

## RESEARCH

# The Response of Leaf Photosynthesis and Dry Matter Accumulation to Nitrogen Supply in an Older and a Newer Maize Hybrid

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## ABSTRACT

Nitrogen use efficiency is higher in newer than in older maize (*Zea mays* L.) hybrids, but the physiological mechanisms underlying differences in N-use efficiency are unknown. The objective of this study was to quantify differences between an older and a newer maize hybrid in their response to N availability throughout the life cycle at both the leaf and the whole-plant level. An older and a newer maize hybrid were grown in a field hydroponic system located near Guelph, ON, in 2005 at a high and a low N level. Leaf carbon exchange rate (CER), chlorophyll index, and the thylakoid electron transport rate (ETR) were measured weekly from 2 wk presilking to 8 wk postsilking. Plant-component dry matter and N content were determined from 1 wk presilking to maturity. At the leaf level, leaf CER declined during the grain-filling period, and the decline was greater under low than high N availability. The decline in leaf CER during the grain-filling period was less in the newer than in the older hybrid under both high and low N availability, and differences in leaf CER were associated most strongly with a reduction in leaf CER per unit absorbed photosynthetic photon flux density. At the whole-plant level, reduction in grain yield in low vs. high N was greater in the older than in the newer hybrid. The hybrid  $\times$  N interaction for grain yield was attributable predominantly to a greater decline in the proportion of dry matter allocated to the grain in the older hybrid.

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**Abbreviations:** CER, carbon exchange rate; CER Abs<sup>-1</sup>, CER per unit absorbed photosynthetic photon flux density; CHU, crop heat units; ETR, thylakoid electron transport rate; HI, harvest index; HLN<sub>1</sub>, high N level switched to low N level at 1 wk presilking; HLN<sub>2</sub>, high N level switched to low N level at 2 wk postsilking; HN, high N availability; LN, low N availability; Na, N content per unit leaf area; PPF, photosynthetic photon flux density; PSII, Photosystem II.

**G**RAIN YIELDS OF NEWER MAIZE (*Zea mays* L.) hybrids are greater than those of older hybrids across nitrogen (N) levels (Castleberry et al., 1984; Duvick, 1984; Sangoi et al., 2001; Ding et al., 2005). Differences among older and newer maize hybrids have been associated, in general, with increased stress tolerance that often results in higher rates of dry matter accumulation during the grain-filling period (Tollenaar and Wu, 1999) and reduced rates of visible leaf senescence in newer hybrids (e.g., Valentinuz and Tollenaar, 2004). Ding et al. (2005) showed that the difference in dry matter accumulation between maize grown under high and low N conditions was associated with sustained higher leaf carbon exchange rate (CER) and chlorophyll content during the grain-filling period and that this response to N availability

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was consistent for a set of older and newer maize hybrids. An understanding of the mechanisms that control the capacity of the photosynthetic apparatus to sustain CER throughout the grain-filling period in both newer vs. older hybrids and under high vs. low N availability could be instrumental to the genetic improvement of N-use efficiency in maize.

Leaf CER is influenced by leaf N content, since a large proportion of the N in leaves is incorporated into photosynthesis-related functions (Evans, 1989). Leaf CER is linearly related to N content per unit leaf area ( $N_a$ ) at low levels of  $N_a$ , and CER levels off when  $N_a$  approaches  $2 \text{ g m}^{-2}$  (Wolfe et al., 1988; Sinclair and Horie, 1989; McCullough et al., 1994a; Vos et al., 2005; Paponov et al., 2005). Differences among maize hybrids in leaf CER might be attributable to differences in either  $N_a$  and/or leaf CER per unit  $N_a$ . Greater N uptake in newer than in older hybrids at low N availability has been reported for the early vegetative phase (McCullough et al., 1994a) and for the postsilking period (Rajcan and Tollenaar, 1999). The capacity of the photosynthetic apparatus to sustain leaf CER under relatively low N conditions could also be related to a more efficient partitioning of N within the leaf. Partitioning of leaf N between photosynthetic and nonphotosynthetic components and, within the photosynthetic apparatus, among light harvesting, electron transport, and carbon reduction can influence the photosynthetic N-use efficiency (Pons et al., 1994). Partitioning of leaf N among the various components is regulated by N supply, available light energy, and the ratio of red to far-red radiation (Lawlor, 1994) and has been shown to vary among plant species (Pons et al., 1994).

The objective of this study was to quantify differences between an older and a newer maize hybrid in their response to N availability throughout the life cycle at both the leaf and the whole-plant level. At the leaf level, CER, thylakoid electron transport rate (ETR), and the chlorophyll index were monitored from two weeks pre-silking to eight weeks postsilking. Plants were grown in a field hydroponic system to ensure that when measurements were made, leaves were sunlit throughout the day. At the whole-plant level, plant-component dry matter and N content were measured throughout the life cycle, and dry matter, N content, and grain yield were measured at maturity. In an accompanying paper, we will report gene expression profiles of the hybrids exposed to the N treatments discussed in this study.

## MATERIALS AND METHODS

### Plant Material, Experimental Design, and Treatments

The study was performed at the Arkell Research Station near Guelph, ON (43°39' N, 80°25' W, and 375 m above sea level), using an older maize hybrid 'Pride 5' (first released in

