A

lthough there has been much research on the impact of the timing of N application on grain yield, the impacts of N application near the onset of the critical period (CP) on plant and ear growth, N accumulation rate, and N partitioning has been less well documented. Previous research has identified the CP in maize (Zea mays L.) as the time when maize ears are the most susceptible to stress (Kiniry and Ritchie, 1985; Uhart and Andrade, 1995b; D’Andrea et al., 2008). The CP is typically defined as the 30-d period around silking (Tollenaar et al., 1992; Andrade et al., 1993, 1999; Pagano et al., 2007; Neiff et al., 2016). During the CP, a wide range of stress conditions including nutrient deficiency, low C assimilates (due to reduced photosynthesis), heat, or drought can limit the number of pollinated ovules, increase kernel abortion after pollination, and decrease final kernel number (Setter and Meller, 1984; Artlip et al., 1995; Uhart and Andrade, 1995b; Zinselmeier et al., 1999; Below et al., 2000).

The impact of management and genotypes on ear growth rate (EGR) has been widely tested (Uhart and Andrade, 1995a; Andrade et al., 1999, 2002; Vega et al., 2001; Echarte et al., 2004; Can Late-Split Nitrogen Application Increase Ear Nitrogen Accumulation Rate During the Critical Period in Maize?

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ABSTRACT

Ear and plant growth rates during the critical period (CP) in maize (Zea mays L.) are known to affect grain yield, but little is known about how CP ear N accumulation and vegetative N dynamics respond to N fertilizer timing. In a 2-yr study, the influences of in-season split N applications on ear N accumulation rate (ENAR), ear growth rate (EGR), and stem and leaf dry matter and N accumulation were determined from 14 d before to 14 d after silking (R1). Up to eight N rate and timing treatments (0, 110, 155, 200, and 245 kg N ha$^{-1}$, either applied in a single application or with the last 45 kg ha$^{-1}$ delayed until V12) were imposed on four hybrids: two modern (P1498HR and P1360HR) and two released 20 yr ago (3335 and 3394). Both EGR and ENAR during the CP were very stable among N treatments and were only uniquely influenced by the zero N after R1. Both leaf N and ear N contents during the CP were highly conserved and were not affected by the timing of N application. Late-split N application resulted in less stem N remobilization in the 14 d after R1 than a single N application at moderate and high N rates. Despite sporadic hybrid group differences in EGR as the CP period progressed, the lower yielding older hybrids realized consistently lower presilking ENAR and higher postsilking ENAR. These data suggest that late-split N applications near the CP onset benefits stem N content, which may enhance N source capacity for developing ears but does not increase either ear or leaf N content during the CP.

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Abbreviations: AEGR, algebraic ear growth rate; CP, critical period; DM, dry matter; EGR, ear growth rate; ENAR, ear nitrogen accumulation rate; H1, older hybrids 3335 and 3394; H2, newer hybrids P1360HR and P1498HR; Nc, nitrogen content; PAR$^{2}$, pseudo-adjusted R$^{2}$; T1, time of whole-plant and ear biomass determination 14 d before silking; T2, time of ear biomass determination 7 d before silking; T3, time of whole-plant and ear biomass determination at silking; T4, time of ear biomass determination 7 d after silking; T5, time of whole-plant and ear biomass determination 14 d after silking; UAN, urea ammonium nitrate.


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Luque et al., 2006; Pagano et al., 2007; Rossini et al., 2011; Ciampitti et al., 2013), with the dominant conclusion that kernel number per plant and EGR are highly correlated. Although ear N accumulation rate (ENAR) has not been explored to the same degree, there is evidence that it may be influenced by management factors. Ciampitti et al. (2013) demonstrated across multiple N rates and plant densities that, over the entire CP, the proportional N content (Nc) allocation to maize ears increased with whole-plant N uptake rate. Other research has examined ENAR at weekly intervals after silking. Using $^{15}$N, Friedrich and Shrader (1979) found that vegetative N was remobilized to the developing ear at the same rate regardless of whether or not N was supplied after silking. Also measuring at weekly intervals, Lemcoff and Loomis (1986, 1994) algebraically calculated ENAR from R1 to 21 d after silking to demonstrate that N flux to the developing ear was higher with 224 vs. 56 kg N ha$^{-1}$, and that N flux to the ear increased with time after silking. In contrast, from ear length and Nc measurements in the 8 d before silking, Camberato et al. (1989) observed a significant main effect of hybrid and a hybrid × N rate interaction, but no impact of N rate alone on ear growth or Nc.

There is evidence that modern hybrids may be more likely to respond to in-season N application than older hybrids. It has been well documented that maize grain yield has improved over time through both breeding and management (Duvick, 2005; Tollenaar and Lee, 2011; Ciampitti and Vyn, 2012; Mueller and Vyn, 2016). At maturity, this increase has been largely attributed to increased kernel number per plant and tolerance of higher plant densities (Duvick, 1984; Tollenaar, 1991; Luque et al., 2006; Ci et al., 2013). During the CP, these traits have been associated with increased partitioning of biomass to the ear in modern compared with older hybrids (Echarte et al., 2004; Luque et al., 2006), and a lower threshold of plant growth rate needed for kernel formation (Echarte et al., 2004; D’Andrea et al., 2008).

