Soil Fertility & Crop Nutrition

A Corn Nitrogen Status Indicator Less Affected by Soil Water Content

Juanjuan Zhu, Nicolas Tremblay,* and Yinli Liang

ABSTRACT

The SPAD-502 chlorophyll (Chl) meter and the Dualex device can estimate crop N status based on the measurement of leaf Chl concentration and polyphenolics (Phen) concentration, respectively. However, soil water status may confound such assessment of N status. This study compared the sensitivity of SPAD, Dualex, and SPAD/Dualex ratio as indicators for assessing corn (*Zea mays* L.) N status and the influence of soil water content (SWC) on the indicators in their absolute and nitrogen sufficiency index (NSI) expression. A greenhouse trial was conducted with five N fertilizer application rates (0, 50, 50+75, 50+150, and 200 kg N ha^{-1}) and three SWC levels (high, medium, and low). A field trial also was performed with six N rates (0, 20+40, 20+80, 20+120, 20+160, and 250 kg N ha^{-1}) and spatially variable SWC as a covariate. The responses of SPAD, Dualex, SPAD/Dualex ratio (and their corresponding NSI) to N rates and SWC levels were compared. The results showed that SPAD, Dualex, and SPAD/Dualex ratio were all influenced significantly by N rates and by SWC levels. When expressed as NSI, however, the parameters' relationships with N were essentially decoupled from SWC. The NSI_{SPAD} was more affected significantly by interactions among N, SWC, and DAS (days after sowing) than were $1/NSI_{Dualex}$ and $NSI_{SPAD/Dualex}$. The latter showed a greater sensitivity to N fertility levels than the other indicators, resulting in a better discrimination of N treatments and under variable SWC conditions in the two trials.

Schepers and Raun (2008) and Samborski et al. (2009) recently reviewed the environmental and economic issues related to N fertilization in agricultural systems and the need to improve the match between N supply and crop requirements, including approaches for assessing crop N status. The Chl concentration in corn is correlated positively with the leaf N concentration and N sufficiency (Blackmer and Schepers, 1995). Minolta SPAD-502 (Soil Plant Analysis Development, Minolta Camera Co., Ltd., Japan) measurements are related strongly to leaf Chl concentration (Markwell et al., 1995) so that it can be used to provide an indication of crop N status. Scharf et al. (2006) reported that Chl meter readings were highly significant predictors of economically optimal nitrogen rates and yield response to N for corn across a wide range of soil types, geography, landscape forms, weather conditions, corn hybrids, and management practices. However, SPAD meters can only detect severe N deficiencies (Ortuzar-Iragorri et al., 2005; Zhang et al., 2008) and some studies have indicated that the measurements are also influenced by crop variety, water and cold stress, location, and insect damage (Blackmer and Schepers, 1995; Samborski et al., 2009).

J. Zhu, College of Life Science, Northwest A&F Univ., No. 3 Taicheng Road, Yangling, Shaanxi, P.R. China, 712100; N. Tremblay, Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, 430 Boul. Gouin, St-Jean-sur-Richelieu, QC, Canada, J3B 3E6; Y. Liang, College of Life Science, Northwest A&F Univ., No. 3 Taicheng Road, Yangling, Shaanxi, P.R. China, 712100, Inst. of Soil Water and Conservation, Chinese Academy of Sciences and Ministry of Water Resources, No.26 Xinong Road, Yangling, Shaanxi, P.R. China, 712100. Received 13 Aug. 2010. *Corresponding author (Nicolas.Tremblay@agr.gc.ca).

Published in Agron. J. 103:890–898 (2011)
Published online 23 Mar 2011
doi:10.2134/agronj2010.0351

To eliminate or reduce the influence of confounding factors, Blackmer and Schepers (1995) indicated that readings should be normalized to plants in a well-fertilized reference plot within the same field. A normalized SPAD index, also called a NSI, is calculated by dividing the SPAD readings for the area being assessed by the highest value from the reference plot (Debaeke et al., 2006). Many studies have reported that a NSI expression is better than an absolute reading for assessing crop N status (Hussain et al., 2000; Debaeke et al., 2006; Hawkins et al., 2007; Samborski et al., 2009).

Alternatives to SPAD can be used for the nondestructive and quick assessment of N status in crops. For example, the Dualex (Force-A, Orsay, France) is a field-portable instrument for the evaluation of leaf Phen compounds (mainly flavonoids) from the measurement of UV (375 nm) absorbance of the leaf epidermis by double excitation of Chl fluorescence (Goulas et al., 2004). Polyphenolics are a diverse class of secondary metabolites and carbon-based compounds (including hydroxycinnamic acids, flavonoids, condensed tannins and lignin [Meyer et al., 2006]) having many functions in leaves (Cartelat et al., 2005; Tremblay et al., 2007). Their synthesis and accumulation in plants is generally stimulated in response to biotic/abiotic stresses (Ksouri et al., 2007) such as N shortage, according to the Protein (N-based compounds) Competition Model (Jones and Hartley, 1999). A good correlation has been obtained between Dualex-derived UV absorbance and the absorbance of a Phen extract (Goulas et al., 2004; Agati et al., 2008). Many reports have shown that Dualex readings decreased with increasing N fertilizer application dose (Cartelat et al., 2005; Tremblay et al., 2007; Demotes-Mainard et al.,2008; Tremblay et al., 2009; Tremblay et al.,

Abbreviations: CAN, calcium ammonium nitrate; Chl, chlorophyll; DAS, days after sowing; NSI, nitrogen sufficiency index; $1/NSI_{Dualex}$, the reciprocal of nitrogen sufficiency index of Dualex readings; NSI_{SPAD}, nitrogen sufficiency index of SPAD readings; $NSI_{SPAD/Dualex}$, nitrogen sufficiency index of SPAD/Dualex ratio; Phen, polyphenolics; SWC, soil water content.

2010). Demotes-Mainard et al. (2008) described Dualex as a better instrument than SPAD for the evaluation of N status in situ because Dualex readings were more strongly correlated with shoot N content in comparison to SPAD readings. Tremblay et al. (2007), however, indicated that Dualex was less reliable than SPAD for N status diagnosis at late growing stages for corn. Zebarth et al. (2009) suggested that Dualex makes a new contribution to the study of N limitations in agricultural crops because it is based on Phen rather than Chl status.

The two instruments can be used together to obtain a Chl/Phen (SPAD/Dualex) ratio, which has a positive correlation with leaf mass-based N content (Demotes-Mainard et al., 2008). Cartelat et al. (2005) suggested that the simple Chl/Phen ratio would relieve, at least partially, the problem of Chl and Phen gradients along leaves. The SPAD/Dualex ratio has been described as a better indicator of N status for wheat (*Triticum aestivum* subsp. *aestivum*) (Cartelat et al., 2005), corn (Tremblay et al., 2007), and broccoli (*Brassica oleracea* var. *italica Plenck*) (Tremblay et al., 2009) than either Dualex or SPAD values alone.

Soil water status is one of the major environmental factors affecting plant N availability from organic N sources (Agehara and Warncke, 2005; Prasertsak and Fukai, 1997). Nitrogen use efficiency (Aulakh and Malhi et al., 2005) and the response of a crop to N fertilizer applications (Tilling et al., 2007) are heavily dependent on available water. Water limitation restricts substantially total N uptake (Haefele et al., 2008). Shangguan et al. (2000) showed that leaf N content was higher under high water availability, regardless of N supply. Crop water status may interact significantly with N fertility conditions in the assessment of crop N status by SPAD (Martínez and Guiamet, 2004; Schlemmer et al., 2005). SPAD readings increased from 44.3 to 47.2 when relative water content (RWC) in tissue was reduced from 94 to 87.5% (Martínez and Guiamet, 2004), and Chl meter threshold values differ between irrigated and nonirrigated crops (Samborski et al., 2009). These findings indicate that SWC can modify SPAD results and cause bias in the estimation of crop N status. It is desirable to find a crop-based indicator that is highly sensitive to N status and as free from the influence of SWC as possible. Estiarte et al. (1999) reported a 10% increase in Phen concentrations in wheat leaves under drought stress, a finding consistent with Dualex measurements on irrigated broccoli (Tremblay et al., 2009). Dualex-based measurements for corn may be affected less than SPAD measurements by variable SWC conditions. At the same time, expressing N status indicators in the form of an NSI may help to reduce the influence of SWC on the assessment of N.

