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# Corn (*Zea mays* L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply

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**Abstract**

Plant nitrogen (N) deficiency often limits crop productivity. Early detection of plant N deficiency is important for improving fertilizer N-use efficiency and crop yield. An experiment was conducted in sunlit, controlled environment chambers in the 2001 growing season to determine responses of corn (*Zea mays* L. cv. 33A14) growth and leaf hyperspectral reflectance properties to varying N supply. Four N treatments were: (1) half-strength Hoagland's nutrient solution applied throughout the experiment (control); (2) 20% of control N starting 15 days after emergence (DAE); (3) 0% N starting 15 DAE; and (4) 0% N starting 23 DAE (0% NL). Plant height, the number of leaves, and leaf lengths were examined for nine plants per treatment every 3–4 days. Leaf hyperspectral reflectance, concentrations of chlorophyll *a*, chlorophyll *b,* and carotenoids, leaf and canopy photosynthesis, leaf area, and leaf N concentration were also determined during the experiment. The various N treatments led to a wide range of N concentrations (11 – 48 g $kg^{-1}$ DW) in uppermost fully expanded leaves. Nitrogen deficiency suppressed plant growth rate and leaf photosynthesis. At final harvest (42 DAE), plant height, leaf area and shoot biomass were 64–66% of control values for the 20% N treatment, and 46-56% of control values for the 0% N treatment. Nitrogen deficit treatments of 20% N and 0% N (Treatment 3) could be distinguished by changes in leaf spectral reflectance in wavelengths of 552 and 710 nm 7 days after treatment. Leaf reflectance at these two wavebands was negatively correlated with either leaf N ($r$ = –0.72 and –0.75**) or chlorophyll ($r$ = –0.60 and –0.72**) concentrations. In addition, higher correlations were found between leaf N concentration and reflectance ratios. The identified N-specific spectral algorithms may be used for image interpretation and diagnosis of corn N status for site-specific N management.

*Abbreviations:* DAE – days after emergence; DW – dry weight; PAR – photosynthetically active radiation; Pn – net photosynthetic rate; SPAR – Soil-Plant-Atmosphere-Research

## Introduction

Nitrogen is an essential element for crop growth, development, and yield and is often a limiting nutrient in agricultural soils. Insufficient N supply reduces crop leaf area (Fernandez et al., 1996; van Delden 2001), photosynthesis (Ciompi et al., 1996; Lu et al., 2001), development, and biomass production (Dev and Bhardwaj, 1995), resulting in a low yield. On the other hand, excessive application of N fertilizer usually increases input cost and reduces environmental quality especially water quality. Therefore, the applic-

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ation of N based on requirements for crop growth and plant and soil N levels is critical in precision agricultural production. The goal of farm managers is to detect crop N status at an early growth stage, and apply the appropriate amount of N fertilizer for optimal yield, high N-use efficiency, and minimal N losses to the environment.

Traditional methods to determine plant tissue nutrient concentrations in a laboratory are time consuming and costly. Furthermore, by the time symptoms of plant nutrient deficiency become clearly visible, many physiological processes have been severely disrupted by nutrient stress. Remote sensing at leaf to landscape scales of crop physiology as affected by environmental stresses has a great potential for timely crop stress assessment and management (Afanasyev et al., 2001; Daughtry et al., 2000; Filella et al., 1995; Zarco-Tejada et al., 2000a, b). Recent studies have found close relationships between plant physiological parameters and spectral reflectance (Chappelle et al., 1992; Penuelas and Filella, 1998; Penuelas and Inoue, 2000). Several studies have documented that N status of field crops can be assessed using leaf or canopy spectral reflectance data (Blackmer et al., 1994; Chappelle et al., 1992; Gausman, 1982; Thomas and Gausman, 1977). Nitrogen deficiency always causes a decrease in leaf chlorophyll concentration, resulting in an increase in spectral reflectance in the visible spectrum (400–700 nm). However, a variety of causes of plant stress may result in increased reflectance due to reduced amounts of chlorophyll (Carter and Knapp, 2001). Furthermore, diagnosing a specific nutrient deficiency with remotely sensed data can be difficult when plants are subjected to deficiencies of multiple elements (Masoni et al., 1996).

When the effects of N supply on crop physiological parameters and reflectance properties are determined under field conditions, the results may be interfered by some other unexpected factors (Masoni et al., 1996). Therefore, in order to investigate responses of corn (*Zea mays* L.) growth, development, and leaf spectral properties to N supply while keeping other conditions optimum, we carried out an experiment in sunlit, controlled environment chambers known as Soil-Plant-Atmosphere-Research (SPAR) units during the 2001 growing season. The specific objectives of this study were to: (i) determine the effects of N deficiency on plant growth parameters, leaf chlorophyll and N concentrations, and photosynthesis and (ii) establish the quantitative relationships between hyperspectral reflectance and leaf pigments and plant N status in corn.

## Materials and methods

### *Soil-Plant-Atmosphere-Research (SPAR) units*

The experiment was conducted at the Mississippi Agricultural and Forestry Experiment Station, Mississippi State, Mississippi, USA using four SPAR units. The SPAR facility has the capability to precisely control temperature and $CO_2$ concentration at predetermined set points for plant growth studies in near natural solar radiation regimes. Details of the SPAR operation and controls have been described by Reddy et al. (2001). Each SPAR unit consists of a steel soil bin (1 m deep by 2 m long by 0.5 m wide), and a Plexiglas chamber (2.5 m tall by 2 m long by 1.5 m wide) to accommodate above ground plant parts, a heating and cooling system, and an environment monitoring and control system. The Plexiglas chamber transmits 97% of incoming photosynthetically active radiation (PAR, 400 – 700 nm). Air temperature and $CO_2$ concentration in each SPAR unit were monitored and adjusted every 10 s throughout the experimental period.

### *Plant culture*

A Pioneer brand hybrid corn, cv. 33A14, was seeded on 1 August 2001 in fine sand medium within the SPAR soil bins. Emergence was observed five days later. Five rows in each SPAR unit were spaced 0.4 m apart with 25 plants $m^{-2}$. All SPAR units were maintained at 30/22 °C (day/night) temperatures and 360 $\mu$L $CO_2$ $L^{-1}$ during the experiment. Plants were irrigated three times a day with defined nutrient solutions, based on N treatments, delivered at 0800, 1200 and 1700 h to ensure favorable water conditions for plant growth and development. Irrigation was provided through an automated and computer-controlled drip system. Variable-density black shade cloths (Hummert Seed Co., St. Louis, Missouri, USA) were placed around the plant canopy and adjusted regularly to simulate natural shading by other plants.