Grain N is derived from a combination of postsilking N uptake and remobilization of vegetative N. Research using labeled $^{15}$N has consistently demonstrated that maize is capable of accumulating N applied to soil shortly before silking and throughout the grain-filling period (Ta and Weiland, 1992; de Oliveira Silva et al., 2017), but remobilized N also plays an important role. Prior to R1, there is little N remobilized amongst vegetative organs (Hanway, 1962; Camberato et al., 1989), but by R6, 27 to 49% of the total plant N present at R1 will be remobilized to the grain (Mueller and Vyn, 2016). Delaying remobilization from the leaves early in the grain-filling period is an important objective because of the crucial role leaf N has in the photosynthetic mechanisms of the plant (Gut and Amasino, 1997). The maintenance of green leaves, and therefore photosynthesis, has often been documented as a source of genetic improvement in maize over time (Fakorede and Mock, 1980; Duvick, 1984; Ma and Dwyer, 1998; Luque et al., 2006; Pommel et al., 2006). If in-season N application near the onset of the CP can ensure that concurrent plant N accumulation meets crop N demand around flowering, N remobilization from both the stem and the leaves may be delayed, allowing leaves to maintain Nc and photosynthesis during the grain-filling period.

In the present experiment, we hypothesized that N application near the onset of the CP would increase ear N accumulation and EGRs, as well as delay N remobilization from the stems and leaves. Additionally, we hypothesized that these physiological responses to late-split N application would be greater in modern hybrids than older hybrids. The specific objectives of this study were (i) to characterize the ear N accumulation and growth rate responses to late-split N application near the onset of the CP, (ii) to determine the impact of late-split N application on N accumulation and remobilization from the vegetative organs during the CP, and (iii) to investigate whether response to late-split N application differed in modern hybrids compared with hybrids released 20 yr ago.

**MATERIALS AND METHODS**

**Experiment Management and Design**

The experimental design is described in detail in Mueller et al. (2017). In brief, this rainfed maize experiment was performed at the Pinney Purdue Agricultural Center in Northwestern Indiana (41.438°N, 86.925°W) on a sandy-loam soil from 2014 to 2016. The analysis described in this manuscript is only from plant measurements conducted in 2015 and 2016, because resource constraints in 2014 did not allow for multiple in-season biomass sampling dates. A split-plot design was used with N rate as the main plot, hybrid as the subplot, and three replications. Plots were six rows wide (0.76-cm rows) and 27 m long. Experiments were planted on 8 May 2015 and 26 Apr. 2016. Fertilizer N rates in 2015 included 0, 155, 200, and 245 kg N ha$^{-1}$ either applied in a single N application at V3, or split with the last 45 kg N ha$^{-1}$ being delayed until V12. In 2016, an additional N rate of 110 kg N ha$^{-1}$ was added. Nitrogen application rate and timing treatments are further clarified in Table 1. All N treatments (including the zero N treatment) received banded starter fertilizer (19–17–0 N–P–K) at planting at a rate of 28 kg N ha$^{-1}$ being delayed until V12. In 2016, an additional N rate of 110 kg N ha$^{-1}$ was added. Nitrogen application rate and timing treatments are further clarified in Table 1. All N treatments (including the zero N treatment) received banded starter fertilizer (19–17–0 N–P–K) at planting at a rate of 28 kg N ha$^{-1}$ and 24 kg P$_2$O$_5$ ha$^{-1}$. The V3 N application was coulter-injected urea ammonium nitrate (UAN) applied on 4 June 2015 and 6 June 2017. The V12 UAN application was surface banded with the use of 360 Yield Center Y-Drops on 11 July 2015 and 8 July 2016.

The hybrids used were Pioneer 3394, 3335, P1498HR, and P1360HR. These hybrids were released in 1991, 1995, 2012, and 2014, respectively, and are of similar relative maturity (112–114 comparative relative maturity). All four hybrids were planted at a common density, and the average established plant population was 8,25 plants m$^{-2}$. To ensure that the older hybrids were not negatively affected by the use of a common plant density, an additional plant density experiment was also conducted in 2015 and 2016. That experiment, described further in Mueller et al.
Dry Matter and Nitrogen Accumulation

The date of 50% silking was determined as described in Mueller et al. (2017). To monitor the crop N and dry matter (DM) accumulation during the CP, whole-plant biomass samples were taken three times in the 28 d centered around silking. The first biomass harvest was conducted at approximately V13, when the experiment was estimated to be ~2 wk before R1. After the first biomass harvest (T1), ear shoots were collected five times at 7-d intervals (T1, T2, T3, T4, and T5) and whole-plant biomass was collected three times at 14-d intervals (T1, T3, and T5).

At each biomass collection, 10 consecutive plants were sampled. Plants were marked for biomass removal early in the growing season to ensure proper bordering between each biomass zone. At T1, T3, and T5, plants were partitioned into stems (including tassel and ear shanks), leaves (including husks), and apical ears (uppermost ear, kernels, and cobs) and secondary ears (kernels and cobs). Analysis of ear growth and Nc over time were based solely on the apical ears. Secondary ears, when present, were included in the calculations of whole-plant DM and whole-plant Nc. Abortion or termination of secondary ear growth typically occurs near or shortly after R1 (Prine, 1971), and no grain-producing secondary ears were observed in either year. All hybrids were sampled on the same day for all sampling dates.

Biomass samples were dried at 60°C to constant weight, weighed, and ground to 1 mm for N analysis. All N analysis was conducted by DuPont Pioneer using the combustion method.