The objectives of this study were (i) to examine the effects of N and water (and their interaction) on SPAD, Dualex, and SPAD/Dualex values; (ii) to determine the usefulness of NSI calculations in minimizing the influence of SWC on N status assessment; and (iii) to recommend an optimal indicator of corn N status under variable SWC conditions.

MATERIALS AND METHODS

Greenhouse Experiment

Experimental Design

The greenhouse experiment was performed at Agriculture and Agri-Food Canada's Horticulture Research and Development Centre in St-Jean-sur-Richelieu (45°18' N, 73°15' W, elevation 47 m), QC, Canada, using the corn cultivar Pioneer 38B84. The soil used in the greenhouse trial was obtained from the L'Acadie experimental farm (0- to 30-cm layer), Quebec, Canada, in 2008, kept at room temperature in sealed barrels, and sieved through a 1-cm mesh. The soil was clay loam (Aquolls, Humaquepts), and presowing soil tests gave the following mean values: soil pH (water) was 7.3; organic matter content was 3.7%; NO_3–N was 48.3 mg kg^{-1}; available P (Mehlich 3) was 112 mg kg^{-1}; and available K (Mehlich 3) was 128 mg kg^{-1}. Such soil type tends to dry fast and get very hard in pots. Previous experience showed that a rockwool block (length, 10 cm; width, 10 cm; height, 5 cm) put on the bottom in each pot is able to buffer moisture conditions within the soil volume. Phosphate and potash fertilization was applied using 2.6 g of triple superphosphate (0–46–0 N–P–K) and 5.8 g of potassium-magnesium sulfate (0–0–22–11 N–P–K–S) per pot. Fertilizers P, K, and N (except the topdressing N) were applied to the surface layer (0–10 cm) of soil and mixed thoroughly with the soil. Corn was sown on 4 Mar. 2009 in plastic pots (o.d. 20 cm; height 16 cm). Eight seeds were sown in each plastic pot containing 2.85 kg of soil. Before the first sampling date, the smallest plants were removed and five were kept, to ensure stand uniformity.

Two experimental factors were studied: N (five rates) and soil moisture (three watering levels), for a total of 15 treatments. A split-plot design was used with four replications. This design was selected to facilitate watering operations. The water treatments were main-plot factor and the N fertilizer application rates were subplot factor. At sowing, calcium ammonium nitrate (CAN) was applied to provide N rates of 0, 0.64, 0.64, 0.64, and 2.55 g of N pot^{-1}. A direct comparison of greenhouse and field N rate is difficult to achieve. Nevertheless, the highest N fertilizer application rate treatment represented the well-fertilized reference plots with a rate roughly equivalent to 200 kg N ha^{-1} [the official recommendation in Quebec is 120–170 kg N ha^{-1} (CRAAQ, 2003)]. Nitrogen per hectare in the field was calculated according to: $N_f = N_{pot}/A_f \times 10{,}000/1000$, where N_f is the N rate (kg ha^{-1}) applied per hectare in the field, N_{pot} is the amount of N (g pot^{-1}) applied per pot in the greenhouse, A_f is the area per plant in the field ($A_f = 0.17$ m × 0.75 m = 0.1275 m^2). The N rates (kg ha^{-1}) applied for the greenhouse trial were therefore estimated as equivalent to 0, 50, 50+75, 50+150, and 200 kg N ha^{-1} applied in the field setting. Topdressing of N was done at 36 DAS using CAN, and the N rates supplied about 0, 0, 0.96, 1.91, and 0 g of N pot^{-1}, which matched with N treatments at sowing.

The soil water regime in all pots was kept at well watered conditions until the seedlings had between three and four leaves. At that point, different soil water regimes were established (Kang et al., 2000): low soil moisture [SWC (θ_v) from 13 to 14% (v/v)], medium soil moisture [SWC (θ_v) from 17 to 20% (v/v)], and high soil moisture [SWC (θ_v) from 25 to 28% (v/v)], as measured by a Type HH2 soil moisture device (Delta-T Devices Ltd., Cambridge, UK). The SWC in the high soil moisture treatment was set higher than that in Kang et al. (2000) because the volume of soil was smaller in pots than in the field and such soil type tends to dry fast in pots. The SWC (0–10 cm layer) was measured at five sampling points in each pot every 2 d before topdressing and every day thereafter, and then averaged. Immediately after topdressing, irrigation was done using three different watering levels. Water was added as to reach 14% (v/v) in low soil moisture, 20% (v/v) in medium soil moisture, and 28% (v/v) in high soil moisture, respectively, when actual SWC levels were reduced to, or almost to, the lower limit of the established range.

Sampling

Plant samples were collected five times [at 20 (V3–V4), 27 (V4–V5), 35, 55, and 62 DAS]. As of 35 DAS, the growth stages started to differ among SWC treatments. On each sampling date, one plant per pot was randomly selected for measurement and destructive sampling.

Field Experiment

Experimental Design

A corn experiment was conducted on six N treatments at L'Acadie (45°17´ N; 73°20´ W, elevation 47 m), Quebec, Canada, using the corn cultivar Pioneer 38M58. A completely randomized block design with four replications was used. The soil type ranged from loam to clay loam (Aquolls, Humaquepts), and a presowing soil (0- to 30-cm layer) test gave the following mean values: soil pH (water) 7.1; organic matter 4.1%; NO_3–N 5.0 mg kg^{-1}; $(P/Al)_{M\text{-}III}$ percentage (extracted by the Mehlich 3 soil testing method) 1.4%; and available K (Mehlich 3) 97.0 mg kg^{-1}. Corn was sown on 6 May 2009 at a density of 79,000 plants ha^{-1}. Plot size was 25 m by 9 m, and 12 rows of corn with a between-row spacing of 75 cm were planted in each plot. Phosphate and potash fertilization was applied at the recommended rates based on soil tests (0–30 cm) using 90 kg ha^{-1} of triple superphosphate (0–46–0 N–P–K), 25 kg ha^{-1} of potassium-magnesium-sulfate (0–0–22–11 N–P–K–S), and 25 kg ha^{-1} of potassium chloride (0–0–60 N–P–K).

Nitrogen treatments consisted of 0, 60, 100, 140, 180, and 250 kg N ha^{-1}, applied as CAN. In the 250 kg N ha^{-1} treatment (well-fertilized reference), 230 kg N ha^{-1} was broadcast and incorporated as a complement to 20 kg N ha^{-1} banded with the seed at sowing. The fertilizer N for the other treatments was applied on 25 June 2009 as N side-dressing banded 15 cm from the seed-row as a complement to 20 kg N ha^{-1} applied at sowing, except in the N0 treatment. A sprinkler irrigation line was used to generate a spatially variable water supply. The sprinkler irrigation pipe was put in the center of the plots. Hence, the areas close to the sprinkler irrigation lines received more water than those away. Rain gauges were distributed to assess the amount of irrigation water actually received at each destructive sampling point. The 2009 season was fairly rainy. Nonetheless, irrigation was provided six times: 16 June (40 DAS), 19 June (43 DAS), 22 June (46 DAS), 25 June (49 DAS), 30 June (54 DAS), and 3 July (57 DAS), providing surplus water for plants around the sprinkler irrigation line in the field.