### *Treatments*

The four treatments included: (1) irrigation with half-strength Hoagland's nutrient solution throughout the

experiment (Control); (2) N reduction to 20% of control levels starting 15 DAE (20% N); (3) 0% N starting 15 DAE (0% N); and (4) 0% N starting 23 DAE (0% NL) until final harvest (42 DAE). The uniformity tests of the SPAR units in previous studies indicated no statistical differences among all SPAR units (Reddy et al., pers. comm., 2000). Therefore, four treatments were randomly arranged in four identical SPAR units. The nutrient solution was modified by substituting $CaCl_2$ for $Ca(NO_3)_2$ to allow for different N concentrations. All plants in the reduced N (20% N) and withheld N (0% N and 0% NL) treatments received normal half-strength Hoagland's nutrient solution before N-stress treatments were imposed. Three individual tanks were used to provide the respective nutrient solutions when different treatments commenced.

*Measurements*

Plant height, number of leaves and leaf lengths were measured at 3- or 4-day intervals from 10 to 42 DAE on nine plants in three center rows per treatment (3 center plants per row). Plant height was measured from ground surface to the base of an uppermost, fully expanded leaf. Leaf area was calculated based on leaf length using the following equation:

$$Y = 0.191\, X^{1.739},$$

where $Y$ is leaf area in $cm^2$ and $X$ is leaf length in cm. The equation was obtained by regressing the lengths and areas of more than 500 leaves ($r^2$ = 0.86***) measured from 36 plants in the four treatments at the final harvest (42 DAE).

Net photosynthetic rates (Pn) of the uppermost, fully expanded leaf from five plants in each treatment were measured between 1000 and 1200 h using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA) at 20, 25, 35, 37, 40, and 42 DAE. When measuring Pn, the PAR, provided by a 6400-02 LED light source, was set to 1500 $\mu$mol $m^{-2}$ $s^{-1}$, temperature inside the leaf cuvette was set to 30 °C, and leaf chamber $CO_2$ concentration was set to 360 $\mu$L $L^{-1}$.

Canopy photosynthesis on a ground area basis was determined using a mass balance approach in each chamber throughout the experiment (Acock et al., 1985; Reddy et al., 1995). Each SPAR growth chamber and a fan-coil box formed a semi-closed system for the measurement of $CO_2$ fluxes. The Plexiglas chamber containing the plants, ducts, and cooling system was sealed. Carbon dioxide concentration within a SPAR unit was monitored at 10 s intervals and adjusted to respective treatment set levels. A dedicated infrared gas analyzer (Model LI-6252, LI-COR Inc., Lincoln, Nebraska, USA), calibrated weekly, was used to monitor and control $CO_2$ concentration to within $\pm$ 10 $\mu$L $CO_2$ $L^{-1}$ air of the set point. Commercial grade $CO_2$ was injected through a system including a pressure regulator, solenoid and needle valves, and a calibrated flowmeter. The flowmeters were calibrated with a Brooks gas displacement meter at the beginning and end of the experiment. The time intervals during which the solenoid valves were open were monitored by the computer indicating the amount of gas injected. Carbon dioxide flow rates were recorded three times a day and converted into mass quantity using gas corrections for temperature and pressure. A leakage test was performed each night to derive a correction factor for losses of $CO_2$ from the chamber (Acock and Acock, 1989). All $CO_2$ exchange rate data were obtained every 10 s and integrated over 900-s intervals throughout the day-lit period. The corresponding incident PAR was also measured by monitoring with a 200 SB pyranometer (LI-COR, Inc., Lincoln, Nebraska, USA) and summarized with a data acquisition system at 900-s intervals. Data for canopy net $CO_2$ exchange rates were summarized over the same time intervals. The curves of canopy net $CO_2$ exchange rates vs. PAR (i.e. light response curves) for each SPAR were fitted with a quadratic equation, and canopy Pn, expressed on a ground area basis, at 1200 $\mu$mol $m^{-2}$ $s^{-1}$ PAR was estimated from the light response curves during the experimental period. Canopy $CO_2$ exchange rate on a leaf area basis was estimated by dividing canopy Pn on a ground area basis by total leaf area.

Three uppermost fully expanded leaves were sampled from each treatment every 3 or 4 days between 1000 and 1200 h. Leaf hyperspectral reflectance was measured immediately after excising leaves using a portable spectroradiometer (Analytical Spectral Devices Inc., Boulder, Colorado, USA) to include wavelengths from 350 to 2500 nm. The optical sensor was mounted in the frame of a supplemental light source (ML 902, Makita Corporation, Aichi, Japan) with a 5-cm distance from target leaf surface. The angle between the sensor and the leaf surface was 70°. A white panel was used to optimize the instrument to 100% reflectance at all wave bands before measurements were taken. When measuring leaf reflectance, the individual leaves were placed adaxial side up on top of a black polyurethane background.

After measuring leaf reflectance, five leaf discs (38.5 $mm^2$ each) were immediately punched from each leaf and placed in a vial with 4 mL of dimethyl sulphoxide. Three replicate leaves were sampled in each treatment, and the leaf discs were incubated at room temperature in dark, for 24 h, to allow for complete extraction of chlorophyll into the solution. Absorbance of the extract was measured using a Pharmacia UltraSpec Pro UV/VIS spectrophotometer (Pharmacia, Cambridge, England) at 470, 648 and 664 nm to calculate concentrations of chlorophyll *a*, chlorophyll *b*, and carotenoids (Chappelle et al., 1992). The area of each individual leaf was determined using a LI-3100 leaf area meter (LI-COR Inc., Lincoln, Nebraska, USA) after collecting the leaf discs. Leaves were then immediately dried at 70 °C for 72 h, weighed, and ground to determine total N concentrations according to standard micro-Kjeldahl procedures (Nelson and Sommers, 1972). Concentrations of leaf chlorophyll were expressed on a leaf area basis in order to determine the relationships between leaf spectral reflectance and concentrations of leaf pigments. Leaf N concentrations were expressed on both leaf area basis and dry weight (DW) basis.

All plants were harvested at 42 DAE and separated into leaves, stems and roots. Length and area of individual leaves were recorded. Plant components were dried at 70 °C until they were consistent in weights and weighed to determine the effects of N treatments on plant dry mass accumulation and partitioning.

### *Data analysis*

Plant height, the number of leaves, leaf area, and concentrations of leaf chlorophyll and N were plotted vs. days after emergence. Best-fit regressions were employed to determine plant growth patterns as affected by N treatments. Simple correlation analysis and linear regression were carried out using the SAS program (SAS Institute, 1997) to determine the relationship between each physiological variable (chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid, and leaf N contents) and either leaf reflectance at a single 1-nm wide waveband or the simple reflectance ratios of 2-band combinations. Reflectance ratios were computed by dividing reflectance at each of the two single wavebands with highest $r^2$ values, when linearly regressed with each physiological variable measured, by reflectance at each of all other wavebands throughout the 400 – 2500 nm.