Plant Biomass and Nitrogen Accumulation Measurements

To more accurately evaluate the influence of hybrid and N treatment on ear growth and N accumulation rates, plateau-quadratic (dry weight) and logistic (Nc) growth curves were fit to each plot. The independent variable (time) was thermal units (°C d), computed using a base temperature of 8°C (Ritchie and Nesmith, 1991). Thermal units were calculated from the initiation of ear elongation where zero equals R1: 200°C d (Otegui and Bonhomme, 1998; D’Andrea et al., 2008), calculated on a per-plot basis where R1 occurs at 200°C d. R1 was set at 200°C d, as opposed to zero, to allow for solving for values of EGR and ENAR at R1. This scaling based on the true R1 date per plot for each treatment allows for comparison of EGR and DM accumulation rates across treatments without the bias of hybrid silking date differences (Supplemental Table S1).

Linear and nonlinear models have been used extensively to model plant growth (Paine et al., 2012; Archontoulis and Miguez, 2015), including maize. It was necessary to fit different models to the ear dry weight and Nc because, although N accumulation rate peaked within the sampling period (resulting in an S-shaped logistic curve), the rate of ear dry weight gain continued to increase. Ear growth was nearly zero until shortly before silking, resulting in a plateau period before rapid growth began. Therefore, the plateau-quadratic model was a better fit for the dry weight data than exponential growth. Additionally, it should be noted that the sampling period evaluated in this manuscript falls within the “lag” period of ear growth (Johnson and Tanner, 1972; Tollenaar and Daynard, 1978; Lafitte and Edmeades, 1995), which precedes the linear growth phase.

Ear dry weight was fit with the plateau-quadratic model

\[
\text{Ear DM}_i = \begin{cases} 
  a + bx + ax^2 & \text{when } x > x_s \\
  a + bx + ax^2 & \text{when } x \leq x_s 
\end{cases} 
\]

where \(ijkl\) represents each unique hybrid (i), N treatment (j), block (k), and year (l) combination, x represents thermal units (°C d) centered at R1 (200°C d) and \(x_s = -0.5b/c\), which allows for the two segments to meet at \(x_s\) and for the first derivative of the plateau and quadratic curves with respect to x to coincide at \(x_s\). Ear Nc was fit with the logistic model

\[
\text{Ear Nc}_ijkl = \frac{Y_{\text{max}}}{1 + e^{-(x-x_{\text{iso}})}}
\]

where \(Y_{\text{max}}\) is the upper asymptote, k determines the steepness of the curve, and \(x_{\text{iso}}\) is the inflection point at which growth rate reaches its maximum. It should be acknowledged that ending ear shoot sampling 2 wk after R1 resulted in some logistic models that did not reach a saturation point. However, both the pseudo-adjusted \(R^2\) (PAR²) (SAS Institute, 2012) and relative RMSE (Wallach, 2006) indicated that these models fit well. Of the 168 separate curves fit (each plot in each year), PAR² ranged from 0.88 to 0.99 (median = 0.99) and 0.83 to 0.99 (median = 0.99) for EGR and ENAR, respectively. The relative RMSE ranged from 1.3 to 44.5% (median = 10.8%) and 0.2 to 43.5% (median = 3.3%) for EGR and ENAR, respectively.

After fitting these models to each plot, the first derivative was used to determine the growth rate at points of interest. The first derivative for DM accumulation (EGR) is given by the function

\[
\text{EGR}_i = \begin{cases} 
  b + 2ax & \text{when } x > x_s \\
  0 & \text{when } x \leq x_s 
\end{cases} 
\]

and the first derivative for N accumulation (ENAR) is given by the function:

\[
\text{ENAR}_i = \begin{cases} 
  c & \text{when } x > x_s \\
  0.5b/c & \text{when } x \leq x_s 
\end{cases} 
\]
ENAR_{plant} = \frac{dy}{dx} = \frac{Y_{max}ke^{k(x-x_0)}}{1 + e^{k(x-x_0)}}^2 \quad [4]

Derivatives were solved for x = 100 through x = 400 at 50°C d intervals where 200°C d represents the true time of 50% silking for a given plot (ijkl). We began at 100°C d because both EGR and ENAR were essentially zero prior to 100°C d.

The N accumulation rates of stems and leaves were calculated algebraically as the change in Nc or DM divided by the number of days between sampling. Whole-plant growth could not be adjusted to reflect true silking date as was done with the ears because there were only three sampling points. The N accumulation rates of the stem and leaves were different before and after silking, making a first-order linear model inappropriate. The disadvantage of algebraically calculated DM or N accumulation rates is that this assumes a constant rate of change between sampling dates. In 2015, there were 14 d between both T1 to T3 and T3 to T5 harvests. In 2016, there were 12 d between T1 and T3 and 13 d between T3 and T5 biomass harvests. Negative N accumulation rates values were assumed to represent N remobilization.

**Statistical Analysis**

Analysis of variance was performed using PROC MIXED in SAS 9.3 (SAS Institute, 2012). Due to the lack of homogeneity of variance between years, each year was analyzed separately with N treatment (main plot), hybrid (subplot), and sampling date treated as fixed effects and block and block × N rate (Error A) treated as random effects. Sampling date was modeled as a repeated measure with the heterogenous compound symmetry variance–covariance matrix. Orthogonal contrasts were used to compare the means of the older hybrids (3335 and 3394, H1) and the newer hybrids (P1360HR and P1498HR, H2). Means separation was accomplished using the LSmeans statement in SAS. Differences were considered significant at α = 0.05. For all plant organs, there were no hybrid × N treatment interactions for DM and Nc within any sampling date (p-values for the F-tests of hybrid × N treatment interactions always exceeded 0.05).