1959) and a newer maize hybrid 'NK N25-J7' (first released in 2004) grown in a field hydroponic system (Ying et al., 2002) during the 2005 growing season. Relative maturity of Pride 5 is 2600 crop heat units (CHU; Brown and Bootsma, 1993), and relative maturity of NK N25-J7 is 2700 CHU. Plants were grown in 22.5-L plastic pails filled with "turface," a baked montmorillonite clay (International Minerals and Chemical, Blue Mountain, MS), and irrigated with a nutrient solution as described below. A timer controlled application of nutrient solution to the pails. The duration of the application was set so that nutrient solution drained from the bottom of all pails. Pails were arranged at 0.35 m between pail centers within a row and 1.42 m between rows. Three seeds were sown per pail on 24 May for NK N25-J7 and 3 June for Pride 5, to match the silking dates of the two hybrids, and pails were thinned at the 4-leaftip stage (Tollenaar et al., 1979) to two plants per pail, resulting in a plant density of  $4.0 \text{ plants m}^{-2}$ . Weeds were effectively controlled by hand. The two hybrids were planted in adjacent blocks to avoid competition effects. Within each hybrid block, four N treatments were randomly assigned to plots comprised of either four or six 8.5-m-long rows. The outside rows of the plots and plants in one pail on both ends of the treatment rows were used as a border. The four N treatments were (i) high N (20 mM N; designated HN); (ii) low N (4 mM N; designated LN); (iii) high N switched to low N at 1 wk pre-silking (designated  $HLN_1$ ); and (iv) high N switched to low N at 2 wk postsilking (designated  $HLN_2$ ). Nutrient solution was supplied to the pails one to four times daily, depending on stage of development, by diluting (100X) a concentrated nutrient mixture. The concentrated nutrient mixture was made by dissolving 3.29 kg (LN) or 16.47 kg (HN) 34-0-0 soluble fertilizer ( $\text{NH}_4\text{NO}_3$ ), 3.74 kg  $\text{HPO}_4$  ( $850 \text{ g kg}^{-1}$ ), 7.5 kg  $\text{KHCO}_3$  (0-0-47), 8.0 kg  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ( $100 \text{ g kg}^{-1}$ ), and 0.6 kg of Plant-Prod chelated micronutrient mix in a 200-L barrel (all nutrients supplied by Plant Products, Bramalea, ON). Concentration of Ca in the water was  $>100 \text{ g kg}^{-1}$ , and the pH of the diluted solution was adjusted to 5.5 to 6.0 by adding HCl to the concentrated nutrient mixture. For the treatments  $HLN_1$  and  $HLN_2$ , N in the turface rooting medium was partly removed when the nutrient solution was switched from high to low N. In this procedure, holes at the bottom of the pail were blocked, pails were filled with water, and water was drained after 3 h. The procedure was repeated 1 h after the water had completely been drained from the pail. Nitrogen content of the turface rooting medium (i.e., N- $\text{NO}_3$  plus N- $\text{NH}_4$ ) of the N treatments was analyzed at 4 wk postsilking using the methodology described by Maynard and Kalra (1993), and results showed that the N concentration in the  $HLN_1$  and  $HLN_2$  treatments was similar to that in the LN treatments (i.e., about 0.4 g N/pail in LN vs. 4 g N/pail in HN).

The field hydroponic system that was used in this study has been utilized for over two decades in various studies to control and optimize growing conditions for maize (e.g., Tollenaar and Migus, 1984), to manipulate abiotic factors influencing the growth of maize (e.g., Echarte and Tollenaar, 2006), and to be able to obtain meaningful estimates of leaf photosynthetic rate of plants grown in a crop canopy (e.g., Ying et al., 2002). The hydroponic

system has also been extensively used in indoor studies (e.g., Tollenaar and Migus, 1984; McCullough et al., 1994a,b).

## Measurements

Silking dates were recorded for each genotype and N treatment as the dates when 50% of the plants ( $n = 20$ ) presented at least one emerged silk from the husks. Silking dates occurred between 22 and 26 July for the older hybrid and between 21 and 23 July for the newer hybrid, depending on the N treatment.

The leaf carbon exchange rate and the ETR were measured during clear-sky days on light-adapted leaves. Measurements were taken on the first leaf above the topmost ear. Leaf CER was measured with a portable, open-flow gas exchange system LI-6400 (LI-COR, Lincoln, NE) at 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD) at the leaf surface using the 6400-02 LED light source (LI-COR). Fluorescence readings to estimate ETR were taken using a Leaf Chamber Fluorometer (Model 6400-40, LI-COR) and CER was measured across the same circular 2-cm<sup>2</sup> leaf area exposed in the gas exchange chamber. The flow rate of air through the sample chamber was set at 350  $\mu\text{mol s}^{-1}$ , and leaf temperature was maintained at  $\sim 28^\circ\text{C}$ , depending on the ambient temperature of the day, using the chamber's thermoelectric coolers. The sample chamber CO<sub>2</sub> concentration was adjusted to 400  $\mu\text{mol CO}_2/\text{mol air}$  using the system's CO<sub>2</sub> injector (model 6400-01, LI-COR). The modulation frequency of the measuring light was 10 kHz under actinic illumination and increased to 20 kHz during saturating pulses. The measuring light intensity was set to its maximum level (Level 10). The saturating pulse was set to a multiple type, with a total duration of 1.2 s and a decreasing intensity of 10, 6, and 4. Electron transport rate (the actual photon-flux-driving Photosystem II [PSII]) was estimated by the LI-6400 as:

$$\text{ETR} = (\text{Fm}' - \text{Fs}/\text{Fm}') \times f \times I \times \alpha_{\text{leaf}} \quad [1]$$

where Fm' is the maximal fluorescence during a saturating light flash, Fs is "steady-state" fluorescence,  $f$  is the fraction of absorbed quanta that is used by PSII (typically assumed to be 0.4 for C4 plants),  $I$  is incident PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and  $\alpha_{\text{leaf}}$  is leaf absorptance. Leaf absorptance was estimated using chlorophyll index values (see below). At the days that leaf gas exchange measurements were taken, designated plants were moved outside the crop canopy at 0900 and positioned such that the measured leaf was sunlit. Two rounds of measurements of leaf CER and ETR were taken per day (from 1045 to 1245 and from 1245 to 1445).

Leaf chlorophyll index was estimated using a SPAD-502 m (Minolta, Plainfield, IL). Measurements were taken during the afternoon and recorded as the mean of 10 measurements taken along the ear blade (5 on each side of the leaf rib). A linear relationship between leaf chlorophyll content and SPAD meter readings was previously determined (Dwyer et al., 1991). Leaf CER, ETR, and SPAD meter readings were measured weekly on the last fully expanded leaf (before 1 wk presilking) or on the leaf subtending the topmost ear (after 1 wk presilking) of 8 plants in the treatment rows of each subplot, with the exception at silking and 4 wk postsilking, when measurements were made on 2 d (means for these dates represented two times 8 plants/subplot).