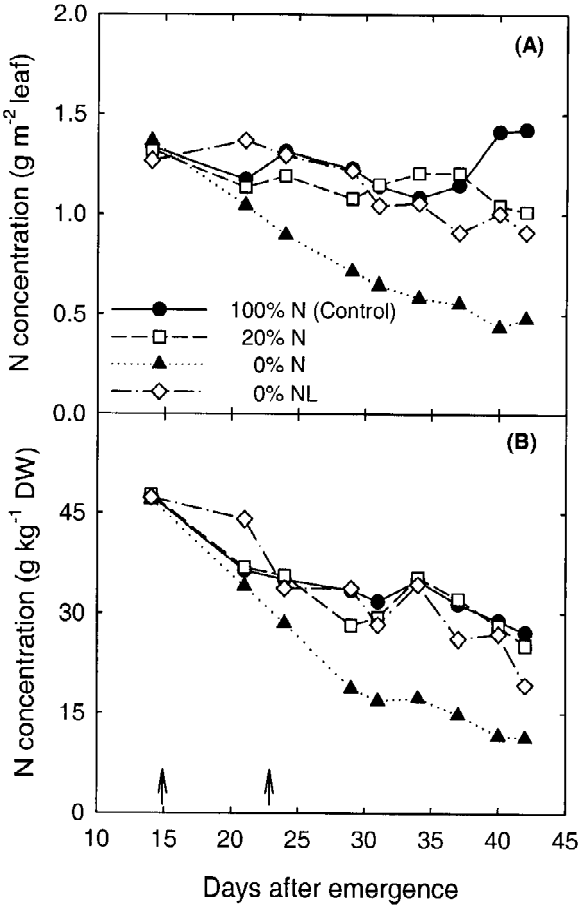


*Figure 1.* Changes in nitrogen concentration of uppermost fully expanded corn leaves for different N treatments during the experiment. Leaf N concentrations are expressed in both (A) leaf area basis and (B) leaf dry weight basis. The two arrows indicate start of N treatment for 20% and 0% N treatments at 15 DAE and 0% NL treatment at 23 DAE.

## Results

### *Leaf nitrogen concentration*

Leaf N concentration expressed on a leaf area basis changed little with days after emergence for the control, 20% N and 0% NL treatments, and there were no consistent differences among the three treatments (Figure 1A). However, leaf N under the 0% N treatment declined linearly with plant growth. Averaged across the nine sampling dates, leaf N concentrations of the control, 20% N, 0% N and 0% NL treatments were 1.25, 1.15, 0.75, and 1.12 g $m^{-2}$ leaf area, respectively.

On a dry weight basis, leaf N concentrations declined under all the treatments as plants aged (Fig-

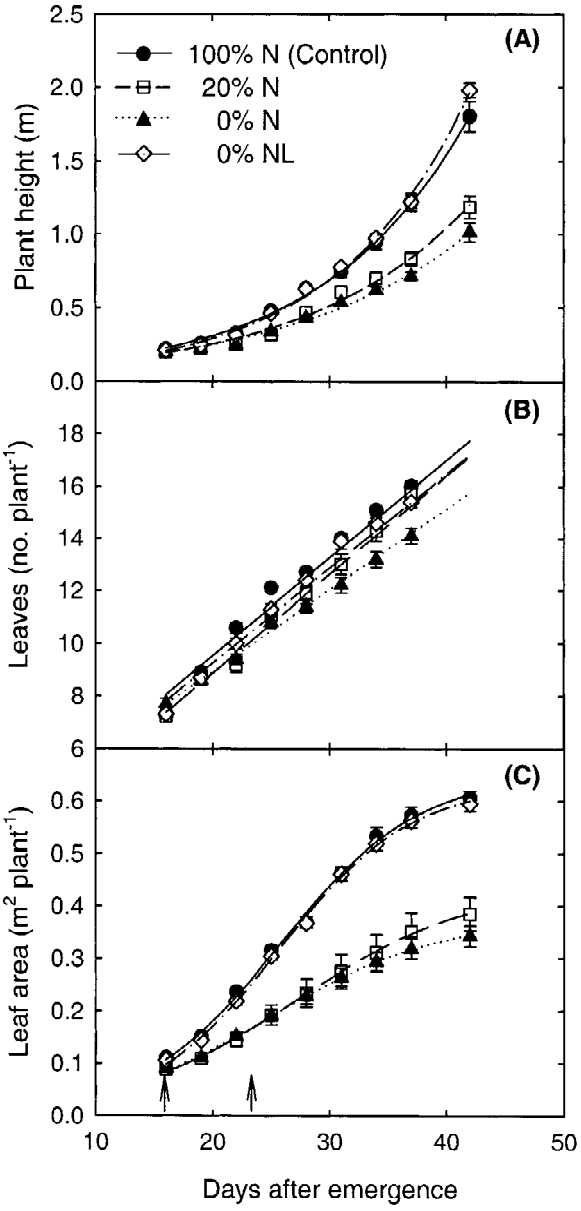


*Figure 2.* Changes in corn (A) plant height, (B) the number of leaves, and (C) leaf area during growth for different nitrogen treatments. The two arrows indicate start of N treatment for 20% and 0% N treatments at 15 DAE and 0% NL treatment at 23 DAE. Each data point is the mean ± SE of nine plants.

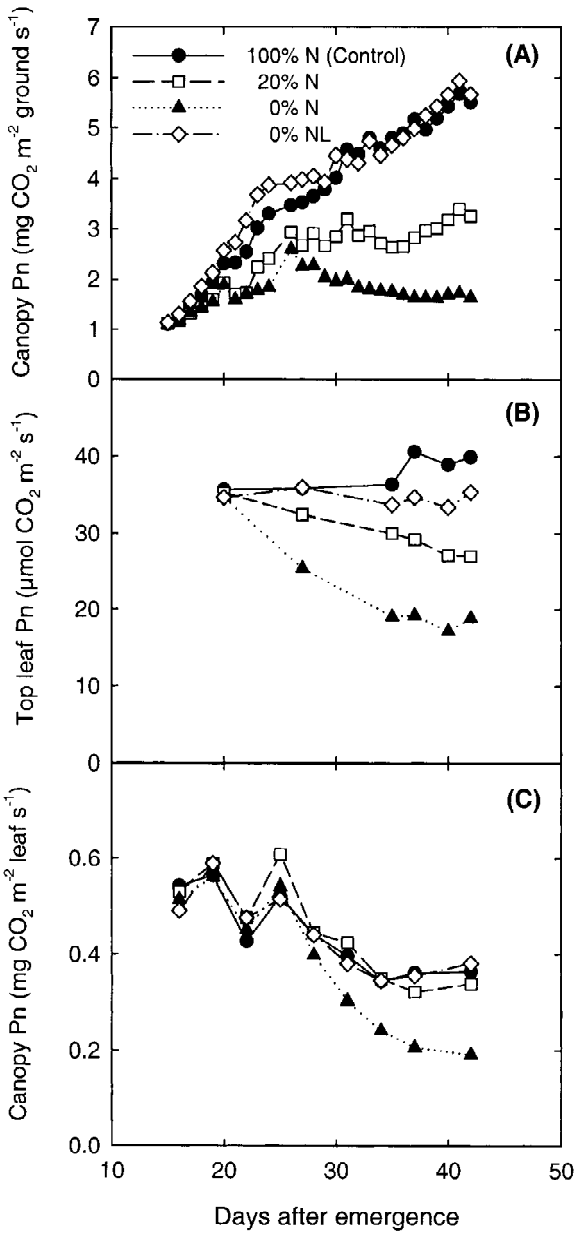


*Figure 3.* Effects of nitrogen deficiency on corn (A) canopy photosynthesis (Pn) on a ground area basis, (B) leaf level Pn of uppermost fully expanded leaves, and (C) canopy Pn on a leaf area basis (canopy Pn per unit ground ÷leaf area) during the experiment.

