



# Physiological constraints to realizing maize grain yield recovery with silking-stage nitrogen fertilizer applications

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## ABSTRACT

Understanding the physiological factors which allow or constrain maize (*Zea mays* L.) recovery from vegetative N stress when N fertilizer is added near flowering is important for N management decisions. To study the capacity of maize to recover from vegetative N stress, we conducted a two-year experiment comparing seven hybrids (released between 1946 and 2015) and five N treatments - a 0 N control (0\_0) and four treatments which received a total of 220 kg N ha<sup>-1</sup> applied either 100% at V4 (220\_0), 100% at R1 (0\_220), 75% at V4 and 25% at R1 (165\_55), or 25% at V4 and 75% at R1 (55\_165). These N treatments created a range of N deficiencies at flowering. There was no indication that yield response to the timing of N application had changed over the past 70 years of hybrid improvement. Compared to 220\_0, grain yield was significantly lowered by the delayed application of 0\_220 (relative yield of 89%) but not by the split applications of 55\_165 or 165\_55 (relative yields of 93 and 100%, respectively), although both 0\_220 and 55\_165 were under N stress at R1, as indicated by the N Nutrition Index. The reasons for the lower grain yield in 0\_220, but not 55\_165, appeared to be 1) low vegetative-stage N accumulation which could not be compensated for by increased post-silking N uptake, 2) lack of recovery of leaf N status following the R1 N application, and 3) significantly reduced biomass accumulation during the vegetative growth. These findings provide a better understanding of the ability of maize to recover from moderate early-season N stress and will aid agronomists in making management decisions regarding the timing of N fertilizer applications.

## 1. Introduction

In order to improve N fertilizer management decisions in maize (*Zea mays* L.), the physiological constraints on N uptake during the post-silking period after low N availability during the vegetative growth stages warrants investigation. Previous research has generally found maize grain yield to be insensitive to the timing of N application during vegetative growth stages where moderate N levels can be provided by soil N mineralization (Jokela and Randall, 1997; Scharf et al., 2002; Kitchen et al., 2017; Mueller et al., 2017). However, there has been little research on the physiological mechanisms which underlie and control maize recovery from early-season N stress. This is of particular interest in areas, such as the Midwestern United States, which often experience excess spring precipitation (Wang et al., 2016) that may prevent early-season N applications (pre-plant or sidedress), cause substantial loss of applied N through leaching or denitrification, or create conditions conducive to minimal soil N mineralization.

Maximum, or nearly maximum, yields have been obtained even

when N applications were delayed until the late-vegetative growth stages (defined here as V12 or later) in locations with adequate soil N mineralization. In a series of 28 experiments, Scharf et al. (2002) found no negative impacts on yield when the total N application was delayed until V11, and only minor yield losses when application was delayed until V12 or V16. Although delaying the entire N application until R1 lowered relative yields to 71–95% of maximum yield, there was still a very strong agronomic benefit to N application at the R1 stage. These observations confirmed the findings of Binder et al. (2000) who investigated an extensive combination of N rates, ratios of early to late applied N, and timings of split N applications. Those authors established that by applying N as late as R1, maximum yield could be obtained in treatments that otherwise would have developed N deficiency during the grain filling period. However, in the same study, final yield was significantly lowered when the full rate of N application was delayed until R3.

A larger impact of the timing of N availability on grain yield has been demonstrated when very severe N deficiencies during specific

**Abbreviations:** Nc, N content; NNI, N nutrition index; UAN, urea ammonium nitrate; %PostN, percent of total N accumulated post-silking

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periods of crop growth could be implemented. In a pot experiment, Subedi and Ma (2005) found that when N was withheld from planting to V8 or from V8 to R6, there were significant reductions in grain yield compared to plants with a continuous supply of N. However, when N was withheld only after R1, there was no negative effect on yield. These results highlight that extreme N stress during the time of floral initiation (around V8) can significantly reduce potential kernel number. Also, because peak N accumulation occurs during V10-R1 (Russelle et al., 1983; DeBruin et al., 2017), an inability to accumulate N during this time was detrimental to yield, but the lack of soil N after R1 had no effect. Pearson and Jacobs (1987) had similar findings in a field experiment on very sandy soil (96% sand) using frequent irrigation (every 1–3 days). Those authors also found that N supply during the period of spikelet initiation had the greatest effect on yield, while supplying N during the grain filling period increased grain N concentration by 1.3-fold but not grain yield. It should be emphasized that both Subedi and Ma (2005) and Pearson and Jacobs (1987) were able to achieve very rapid soil N depletion, and therefore also severe N stress, during targeted periods of the growing season. Maize grown across the U.S. Corn Belt is normally not subject to such extremes due to substantial soil N mineralization.

The quantity and timing of N accumulation in maize is a key concern because of the strong relationship between crop N accumulation and crop dry matter production. Nitrogen deficiency has been shown to reduce both leaf area and leaf N concentration, therefore reducing light capture and photosynthetic capacity (Vos et al., 2005). Furthermore, low biomass accumulation during the vegetative growth may also limit grain yield as harvest index has been shown to be fairly stable in maize (Duvick, 2005). Lastly, limits in crop growth rate during the critical period may lower established kernel number (Andrade et al., 1999) while N deficiency during the grain filling period causes reduction in kernel weight (Borrás and Otegui, 2001).