Plants were destructively sampled at 1 wk presilking, silking, 3 wk postsilking, and at physiological maturity and separated into stem plus tassel, leaves, ears, and roots. Number of plants sampled at each of the harvest dates was 8 plants per treatment, except for the final harvest, when 16 plants per treatment were sampled. Samples were dried at 80°C until constant weight. Dried samples were ground to pass through a 1-mm screen and analyzed for total N. Total N was determined using the methodology described in AOAC (1995). Leaf area of the sampled plants was recorded at silking and at 3 wk postsilking using a LI-3000 area meter (LI-COR).

## Calculations and Data Analysis

Differences in CER between hybrids, N treatments, and stages of development that could be attributed to differences in absorptance of incident PPFD or differences in the efficiency by which the absorbed PPFD was utilized in leaf photosynthesis were estimated using CER, chlorophyll index, and ETR data. The relationship between PPFD absorptance by a leaf and its mean chlorophyll index (SPAD) value was quantified according to Earl and Tollenaar (1997) as:

$$\text{Absorptance} = 0.409 + 0.528 \times (1 - e^{-0.0429 \times \text{SPAD}}) \quad [2]$$

The efficiency by which absorbed photons was utilized in photosynthesis was estimated both by chlorophyll fluorescence (i.e., ETR) and by CER per unit absorbed PPFD (CER Abs<sup>-1</sup>). To account for the effect of differences in visual leaf senescence among plants within a plot, CER, SPAD, and ETR measurements were made only on the green portion of nonsenescent leaves (i.e., leaves that remained visually green for >50% of their maximum area), and means per treatment after 6 wk postsilking were multiplied by the proportion of plants in a plot of 20 plants that had a nonsenescent ear leaf. Plant-component N content and N content per unit of leaf area (Na) were estimated from plant-component dry matter, plant-component N concentration, and leaf area per plant.

Differences between hybrids, N treatments, and stages of development in any of the measured and estimated variables were assessed using  $t$  tests. The experimental unit for the CER, SPAD, and ETR measurements was 1 plant, and the experimental unit for data collected in the destructive harvests was one pail (2 plants).

## RESULTS AND DISCUSSION

### Leaf Carbon Exchange Rate

Leaf CER at high N availability was high and similar between hybrids until approximately 4 wk postsilking (mean = 48  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $p > 0.05$ ; Fig. 1), and subsequently, leaf CER declined at a greater rate in the older than in the newer hybrid (Fig. 1). Linear rate of decline in leaf CER from 4 wk postsilking until 8 wk postsilking were approximately two times greater in the older hybrid ( $7.45 \pm 1.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  per week) than in the newer hybrid ( $3.34 \pm 0.16 \mu\text{mol m}^{-2} \text{s}^{-1}$  per week;  $p < 0.05$ ). This is in agreement with previous reports showing greater rate of CER reductions in older than in newer hybrids (Ying et al., 2002). In contrast, leaf CER at low N availability was greater in the

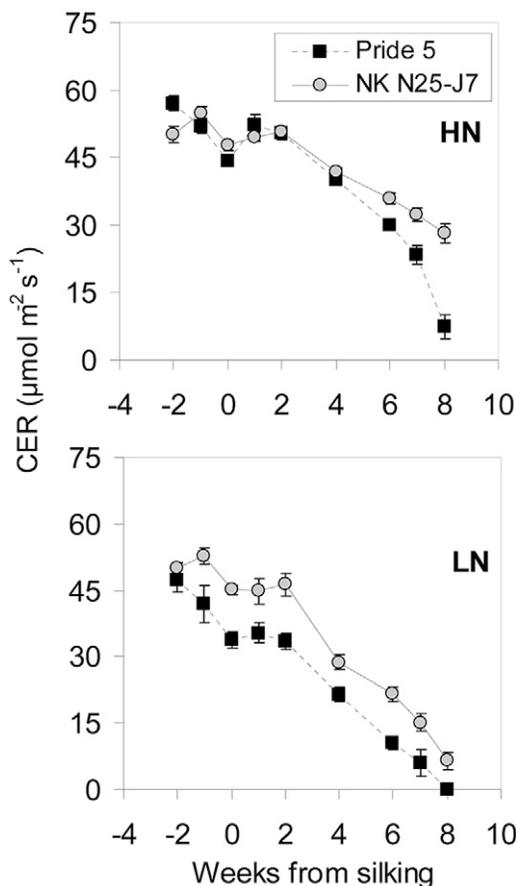


Figure 1. Leaf carbon exchange rate (CER) as a function of weeks from silking for an older ('Pride 5') and a newer ('NK N25-J7') maize (*Zea mays* L.) hybrid exposed to high N (HN) and low N (LN). Vertical bars represent  $\pm$  SE and are not shown when smaller than the symbol size.

newer than in the older hybrid starting at 1 wk presilking ( $p < 0.05$ ; Fig. 1), but the linear rate of decline in leaf CER from 1 wk presilking was similar for the two hybrids ( $4.42 \pm 0.31$  and  $4.45 \pm 0.41 \mu\text{mol m}^{-2} \text{s}^{-1}$  per wk for the older and the newer hybrid, respectively;  $p > 0.05$ ). Although leaf CER was lower at low than at high N availability in both hybrids ( $p < 0.05$ ), reductions in leaf CER were greater in the older than in the newer hybrid (Fig. 2). When plants were switched from high to low N availability at 1 wk presilking or at 2 wk postsilking, leaf CER at the low N level was also reduced relative to that at the high N level in both hybrids, and significant reductions were apparent first in the older hybrid (Fig. 2). A decline in maize leaf CER during the grain-filling period under high N availability and a reduction in leaf CER when N availability was low during this period has been previously reported by Paponov and Engels (2003) and Ding et al. (2005).