**RESULTS AND DISCUSSION**

**Weather**

The 2015 and 2016 growing seasons were similar in terms of temperature but differed greatly in distribution of precipitation (Table 2). In 2016, there was a severe shortage of rainfall during the vegetative growth stages encompassing the sensitive floral initiation period (Pearson and Jacobs, 1987; Otegui and Bonhomme, 1998). Although 2015 and 2016 were both similar to the 30-yr average for total precipitation in the month of May, in 2016, almost all the May precipitation fell in the first 15 d (Table 2). Therefore, precipitation from 16 May through 30 June 2016 was only 51 mm, or approximately one-third of the precipitation in 2015 (157 mm) and the 30-yr average (162 mm) for the same period. The 2016 precipitation returned to near-normal levels beginning the second week of July, corresponding with the onset of the CP.

Cumulative precipitation between the time of the split N application and T3 biomass sampling was 65 mm in 2015 and 18 mm in 2016. By T5, cumulative precipitation was 97 and 105 mm for 2015 and 2016, respectively.

Mean air temperature during the CP was 22 and 23°C for 2015 and 2016, respectively (CP spanned from 15 July 2015 to 12 Aug. 2015 and from 8 July 2016 to 2 Aug. 2016). The comparable 30-yr average temperature for 8 July through 12 August at this location was 22°C. Additionally, cumulative growing degree days for the season are reported in Mueller et al. (2017).

**General Impact of Treatments on Kernel Number and Final Grain Yield**

The effects of hybrid and N treatment in this experiment on final kernel number and grain yield are discussed extensively in Mueller et al. (2017). Briefly, overall average grain yields were 14 Mg ha⁻¹ in 2015 and 10 Mg ha⁻¹ in 2016, with the discrepancy between years being attributed to the vegetative drought stress in 2016. In both years, H2 outyelled H1 by ~10% (Supplemental Table S1). The higher grain yield in H2 was due to a 25 to 30% increase in kernel number per plant despite a 15% decrease in kernel weight relative to H1 (Supplemental Table S1). The increase in final kernel number in H2 vs. H1 appeared to be due to morphogenetic differences during pollination and grain filling, as there was no difference among hybrid groups in potential kernel number measured before pollination (hybrid means ranged from 736 to 772 in 2015 and from 710 to 814 in 2016).

Grain yield plateaued at the first nonzero N rate (155 or 110 kg N ha⁻¹) in both years. In 2016, the higher split N application treatments of 200 and 245 kg N ha⁻¹ in split applications significantly outyielded both 200 and 155 kg N ha⁻¹ applied in a single, early N application (Mueller et al., 2017). However, the lack of difference in yield response between the lowest and highest nonzero N rates (110 and 245 kg N ha⁻¹) in 2016 indicated that increased spatial variability due to the early-season moisture stress may have influenced grain yield results more than N rate.

**Leaf Dry Matter and Nitrogen Accumulation**

In both years, leaf DM (g plant⁻¹) more than doubled in the 2 wk before silking, increasing by 54 and 65% from T1 to T3 in 2015 and 2016, respectively (Table 3). After silking, leaf DM gain was much lower, increasing just 10% in 2015 and 19% in 2016 (averaged over all N treatments) from T3 to T5. There was no consistency in hybrid group impacts on leaf DM between 2015 and 2016, and there was little impact of N treatment on leaf DM.

Leaf Nc (g plant⁻¹), averaged over all N treatments, increased 30 and 37% in the 2 wk before R1 in 2015 and
Table 2. Cumulative precipitation and maximum and minimum temperatures at the Pinney Purdue Agriculture Center in 2015 and 2016 relative to the 30-yr average. The weather station was not recording temperature properly during 16 to 30 Sept. 2015, resulting in missing data.

<table>
<thead>
<tr>
<th>Month</th>
<th>Days</th>
<th>Precipitation (mm)</th>
<th>Temperature (max./min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>1–15</td>
<td>55</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>16–31</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>June</td>
<td>1–15</td>
<td>70</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>16–30</td>
<td>47</td>
<td>17</td>
</tr>
<tr>
<td>July</td>
<td>1–15</td>
<td>59</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>16–31</td>
<td>54</td>
<td>89</td>
</tr>
<tr>
<td>Aug.</td>
<td>1–15</td>
<td>36</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>16–31</td>
<td>50</td>
<td>169</td>
</tr>
<tr>
<td>Sep.</td>
<td>1–15</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>16–30</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Season total</td>
<td></td>
<td>438</td>
<td>429</td>
</tr>
</tbody>
</table>

Table 3. Dry matter accumulation for stems and leaves sampled ~14 d before R1 (T1), at R1 (T3), and 14 d after R1 (T5), plus that for ears collected at 7-d intervals beginning at T1 (T1, T2, T3, T4, and T5), in 2015 and 2016. Least squares means are presented as the average of four hybrids.