Sampling

Six destructive samplings were performed: 41 (V3–V4), 48 (V4–V5), 60, 68, 82, and 90 DAS (VT). One sampling point was selected randomly in each plot on each sampling date for measurement and destructive testing. Destructively sampled areas consisted of 2-m-long strips.

Plant Measurements

Minolta SPAD-502 (Soil Plant Analysis Development, Minolta Camera Co., Ltd., Japan) and Dualex (Force-A, Orsay, France) measurements were made on the uppermost fully expanded leaves of the plants any time of day in the greenhouse or field experiment. Measurements were made at the center along the length of the leaf adaxial side (Tremblay et al., 2007), avoiding midribs, except at V3–V4 because the leaves were too narrow. A minimum of 20 leaves of uniform appearance were measured in each plot, and the values were averaged for the field experiment. In the greenhouse experiment, 20 readings from different positions on the same leaf were averaged to ensure consistency with field procedures. Values of NSI_{SPAD} and $NSI_{SPAD/Dualex}$ were between 0 and 1, but NSI_{Dualex} values were >1. The reciprocal of NSI_{Dualex} ($1/NSI_{Dualex}$) was used to facilitate comparison with NSI_{SPAD} and $NSI_{SPAD/Dualex}$. The N sufficiency index (NSI) was calculated by dividing all of the N status values by the values obtained from the reference plot in the two experiments (N200 in the greenhouse trial and N250 in the field trial).

Laboratory Analyses

Shoot biomass data were collected to check if treatments effects met the expectations, and to compare with N status indicators responses. In the greenhouse or field experiment, shoots were cut at ground level and oven-dried at 70°C for 7 d, after which the dry biomass was weighed. Samples were passed through a 1-mm screen in a Wiley mill, and stored at room temperature before laboratory analyses. Samples of 0.5 g of dried biomass were mineralized using a mixture of sulfuric and selenious acids, as described by Isaac and Johnson (1976). Total N concentrations were measured on a QuikChem 8000 Lachat autoanalyzer (Lachat Instruments, Milwaukee, WI) using the Lachat method 15-107-06-4-A (Lachat Instruments, 2010). Soil water content was measured gravimetrically only in the field experiment. Soil samples were taken from the 0- to 30-cm depth with a punch from sampling points in each plot throughout the experimental periods. The fresh weight of soil samples was determined; the samples were then oven-dried at 80°C for 12 h and weighed thereafter. The SWC values were calculated as follows: SWC (kg kg^{-1}) = (fresh soil weight – dry soil weight)/dry soil weight.

Statistical Analyses

The greenhouse trial data were subjected to correlation analysis, ANOVA, and orthogonal contrast analyses of linear (L), quadratic (Q), and cubic (C) effects for quantitative treatments (Little and Hills, 1978) with the PROC MIXED procedure of SAS (SAS Institute, 2003). First, an ANOVA was conducted to evaluate the effects of treatments. When significant influences of treatments were found, orthogonal polynomial coefficients for each treatment were calculated with the Khanizadeh and Fanous (1992) software and introduced in the PROC MIXED model of SAS. When both linear and quadratic analyses were significant, the trend was labeled as curvilinear (Hedeker and Gibbons, 2006). Since the water treatments were not fixed values, the average values of SWC established for each treatment were used to calculate contrast coefficients. Time-repeated measures analysis (repeated ANOVA) was used to determine the influence of N rates, SWC, and DAS on dry shoot biomass, SPAD, Dualex, and SPAD/Dualex ratio, as well as the corresponding NSI values, using the PROC MIXED procedure of SAS (Klaus and Oscar, 2008).

In the field trial, since soil moisture was not controlled, SWC measurements were treated as a covariate in a covariance analysis (ANCOVA) performed to evaluate the effects of N rates and

Table 1. Pearson's correlation coefficients (R) between N status indicators and tissue N concentration (g kg^{-1}) in the greenhouse experiment.

Sampling date†	SPAD	Dualex	SPAD/Dualex	NSI_{SPAD}‡	$1/NSI_{Dualex}$	$NSI_{SPAD/Dualex}$
20 DAS (-15)	0.84***	-0.81***	0.64***	0.47***	0.50***	0.54***
27 DAS (-8)	0.70***	-0.76***	0.42***	0.37*	0.58***	0.60***
35 DAS (0)	0.88***	-0.66***	0.49***	0.64***	0.57***	0.62***
55 DAS (+20)	0.84***	-0.80***	0.72***	0.26	0.37*	0.53***
62 DAS (+27)	0.40**	-0.82***	0.69***	0.42**	0.39**	0.42**

* Indicates difference at the $P \leq 0.05$ probability level.
** Indicates difference at the $P \leq 0.01$ probability level.
*** Indicates difference at the $P \leq 0.001$ probability level.
† DAS, days after sowing. The number in the parentheses is the days before (-) or after (+) topdressing.
‡ Rich reference plots (N200) excluded in statistical analysis for the nitrogen sufficiency index (NSI).

SWC on each sampling date, using the general linear model (GLM) procedures in SAS (Milliken and Johnson, 2001). The first step was to determine whether the covariate had a significant influence on the parameter of interest or not. When the influence of the SWC covariate was not significant, a contrast analysis was done to estimate the effects of N rates with the SWC covariate excluded from the model. When the influence of the SWC covariate was significant, a contrast analysis was done to estimate the effects of N rates with the data adjusted for the SWC covariate included in the model. Correlation analysis was used to show the relationship between SWC and the parameters of interest. Pearson's linear correlation coefficients (*R*) indicated that the parameters of interest varied positively or negatively with increasing SWC levels while SWC effects were deemed significant based on ANCOVA. Analyses of covariance for repeated measures (repeated ANCOVA) were performed to estimate the effects of N rates, SWC, and DAS using the PROC MIXED procedure of SAS, as described by Milliken and Johnson (2001). If SWC alone or any interaction between SWC and other factors was significant, a repeated ANCOVA was conducted; otherwise a repeated ANOVA was conducted.

RESULTS AND DISCUSSION

Greenhouse Experiment

Relationships between Nitrogen Status Indicators and Nitrogen Tissue Concentration

For each sampling date, the linear correlations between SPAD, Dualex, and their respective NSI, on one hand, and tissue N concentration, on the other hand, were significant, with the exception of NSI_{SPAD} at 55 DAS (Table 1). Positive correlations (negative for the Dualex) were found for all parameters. Similar results were obtained by Cartelat et al. (2005), Meyer et al. (2006), Tremblay et al. (2007), and Esfahani et al. (2008).

Influence of Nitrogen Application Rates and Days after Sowing

Shoot biomass accumulation was strongly dependent on N application rates (data not shown). It increased slowly before topdressing, and then rapidly. Before topdressing, shoot biomass was related quadratically to the N rate because N50 increased biomass more than N200. After topdressing, the N rate/shoot biomass relationship was curvilinear.

Tissue N concentration increased progressively with DAS before topdressing, but decreased gradually thereafter (Fig. 1). The N0 (and N50 after topdressing) treatment resulted in relatively low N concentrations.

SPAD readings and SPAD/Dualex ratio increased in response to N rates, whereas Dualex readings decreased (Fig. 2). Similar effects were found in previous studies (Cartelat et al., 2005; Tremblay et al., 2007, 2009, and 2010; and Demotes-Mainard et al., 2008). The strong quadratic components of the relationships were due to the fact that the N0 treatment resulted in much different readings than the other treatments.

Nitrogen rates increased linearly the NSI_{SPAD}, $1/NSI_{Dualex}$, and $NSI_{SPAD/Dualex}$ on each sampling date before topdressing, whereas quadratic components were also present after topdressing (Fig. 3). On all measurement dates, NSI values were significantly lower in the unfertilized control (N0). After topdressing, only $NSI_{SPAD/Dualex}$ maintained levels consistent with the N treatments.