Reductions in leaf CER associated with low N availability and through the course of development during the grain-filling period were associated with variations in the chlorophyll index and, consequently, leaf absorptance. The chlorophyll index of plants grown at high N availability was relatively stable across the life cycle until at

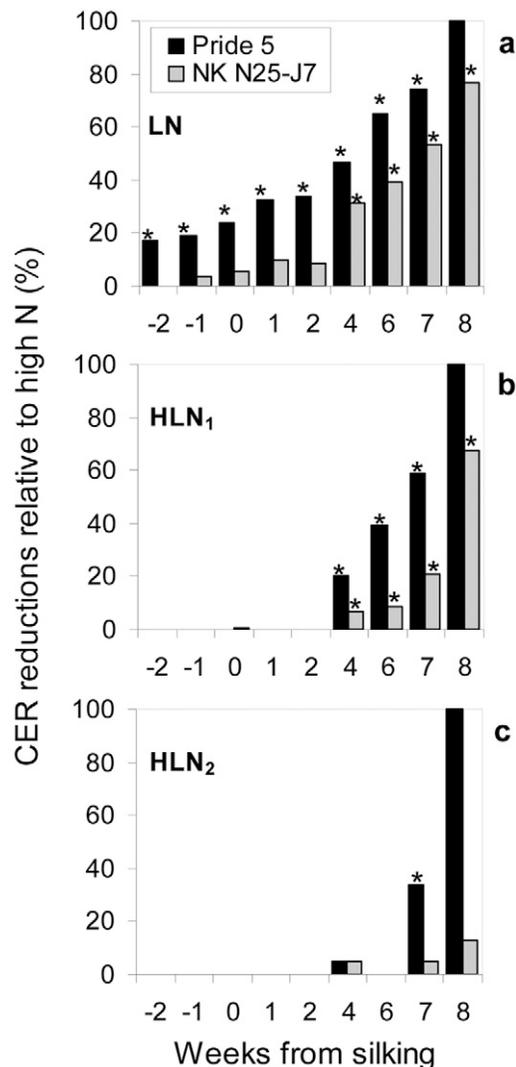


Figure 2. Reduction in leaf carbon exchange rate (CER) relative to leaf CER at high N as a function of weeks from silking for an older ('Pride 5') and a newer ('NK N25-J7') maize (*Zea mays* L.) hybrid exposed to (a) low N (LN), (b) HN switched to LN at 1 wk presilking (HLN<sub>1</sub>), and (c) HN switched to LN at 2 wk postsilking (HLN<sub>2</sub>). \* Indicates when CER values were significantly lower ( $p < 0.05$ ) under LN than under HN levels for each hybrid and moment of measurement.

least 8 wk postsilking for the newer hybrid and until 6 wk postsilking for the older hybrid, but the chlorophyll index of both hybrids started to decline after silking under low N availability (Fig. 3). Maize leaf absorptance of incident PPFD is closely associated with the chlorophyll index (Earl and Tollenaar, 1997), and results depicted in Fig. 3 show, therefore, that leaf absorptance does not vary greatly across the life cycle in the newer hybrid NK N25-J7 under high N availability. In contrast, leaf absorptance of the older hybrid Pride 5 started to decline at 6 wk postsilking under high N availability, and leaf absorptance declined immediately after silking under low N availability in both hybrids, although leaf absorptance of the newer hybrid continued to be greater than that of the older hybrid throughout the grain-filling period (except at 4 wk postsilking).

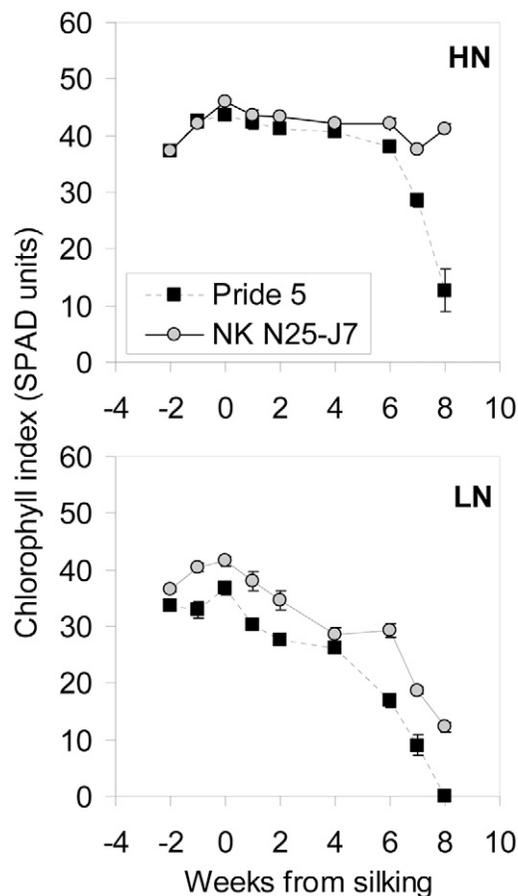


Figure 3. Leaf chlorophyll index (SPAD units) as a function of weeks from silking for an older ('Pride 5') and a newer ('NK N25-J7') maize (*Zea mays* L.) hybrid exposed to high (HN) and low (LN) N levels. Vertical bars represent  $\pm$  SE and are not shown when smaller than the symbol size.

Reductions in leaf CER are attributable to either reductions in PPFD absorptance or a lower efficiency of the use of absorbed photons in photosynthesis (i.e., CER  $\text{Abs}^{-1}$ ). We will examine the relative contributions of absorptance and CER  $\text{Abs}^{-1}$  to the decline in CER in three scenarios:

1. CER reductions due to stage of development under high N conditions. Leaf absorptance of the newer hybrid NK N25-J7 during the period from 2 wk presilking to 8 wk postsilking was 0.83 at 2 wk presilking and 7 wk postsilking and either 0.85 or 0.86 at the other dates (data not shown). The decline of CER during the grain-filling period of this hybrid under high N availability (Fig. 1) can therefore be attributed entirely to the decline in CER  $\text{Abs}^{-1}$ . Leaf absorptance by the older hybrid Pride 5 declined substantially during Week 7 (0.78) and Week 8 (0.63) from a mean of 0.845 during the preceding period (i.e., about 12%  $\text{wk}^{-1}$ ). The CER  $\text{Abs}^{-1}$  of this hybrid started to decline at 4 wk postsilking, and the rate of decline was about 20%  $\text{wk}^{-1}$  of the value of CER  $\text{Abs}^{-1}$  at 4 wk postsilking.

