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Remote Sensing of Nitrogen Stress in Creeping Bentgrass

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ABSTRACT

Development of a remote sensing system that can reliably identify nutrient deficiencies may reduce time spent sampling turfgrass areas and allow for site-specific applications of fertilizers. The objectives of this research were to evaluate the use of a ground-based remote sensing system and partial least-squares (PLS) regression to predict the N concentration, biomass production, chlorophyll content, and visual quality of creeping bentgrass (*Agrostis stolonifera* L. 'Penncross') growing under varying N rates, and to compare PLS regression to other vegetative indices. The study consisted of three N treatments (0.0, 12.2, and 24.4 kg ha^{-1} 15 d^{-1}) arranged in a randomized complete block design. Spectral radiance measurements were obtained from plots using a fiber-optic spectrometer to calculate vegetative indices. The PLS regression analysis yielded a strong relationship between actual and predicted N concentration of creeping bentgrass plant tissue during 2002 and 2003 (r^2 = 0.95 and 0.71 respectively). However, PLS regression failed to produce a prediction for the chlorophyll concentration. Regressing the normalized vegetation index (NDVI), Stress1 (R_{706}/R_{760}), and Stress2 (R_{706}/R_{813}) ratios against N concentration yielded better results in 2003 when there were distinct differences in N concentration between the N rates. These results indicate that the traditional vegetation indices like NDVI might be better suited for determining the relative N status of turfgrass plants when compared against a well-fertilized control. More research will be required to determine if the PLS regression analysis produces prediction models that are able to specifically identify a particular nutrient deficiency or plant stress, and how the results will vary between grass species.

Traditional plant tissue sampling and analysis is time and labor intensive and requires collection of several samples from representative areas to adequately characterize variability found on turfgrass sites. In addition, turfgrass quality may decline because of nutrient deficiencies in the interim between sampling and the availability of sample results. Therefore, many turfgrass managers make scheduled applications of N to prevent a deficiency. Unnecessary applications of fertilizer N may result in nutrient runoff and leaching with ultimate contamination of surface and groundwater. Remote sensing systems have the potential to be used as a monitoring tool for scheduling nutrient applications. Coupling a remote sensing system with mapping software to monitor the nutrient status of turfgrass areas may allow for site-specific applications of fertilizer only to areas that require supplemental nutrition.

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Handheld chlorophyll meters have been used to rapidly assess plant N status in agronomic crops (Piekielek and Fox, 1992; Schepers et al., 1996; Wood et al., 1992) by measuring optical density at two wavelengths and converting to a value that has been positively correlated with chlorophyll and N. Rodriguez and Miller (2000) reported that handheld chlorophyll meter readings are correlated with chlorophyll and N concentrations of greenhouse-grown St. Augustinegrass [*Stenotaphrum secondatum* (Walt.) Kuntze]. While handheld chlorophyll meters are an attractive option for monitoring turfgrass health, they are limited in the amount of spectral information collected from the turfgrass canopy since they collect reflectance values at a limited number of wavelengths.

An alternative to chlorophyll meters is to measure light reflected from the turfgrass canopy with a multispectral radiometer. A major advantage of canopy analysis is that a single measure of reflected radiation can characterize the N status of many plants within a selected area. Multispectral radiometry assesses reflectance of light at various wavelengths where the percentage of light not reflected is either absorbed by the plant or transmitted to the soil surface.

Leaf reflectance in the visible portion of the spectrum (400–700 nm) is relatively low due to increased absorption by chlorophyll and is correlated ($r^2 > 0.97$) with concentration of leaf pigments (Gitelson and Merzlyak, 1994; Horler et al., 1983). As plants become stressed, they exhibit decreased reflectance in the near-infrared (NIR) spectral region due to decreased cell layers and increased reflectance in the red spectral region due to decreased chlorophyll content (Guyot, 1990). Near-infrared reflectance spectroscopy is a rapid analytical method for measuring the chemical composition of materials. Covalent bonds between atoms such as C, N, H, and O absorb energy in the infrared region (IR) and have vibrational frequencies and overtones that are detectable in the NIR region (750–2500 nm) (Malley et al., 2000; Gillon et al., 1999). Monitoring these changes in spectral reflectance may reliably indicate changes in plant growth or physiological status (Carter, 1994; Carter and Miller, 1994). The normalized difference vegetation index (NDVI), defined as the NIR minus visible reflectance divided by NIR plus visible reflectance, has been widely used for remote sensing of vegetation for nearly three decades (Rouse et al., 1973). This index has been used in many different ways, including estimation of crop yields and end-of-season aboveground dry biomass (Tucker et al., 1986). Several studies have identified the use of NDVI