ure 1B), but this decline was more pronounced in plants supplied with 0% N compared with any other treatments. Similar to leaf area basis N content, dry weight basis N concentrations of 20% N and 0% NL treatments did not differ from the control at most sampling dates, but 0% N supplied plants had much lower leaf N concentrations than the control plants from 24 DAE.

*Plant growth*

From 15 to 42 DAE, plant height increased in an exponential fashion, while the number of leaves increased linearly in all treatments (Figure 2A, B). Leaf area expansion, on the other hand, showed a sigmoid relationship with time (Figure 2C). Plants supplied with 0% N and 20% N from 15 DAE were characterized by

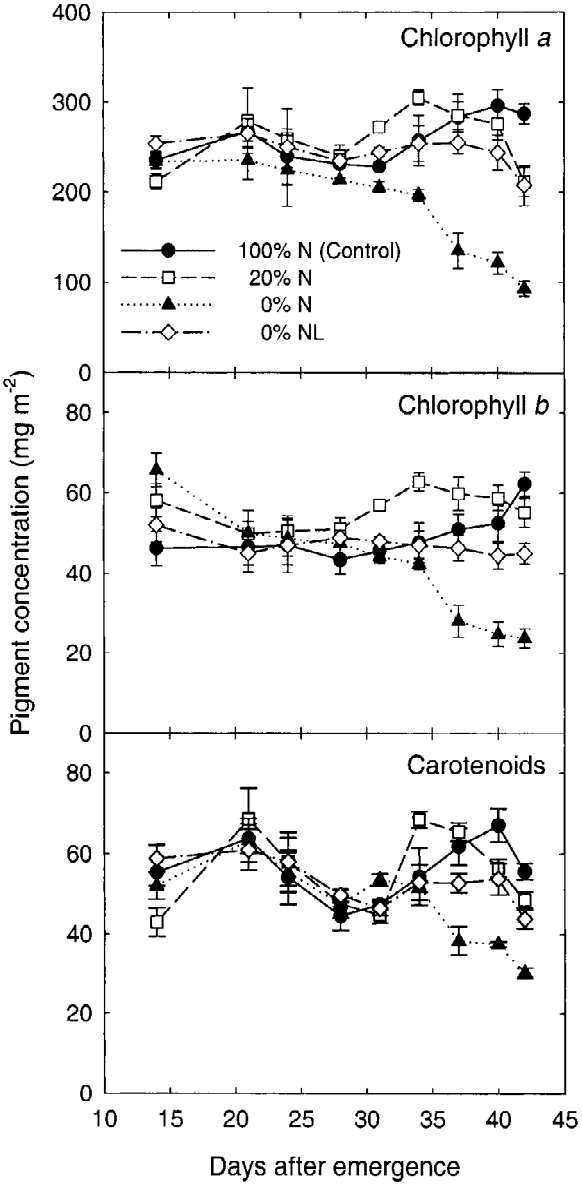


*Figure 4.* Changes in chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations of uppermost fully expanded corn leaves for different treatments during the experiment. Each data point is the mean ± SE of three plants.

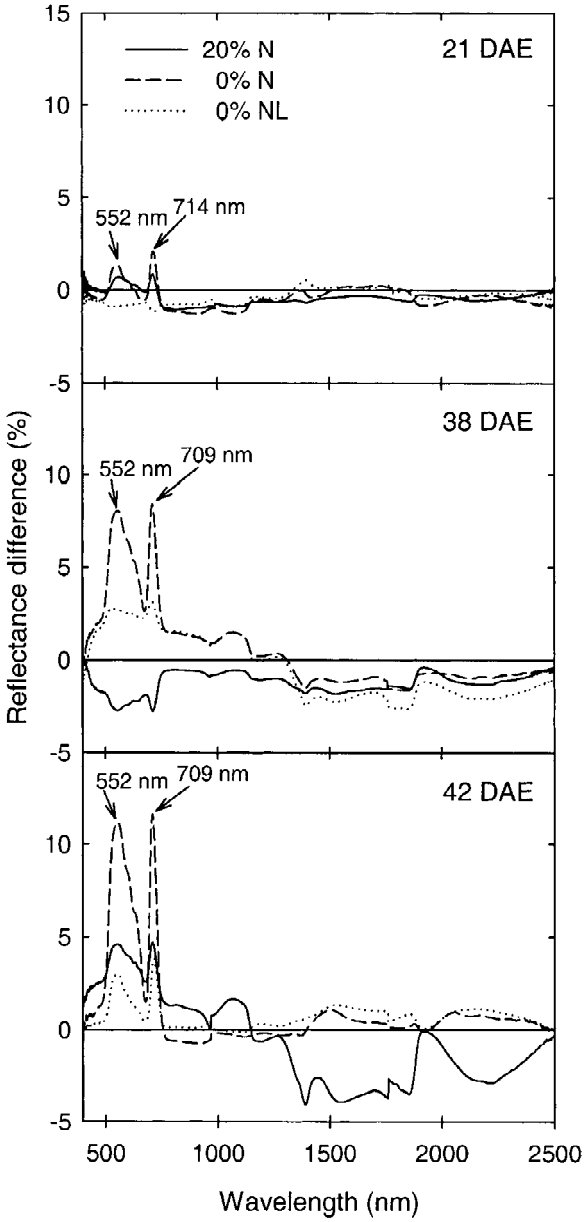


*Figure 5.* Corn leaf reflectance differences (Reflectance of N deficit treatments – Reflectance of the control at each waveband) at all wavelengths (400 – 2500 nm) for the three N deficit treatments of 20% N, 0% N and 0% NL, compared with the control (100% N), at 21, 38, and 42 days after emergence (DAE). Note that 20% and 0% N treatments were initiated at 15 DAE, and 0% NL treatment was initiated 23 DAE.

significantly lower rates of stem elongation and leaf area expansion, but produced only a slightly lesser number of leaves than the control plants. The 0% NL treatment did not differ from the control in any of the growth parameters measured. Time required to reach maximum leaf area expansion rate was similar (between 25 and 30 DAE) for all treatments, but leaf area expansion rates under 0% N and 20% N were only about 47% and 54% of control values, respectively (data not shown). At the final harvest, plant height and leaf area were decreased by 44% and 43%, respectively, for the 0% N treatment and by 34% and 36%, respectively, for the 20% N treatment, compared with control values. The number of leaves under the 20% N and 0% NL treatments did not differ from that of

the control plants, but significantly fewer leaves were produced under 0% N treatment at the final harvest (Figure 2B).