Table 1. Relative difference between 'NK N25-J7' and 'Pride 5' for carbon exchange rate (CER), CER per unit absorbed photosynthetic photon flux density (CER  $\text{Abs}^{-1}$ ), and leaf absorptance from 2 wk presilking to 8 wk postsilking for plants grown at low N level.

Weeks from silking	(NK N25-J7 – Pride 5)/NK N25-J7 $\times$ 100%		
	CER	CER $\text{Abs}^{-1}$	Absorptance
-2	5	3	2
-1	20	17	4
0	25	28	2
1	21	17	5
2	28	24	5
4	26	19	2
6	54	53	13
7	60	52	17
8	100	100	100

Consequently, CER  $\text{Abs}^{-1}$  during the grain-filling period of the older hybrid Pride 5 started to decline earlier and the rate of decline was steeper than that of leaf absorptance.

2. CER reductions due to low vs. high N availability. The reduction in CER  $\text{Abs}^{-1}$  due to N availability in the older hybrid Pride 5 increased from 15% at 1 wk presilking to 66% at 7 wk postsilking. Reduction in absorptance increased from 5 to 26% during the same period, indicating a 3:1 ratio between reductions in CER  $\text{Abs}^{-1}$  and reductions in absorptance. The reduction in CER  $\text{Abs}^{-1}$  due to N availability in the newer hybrid NK N25-J7 increased from 30% at 4 wk postsilking to 69% at 8 wk postsilking. Reduction in absorptance during this period increased from 8 to 26%, showing a substantially greater reduction in CER  $\text{Abs}^{-1}$  than in absorptance.
3. Difference in leaf CER between the older and newer hybrid grown under low N availability (Fig. 1) was also predominantly associated with CER  $\text{Abs}^{-1}$  (Table 1). Differences between the two hybrids in CER  $\text{Abs}^{-1}$  paralleled differences between the two hybrids in ETR across a large range of CER values (Fig. 4 and 5). The ratio between gross  $\text{CO}_2$  uptake and the noncyclic electron transport rate in the thylakoid membrane (cf. Earl and Tollenaar, 1998b) cannot be assessed in this study, however, because leaf respiration was not measured, and it is known that respiration rate per unit leaf area varies with hybrid, stage of development, and soil N level (Earl and Tollenaar, 1998a). The association between relative changes in CER  $\text{Abs}^{-1}$  and ETR indicates, however, that reductions in CER  $\text{Abs}^{-1}$  are attributable in part to reductions in the efficiency of the electron flux in PSII.

The differences in CER  $\text{Abs}^{-1}$  and ETR between the two hybrids grown under low N conditions suggest that

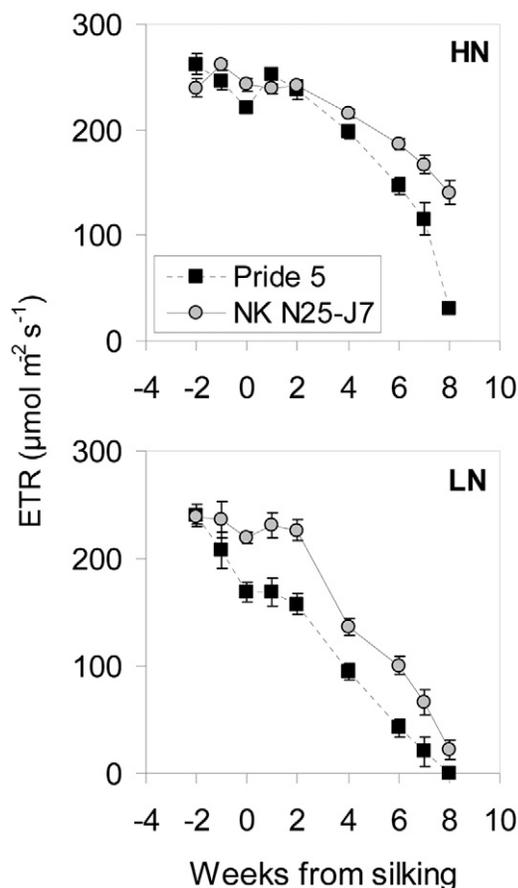


Figure 4. Electron transport rate (ETR) as a function of weeks from silking for an older ('Pride 5') and a newer ('NK N25-J7') maize (*Zea mays* L.) hybrid exposed to high N (HN) and to low N (LN). Vertical bars represent  $\pm$  SE and are not shown when smaller than the symbol size.

nitrogen-use efficiency at the leaf level (CER/Na) was greater in the newer than in the older hybrid under low N availability, particularly during later phases of the grain-filling period. Mean CER/Na at 1 wk presilking, silking, and 3 wk postsilking in the LN treatment was  $23.2 \mu\text{mol CO}_2 \text{ s}^{-1}(\text{g N})^{-1}$  for the older hybrid and  $26.1 \mu\text{mol CO}_2 \text{ s}^{-1}(\text{g N})^{-1}$  for the newer hybrid ( $p > 0.05$ ). Differences in CER/Na between the two hybrids can also be inferred from the relationships between the chlorophyll index and Na, leaf absorptance and the chlorophyll index, and CER  $\text{Abs}^{-1}$ . The chlorophyll index declined linearly with Na for values of  $\text{Na} < 2.0 \text{ g m}^{-2}$  (Fig. 6). Dwyer et al. (1995) reported a similar relationship between the chlorophyll index and leaf N concentration of field-grown maize, and the leaf N concentration at which the chlorophyll index plateaued declined with the advance of development from presilking to the middle of the grain-filling period in their study. Leaf absorptance also decreases with a decline in the chlorophyll index (Eq. [2]). Mean CER  $\text{Abs}^{-1}$  for the three stages of development in the LN treatment was  $40.1$  and  $50.3 \mu\text{mol m}^{-2} \text{ s}^{-1} (\%)^{-1}$  for Pride 5 and NK N25-J7, respectively ( $p > 0.05$ ). Consequently, CER  $\text{Abs}^{-1}$  is associated with CER/Na, and differences in CER  $\text{Abs}^{-1}$