Abbreviations: CST, central standard time; IR, Infrared; MLR, multiple linear regression; NDVI, normalized difference vegetation index; NIR, near infrared; PLS, partial least-squares; PRESS, predicted residual sum of squares; R, reflectance; SEP, standard error of prediction; Stress1, R_{706}/R_{760}; Stress2, R_{706}/R_{803}; WL550, spectral reflectance at 550 nm; WL710, spectral reflectance at 710 nm.

as a tool in identifying turfgrass plants exhibiting signs of stress (Fenstermaker-Shaulis et al., 1997; Trenholm et al., 1999a). Reflectance in the NIR range divided by reflectance in the red range (IR/R) has been associated with shoot biomass in corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] by Daughtry et al. (1982) and turfgrass quality and density in seashore paspalum (*Paspalum vaginatum,* Swartz) and bermudagrass [*Cynodon dactylon* (L.) × *C. transvaalensis* Burtt Davy] (Trenholm et al., 1999b). Blackmer et al. (1996) identified reflectance at 550 and 710 nm as being the best indicators of N deficiency in corn.

Despite the growing interest in using remote sensing to manage a turfgrass system, spectral analysis has not yet received widespread acceptance by turfgrass managers. One reason for this may be that after more than two decades of research in the field of agronomy, we cannot yet make quantitative, or even qualitative, translations from the raw spectral data without first calibrating some sort of empirical model (Richardson et al., 2004). Given the successful use of NIR spectroscopy in predicting biochemical composition of samples, it follows that the visible/NIR spectra could potentially provide information about the chemical composition of samples as if we were to perform a full set of laboratory analysis.

Research often involves the use of controllable variables (factors) to explain or predict other variables (responses). For instance, we may be interested in the influence of N concentration on the biomass production of a particular turfgrass. When these factors are few in number, not highly collinear, and have a well-understood relationship to the responses, multiple linear regression (MLR) can be a good way to turn data into useable information (Tobias, 1997). However, in the case of remote sensing, the factors used are the measurements from the spectrum that can number in the hundreds or thousands and are likely to be highly collinear. When using MLR in cases such as this, it is easy to produce a model that fits the data perfectly from the sample set, while having limited ability in predicting values from non-modeled samples. When this occurs, the model is said to be "over-fitting" the data set. Over-fitting occurs when there are many factors, but only a small number of the factors account for most of the variation in the response.

Partial least-squares regression (PLS) is a method developed for constructing predictive models when there are a large number of highly collinear factors (Tobias, 1997). During the calculation of PLS, the X- and Y-scores are chosen so that the relationship between successive pairs of scores are as strong as possible. The PLS factors are computed as linear combinations of spectral amplitudes and the responses are predicted linearly based on these extracted factors. As a result, a PLS regression is not based on a single or even a few frequencies as would be the case with MLR or stepwise regression. In comparison, the factors used in the PLS regression are computed as linear combinations of the spectral amplitudes, and the response variable is predicted based on these linear extractions. Instead of being based on a small group of frequencies as would be the case in using MLR, the PLS regression is based on all of the input factors.

Ideally, the use of a remote sensing in turfgrass systems will predict nutritional deficiencies early enough to allow for site-specific fertilization before the decline in turfgrass health and the associated visual symptoms. Accurate prediction of nutrient deficiencies through the use of spectral reflectance requires the use of a statistical method that considers the number of variables involved and tests for multicollinearity. Traditional multiple-regression techniques do not compensate for collinearity and can increase the risk of overfitting if the reflectance at each wavelength is considered as an explanatory (X) variable (Helland, 1988). The PLS regression can be used to develop predictive models in cases where the number of factors exceeds sample numbers and are highly collinear (Tobias, 1997). In contrast to traditional multiple-regression techniques that only consider the influence of the independent variables, PLS regression utilizes the influence of both the independent and dependent variables in the formation of the factors (Garthwaite, 1994; Tobias, 1997).

The specific objectives of this research were: (i) to determine if PLS regression of reflectance data may accurately be used to determine the N concentration in a turfgrass canopy; (ii) to investigate the relationship between multispectral reflectance data, N concentration, chlorophyll content, and turfgrass quality; and (iii) compare PLS regression to other vegetative indices for prediction of N concentration in a turfgrass system.