The 0% NL treated plants did not differ from the control plants in biomass production, but plants supplied with 0% N and 20% N had a 54% and 36% lower total biomass, respectively, than the control at final harvest (Table 1). The influence of N deficiency on shoot dry weight was greater than the influence on root dry weight, resulting in an increase in the root:shoot ratio.

*Canopy and leaf photosynthesis*

Canopy photosynthesis per unit ground area increased linearly over time as plants grew in the control and 0% NL treatments, but did not change much in the 0% N and 20% N treatments between 25 and 42 DAE (Figure 3A). Both the 0% N and 20% N treatments had much lower canopy photosynthesis than the control starting from 20 DAE, and it decreased by 43% and 67%, respectively, between 35 and 42 DAE as compared to controls.

The Pn of the uppermost fully expanded leaves in the control and 0% NL treatments changed little during plant growth, but large reductions were evident in the 20% N and 0% N treatments (Figure 3B). Between 35 and 42 DAE, the 20% N, 0% N and 0% NL treated plants had 27, 52, and 12% lower leaf Pn, respectively, than did the control plants. Canopy Pn per unit leaf area (canopy Pn per unit ground area ÷ leaf area) declined with canopy enlargement, but did not differ among treatments except for the 0% N treatment (Figure 3C). Compared with the control, the relative decrease in leaf area was more than in leaf Pn for 20% N treatment, but proportional decreases in leaf Pn and leaf area were evident for the 0% N treatment. Averaged across the last four measurements (35 to 42 DAE), the 20% N treatment had a 38% smaller leaf area (Figure 2C), but only 8% lower canopy Pn per unit leaf area than the control (Figure 3C). In contrast, both leaf area and the Pn of the 0% N treatment were 42–44% lower than those of the control.

*Leaf chlorophyll concentrations*

Changes in leaf concentrations of chlorophyll *a*, chlorophyll *b*, and carotenoids during the treatment period were similar to those in leaf N concentrations as expressed on a leaf area basis (Figure 1A), as these pigments did not differ consistently among the control, 20% N and 0% NL treatments during the experiment (Figure 4). Averaged across sampling dates, total chlorophyll (chlorophyll $a + b$) concentrations of the control, 20% N and 0% NL treatments were very similar at 310, 320, and 290 mg $m^{-2}$, respectively. Starting from 30 DAE, however, total chlorophyll concentration in 0% N treated plants declined linearly as plants aged. By 42 DAE (final harvest), the 0% N treated plants had a 67% lower total chlorophyll, 68% lower chlorophyll *a*, 62% lower chlorophyll *b*, and 46% lower carotenoids than the control plants. Nitrogen deficiency also decreased values for the chlorophyll *a:b* ratio, which was 5.29, 4.67, 4.49, and 5.24, respectively, for the control, 20% N, 0% N, and 0% NL treatments, averaged across the sampling dates.

*Leaf hyperspectral reflectance*

Leaf reflectance of corn plants was sensitive to changes in leaf N concentrations (Figure 5). Nitrogen deficiency mainly affected leaf reflectance in the visible range (400 – 720 nm) and especially caused the greatest increase in leaf reflectance near 550 and 710 nm (Figure 5). About one week after N was withheld or reduced from the nutrient solution, a difference of 2% in leaf reflectance spectra was detected and the spectral differences increased to 12% by 42 DAE.

In order to determine relationships between leaf spectral reflectance and leaf chlorophyll or leaf N concentration, data of leaf reflectance, as well as the pigment and nutrient concentrations, were first averaged across replicate leaves and then pooled across the N treatments and sampling dates ($n = 36$). Simple correlation analysis and linear regression indicated that although leaf reflectance in most wavebands was negatively and significantly correlated with leaf N or chlorophyll ($P \leq 0.05$), correlations were strongest in the 554 – 575 and 702 – 712 nm ranges ($r^2 = 0.32$ – 0.58) (Figure 6).

The reflectance values at two wavebands of 554 and 712 nm (for chlorophyll and leaf area basis N concentrations) or 575 and 702 nm (for leaf DW basis N concentration), which had highest $r^2$ values (see Figure 6) with the physiological measurements, were used as numerators to calculate 2-band reflectance ratios with reflectance values at each of all other wavebands ($R_i$) from 400 to 2500 nm. Linear regression was used to determine $r^2$ values of all the simple reflectance ratios with pigment or N concentration (Figure 7). Most ratios of reflectance at both 554 and 712 nm to reflectance at 760–1300 nm were highly correlated to

*Table 1.* Effect of Nitrogen deficiency on the accumulation and partitioning of dry matter in corn. Plants were harvested 42 days after emergence and each value is the mean ± SE of nine plants

| Treatment† | Leaves | Stems | Shoots | Roots | Root/shoot |
|---|---|---|---|---|---|
| | (g plant$^{-1}$) | | | | |
| 100% N (Control) | 23.9 ± 1.3 a‡ | 38.6 ± 4.2 a | 62.5 ± 5.4 a | 9.7 | 0.155 |
| 20% N | 15.1 ± 1.5 b | 24.8 ± 3.7 b | 39.9 ± 5.2 b | 6.8 | 0.170 |
| 0% N | 11.9 ± 1.2 b | 16.7 ± 2.0 b | 28.6 ± 3.1 b | 5.5 | 0.192 |
| 0% NL | 22.2 ± 1.2 a | 38.8 ± 3.2 a | 60.8 ± 4.2 a | 12.2 | 0.201 |

†The 20% and 0% N treatments were imposed 15 days after emergence and the 0% NL treatment was imposed 23 days after emergence.
‡Means followed the same letter in a column are not significant at $P = 0.05$ level.

chlorophyll and N concentrations ($r^2$ = 0.45 – 0.60). When leaf N concentration was expressed on a DW basis, most ratios of reflectance at both 575 and 702 nm to reflectance at 715–1300 nm were highly correlated to leaf N ($r^2$ = 0.60–0.63). Additionally, one ratio of $R_{575}/R_{526}$ was strongly related to leaf DW basis N concentration ($r^2$ = 0.69). Compared with leaf reflectance at a single waveband, reflectance ratios improved the precision (higher $r^2$ values) of estimating chlorophyll and N concentrations of corn leaves (Figures 6 and 7).