<table>
<thead>
<tr>
<th>N treatment</th>
<th>Ear</th>
<th>Leaf</th>
<th>Stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg N ha⁻¹</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>0</td>
<td>0.03</td>
<td>0.41</td>
<td>4.80</td>
</tr>
<tr>
<td>155</td>
<td>0.03</td>
<td>0.51</td>
<td>5.85</td>
</tr>
<tr>
<td>200</td>
<td>0.03</td>
<td>0.54</td>
<td>6.47</td>
</tr>
<tr>
<td>245</td>
<td>0.04</td>
<td>0.48</td>
<td>6.12</td>
</tr>
<tr>
<td>Mean</td>
<td>0.03D†</td>
<td>0.49D</td>
<td>5.76C</td>
</tr>
</tbody>
</table>

† Uppercase letters signify differences within sampling date at the significance level of p < 0.05.
‡ Uppercase letters signify differences between sampling dates at the significance level of p < 0.05.

2016, respectively (Table 4). In the 2 wk after silking, leaf Nc was mostly stable as leaf N concentration decreased in response to the continued increase in leaf DM. Leaf Nc in the 0 kg N ha⁻¹ treatment was lower than all other treatments at all three sampling dates in 2015, and within sampling dates, there were few differences among the nonzero N rates until T5 (Table 4). In 2015, a significant N treatment × sampling date interaction occurred due to 0 kg N ha⁻¹ being the only treatment to significantly decrease leaf Nc from T3 to T5, whereas the split treatment of 245 kg N ha⁻¹ was the only treatment to significantly increase leaf Nc during the same period. In 2016, there was no meaningful pattern in leaf Nc with N treatment during the CP. There was no difference between hybrid groups in leaf Nc in 2015, but in 2016, H2 achieved significantly higher leaf Nc than H1 at T3 and T5 (Supplemental Table S2).

The lack of leaf DM response to either N rate or timing during the CP, with the exception of the zero N control in 2015, was likely due to the overall lack of response to N rate in this experiment. Consistently across both years, leaf DM continued to accumulate in the 14 d after
Table 4. Nitrogen content for stems and leaves sampled approximately 14 d before R1 (T1), at R1 (T3), and 14 d after R1 (T5), plus that for ears collected at 7-d intervals beginning at T1 (T1, T2, T3, T4, and T5), in 2015 and 2016. Least squares means are presented as the average of four hybrids.

<table>
<thead>
<tr>
<th>N treatment</th>
<th>Ear N content</th>
<th>Leaf N content</th>
<th>Stem N content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>kg N ha⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.79</td>
<td>1.67</td>
<td>1.12</td>
</tr>
<tr>
<td>155</td>
<td>2.25</td>
<td>2.18</td>
<td>1.54</td>
</tr>
<tr>
<td>200</td>
<td>2.39</td>
<td>2.63</td>
<td>1.71</td>
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<td>200 Split</td>
<td>2.17</td>
<td>2.37</td>
<td>1.46</td>
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<tr>
<td>245</td>
<td>2.52</td>
<td>2.15</td>
<td>1.63</td>
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<tr>
<td>245 Split</td>
<td>2.68</td>
<td>2.04</td>
<td>1.62</td>
</tr>
<tr>
<td>Mean</td>
<td>2.30</td>
<td>2.13</td>
<td>1.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ear N content</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg N ha⁻¹</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>110</td>
<td>–</td>
</tr>
<tr>
<td>155</td>
<td>–</td>
</tr>
<tr>
<td>155 Split</td>
<td>–</td>
</tr>
<tr>
<td>200</td>
<td>–</td>
</tr>
<tr>
<td>200 Split</td>
<td>–</td>
</tr>
<tr>
<td>245</td>
<td>–</td>
</tr>
<tr>
<td>245 Split</td>
<td>–</td>
</tr>
<tr>
<td>Mean</td>
<td>–</td>
</tr>
</tbody>
</table>

† Lowercase letters signify differences within sampling date at the significance level of p < 0.05.
‡ Uppercase letters signify differences among sampling dates at the significance level of p < 0.05.

Silking, whereas leaf Nc did not, implying a decrease in leaf N concentration, an observation also noted by Kosgey et al. (2013). Anderson et al. (1984) found that leaf Nc remained stable as late as 30 d after silking in single-eared hybrids. Because leaf N remobilization reduces whole-plant photosynthesis (Mu et al., 2016), the conservation of leaf N shortly after silking is usually considered a positive trait. Collectively, our data and earlier data suggest that leaf Nc is highly conserved in the early postsilking period and little affected by N management except in extreme cases. Large losses in leaf Nc during the CP were only found under the zero N control.

Stem Dry Matter and Nitrogen Accumulation

In both years, stem DM increased rapidly from T1 to T3 (an increase of 75% in 2015 and 90% in 2016) and then continued to increase (8% gain, 2015) or plateau (2% gain, 2016) from T3 to T5. In contrast with previous research finding older hybrids to have higher R1 stem DM (Chen et al., 2015), we found no consistent influence of hybrid group on stem DM during the CP (Supplemental Table S2).

Stem Nc was much more sensitive to N treatment than the leaves or ears, as it was significantly affected by both N rate and timing in both years (Fig. 1, Table 4). In general, over the course of the CP, the impact of the N treatments became clearer as the highest rate and split N treatments achieved higher stem Nc than the single-timing applications with lower N rates. Although the time of peak stem Nc differed by year, in both years we observed rapid stem Nc remobilization in the 2 wk after R1. In 2015, stem Nc decline was much more pronounced in the treatments of 0, 155, and 200 kg N ha⁻¹ (dropping by 24–27% in the 2 wk postsilking). In contrast, the highest N rate and split N treatments (200 [split], 245, and 245 kg N ha⁻¹ [split]) maintained higher stem Nc status, decreasing only 11 to 14% during the same 14-d period. Thus, the split application of 200 kg N ha⁻¹ achieved the same stem Nc as the higher N treatment of 245 kg N ha⁻¹ (in a single application or split) (Table 4). In 2016, a similar pattern was evident in which the 0 kg N ha⁻¹ and single N application treatments realized greater remobilization from T3 to T5 (ranging 18–29%) than the split N application plots (ranging 6–14%). Split application of 200 kg N ha⁻¹ was the only N treatment that did not significantly decline in stem Nc from T3 to T5 in 2016 (Fig. 1, Table 4).