MATERIALS AND METHODS

Experimental Setup

A 2-yr field experiment was conducted at the Iowa State University Horticulture research station in Gilbert, IA, on a creeping bentgrass putting green constructed according to U.S. Golf Association specifications (USGA, 1993) to determine the correlation between N concentration of plant tissue and remotely sensed multispectral scanner data. Plots were 1.52 by 1.52 m in size and treatments were arranged in a randomized complete block design with four replications.

Three N fertilizer treatments were applied at 0, 12.2, and 24.4 kg ha^{-1}, fourteen times on a 15-d interval as urea in solution with a CO_2 sprayer. Spray volume was 283 mL and spray pressure was 207 kPa. In addition to N, all plots received uniform P at 2.44 kg ha^{-1} 15 d^{-1} applied as phosphoric acid and K at 5.0 kg ha^{-1} 15 d^{-1} applied as potassium chloride.

Treatments were applied from 25 March to 8 October 2002 and from 9 June to 23 Sept. 2003 on a 15-d interval. Plots were mowed four times a week at a height of 3.8 mm, removing clippings after each mowing. Irrigation was applied as needed to maintain optimum turfgrass quality.

Biological Parameters

Plots were evaluated for visual quality based on color, shoot density, and uniformity of stand, where 1 was live grass, 6 was minimally acceptable turfgrass quality, and 9 was dark-green, dense, uniform grass. Samples collected for nutrient analysis and biomass production were collected by removing clippings from a 1.74-m^2 area in conjunction with collection of reflectance data once every 30 d during the growing season. One gram of fresh tissue from each plot was analyzed for total chlorophyll content according to the method of Arnon (1949) as modified by Bruinsma (1961). The remaining plant tissue was oven-dried at 60°C for 4 d and weighed to determine biomass production. Samples were analyzed for P, K, and micronutrient concentration using inductively coupled argon plasma spectroscopy. Total N concentration was determined by using a LECO FP-2000 nitrogen/protein analyzer (LECO Corp., St. Joseph, MI).

Reflectance Measurements

Remotely sensed data was collected with a field portable fiber optic spectrometer (Model S2000, Ocean Optics, Winter Park, FL) fitted with 30-degree field-of-view optics mounted 29.8 cm above the ground, resulting in a sample area of 182 cm^2. Spectrometer data was collected on a 30-d interval corresponding with the collection of biological parameter data. To reduce variability due to cloud cover and solar zenith angle, the tip of the fiber was mounted inside a rectangular plastic hood that extended down to the turf canopy. Auxiliary lighting was provided by two 12-V halogen lights with an irradiance of 2250 μmol m^{-2} s^{-1}. Radiance values were expressed as percentage spectral reflectance after standardization with a white ceramic tile standard. The spectrometer has a nominal spectral range from 200 to 1200 nm with approximately 0.3-nm nominal bandwidth. Thus, for each measurement the spectrometer program automatically collects 2500 data points covering the entire spectral range. A linear interpolation routine was used to estimate values at 1-nm interval before calculation of indices from the reflectance data. Eight scans were averaged for every measurement and approximately 10 measurements were collected and averaged for each plot. Recalibration of the instrument with the white standard was conducted immediately before collecting measurements from each replication. Canopy reflectance was measured on days with minimal cloud cover between 1100 and 1400 h central standard time (CST).

Reflectance at individual wavelengths and several spectral indices were examined for comparison to PLS regression results. They included: NDVI = $(R_{800} - R_{600})/(R_{800} + R_{600})$; IR/$R$ = (R_{780}/R_{600}); Stress1 = (R_{706}/R_{760}) (Trenholm et al., 1999b); Stress2 = (R_{706}/R_{813}) (Trenholm et al., 1999b); and WL550 = R_{550} (Blackmer et al., 1996); and WL710 = R_{710} (Blackmer et al., 1996), where R_x is the reflectance value at the x wavelength.

Statistical Analysis

An analysis of variance (ANOVA) was performed to test fertilizer N rate effects on leaf N, chlorophyll content, biomass production, and visual quality using PROC ANOVA in SAS (SAS Institute, 1999). Correlation coefficients were calculated using PROC CORR in SAS while prediction equations for tissue N concentration, biomass production, chlorophyll concentration, and visual quality were developed by regressing field data against derived spectra using PROC REG. Mean separation was determined using LSD at $P = 0.05$. The procedure for spectral calibration was PLS regression as performed by SAS (SAS Institute, 1999). Equations were validated through single sample cross-validation. For the cross-validation, 10% of the sample was left out for prediction at a time and the number of factors that minimized the predicted residual sum of squares (PRESS) was chosen (see Fig. 1 for graphical illustration). This process was repeated so that every observation was used exactly once for cross-validation.