The reflectance ratio with greatest $r^2$ value for each chlorophyll or N concentration variable was selected from data in Figure 7. These reflectance ratios were plotted vs. chlorophyll or N concentrations (Figure 8). Results indicated strong linear relationships between the reflectance ratios and these physiological variables measured in the individual leaves of corn.

## Discussion

### *Plant growth and photosynthetic responses to nitrogen supply*

Nitrogen deficiency during early growth suppressed plant growth and dry matter accumulation in corn (Figure 2 and Table 1). Decreased biomass production under limited N supply was mainly attributed to smaller leaf area rather than leaf photosynthetic rate. However, both leaf area expansion rate and leaf photosynthesis decreased significantly and led to significantly lower biomass production when plants were subjected to severe N deficiency. Corn leaf Pn was closely related to leaf N level and decreased linearly as leaf N concentration decreased ($r^2$ = 0.79**). These results agree with earlier reports by Wolfe et al. (1988) and Settimi and Maranville (1998). When canopy Pn was expressed on a ground basis, the canopy Pn of 0% N and 20% N treatments were much lower than that of the control (Figure 3A). When canopy Pn was expressed on a leaf area basis, however, the Pn did not differ between the 20% N treatment and the control (Figure 3C). The difference in responses of canopy photosynthesis to N treatments between ground basis and leaf area basis was mainly associated with plant competition to light condition. Since the control had much larger leaf area than the 20% N treatment, this competition was more severe than the low N treatment. The increase in PAR in the canopy of the 20% N treatment in part compensated canopy photosynthesis based on leaf area.

Lower leaf chlorophyll content under severe N deficient conditions (0% N treatment) was associated with decreased photosynthesis (Figure 4). Limited N supply at an early growth stage (i.e. 20% N treatment) mainly led to reductions in plant size or leaf area rather than leaf chlorophyll concentration or leaf Pn (Figures 2 and 3). Tóth et al. (2002) reported that leaf chlorophyll concentration of field-grown corn decreased, whereas carotenoid and chlorophyll *a:b* ratio increased with the reduction in N supply. In contrast, our results indicated that carotenoid concentration decreased during corn N deficiency. This pattern was similar to that of chlorophyll concentration response to N supply, but the decline of carotenoid was slower than that of chlorophyll. Our results also indicate that N deficiency in corn decreased, rather than increased chlorophyll *a:b* proportion because reduction in leaf chlorophyll *a* was more than that of chlorophyll *b* under N deficiency (Figure 4). In the present study, most growth and physiological variables measured for 0% NL treatment did not differ from the control plants. This is probably because accumulation of N in the sand medium was sufficient to meet N requirements for growth during a short period (19 days) of N deficit treatment.

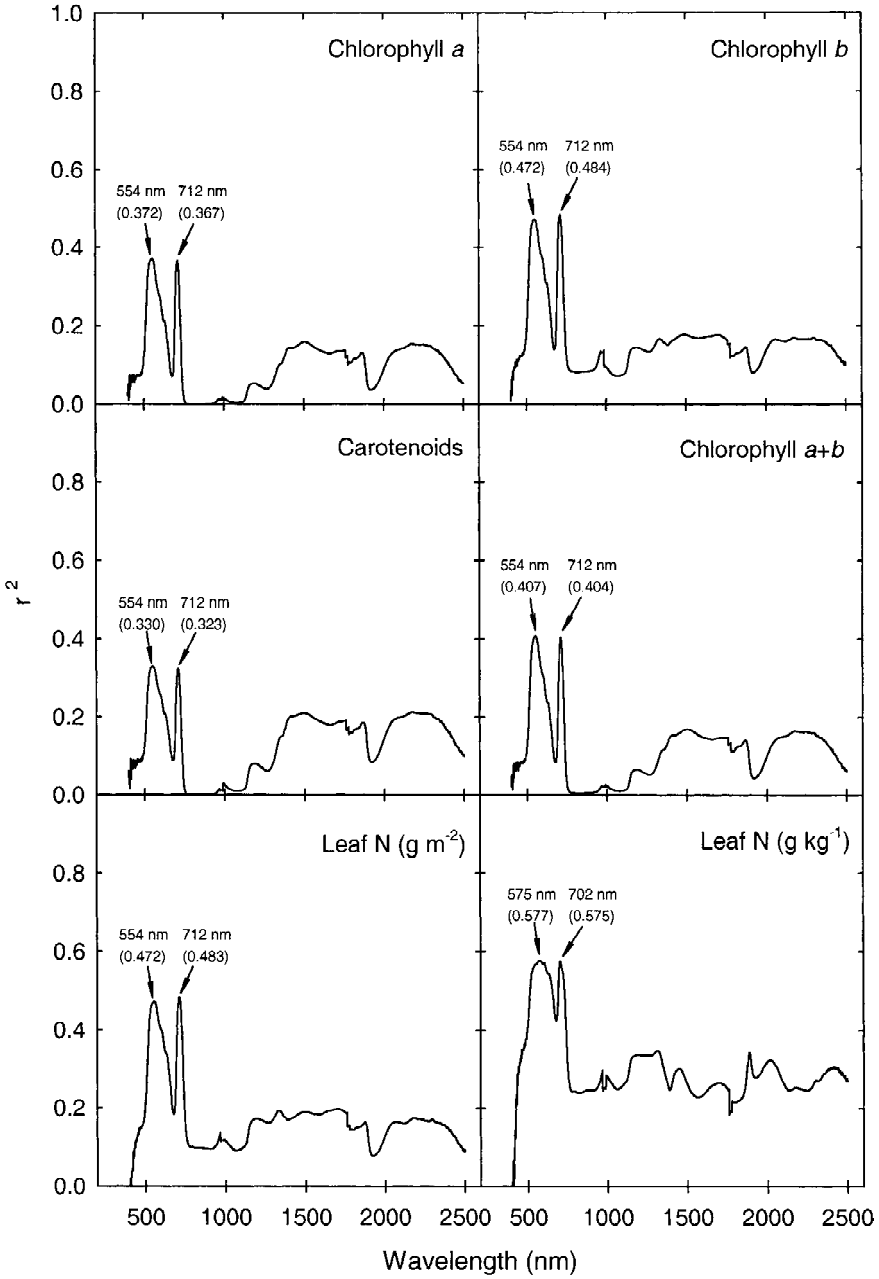


*Figure 6.* Coefficient of determination ($r^2$) versus wavelength for relationships of corn leaf chlorophyll and N concentrations with leaf reflectance at all wavelengths (400 – 2500 nm). The $r^2$ values were based on linear model and data combined among the four treatments and nine sampling dates ($n$ = 36). Wavebands with the highest $r^2$ values ($r^2$ values are in parenthesis) are presented in the Figure.