Influence of Soil Water Content and Days after Sowing

Shoot biomass was linearly related to SWC levels before topdressing, but curvilinearly thereafter. A quadratic trend appeared after topdressing as the positive effect of the fully watered treatment became more obvious (data not shown).

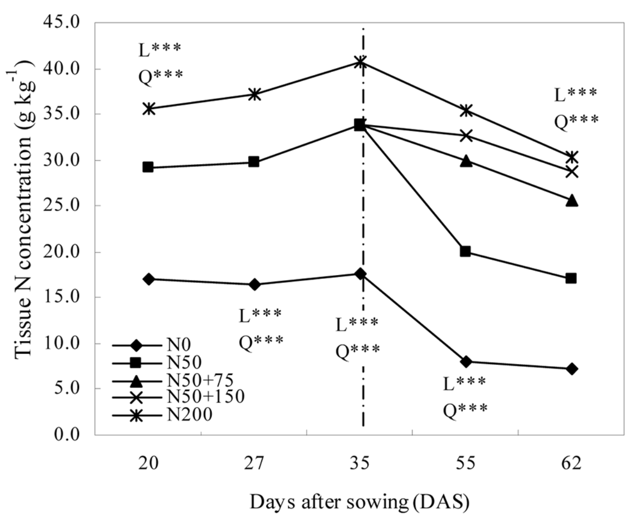

Fig. 1. Influence of N rates on tissue N concentration (g kg^{-1}) at different days after sowing (DAS) in the greenhouse experiment. Nitrogen topdressing was done at 36 DAS (vertical broken line). The asterisks indicate significant differences at the 0.001 (***) probability level. L and Q indicate linear and quadratic component among N treatments, respectively, based on orthogonal contrast analyses.

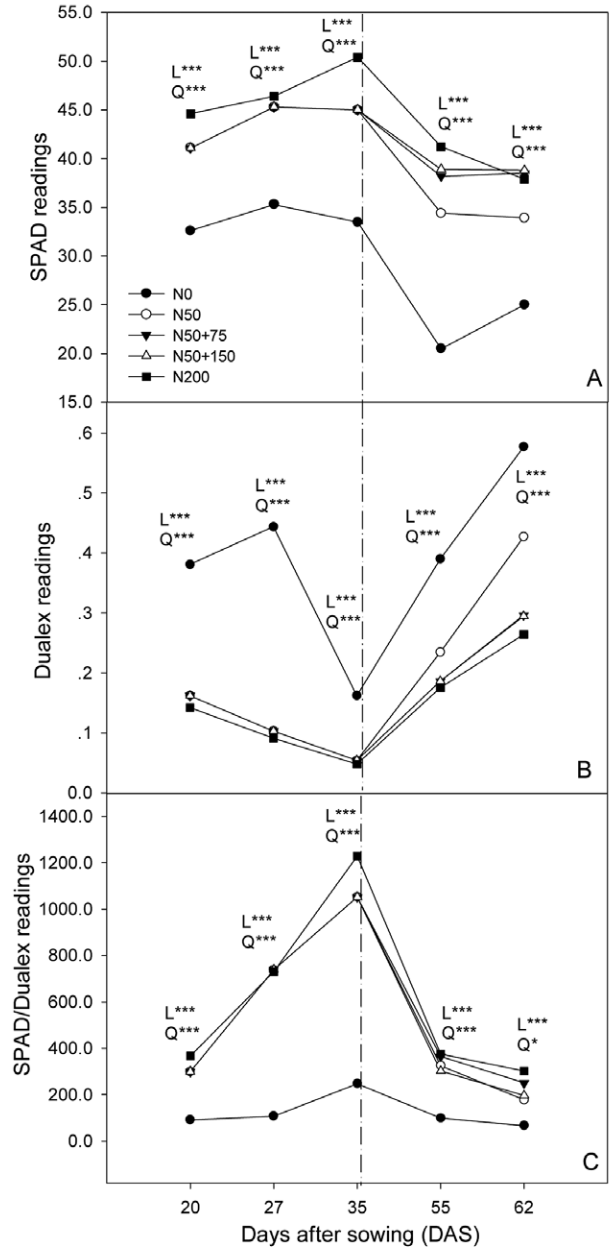

Fig. 2. Influence of N rates on (A) SPAD, (B) Dualex, and (C) SPAD/Dualex at different days after sowing (DAS) in the greenhouse experiment. Nitrogen topdressing was done at 36 DAS (vertical broken line). The asterisks indicate significant differences at the 0.05 (*) and 0.001 (***) probability levels. L and Q indicate linear and quadratic component among N treatments, respectively, based on orthogonal contrast analyses.

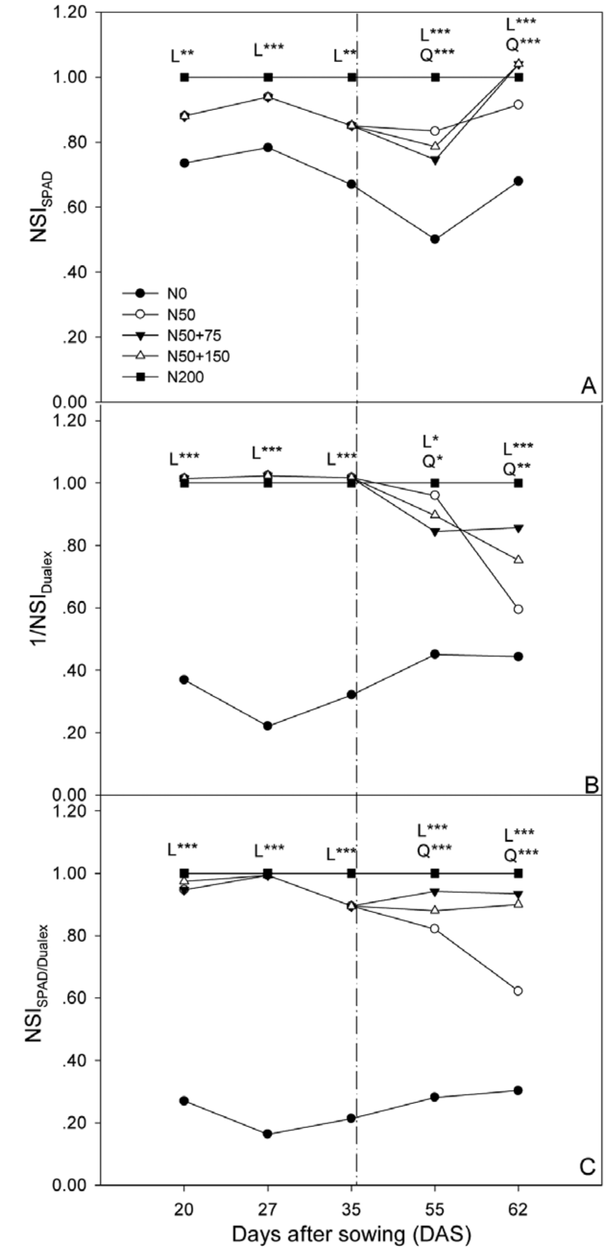

Fig. 3. Influence of N rates on NSI_{SPAD} (A), $1/NSI_{Dualex}$ (B), and $NSI_{SPAD/Dualex}$ (C) at different days after sowing (DAS) in the greenhouse experiment. N topdressing was done at 36 DAS (vertical broken line). The asterisks indicate significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability levels. L and Q indicate linear and quadratic component among N treatments, respectively, based on orthogonal contrast analyses. N200 was excluded from the statistical analyses as it is included by default in the NSI calculation.