RESULTS AND DISCUSSION

A weak relationship was observed between actual biomass production and the predicted biomass production as calculated through partial least-squares regression (Table 1). This is likely due to a reduction in turfgrass growth during the summer months without a corresponding change in spectral properties. Previous research by Osborne et al. (2002) has shown that the spectral properties of plants are influenced primarily by the biochemical content and moisture status of the plant tissue. We theorize that the changes in spectral properties resulting from increased heat and/or drought stress made it more difficult to observe a relationship between spectral reflectance and actual biomass production in our results.

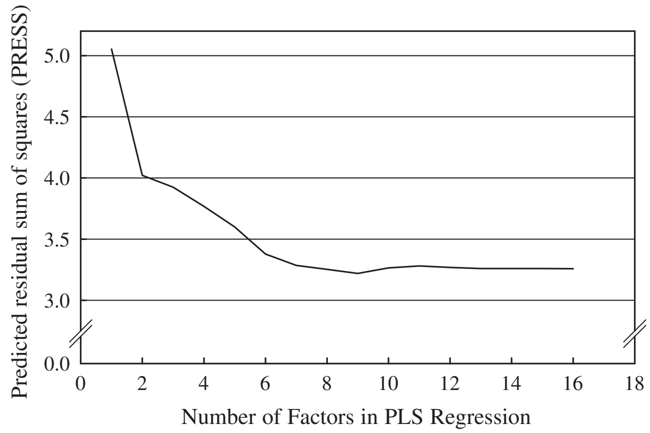

Fig. 1. Graphical illustration of the predicted residual sum of squares (PRESS) vs. the number of factors used in the partial least-squares regression model.

Changes in tissue pigment concentrations translated to variations in the spectral signatures at each N rate. Figure 2 illustrates the mean reflectance spectra curves of creeping bentgrass tissue for each N rate at the 22 July 2002 sampling date. Minimum reflectance in the blue (400–500 nm) and red (650–690 nm) regions is characteristic of maximum light absorption by chlorophyll. The broad peak centering at 550 nm in the green region (500–600 nm) is indicative of the minimal chlorophyll absorption. Bentgrass tissue receiving no N (0 kg ha^{-1} N) showed a greater increase in reflectance near 550 nm compared to the other N rates, agreeing with the findings reported by Buscaglia and Varco (2002) and Fridgen and Varco (2004) from research conducted on

Table 1. Effect of fertilizer N on canopy N concentration, biomass production, chlorophyll concentration, and visual quality ratings for creeping bentgrass in Gilbert, IA, during 2002 and 2003.

N rate	N conc.	Biomass	Chlorophyll conc.	Visual quality†
kg ha^{-1}	g kg^{-1}	g m^2 d^{-1}	g kg^{-1}	
			2002	
0.0	30.30	1.82	0.99	4.55
12.2	33.96	2.82	1.13	6.25
24.4	38.57	4.26	1.21	7.2
LSD(0.05)	3.96	0.81	0.10	0.81
			2003	
0.0	35.05	1.40	1.10	4.69
12.2	39.41	2.15	1.12	6.59
24.4	43.32	3.04	1.21	8.47
LSD(0.05)	3.49	0.85	0.05	0.54

† Visual quality was based on color, shoot density, and uniformity of stand, where 1 was no live grass, 6 was minimally acceptable turfgrass quality, and 9 was dark-green, dense, uniform grass.