*Relationships between leaf reflectance and concentrations of leaf chlorophyll or N*

The results of N deficiency increasing corn leaf reflectance in two narrow ranges at 540–560 and 700–720 nm in our study are consistent with several earlier reports (Blackmer et al., 1994, 1996; Carter and Estep, 2002; Masoni et al., 1997; Meyer et al., 1992). Blackmer et al. (1996) found that reflected radiation near 550 and 710 nm was superior to reflected radiation near 450 and 650 nm for detecting corn N deficiencies. Generally, the effects of N deficiency on corn leaf reflectance at these wavebands can be attributed to changes in leaf chlorophyll levels (Carter and Knapp,

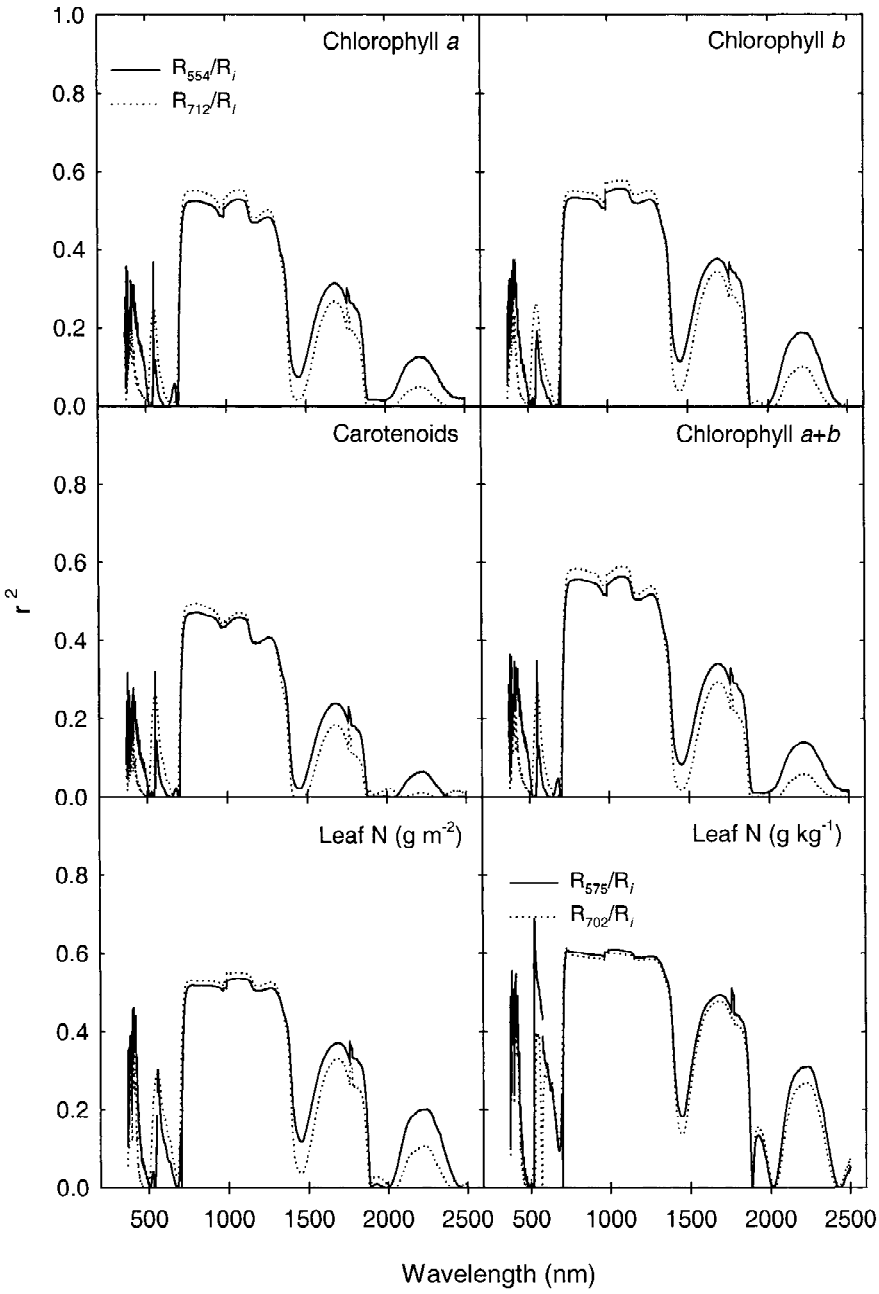


*Figure 7.* Coefficient of determination ($r^2$) versus denominator wavelength for relationships of corn leaf chlorophyll and nitrogen concentrations with leaf reflectance ratios. The $r^2$ values were based on linear model and data combined among the four treatments and nine sampling dates ($n = 36$). Ratios were computed by dividing reflectance at the best-fit wavelength for each physiological variable in Figure 6 by reflectance ($R_i$) at all other wavelengths from 400 to 2500 nm.

2001; Carter and Estep, 2002). In the present study, leaf total chlorophyll concentration (Figure 4) was closely related ($r = 0.79^{**}$, $n = 36$) to leaf N content (Figure 1). Reddy and Rao et al. (2001) found that chlorophyll concentration of maize, groundnut and soybean crops mainly affected leaf spectral reflectance at 450-520 and 620-680 nm. However, our analysis of hyperspectral data indicated that chlorophyll correlated most strongly with reflectance at 554 and 712 nm (Figure 6). Similarly, Jacquemoud and Baret (1990) found that reflectance at 548 nm was a good predictor of chlorophyll in several crops. A recent study