Before topdressing, a significant increase of tissue N with SWC levels was found on the first sampling date (20 DAS). After topdressing, the inverse was observed as tissue N declined with SWC (Fig. 4).

SPAD and SPAD/Dualex decreased (and Dualex increased) with SWC levels (data not shown), behaving similarly as tissue N concentration after topdressing. This decrease for SPAD is in agreement with Martínez and Guiamet (2004) and Gianquinto et al. (2004). This is likely attributable to leaf shrinkage, which modifies the Chl concentration per leaf area (Samborski et al., 2009), and to the fact that SWC treatments were more effective at later stages of development. Schlemmer et al. (2005) found that SPAD readings were affected significantly by either N or water status, whereas laboratory-analyzed Chl concentrations were affected significantly only by N treatments. SPAD measurement is based on the principle of relative transmittance, which may be more sensitive to water stress than to N stress (Elwadie et al., 2005). Reduced cell turgor caused by the variations of intercellular air spaces in the leaf tissue affects the transmittance of NIR energy (Samborski et al., 2009). Cartelat et al. (2005) found that Phen decreased on the adaxial side of wheat leaves under severe water deficit, in agreement with results for Dualex in the present study, but Estiarte et al. (1999) and Tremblay et al. (2009) obtained

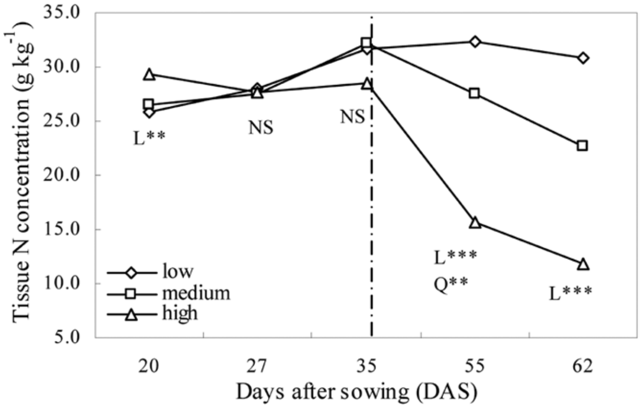

Fig. 4. Influence of SWC levels (low, medium, or high) on tissue N concentration (g kg^{-1}) at different days after sowing (DAS) in the greenhouse experiment. Nitrogen topdressing was done at 36 DAS (vertical broken line). The asterisks indicate significant differences at the 0.01 (**) and 0.001 (***) probability levels. NS indicates no significant difference. L and Q indicate linear and quadratic component among SWC levels, respectively, based on orthogonal contrast analyses.

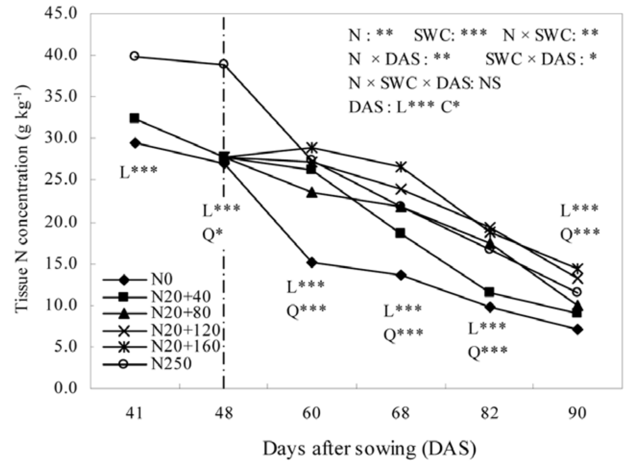

Fig. 5. Influence of N rates on tissue N concentration (g kg^{-1}) at different days after sowing (DAS) in the field trial. N side-dressing was done at 48 DAS (V4–V5; vertical broken line). Repeated analyses of covariance were used after side-dressing. The asterisks indicate significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability levels. NS indicates no significant difference. L, Q, and C denote the linear, quadratic, and cubic components, respectively.

different results. This apparent contradiction is due to observed leaf rolling by Cartelat et al. (2005), which resulted in shading of the measured epidermis and a reduction in the influence of direct sunlight which normally promotes Phen accumulation.

Almost no significant influence of SWC was observed on NSI_{SPAD}, $1/NSI_{Dualex}$, and $NSI_{SPAD/Dualex}$ during the whole experiment (data not shown). This is an indication that NSI calculations can minimize the effects of SWC on N status assessment. A significant SWC × DAS interaction was observed on NSI_{SPAD}.

Interactions among Nitrogen Application Rates, Soil Water Content, and Days after Sowing

Shoot biomass was affected by N × SWC and by N × SWC × DAS interactions, given that the biomass level increased with N rates after topdressing, but only for the fully watered treatment (data not shown). Significant N × SWC and/or N × SWC × DAS interactions were also found for tissue N concentration, SPAD, Dualex, and SPAD/Dualex, as well as NSI_{SPAD}, but not for $1/NSI_{Dualex}$ or $NSI_{SPAD/Dualex}$ (data not shown). The reduced influence of interactions on $1/NSI_{Dualex}$ and $NSI_{SPAD/Dualex}$ suggests that they would be more stable among variable SWC and DAS conditions.

Field Experiment

Relationships between Nitrogen Status Indicators and Nitrogen Tissue Concentration

In general, positive correlations (negative for Dualex) with tissue N concentration were observed for all indicators, except for $1/NSI_{Dualex}$ (Table 2). Dualex was less correlated than SPAD with tissue N concentrations.

Influence of Nitrogen Application Rates and Days after Sowing

As for the greenhouse experiment, shoot biomass increased slowly before side-dressing, but rapidly thereafter. In general, N rates resulted in a linear increase of shoot biomass at each DAS (data not shown). The N0 treatment became more and more divergent from the other treatments with increasing DAS.

During the corn growing season, tissue N concentration decreased gradually with increasing DAS (Fig. 5). Nitrogen rates increased tissue N concentration curvilinearly at all DAS but the first. There was a significant N × DAS interaction after side-dressing as tissue N concentration in the *all-at-sowing* N250 treatment became lower than the two highest N side-dressing rates.

SPAD readings varied overall from 21.2 to 54.2 (Fig. 6A). They reached a peak and then decreased, in agreement with Dwyer et al. (1995) and Ziadi et al. (2008). SPAD readings and SPAD/Dualex

Table 2. Pearson's correlation coefficients (*R*) between N status indicators and tissue N concentration (g kg^{-1}) in the field experiment.

Sampling date†	SPAD	Dualex	SPAD/Dualex	NSI_{SPAD}‡	$1/NSI_{Dualex}$	$NSI_{SPAD/Dualex}$
41 DAS (-7)	0.78***	-0.52**	0.80***	0.77***	0.06	0.74***
48 DAS (0)	0.75***	-0.71***	0.79***	0.54*	0.27	0.46*
60 DAS (+12)	0.70***	-0.37	0.55***	0.79***	0.41	0.72***
68 DAS (+20)	0.75***	-0.38***	0.61***	0.86***	0.28	0.70***
82 DAS (+34)	0.86***	-0.63***	0.73***	0.86***	0.76***	0.85***
90 DAS (+42)	0.84***	-0.41*	0.87***	0.73***	0.46*	0.76***

* Indicates difference at the $P \leq 0.05$ probability level.

** Indicates difference at the $P \leq 0.01$ probability level.

*** Indicates difference at the $P \leq 0.001$ probability level.

† DAS, days after sowing. The number in the parentheses is the days before (-) or after (+) topdressing.

‡ Rich reference plots (N250) excluded in statistical analysis for the nitrogen sufficiency index (NSI).