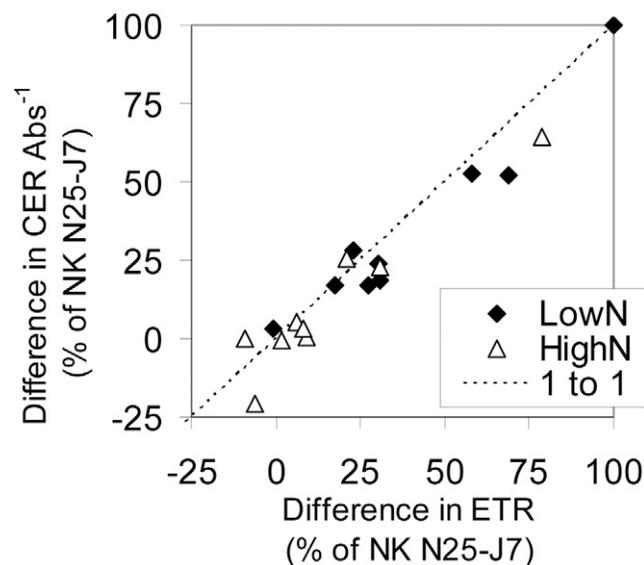


Figure 5. Relationship of the difference between the newer ('NK N25-J7') and older ('Pride 5') maize (*Zea mays* L.) hybrid in carbon exchange rate per unit absorbed photosynthetic photon flux density (CER  $\text{Abs}^{-1}$ ) vs. the difference between the newer and older hybrid in thylakoid electron transport rate (ETR) for plants grown at low and high N levels. Differences are expressed as a proportion of the value of the newer hybrid (i.e.,  $100\% \times [\text{NK N25-J7} - \text{Pride 5}]/\text{NK N25-J7}$ ), and the dashed line indicates the 1:1 ratio.

between the two hybrids increased during later phases of the grain-filling period (Table 1).

Although the reduction of leaf chlorophyll content during the grain-filling period due to low N availability was associated with a reduction in postsilking N uptake, it is not clear whether differences in the response of the chlorophyll index to low N between hybrids were the result or the cause of differences in N uptake between the hybrids. Shoot N content was similar for the hybrids at silking ( $p > 0.05$ , Fig. 7), was reduced due to low N availability in both hybrids at silking and at final harvest ( $p < 0.05$ ; Fig. 7), and was higher in the newer than in the older hybrid at physiological maturity ( $p < 0.05$ ; Fig. 7). Postsilking N uptake, in other words, the difference between N content at physiological maturity and silking (Fig. 7), was lower in the older than in the newer hybrid (48% for HN, 49% for HLN<sub>1</sub>, and 65% for LN). Rajcan and Tollenaar (1999) also reported greater postsilking N uptake in newer than in older maize hybrids. Leaf CER and the chlorophyll index were significantly ( $p < 0.05$ ) correlated with postsilking N uptake at 4 wk postsilking ( $r = 0.8$  and  $r = 0.79$ , respectively), 6 wk postsilking ( $r = 0.89$  and  $r = 0.87$ , respectively), and 7 wk postsilking ( $r = 0.94$  and  $r = 0.93$ , respectively). Greater postsilking N uptake in the newer than in the older hybrid (Fig. 7) may have resulted in a longer duration of leaf greenness in the newer hybrid (Fig. 3) and, consequently, higher leaf CER during the grain-filling period (Fig. 1). Alternatively, a greater postsilking N uptake could have been a consequence of maintenance of a higher leaf CER (Fig. 1) and

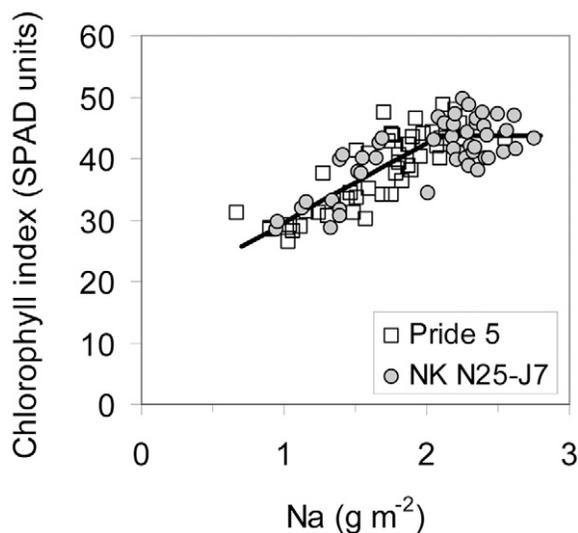


Figure 6. Relationship between leaf chlorophyll index (SPAD units) and N content per unit of leaf area (Na) for an older ('Pride 5') and a newer ('NK N25-J7') maize (*Zea mays* L.) hybrid exposed to high N, low N, high N switched to low N at 1 wk presilking, and high N switched to low N at 2 wk postsilking. Data are from harvests at 1 wk presilking, silking, and 3 wk postsilking. A linear with plateau model (Jandel TBLCURVE, 1992) was fitted to the relationship between leaf chlorophyll index and Na for the two hybrids: Leaf chlorophyll index =  $a + b \times \text{Na}$  if  $\text{Na} < c$  and leaf chlorophyll index =  $a + b \times c$  if  $\text{Na} \geq c$ , where  $a$  is the  $y$ -intercept or leaf chlorophyll index when  $\text{Na} = 0$ ,  $b$  is the slope of the chlorophyll index–Na relationship, and  $c$  is Na at maximum leaf chlorophyll index.

a higher CER  $\text{Abs}^{-1}$  (Table 1) of the newer hybrid NK N25-J7. N uptake is related, in part, to the demand of N within the plant and, in part, to the availability of soluble carbohydrates in the roots (Tolley-Henry and Raper, 1991). Hybrids expressing the late-senescing characteristic during the grain-filling period accumulate more dry matter than early-senescing hybrids (e.g., Valentinuz and Tollenaar, 2004; Pommel et al., 2006), but the cause for increased leaf longevity is not clear. Pommel et al. (2006) showed that dry matter accumulation, N uptake, and leaf longevity were positively associated in two contemporary maize hybrids that differed in rate of leaf senescence when grown under high N conditions. At the molecular level, timing of the expression of six senescence-enhanced marker genes was similar in the two hybrids used in the study reported by Pommel (Martin et al., 2005).