Driven by the stem N remobilization after R1, stem N concentration in the 0 kg N ha⁻¹ control dropped from 0.48% at T1 to 0.18% at T5 in 2015 and from 0.69 to 0.31% during the same time period in 2016. For comparison, averaged over all nonzero N rates, stem N concentration was 1.3 and 1.1% at T1 and 0.52 and 0.68% at T5 in 2015 and 2016, respectively. These stem N concentrations are similar to those reported by Soufizadeh et al. (2017) for the bowing hybrid maize. There was no hybrid influence on stem Nc (Supplemental Table S2).

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The delay in stem Nc remobilization in the highest N rates and split N applications agrees with Crawford et al. (1982), who found that presence of N in the rooting medium after silking delayed remobilization from the vegetative organs. Our data confirm the stem’s role as an important transitory organ and N source for the developing ear (Swank et al., 1982; Pearson and Jacobs, 1987; Cliquet et al., 1990; Ta and Weiland, 1992; Kosgey et al., 2013).

The rapid loss of stem N in the 14 d after silking in both years indicated net Nc remobilization from the stem to the developing ear. This confirms the results previously reported by Swank et al. (1982) and Kosgey et al. (2013). Swank et al. (1982) showed little increase in stem Nc from 5 d before silking until 12 d after silking, followed by remobilization from 12 to 18 d after silking and for the duration of the postsilking period. Similarly, Kosgey et al. (2013) also reported a rapid loss of stalk Nc in the 400°C d interval after silking when 270 kg N ha⁻¹ was applied. However, under a zero N control, Kosgey et al. (2013) found no evidence of stem Nc remobilization in the 12 d after silking and instead noted a slight increase during the early grain-filling period. In contrast, in the zero N control treatments, we found a decline in stem Nc of 27 and 29% in the 14 d after silking in 2015 and 2016, respectively.

Although husks, cobs, and shanks were not analyzed separately in this experiment, we did not find evidence of N flux out of the stems greater than that into the ear, as was suggested by Crawford et al. (1982) and Cliquet et al. (1990). In the 14 d after R1, average stem N loss was 101.2 and 75.0 mg N plant⁻¹ for 2015 and 2016, respectively, compared with ear N gains of 532.7 and 352.0 mg N plant⁻¹ during the same period (average of all four hybrids, Table 4). This suggests that stem Nc remobilization contributed 19 and 21% of the ear N accumulation in the first 2 wk after silking in 2015 and 2016, respectively. The significant impacts of N treatments before and after silking are evidence that stem Nc is the most sensitive indicator of plant N status during the CP, a preferred site of excess N accumulation prior to grain filling, and the most important source of remobilized N during the key period of pollination, fertilization, and kernel set.

Driven by the increase in stem Nc, whole-plant Nc was significantly higher at the highest N rate (245 kg N ha⁻¹, split and single) in 2015 and under the two high-rate split applications (200 and 245 kg N ha⁻¹) in 2016. These gains contributed to higher total Nc and N recovery efficiencies at R6 with the use of the split N application compared with single N timing at N rates of 200 (2015 and 2016) and 245 (2016) kg ha⁻¹ (Mueller et al., 2017). However, because only the zero N control was yield limiting in this experiment, these differences in whole-plant Nc and N recovery efficiency did not result in increased grain yield among the nonzero N treatments.

### Ear Growth Rate

As expected, ear DM and Nc increased significantly between each biomass sampling beginning from T2 (DM, Table 3) or T1 (Nc, Table 4). The hybrid effect on ear DM and Nc, as measured through biomass sampling, was heavily influenced by silking date (Supplemental Table S2), which differed among hybrid groups but not among N treatments (Supplemental Table S1). In both years, H1 reached R1 about 2 d before H2, resulting in H1 having higher ear dry weight and ear Nc at all sampling points from T3 to T5 (Supplemental Table S2). By correcting to the true silking date for each plot, a more meaningful pattern could be observed that was not biased by differences in phenology.

There were some small EGR differences between the hybrid groups in 2015 and 2016 (Table 5). However, the lack of consistency between the 2 yr and the small differences suggest that EGR was not meaningfully different between these two hybrid groups in the 2 wk after flowering.

Several previous authors have explored the impact of genotype on the partitioning of DM to the ear during the CP. Comparing hybrids released from 1965 through
Table 5. Estimated ear growth rate for ears in 2015 and 2016 based on plateau-quadratic model for H1 (3335 and 3394) and H2 (P1360HR and P1498HR). Least squares means are presented as the average of either six (2015) or eight (2016) N treatments.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>x_c †</th>
<th>Ear growth rate</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(°C d)</td>
<td>100°C d</td>
</tr>
<tr>
<td>H1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>157.6a</td>
<td>0.00</td>
<td>1.35b‡</td>
</tr>
<tr>
<td>H2</td>
<td>158.0b</td>
<td>0.43</td>
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</table>

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>x_c †</th>
<th>Ear growth rate</th>
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<tr>
<td></td>
<td>(°C d)</td>
<td>100°C d</td>
</tr>
<tr>
<td>H1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>166.2</td>
<td>0.77</td>
<td>6.64</td>
</tr>
<tr>
<td>H2</td>
<td>153.3</td>
<td>1.14</td>
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</tbody>
</table>

† Least squares means assigned different letters within year and sampling date are significantly different from each other at the significance level of p < 0.05.