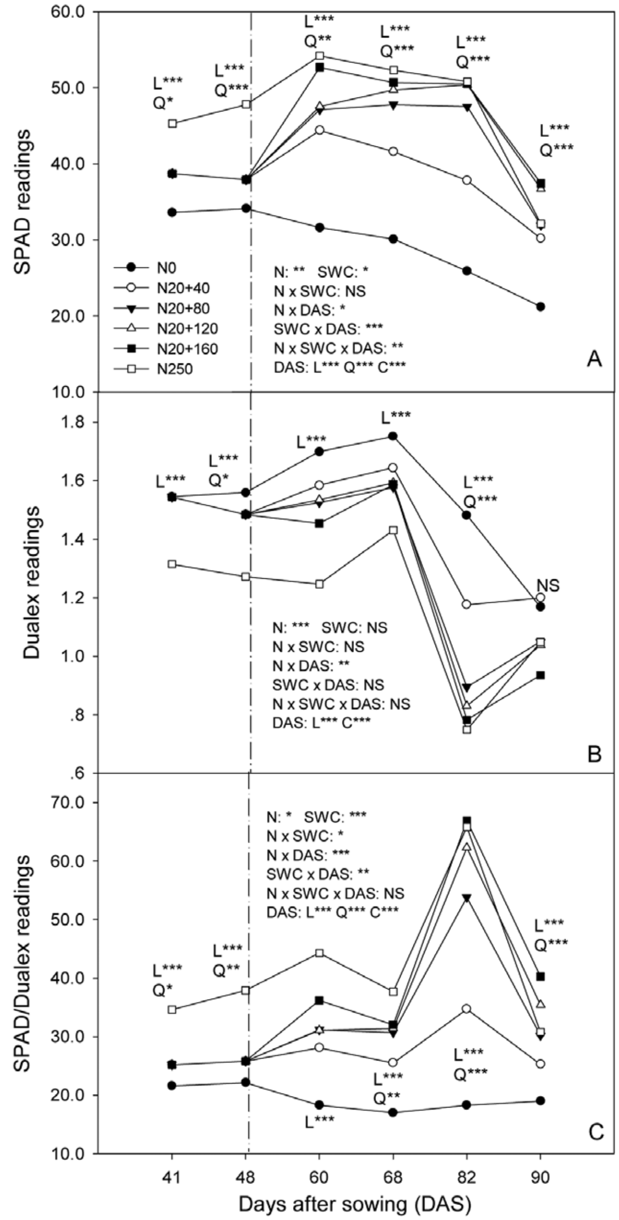

Fig. 6. Influence of N rates on (A) SPAD, (B) Dualex, and (C) SPAD/Dualex at different days after sowing (DAS) in the field trial. Nitrogen side-dressing was done at 48 DAS (V4–V5; vertical broken line). Repeated analyses of covariance were used after side-dressing. The asterisks indicate significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability levels. NS indicates no significant difference. L, Q, and C denote the linear, quadratic, and cubic components, respectively.

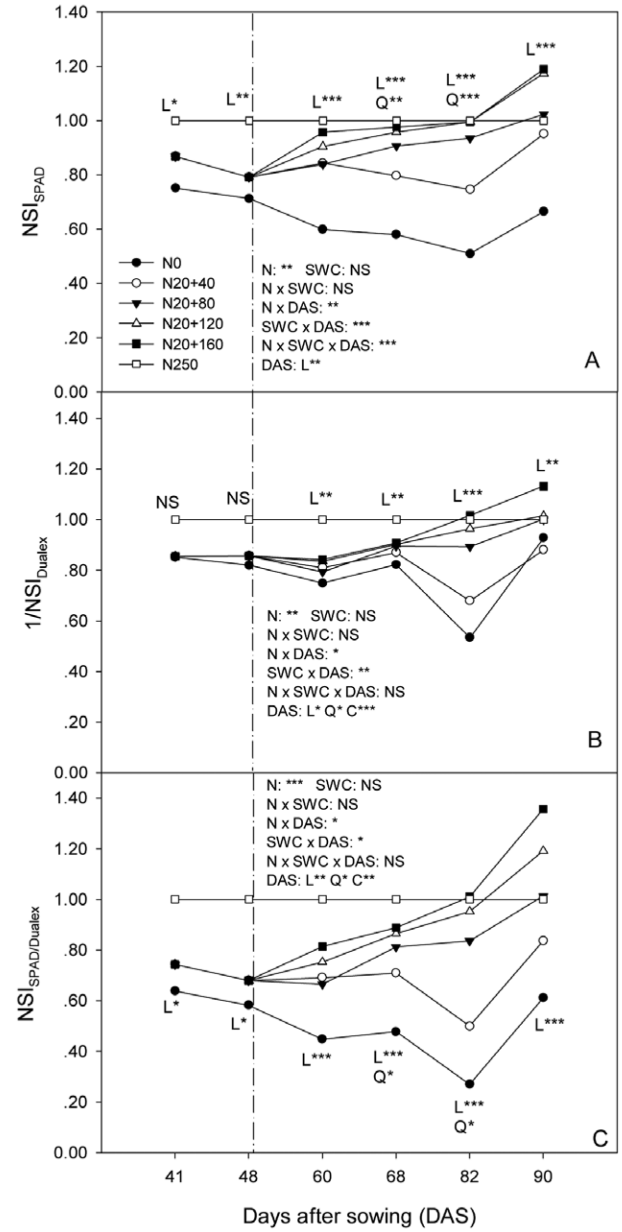

Fig. 7. Influence of N rates on (A) NSI_{SPAD} (nitrogen sufficiency index of SPAD readings), (B) $1/NSI_{Dualex}$ (the reciprocal of nitrogen sufficiency index of Dualex readings), and (C) $NSI_{SPAD/Dualex}$ (nitrogen sufficiency index of SPAD/Dualex ratio) at different days after sowing (DAS) in the field trial. Nitrogen side-dressing was done at 48 DAS (V4–V5; vertical broken line). Repeated ANCOVAs were used after side-dressing. The asterisks indicate significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability levels. NS indicates no significant difference. L, Q, and C denote the linear, quadratic, and cubic components, respectively. N250 was excluded in the statistical analyses as it is included by default in the NSI calculation.

ratio increased curvilinearly with N rates (Fig. 6). SPAD readings for the unfertilized control increasingly stood apart from the other treatments with increasing DAS. Dualex readings showed an inverse linear relationship with N rates. These changes with DAS may be due either to the particular leaf selected for sampling or to changes in the parameters themselves, such as the Phen concentration (Estiarte et al., 1999; Cartelat et al., 2005).

With the SPAD/Dualex ratio, the relative difference between the lowest and the highest N levels was always greater (and notably before N topdressing when an assessment of N status is required) than with SPAD in both the greenhouse (Fig. 2A and C) and the field trial (Fig. 6A and C). For the whole experimental period, the mean value of the SPAD/Dualex ratio was 383% greater for the highest N as compared with the lowest N level in the greenhouse trial (Fig. 2C) and 120% greater in the field trial (Fig. 6C). These values correspond to 54% (Fig. 2A) and 61% (Fig. 6A), respectively, for SPAD.

The NSI_{SPAD} increased linearly with N rates on all sampling dates, with occasional quadratic components (Fig. 7). $1/NSI_{Dualex}$ increased linearly with N rates but only after side-dressing. $NSI_{SPAD/Dualex}$ behaved similarly to NSI_{SPAD} but with greater amplitude and better segregation of treatments. On the last

Table 3. The effects of soil water content (SWC) levels (kg kg^{-1}) on N status indicators at different days after sowing (DAS) based on ANCOVA in the field experiment.