### Grain Yield, Shoot Dry Matter, and Harvest Index

Grain yield of the newer hybrid was greater than that of the older hybrid at all N levels ( $p < 0.05$ ; Fig. 8a), which is in accordance with previous reports (Castleberry et al., 1984; Duvick, 1984; Ma and Dwyer, 1998; Sangoi et al., 2001; Ding et al., 2005). Grain yield was lower at low N levels in both hybrids, but the reduction in grain yield of plants grown under low vs. high N during the entire life cycle was greater in the older (53%) than in the newer hybrid

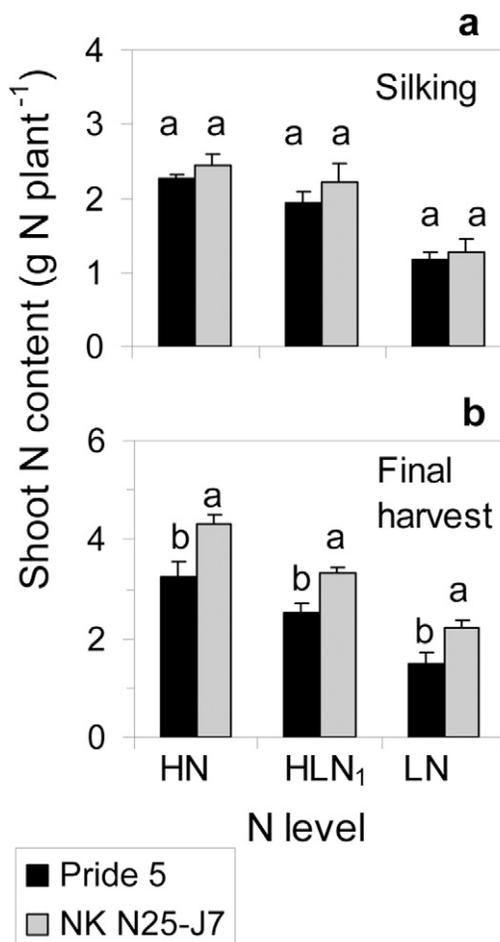


Figure 7. Shoot N content ( $\text{g N plant}^{-1}$ ) for an older ('Pride 5') and a newer ('NK N25-J7') maize (*Zea mays* L.) hybrid grown at high N (HN), low N (LN), and high N switched to low N at 1 wk presilking ( $\text{HLN}_1$ ) at (a) silking and (b) physiological maturity. Different letters indicate significant differences between hybrids per N level ( $p < 0.05$ ) by  $t$  test.

(35%;  $p < 0.05$ ; Fig. 8a). Previous reports also indicated that newer maize hybrids had a greater tolerance to low N conditions than older hybrids (Tollenaar and Wu, 1999; Bänziger et al., 2002; Echarte et al., 2004). The impact of low N availability on grain yield was less when N level was switched from high to low later during the growing season (i.e.,  $\text{HLN}_1$  and  $\text{HLN}_2$  treatments) in comparison to low N during the entire growing season (Fig. 8a). The reduction in grain yield from HN to  $\text{HLN}_1$  was 3% ( $p > 0.05$ ) in the older hybrid and 14% ( $p < 0.05$ ) in the newer hybrid. Both yield determinants (total shoot dry matter and harvest index [HI]) contributed to the yield response to N availability.

Total shoot dry matter was greater in the newer than in the older maize hybrid at all N levels ( $p < 0.05$ ; Fig. 8b), and the reduction in shoot dry matter at maturity due to low N conditions during the whole life cycle was similar in both hybrids (reduction was 34% for the older and 29% for the newer hybrid). Leaf area per plant at silking did not differ between the two hybrids at either the high or

the low N level ( $p > 0.05$ ), but mean leaf area of the two hybrids was 20% lower at low than at high N availability (data not shown). Greater dry matter accumulation in the newer than the older hybrid (Fig. 8b) was attributable, in part, to the maintenance of a relatively high leaf CER (Fig. 1). Higher rates of dry matter accumulation in newer than older maize hybrids during the grain-filling period have been associated with increased “stay green,” higher leaf chlorophyll concentration, and higher N uptake during this period (Rajcan and Tollenaar, 1999). The ability of newer hybrids to maintain relatively high leaf CER  $\text{Abs}^{-1}$  during the grain-filling period (i.e., functional “stay green”) under N stress may not be specific to N availability, as enhanced functional “stay green” was also apparent under high N availability (Fig. 1a) and in newer and older hybrids exposed to cold stress during the grain-filling period (Ying et al., 2002).

Dry matter partitioning to the roots influenced to some extent differences in shoot dry matter between the two hybrids. The root:shoot ratio at maturity was greater in the newer than in the older hybrid except for the LN treatment (Fig. 8d). The root:shoot ratio was greater under low N than under high N conditions, which is consistent with the contention that the root:shoot ratio increases when maize plants experience N stress (e.g., McCullough et al., 1994b). The difference in root:shoot ratio between the two hybrids, however, was greater in  $\text{HLN}_1$  than in the other N treatments (Fig. 8d), and the lower shoot dry matter accumulation of NK N25-J7 in  $\text{HLN}_1$  relative to HN was attributable, in part, to the relatively high root:shoot ratio of this hybrid in  $\text{HLN}_1$ .