The lack of treatment impacts on EGR during the CP indicates that differences in final kernel number are likely due to differences in kernel abortion, rather than differences in floret number and pollinated kernels (Rossini et al., 2012). Rattalino Edreira and Otegui (2013) reported that loss in kernel number from environmental stress can be caused by reduced plant growth rate during the CP, change in biomass partitioning to the ear during the CP, and other constraints not directly related to assimilate allocation to the ear. Our data indicate that the differences in final kernel number were affected by constraints not related to assimilate allocation to the ear during the CP.

An alternative explanation for the lack of hybrid group differences realized in this experiment was proposed by Egli (2015), which is that hybrid improvement has resulted from increased kernel number per ear in hybrids in Argentina and China, but not in the United States or Canada. Several US and Canadian studies have found that kernel number per plant has remained stable and increased grain yield has been driven by higher plant densities and higher kernel weight (Tollenaar et al., 1992; Duvick, 2005; Chen et al., 2017; DeBruin et al., 2017). Although the H2 hybrids used in this experiment achieved significantly higher kernel number per plant, and significantly lower 200-kernel weight (Supplemental Table S1), it is possible that these differences between hybrids groups were too small to be detected during the CP. Gonzalez et al. (2018) also found that genetic differences in the relationship between kernel number per plant and plant growth rate at R1 were only evident under very low plant populations (3.5 plants m⁻²) and differences among hybrids were not detected at moderate (7 plants m⁻²) or high (14 plants m⁻²) plant densities.

Nitrogen treatments did not impact EGR during the CP in 2015 with the exception of the zero N rate being lower, but even then not until 300°C d (data not shown). There was no N treatment effect on EGR in 2016.

Other authors have also investigated the impact of N availability on biomass allocation to the ear with mixed conclusions. Comparing 0 and 200 kg N ha⁻¹ applied at V6, Rossini et al. (2011) reported that biomass allocation to the ear during the CP was at least partially attributable to increased biomass partitioning to the ear during the CP. Studies comparing crowding-tolerant and intolerant hybrids found that crowding-tolerant hybrids realized higher biomass allocation to ears at silking (Pagano et al., 2007; Rossini et al., 2011). In the present study, after adjusting for silking date, H2 achieved ear dry weights ~0.5 g plant⁻¹ greater than H1 at R1 in 2015 (Eq. [1], data not shown), but there was no difference in R1 ear dry weights among hybrids in 2016.

The most common method of evaluating EGR in previous literature has been calculating the algebraic EGR over the entire CP (AEGR). Using this method, many experiments, including Andrade et al. (1999), Echarte et al. (2004), Maddonni and Otegui (2004), and Pagano and Maddonni (2007), reported a strong relationship between AEGR and final kernel number. For comparison, we solved for ear dry weight at 400°C d (Eq. [1]) to evaluate whether there was a hybrid group effect on ear DM at the end of the lag period. There was no significant difference in 400°C d ear DM between hybrid groups (overall average of 34.4 and 22.8 g plant⁻¹ in 2015 and 2016, respectively). The absence of hybrid group effect on ear DM at the end of the lag phase and onset of the grain-filling period agrees with the overall lack of difference in total plant DM between hybrid groups and is supported by Maddonni and Otegui (2004), who reported that ear DM at the onset of active kernel growth was strongly related to whole-plant DM at the beginning of the CP.

To compare our results with previous research, we evaluated the correlation of ear DM at 400°C d with final kernel number (Supplemental Fig. S1). This is comparable because AEGR has a 1:1 relationship with ear DM at the end of the CP due to the starting ear DM being approximately zero. We found no relationship between 400°C d ear DM and final kernel number for either hybrid group (Supplemental Fig. S1), further supporting the overall lack of hybrid group influence on the EGRs for the genotypes used in this experiment.

1997, Luque et al. (2006) and Echarte et al. (2004) both concluded that increased kernel number in newer hybrids was at least partially attributable to increased biomass partitioning to the ear during the CP. Studies comparing cropping-tolerant and intolerant hybrids found that cropping-tolerant hybrids realized higher biomass allocation to ears at silking. Maddonni and Otegui (2004), reported a strong relationship between AEGR and final kernel number. For comparison, we solved for ear dry weight at 400°C d (Eq. [1]) to evaluate whether there was a hybrid group effect on ear DM at the end of the lag period. There was no significant difference in 400°C d ear DM between hybrid groups (overall average of 34.4 and 22.8 g plant⁻¹ in 2015 and 2016, respectively). The absence of hybrid group effect on ear DM at the end of the lag phase and onset of the grain-filling period agrees with the overall lack of difference in total plant DM between hybrid groups and is supported by Maddonni and Otegui (2004), who reported that ear DM at the onset of active kernel growth was strongly related to whole-plant DM at the beginning of the CP.