N status indicators	41 DAS (–7) †	48 DAS (0)	60 DAS (+12)	68 DAS (+20)	82 DAS (+34)	90 DAS (+42)
Tissue N concentration	* (+0.24)‡	** (+0.16)	*** (-0.49)	** (-0.15)	ns§	ns
SPAD	*** (+0.52)	*** (+0.54)	** (+0.13)	** (+0.17)	ns	ns
Dualex	ns	** (-0.55)	** (-0.31)	** (-0.30)	* (-0.18)	ns
SPAD/Dualex	*** (+0.44)	*** (+0.53)	*** (+0.23)	*** (+0.23)	ns	ns
NSI_{SPAD}¶	*** (+0.49)	* (+0.30)	ns	ns	ns	ns
$1/NSI_{Dualex}$	ns§	ns	ns	ns	* (+0.03)	ns
$NSI_{SPAD/Dualex}$	** (+0.32)	* (+0.23)	ns	ns	* (-0.07)	ns

* Indicates difference at the $P \le 0.05$ probability level.

** Indicates difference at the $P \le 0.01$ probability level.

*** Indicates difference at the $P \le 0.001$ probability level.

† The number in parentheses is the days before (-) or after (+) side-dressing.

‡ The data in parentheses are Pearson's linear correlation coefficients (R) between N status indicators and SWC levels, indicating the positive (+) or negative (-) trend of N status indicators with SWC levels.

§ ns indicates no significant difference.

¶ Rich reference plots (N250) excluded in statistical analysis for the nitrogen sufficiency index (NSI).

sampling date after side-dressing, the highest N rates resulted in NSI values >1, since N saturation decreased in the N250 reference treatment as the season progressed.

Influence of Soil Water Content and Days after Sowing

Shoot biomass was positively related to SWC at 48, 60, 68, and 90 DAS (data not shown). Tissue N concentration varied positively with SWC levels before 48 DAS but negatively after side-dressing on 60 and 68 DAS (Table 3). There was a significant N × SWC interaction after side-dressing (Fig. 5).

SPAD and SPAD/Dualex ratio were positively related to SWC levels before 68 DAS, in agreement with Schröder et al. (2000) for SPAD. Dualex was negatively related to SWC before 82 DAS (except for 41 DAS) (Table 3). However, the degree of correlation generally decreased with DAS ($R_{Pearson}$ went from 0.52 to 0.17 for SPAD, from 0.44 to 0.23 for SPAD/Dualex ratio, and from −0.55 to −0.18 for Dualex). SPAD and SPAD/Dualex were affected by SWC × DAS interactions but Dualex was not (Fig. 6). There was a significant N × SWC interaction in SPAD/Dualex ratio after side-dressing (Fig. 6C).

NSI_{SPAD} and $NSI_{SPAD/Dualex}$ showed a positive trend with SWC before side-dressing (Table 3). There was no significant SWC effects on NSI_{SPAD}, $1/NSI_{Dualex}$, and $NSI_{SPAD/Dualex}$ after side-dressing (Table 3, Fig. 7), as was the case in the greenhouse trial.

Performance of the Dualex in a Greenhouse and a Field Environment

Dualex measurements depend on how much UV light crosses the leaf epidermis exciting Chl fluorescence (Goulas et al., 2004), which is used as a remote and quick measure of leaf Phen accumulation, itself triggered by stress level (such as UV rays from the sun or N deficiency). Dualex levels ranged from 0.05 to 0.60 in the greenhouse trial (Fig. 2B) and from 0.75 to 1.75 in the field trial (Fig. 6B). This difference is attributable to the reduced UV levels reaching corn leaves through the greenhouse glass (Lau et al., 2006). Dualex values were on average 58% higher in the zero-N control than in N fertilized treatments in the greenhouse trials and only 14% higher in the field trial. Dualex measurements were overall better correlated with tissue N in a greenhouse (Table 1) than in a field environment (Table 2). This indicates that, despite the overall limited Phen accumulation in a greenhouse setting, Dualex is still able to capture N treatment effects since they are better controlled within pots than in a field plot setting, with soil N mineralization occurring even in the zero-N control.

CONCLUSION

A good indicator requires specificity, sensitivity, and robustness for N status assessment. The SPAD-502 can estimate leaf Chl content and the Dualex can estimate Phen content—two recognized indicators of crop N status. This study showed, on one hand, that SPAD, Dualex, and SPAD/Dualex were highly related to N fertilizer application rates and were significantly correlated to tissue N. However, they were also affected strongly by soil moisture. On the other hand, the significant influence of N rates on NSI_{SPAD}, $1/NSI_{Dualex}$, and $NSI_{SPAD/Dualex}$, and their correlation with tissue N concentration were also confirmed. $NSI_{SPAD/Dualex}$ was better correlated with tissue N concentration than NSI_{SPAD} in the greenhouse trial and was close to NSI_{SPAD} in the field trial. $NSI_{SPAD/Dualex}$ was better correlated with tissue N concentration than $1/NSI_{Dualex}$ in both trials. $NSI_{SPAD/Dualex}$ showed a significant linear correlation with N rates and a better discrimination of N fertility conditions than NSI_{SPAD} or $1/NSI_{Dualex}$. $NSI_{SPAD/Dualex}$ was therefore the best indicator of corn N status.

In this study, SPAD and Dualex measurements were made with two different instruments but a new version of the Dualex has been recently released that provides a one-shot assessment of the SPAD/Dualex ratio.

ACKNOWLEDGMENTS

The authors would like to thank Edith Fallon, Carl Bélec, Marcel Tétreault, Min Xie, the summer students, the greenhouse crew, and the L'Acadie Experimental farm.

REFERENCES

Agati, G., Z.G. Cerovic, A.D. Marta, V.D. Stefano, P. Pinelli, M.L. Traversi, and S. Orlandini. 2008. Optically-assessed preformed flavonoids and susceptibility of grapevine to *Plasmopara viticola* under different light regimes. Funct. Plant Biol. 35:77–84.

Agehara, S., and D.D. Warncke. 2005. Soil moisture and temperature effects on nitrogen release from organic nitrogen sources. Soil Sci. Soc. Am. J. 69:1844–1855.

Aulakh, M.S., and S.S. Malhi. 2005. Interaction of nitrogen with other nutrients and water: Effect on crop yield and quality, nutrient use efficiency, carbon sequestration and environmental pollution. Adv. Agron. 86:341–409.

Blackmer, T.M., and J.S. Schepers. 1995. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. J. Prod. Agric. 8:56–60.

Cartelat, A., Z.G. Cerovic, Y. Goulas, S. Meyer, C. Lelarge, J.L. Prioul, A. Barbottinc, M.H. Jeuffroy, P. Gate, G. Agati, and I. Moya. 2005. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). Field Crops Res. 91:35–49.

Centre de Références en Agriculture et Agroalimentaire du Québec. 2003. Fertilisation reference guide (In French). CRAAQ, Québec, QC, Canada.

Debaeke, P., P. Rouet, and E. Justes. 2006. Relationship between the normalized SPAD index and the nitrogen nutrition index: Application to durum wheat. J. Plant Nutr. 29:75–92.

Demotes-Mainard, S., R. Boumaza, S. Meyer, and Z.G. Cerovic. 2008. Indicators of nitrogen status for ornamental woody plants based on optical measurements of leaf epidermal polyphenol and chlorophyll contents. Sci. Hortic. 115:377–385.

Dwyer, L.M., A.M. Anderson, B.L. Ma, D.W. Stewart, M. Tollenaar, and E. Gregorich. 1995. Quantifying the nonlinearity in chlorophyll meter response to corn leaf nitrogen concentration. Can. J. Plant Sci. 75:179–182.

Elwadie, M.E., F.J. Pierce, and J. Qi. 2005. Remote sensing of canopy dynamics and biophysical variables estimation of corn in Michigan. Agron. J. 97:99–105.

Esfahani, M., H.R.A. Abbasi, B. Rabiei, and M. Kavousi. 2008. Improvement of nitrogen management in rice paddy fields using chlorophyll meter (SPAD). Paddy Water Environ. 6:181–188.