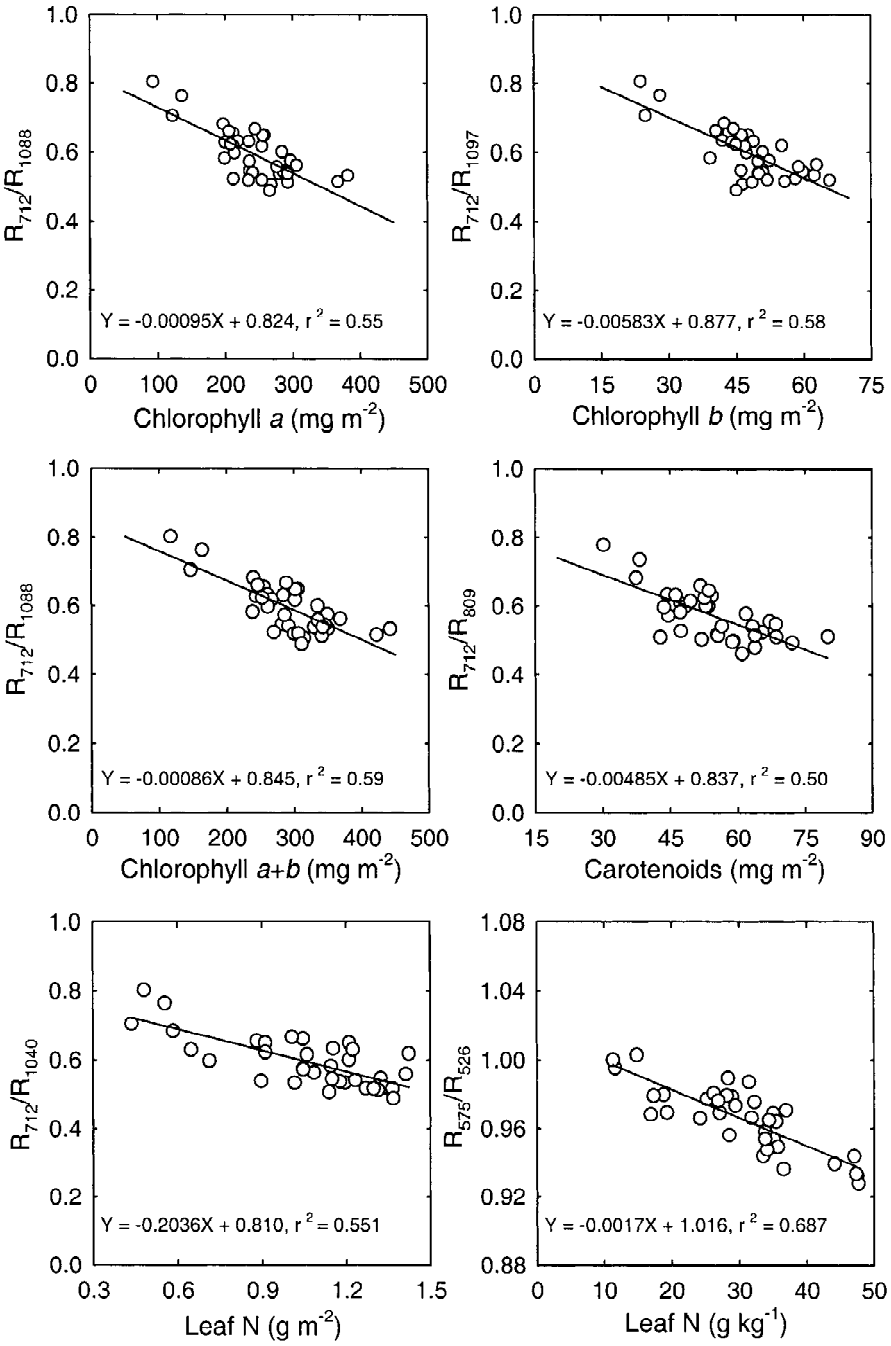


*Figure 8.* Linear regression of corn leaf pigment or N concentration with a specific reflectance ratio that has the maximum $r^2$ value with chlorophyll or N concentration in Figure 7. Data are combined among the four treatments and nine sampling dates ($n$ = 36).

also showed that corn leaf reflectance at 550 nm is closely related to chlorophyll concentration (Daughtry et al., 2000). In addition, we found that changes in reflectance at 712 nm were also highly correlated with chlorophyll concentration ($r$ = –0.60 ~ –0.70**, $n$ = 36). Therefore, corn leaf chlorophyll concentration could be estimated using spectral reflectance at 554 or 712 nm. Our results agree with findings in several tree species by Carter and Spiering (2002). Compared with the reflectance at a single waveband, the ratios of reflectance at both 554 and 712 nm to reflectance in near infrared range (760-1300 nm) improved precision, and

could be used to estimate changes in leaf chlorophyll concentration in corn (Figures 7 and 8).

Although several studies have found that nondestructive measurements of leaf or canopy reflectance can be used as an indicator of plant N status (Ma et al., 1996; Voullot et al., 1998; Wang et al., 1998), the functional relationships between leaf reflectance or reflectance ratios and plant growth or physiological variables have been established in only a few studies. Yoder and Pettigrew-Crosby (1995) reported that leaf reflectance at visible bands could be used to predict chlorophyll content, and short-wave infrared bands were sensitive to leaf N concentration in big leaf maple (*Acer macrophyllum*). Fouche (1999) suggested that reflectance at the 779 nm wavelength may provide the best detection of N deficiency in cotton, tobacco and wheat. Carter and Estep (2002) reported that a simple linear relationship existed between leaf N (%) and reflectance at 721 nm in corn. In our study, leaf reflectance in two narrow waveband ranges of 550 – 580 and 700 – 720 nm had the largest negative correlations ($r = -0.70 \sim -0.75^{**}$) with leaf N concentration. However, the linear regression model of leaf N level and the single band reflectance at 575 or 712 nm with greatest $r^2$ values only explained 44% (on a leaf area basis, $r^2 = 0.44$) or 57% (on a leaf dry weight basis, $r^2 = 0.57$) of corn leaf N concentration variations (Figure 6).

Blackmer et al. (1996) reported that ratio of reflectance between 550 and 600 nm to reflectance in the 800 – 900 nm range provided sensitive detection of N stress in corn. In cotton, Tarpley et al. (2000) similarly found that ratios of leaf reflectance at a red edge (700 or 716 nm) with a waveband of very near infrared region (755 – 920 and 1000 nm) provided good precision and accuracy for predicting leaf N concentration. Our results indicated that a simple reflectance ratio ($R_{712}/R_{1040}$ or $R_{575}/R_{526}$) improved precision of estimating corn leaf N concentration (Figure 8), although the wavebands did not exactly match the findings of either Blackmer et al. (1996) or Tarpley et al. (2000). We found that when leaf N level was expressed on a dry weight basis, the best reflectance ratio for estimating corn leaf N was $R_{575}/R_{526}$ ($r^2 = 0.69$), and when leaf N concentration was expressed on a leaf area basis, the best ratio was $R_{712}/R_{1040}$ ($r^2 = 0.55$). Therefore, leaf reflectance ratio of $R_{575}/R_{526}$ or $R_{712}/R_{1040}$ may be used for predicting corn leaf N concentration.

## Conclusions

Nitrogen deficiency decreased stem elongation rate, leaf area, and leaf or canopy Pn of corn, resulting in shorter plants with less dry matter accumulation. Leaf hyperspectral reflectance was very sensitive to plant N status. Nitrogen deficiency mainly increased leaf reflectance in two spectrally narrow waveband ranges of green (550–580 nm) and far red (700–720 nm), which were closely related to either leaf N or chlorophyll concentration. On a leaf area basis, chlorophyll *a*, chlorophyll *b*, carotenoid, and chlorophyll *a*+*b* concentrations could be estimated using reflectance ratios in the near infrared region of 712 nm to 1088, 1097, 809, and 1088 nm, respectively. The N concentration based on leaf area (g $m^{-2}$) and leaf DW (g $kg^{-1}$ DW) could be estimated using the ratio of reflectance at 712 – 1040 nm and at 575 – 526 nm, respectively. Therefore, nondestructive measurements of leaf spectral reflectance at these narrow wavebands could provide a rapid, easy and inexpensive tool for detecting corn plant N status. The identified corn-N-specific spectral signatures may be used for image interpretation and diagnosis of corn N deficiency in a production environment for site-specific management.

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