Hybrids did not differ for HI at high N availability or when N reductions occurred later in the growing season ( $\text{HLN}_1$  and  $\text{HLN}_2$ ), but HI was greater in the newer (0.48) than in the older hybrid (0.35) at low N availability ( $p < 0.05$ ; Fig. 8c). Therefore, the differential impact of low N level on grain yield in the two hybrids was attributable more to HI than to dry matter accumulation. In contrast, Ding et al. (2005) and Ma and Dwyer (1998) reported that the main effect of N deficiency on differences between older and newer maize hybrids was a greater reduction in dry matter accumulation rather than a reduction in HI. Our results show, however, that the response depends on level and timing of the N stress, as HI in the intermediate treatments ( $\text{HLN}_1$  and  $\text{HLN}_2$ ) was not influenced by N stress. The greater reduction in HI due to low N availability in the older than in the newer hybrid (Fig. 8c) results from a lower kernel set in the older hybrid due to either a greater reduction in leaf CER around silking under low N availability (Fig. 2) or a higher threshold plant growth rate for kernel set in the older maize hybrid. Kernel set has been quantified by a curvilinear relationship between kernel number and leaf CER at silking (Edmeades and Daynard., 1979) or kernel number and plant growth rate

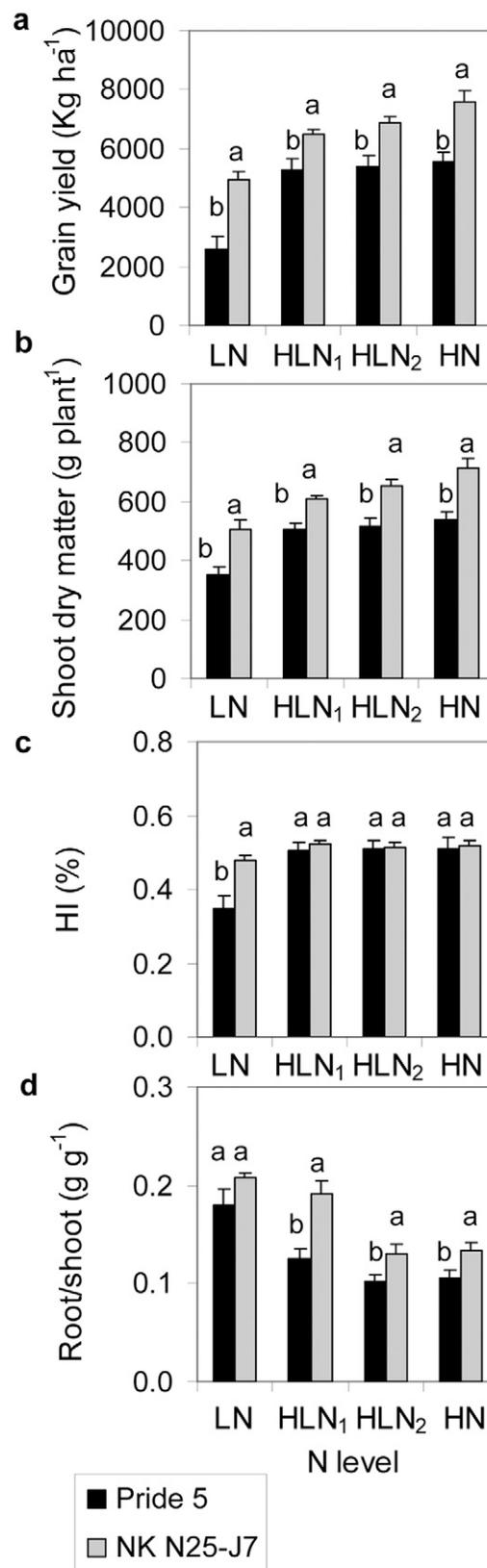


Figure 8. (a) Grain yield ( $\text{kg ha}^{-1}$ ), (b) shoot dry matter ( $\text{g plant}^{-1}$ ), (c) harvest index (HI) and (d) root:shoot ratio for an older (“Pride 5”) and a newer (“NK N25-J7”) maize (*Zea mays* L.) hybrid exposed to low N (LN), high N switched to low N at 1 wk presilking ( $\text{HLN}_1$ ), high N switched to low N at 2 wk postsilking ( $\text{HLN}_2$ ), and high N (HN). Different letters indicate significant differences between hybrids per N level ( $p < 0.05$ ) by *t* test.

during the period bracketing silking (Tollenaar et al., 1992; Andrade et al., 1999; Echarte et al., 2004; Echarte and Tollenaar, 2006). The relationship has a positive x-intercept, a threshold leaf CER or plant growth rate at which kernel set is zero. Consequently, kernel set per plant will decline when plant CER (or plant growth rate) during silking decreases, and the decline in kernel set will accelerate when plant growth rate approaches the threshold plant growth rate for kernel set. Since the threshold plant growth rate for kernel set is higher in older than in newer hybrids (Echarte et al., 2004), kernel set of an older hybrid will be influenced more than a newer hybrid by a reduction in plant CER at relatively low plant growth rates.

## CONCLUSIONS

The response of an older and a newer maize hybrid to N availability was examined at both the leaf and the whole-plant level in an attempt to elucidate physiological mechanisms that contribute to differences in N-use efficiency between maize hybrids. The differential response of the two hybrids to N availability were associated with differences in both leaf CER and dry matter partitioning to the grain (i.e., HI), but the specific response of both CER and HI to N availability varied with stage of development and level of N availability.

The major impact of N stress on the difference in CER between the older and newer hybrid was on advancing the stage at which CER of the two hybrids first differed. Leaf CER of the older hybrid was less than that of the newer hybrid starting at 6 wk postsilking under high N availability and starting at 1 wk presilking under low N availability. The higher tolerance of the newer maize hybrid to low N conditions is consistent with other reported studies in which older and newer hybrids were exposed to various abiotic stresses (McCullough et al., 1994a; Nissanka et al., 1997; Rajcan and Tollenaar, 1999; Ying et al., 2002; Ding et al., 2005). Although both leaf absorptance (indicated by the chlorophyll index) and photosynthesis per unit absorbed PPFD (i.e., CER Abs<sup>-1</sup>) contributed to the response of leaf CER to N availability, the contribution of CER Abs<sup>-1</sup> to the reduction was much greater than that of absorptance. Reductions of CER Abs<sup>-1</sup> were associated with reductions in efficiency of electron flux in PSII, indicating that improved N-use efficiency in the newer hybrid was associated with a higher tolerance of electron flux in PSII to low N conditions.

At the whole-plant level, grain yield of the newer hybrid was greater than that of the older hybrid at all N levels, and the difference between the two hybrids was significantly greater at low than at high N availability. The hybrid × N interaction for grain yield was predominantly a consequence of the lower HI of the older hybrid at low N availability, whereas the HI of the newer hybrid was not

affected by N level. Shoot dry matter at maturity of the newer hybrid was greater than that of the older hybrid at all N levels, which was generally consistent with differences in leaf CER between the two hybrids at the leaf level.

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