Estiarte, M., J. Penuelas, B.A. Kimball, D.L. Hendrix, P.J. Pinter, G.W. Wall, R.L. LaMorte, and D.J. Hunsaker. 1999. Free-air CO_2 enrichment of wheat: Leaf flavonoid concentration throughout the growth cycle. Physiol. Plant. 105:423–433.

Gianquinto, G., J.P. Goffart, M. Olivier, G. Guarda, M. Colauzzi, L.D. Costa, G.D. Vedove, J. Vos, and D.K.L. Mackerron. 2004. The use of hand-held chlorophyll meters as a tool to assess the nitrogen status and to guide nitrogen fertilization of potato crop. Potato Res. 47:35–80.

Goulas, Y., Z.G. Cerovic, A. Cartelat, and I. Moya. 2004. Dualex: A new instrument for field measurements of epidermal ultraviolet absorbance by chlorophyll fluorescence. Appl. Opt. 43:4488–4496.

Haefele, S.M., S.M.A. Jabbar, J.D.L.C. Siopongco, A. Tirol-Padre, S.T. Amarante, P.C.S. Cruz, and W.C. Cosico. 2008. Nitrogen use efficiency in selected rice (*Oryza sativa* L.) genotypes under different water regimes and nitrogen levels. Field Crops Res. 107:137–146.

Hawkins, J.A., J.E. Sawyer, D.W. Barker, and J.P. Lundvall. 2007. Using relative chlorophyll meter values to determine nitrogen application rates for corn. Agron. J. 99:1034–1040.

Hedeker, D., and R.D. Gibbons. 2006. Longitudinal data analysis. John Wiley & Sons, Hoboken, NJ.

Hussain, F., K.F. Bronson, S. Yadvinder, S. Bijay, and S. Peng. 2000. Use of chlorophyll meter sufficiency indices for nitrogen management of irrigated rice in Asia. Agron. J. 92:875–879.

Isaac, R.A., and W.C. Johnson. 1976. Determination of total nitrogen in plant tissue using a block digester. J. Assoc. Off. Anal. Chem. 59:98–100.

Jones, C.G., and S.E. Hartley. 1999. A protein competition model of phenolic allocation. Oikos 86:27–44.

Kang, S.Z., W.J. Shib, and J.H. Zhang. 2000. An improved water-use efficiency for maize grown under regulated deficit irrigation. Field Crops Res. 67:207–214.

Khanizadeh, S., and M.A. Fanous. 1992. Statistical methods—A computer program to calculate orthogonal polynomial coefficients. HortScience 27:367.

Klaus, H., and K. Oscar. 2008. Design and analysis of experiments. John Wiley & Sons, Hoboken, NJ.

Ksouri, R., W. Megdiche, A. Debez, H. Falleh, C. Grignon, and C. Abdelly. 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte cakile maritime. Plant Physiol. Biochem. 45:244–249.

Lachat Instruments. 2010. Methods list for automated ion analyzers (flow injection analyses, ion chromatography). Available at http://www.lachatinstruments.com/download/LL022-Methods-List_5-10.pdf [updated May 2010; verified 1 Mar. 2011]. Lachat Instruments, Milwaukee, WI.

Lau, T.S.L., E. Eno, G. Goldstein, C. Smith, and D.A. Christopher. 2006. Ambient levels of UV-B in Hawaii combined with nutrient deficiency decrease photosynthesis in near-isogenic maize lines varying in leaf flavonoids: Flavonoids decrease photoinhibition in plants exposed to UV-B. Photosynthetica 44(3):394–403.

Little, T.M., and F.J. Hills. 1978. Agricultural experimentation: Design and analysis. Wiley, New York.

Markwell, J., J.C. Osterman, and J.L. Mitchell. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynth. Res. 46:467–472.

Martínez, D.E., and J.J. Guiamet. 2004. Distortion of the SPAD-502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie 24:41–46.

Meyer, S., Z.G. Cerovic, Y. Goulas, P. Montpied, S. Demotes-Mainard, L.P.R. Bidel, I. Moya, and E. Dreyer. 2006. Relationships between optically assessed polyphenols and chlorophyll contents and leaf mass per area ratio in woody plants: A signature of the carbon–nitrogen balance within leaves? Plant Cell Environ. 29:1338–1348.

Milliken, G.A., and D.E. Johnson. 2001. Analysis of messy data: Volume III. Analysis of covariance. Chapman & Hall/CRC, Boca Raton, FL.

Ortuzar-Iragorri, M.A., A. Alonso, A. Castellon, G. Besga, J.M. Estavillo, and A. Aizpurua. 2005. N-tester use in soft winter wheat: Evaluation of nitrogen status and grain yield prediction. Agron. J. 97:1380–1389.

Prasertsak, A., and S. Fukai. 1997. Nitrogen availability and water stress interaction on rice growth and yield. Field Crops Res. 52:249–260.

Samborski, S.M., N. Tremblay, and E. Fallon. 2009. Strategies to make use of plant sensors-based diagnostic information for nitrogen recommendations. Agron. J. 101:800–816.

SAS Institute. 2003. SAS for Windows. v. 9.1. SAS Inst., Cary, NC.

Scharf, P.C., S.M. Brouder, and R.G. Hoeft. 2006. Chlorophyll meter readings can predict nitrogen need and yield response of corn in the North-Central USA. Agron. J. 98:655–665.

Schepers, J.S., and W.R. Raun. 2008. Nitrogen in agricultural systems. ASA, CSSA, and SSSA, Madison, WI.

Schlemmer, M.R., D.D. Francis, J.F. Shanahan, and J.S. Schepers. 2005. Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. Agron. J. 97:106–112.

Schröder, J.J., J.J. Neeteson, O. Oenema, and P.C. Struik. 2000. Does the crop or the soil indicate how to save nitrogen in maize production? Reviewing the state of the art. Field Crops Res. 66:151–164.

Shangguan, Z.P., M.A. Shao, and J. Dyckmans. 2000. Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. Environ. Exp. Bot. 44:141–149.

Tilling, A.K., G.J. O'Leary, J.G. Ferwerda, S.D. Jones, G.J. Fitzgerald, D. Rodriguez, and R. Belford. 2007. Remote sensing of nitrogen and water stress in wheat. Field Crops Res. 104:77–85.

Tremblay, N., É. Fortier, R. Mellgren, C. Bèlec, and S. Jenni. 2009. The Dualex—A new tool to determine nitrogen sufficiency in broccoli. Acta Hortic. 824:121–131.

Tremblay, N., Z. Wang, and C. Bèlec. 2007. Evaluation of the Dualex for the assessment of corn nitrogen status. J. Plant Nutr. 30:1355–1369.

Tremblay, N., Z. Wang, and C. Bélec. 2010. Performance of Dualex in spring wheat for crop nitrogen status assessment, Yield prediction and estimation of soil nitrate content. J. Plant Nutr. 33:57–70.

Zebarth, B.J., C.F. Drury, N. Tremblay, and A.N. Cambouris. 2009. Opportunities for improved fertilizer nitrogen management in production of arable crops in eastern Canada: A review. Can. J. Soil Sci. 89:113–132.

Zhang, J., A.M. Blackmer, J.W. Ellsworth, and K.J. Koehler. 2008. Sensitivity of chlorophyll meters for diagnosing nitrogen deficiencies of corn in production agriculture. Agron. J. 100:543–550.

Ziadi, N., M. Brassard, G. Bélanger, A. Claessens, N. Tremblay, A. N. Cambouris, M. C. Nolin, and L.É. Parent. 2008. Chlorophyll measurements and nitrogen nutrition index for the evaluation of corn nitrogen status. Agron. J. 100:1264–1273.