilization (kg grain/kg plant N)	N/A	37.7 (17.9 - 56.1)
N utilization (kg grain/kg plant N)	N/A	37.7 (17.9 - 56.1)

# **Current N Response Curve**

The response curve shown in Figure 3 was generated on-farm using commercial variable rate applicators, and yields obtained using as-applied N maps and yield monitors. It represents 17 on-farm sites in Illinois and Indiana in 2005, and 20 sites over five states in 2006. All sites were managed according to local recommendations for high yields and included a mixture of crop rotations and tillage practices. We believe this curve represents a reasonable 'real world' picture of fertilizer N use in commercial corn production.

Three important aspects can be gleaned from this curve including: 1) the check plot yield, or the yield without added fertilizer N; 2) the delta yield, or the magnitude of increase in yield from applying the optimal N rate; and 3) the minimum N level required for maximum yield, based on the inflection point of the yield response function. Surprising to many or our farmer cooperators are the check plot yields, averaging 112 bushels/acre without any fertilizer N. Based on this function, 130 lbs of N produced a maximum yield of 173 bushels/acre. This represents a biological N requirement of 0.75 lbs N/bushel, which is considerably less than the 1.0 to 1.2 lbs of N/bushel typically used by these growers. This is why yields have continued to increase even though N rates have remained constant, and why we are seeing new recommendation systems for fertilizer N, like the maximum return to N (MRTN).



Figure 3. The grain yield response of corn to fertilizer N rate, and the maximum return to N (MRTN) curve averaged over 37 on-farm locations in 2005 and 2006. The yield response curve was used to generate the MRTN curve using \$3.30/bushel grain price and 0.35 lb/N fertilizer cost. The dashed line at the inflection points indicate the N rate associated with maximum biological yield, and the N rate associated with the maximum dollar return to fertilizer N.

We show the MRTN concept graphically in Figure 3 using our on-farm N response curve and a grain price of \$3.30/bu and N cost of 0.35/lb N. A maximum return to N of \$160/acre was achieved with 110 lb/acre of N, which was 20 lbs less than the amount needed to produce maximal yield, and at least 50 lb less than the amount used by each of these farmers. The lost profit from either under or over fertilization is readily apparent.

#### **Strategies for Improvement**

While there is a lot of interest in improving NUE with biotechnology, the strategy for this improvement is less certain. Two types of potential improvement scenarios that have been reported include: 1) the same yield with 25% less fertilizer N or, 2) 25% more yield with the same level of fertilizer N. Although sounding similar these approaches are actually very different, and have different implications on which NUE components need to be altered. A hybrid producing the same yield with 25% less N is depicted in Fig. 4A. For this response to occur, the plant would have to be improved in its ability to acquire N at low levels (i.e. have a high genetic utilization), but not able to utilize additional fertilizer N should it be made available. Such hybrids would be most suited to developing countries where the high cost of fertilizer N prevents adequate use.

A hybrid producing 25% more yield with the same level of N is shown in Fig. 4B. These hybrids have to be improved in their ability to acquire N at low levels, and be able to utilize this N for additional yield production. This is similar to how modern hybrids use N compared to older ones (see Fig. 2). An even better NUE hybrid is depicted in Fig. 4C. This hybrid has improved yield at low levels of N, but yields even more with additional N. This response involves high genetic utilization and high NUE. Such a hybrid would assure against yield losses due to insufficient N, but also allow for even higher yields when adequate N is available. Even though such a hybrid would have a lower N requirement, it would actually require more total N to achieve its maximum yield.

# **Root Worm bt and N Response**

There is a possibility that the biotech industry has already altered corn's N requirement with the bt-rootworm trait. We have seen large yield advantages conferred by the rootworm trait that are hard to attribute entirely to rootworm control. One of these early studies is shown in Figure 5 where we compared N response for the same hybrid with varying biotech trait combinations. Plots had fairly low rootworm pressure and were treated with soil insecticide at planting. There was a large yield advantage (over 50 bushels) of the biotech combination containing the bt root worm, but with almost a 100 lb/acre increase in the fertilizer N rate needed for maximum yield. This pattern is similar to the strategy for NUE improvement depicted in Fig. 4C, which also increases the overall need for fertilizer N.

Additionally, we have also seen cases where inclusion of the rootworm bt trait increased yield without altering the N level needed for maximum yield, and this was associated with an increase in N utilization (data not shown). In another instance we observed an increase in yield with a decrease in the N level needed to achieve maximum yield (data not shown). Obviously, more work needs to be none to ascertain the full impact of the room worm bt trait in the fertilization and use of N by the corn plant. Happily, in none of our trials to date have we observed a yield decrease attributed to the root worm bt trait, and in most cases there has been a substantial increase. We think this fact bodes well for future improvements in N use of corn with biotechnology.



Figure 4. Graphic demonstration of three potential ways that the corn yield response to N could be improved with biotechnology. The solid line represents the current N response curve from the 37 on-farm sites, while the dashed line shows different scenarios that might occur with an improved hybrid. (A) Similar grain yields with 25% less fertilizer N; (B) 25% higher yield with the same level of fertilizer N; and (C) improved yield at low N, but even higher yield at high N. The dashed lines at the inflection points indicate the N rate associated with maximum yield.



Figure 5. Influence of combinations of biotech traits on the response of corn grain yield to fertilizer N. The same hybrid genetics was used for the single (herbicide resistant only), the double (herbicide resistance and corn borer bt), and the triple (herbicide resistance, corn borer, and rootworm bt) stack versions. All plots also received soil insecticide at planting. The dashed lines at the inflection points indicate the N rate associated with maximum yield. Data are from a 2006 experiment conducted at Urbana, IL.

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