The timing of N application may impact other aspects crop development besides final yield, such as the source of grain N. The N needed to support kernel growth and grain yield originates from both remobilization of N taken up during the vegetative growth and N accumulated during the post-silking period (Christensen et al., 1981; Cliquet et al., 1990; Ta and Weiland, 1992; Moll et al., 1994; Ciampitti and Vyn, 2013; Chen et al., 2015a). Relative contributions of these two sources to grain N vary with management and environment; however, in a review Mueller and Vyn (2016) found that 57% of the grain N could be attributed to post-silking N uptake in hybrids released since 1991. Generally, post-silking N and N remobilization appear to have an inverse relationship (Pan et al., 1984, 1995; Coque and Gallais, 2007), but studies which prevented pollination have shown that high rates of post-silking N uptake occurred even when the sink was effectively removed (Pan et al., 1995; Yang et al., 2016). This suggests that post-silking N may be more strongly controlled by soil N availability than by crop N demand.

There is reason to believe modern hybrids may respond differently than older hybrids to the timing of N fertilizer application. It is known that there are genotypic differences in the timing of N accumulation (Beauchamp et al., 1976; Ma and Dwyer, 1998). Furthermore, there has been substantial evidence that modern hybrids accumulate more total N, and more N during the post-silking period (Mi et al., 2003; Ciampitti and Vyn, 2012; Haegele et al., 2013; Chen et al., 2015a; Woli et al., 2017). This increase in total N accumulation has likely contributed to the 4-fold increase in grain yields that have occurred in the United States since the 1930's (Duvick, 2005). Other sources of increased grain yield have been related to improved resilience to stresses such as high plant population, drought, and low soil N (McCullough et al., 1994; Byrne et al., 1995; Tollenaar and Lee, 2002; Reyes et al., 2015; DeBruin et al., 2017).

In a recent review study, Mueller and Vyn (2016) found that genotypes released after 1991 increased the proportion of their total N accumulation taken up post-silking (%PostN) when under increasing

levels of N stress at R1. However, in genotypes released prior to 1990, there was no change in %PostN with increased R1 N stress. Based on this, the authors concluded that the ability to continue to accumulate N post-silking, if N fertilizer becomes available, may be another mode of increased resilience to stress in modern maize hybrids.

Although it has been shown that N deficiency during vegetative growth can be remedied with N applications made just prior to R1, the mechanisms which allow or prevent maize yield recovery from vegetative N stress are not well understood. To answer these questions, the objectives of this research were to i) elucidate the physiological mechanisms that allow or constrain maize plant recovery from vegetative N stress if new fertilizer N becomes available at silking, and ii) determine whether the ability to recover from vegetative N stress has increased with hybrid improvement.

## 2. Materials and methods

### 2.1. Experimental design and site description

A two-year experiment was conducted in 2016 and 2017 at the Purdue Agriculture Center for Research and Education in West Lafayette, Indiana (40.471, –86.992) on a silty clay loam soil (fine-silty, mixed, mesic Typic Haplaquolls). The experiment was rain-fed and managed in a maize-soybean (*Glycine max* L.) rotation. A split-plot design was used with N treatment as the main plot and hybrid as the sub-plot with three replications. To establish a range of N stress levels at R1, N treatments included a 0 N control (0,0) with no N application and a conventional treatment of 220 kg N ha<sup>-1</sup> all applied as a V4 sidedress (220\_0, high control). Three additional N treatments all received a total of 220 kg N fertilizer ha<sup>-1</sup> applied either at V4 and/or R1. These treatments were: 0 kg N applied at V4 and 220 kg N applied at R1 (0\_220), 55 kg N applied at V4 and 165 kg N applied at R1 (55\_165), and 165 kg N applied at V4 and 55 kg N applied at R1 (165\_55). A total N rate of 220 kg N ha<sup>-1</sup> was used to ensure that the final N rate was not yield limiting (Camberato and Nielsen, 2017). Seven hybrids were included representing a subset of the DuPont Pioneer ERA hybrids (DeBruin et al., 2017) plus an additional more recent hybrid. Hybrids used in this experiment were (year of release): 352HYB (1946), 354A (1958), 3390 (1967), 3382 (1976), 3335 (1995), 34N42 (2003), and P1311 (2015). Hybrids will subsequently be referred to by their year of release. All hybrids were similar in crop relative maturity (111–114 days) and were planted at a common seeding rate resulting in an average established plant density of 78,500 plants ha<sup>-1</sup>. Although this density is above the common plant population used at the time of commercialization for many of these hybrids, a single “modern-era” plant population has been used in several other ERA studies (Campos et al., 2006; Haegele et al., 2013; Reyes et al., 2015).

The experiments were planted on 20 May 2016 and 18 May 2017. Plots were 4 rows wide (0.76 m row spacing) and 17 m long. There was no starter fertilizer used at planting but an in-furrow insecticide [Tefluthrin, (2,3,5,6-tetrafluoro-4-methylphenyl) methyl-(1a,3a)-(Z)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] was applied at planting to all hybrids to protect against corn rootworm (*Diabrotica virgifera virgifera*). Sidedress N applications were made at V4 and R1. At V4, N was applied as coulter-injected 28% urea ammonium nitrate (UAN). At R1, 28% UAN was surface banded by hand. To prevent lateral movement of applied N between treatments, 8 rows of border separated each N treatment. The V4 and R1 N applications were conducted 21 and 63 days after planting in 2016 and 16 and 59 days after planting in 2017.

Standard soil fertility samples were collected shortly after planting at a depth of 20 cm. Available phosphorous and exchangeable potassium were well above critical levels (49 and 173 ppm, respectively, as determined by Mehlich-3 extraction). Average pH was 6.5 and average organic matter was 4.7%. Residual soil nitrate-N concentration prior to the V4 sidedress application was 13 mg kg<sup>-1</sup> in the top 0–30 cm as determined by colorimetry after extraction with 2 M potassium

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