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ear during the CP was not reduced by the lower N rate. In contrast, Peng et al. (2016) recently demonstrated increased ear DM 6 d before silking and 8 d after silking with a split application of 300 kg N ha\(^{-1}\) (150 kg ha\(^{-1}\) at planting, 75 kg ha\(^{-1}\) at V6, and 75 kg ha\(^{-1}\) at V10) compared with either 0 or 150 kg N ha\(^{-1}\) applied at planting. Examining N rates of 0 to 180 kg N ha\(^{-1}\) applied either at planting or V6, Uhart and Andrade (1995a) found that AEGR tended to increase with increasing N rate, but the increase in AEGR was proportional to the increase in crop growth rate.

**Ear Nitrogen Accumulation Rate**

Ear N accumulation rate was influenced by hybrid and N treatments (Fig. 2 and 3). The hybrid groups differed primarily in the temporal pattern of ENAR, which was consistent in both years despite the presence of moisture stress in 2016. The modern hybrids (H2) achieved higher ENAR until 200°C d, whereas H1 realized higher ENAR after silking. Additionally, H1 ENAR peaked at significantly higher maximum values (\(x_{m}\), Fig. 2). All hybrids reached peak ENAR between 314 and 367°C d, which coincides with the end of the ear elongation period (Otegui and Bonhomme, 1998).

Quantifying the continuous ENAR provides important insight into the impact of N availability on N allocation to the ear during the CP (Fig. 3). The zero ENAR was significantly lower than all other N treatments, but not until R1 (2015) or 350°C d (2016). The delay in the zero N rate differentiating from the other N treatments in 2016 may be partially explained by the lower overall EGR and ENAR (Sousifadegh et al., 2017) due to limited precipitation during the vegetative growth. Among the nonzero N treatments, neither N timing nor N rate affected ENAR or maximum ENAR (\(x_{m}\)). Peak ENAR occurred at nearly the same thermal time in both years, indicating that this trait is not highly influenced by environment.

The impact of genotype or N treatments on the ENAR beginning before R1 at 100°C d (approximately half-way between the initiation of ear elongation and R1) until 400°C d has not been previously reported. Earlier literature has focused on the ENAR either before or after silking, or

![Fig. 2. Effect of hybrid groups on ear N accumulation rates in (A) 2015 and (B) 2016. H1 denotes the older hybrids (3335 and 3394) and H2 denotes the newer hybrids (P1360HR and P1498HR). The x axis represents cumulative growing degree day units since the initiation of ear elongation. R1 occurred at 200°C d. The LSD values are represented with bars. Means are presented as the average of six (2015) or eight (2016) N treatments. ns, nonsignificant.](image)

![Fig. 3. Effect of N treatments on ear N accumulation rates in (A) 2015 and (B) 2016. The x axis represents cumulative growing degree day units since the initiation of ear elongation. R1 occurred at 200°C d. The LSD values are represented with bars. Means are presented as the average of four hybrids. An S following a treatment rate indicates a split application. ns, nonsignificant.](image)
generalized over the entire period. Crawford et al. (1982) used a similar method as employed in this manuscript by using the first derivative of polynomial equations to represent N flux into and out of plant organs during the postsilking period. However, measurement of ear N accumulation did not begin until R1, and biomass measurements were only conducted at 12-d intervals. This may explain why these authors did not detect a peak in ENAR shortly after R1 as observed in the present experiment. Another key difference between this experiment and that of Crawford et al. (1982) was the separation of the cob and kernels beginning 12 d after silking in the latter study, which may have caused the difference in timing of ENAR peaks.

Crawford et al. (1982) reported that postsilking fluxes of N into developing ears were identical regardless of N supply to the roots in the 36 d after silking. Although the present study did not find a significant effect of late-split N application on ENAR in the 400°C d after the beginning of ear elongation, Pearson and Jacobs (1987) demonstrated that the application of 15 kg N ha⁻¹ wk⁻¹ after silking nearly doubled the N accumulation rate in the kernels during the linear phase of grain filling in a single hybrid grown under irrigation on a coarse sandy soil. This response occurred regardless of N fertilization rate (either 20 or 60 kg N ha⁻¹) split into weekly N applications from 30 d after sowing until anthesis. This may indicate that increased ENAR after split N applications might have been detected if ear N sampling had continued into the linear phase of ear growth. However, biomass sampling in the present experiment was stopped at 2 wk after silking because we were primarily interested in the impact of treatments during the CP.

CONCLUSIONS

It is widely recognized that the CP in maize is crucial for kernel establishment. We set out to determine the impacts of late-split N application on ear N accumulation and growth rates, to study N dynamics in the vegetative organs during the CP, and to understand if modern hybrids responded differently to late-split N applications than hybrids released 20 yr ago. To our knowledge, this is the first characterization of ENAR and EGR measured at multiple points before and after R1 prior to the linear period of ear growth in maize.

Our results showed that in the 14 d after R1, split N applications increased total N accumulation and decreased stem Nc remobilization. However, neither N rate (with the exception of the zero N control), nor the application of 45 kg N ha⁻¹ near the onset of the CP, influenced leaf Nc, EGR, or ENAR during the 2 wk before and after silking. We demonstrated that both ENAR and EGR are highly conserved during the CP and are not likely to be influenced by non-yield-limiting differences in N rate or relatively small differences in hybrid year of release. Lastly, we did not find evidence of changes in N allocation or DM distribution among the stems, leaves, and ears during the CP in the last 20 yr of hybrid improvement.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Acknowledgments

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