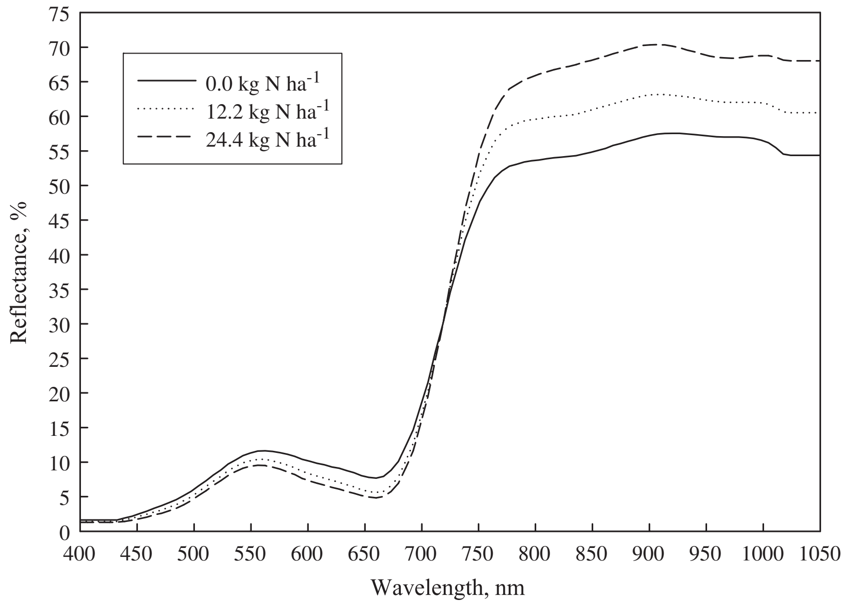

Fig. 2. Mean spectral reflectance for each of the three N treatments on 22 July 2002 in Gilbert, IA, on creeping bentgrass (*Agrostis stolonifera* L.). Similar trends were observed for all sampling dates in 2002 and 2003.

cotton (*Gossypium hirsutum* L.). Similar results were observed at all sampling dates in 2002 and 2003.

Nitrogen treatments resulted in a wide range of responses for N concentration, biomass production, chlorophyll concentration, and turfgrass quality in creeping bentgrass plots during 2002 and 2003. The N treatments resulted in different N concentrations that increased from 30.30 g kg^{-1} in the 0 kg ha^{-1} treatment to 38.57 g kg^{-1} in the 24.4 kg ha^{-1} treatment during 2002 (Table 1). Nitrogen treatments succeeded in establishing tissue concentrations that ranged from low to sufficient according to the sufficiency values reported by Jones et al. (1991). Similar results were observed during 2003. Biomass production, chlorophyll concentration, and visual quality ratings also increased with increasing N rate during 2002 and 2003 (Table 1). The 0 kg ha^{-1} N treatment resulted in visual quality that was below the minimally acceptable level of 6.0 along with the lowest chlorophyll concentration during both 2002 and 2003 due to increased chlorosis and low plant density. While the 12.2 kg ha^{-1} N treatment resulted in acceptable quality during 2002 and 2003, the 24.4 kg ha^{-1} N treatment yielded the highest quality and chlorophyll concentration characterized by a dense, dark green turf canopy.

Coefficients of determination for the regression analyses relating NDVI, IR/R, Stress1, Stress2, WL550, and WL710 to the N concentration, biomass production, chlorophyll concentration, and visual quality are shown in Table 2. No relationships were observed between WL550 or WL710 and the N concentration, biomass production, chlorophyll concentration, or visual quality. This contradicts the results of Blackmer et al. (1996) who reported

Table 2. Coefficient of determination for regressions of N concentration, biomass production, chlorophyll concentration, and visual quality of creeping bentgrass regressed on normalized difference vegetation index (NDVI), infrared/red (IR/R), Stress1, Stress2, spectral reflectance at 550 nm (WL550), and spectral reflectance at 710 nm (WL710), Gilbert, IA, 2002–2003.

Calibration	NDVI†	IR/R‡	Stress1§	Stress2¶	WL550#	WL710††
			R^2			
			2002			
N concentration, g kg^{-1}	0.23	0.32	0.58	0.51	NS‡‡	0.16
Biomass production, g m^2 d^{-1}	0.35	0.24	0.25	0.17	0.28	0.30
Chlorophyll content, mg kg^{-1}	0.32	0.26	0.29	0.29	0.20	0.19
Visual quality	0.54	0.71	0.71	0.67	0.25	0.32
			2003			
N concentration, g kg^{-1}	0.63	0.48	0.63	0.68	0.16	0.39
Biomass production, g m^2 d^{-1}	0.34	0.23	0.27	0.35	NS	0.16
Chlorophyll content, mg kg^{-1}	0.15	0.22	0.24	0.16	0.34	0.30
Visual quality	0.40	0.45	0.54	0.44	0.43	0.38

† Normalized difference vegetation index (NDVI) = $(R_{800} - R_{600})/(R_{800} + R_{600})$.
‡ Infrared/red (IR/R) = (R_{780}/R_{600}).
§ Stress1 = (R_{706}/R_{760}).
¶ Stress2 = (R_{706}/R_{813}).
# WL550 = R_{550}.
†† WL710 = R_{710}.
‡‡ Not significant (NS).

that reflectance centered on 550 nm and 710 nm yielded some of the best relationships with N deficiency in corn. The best relationship between IR/R, Stress1, and Stress2 vegetation indices when regressed against visual quality was observed during 2002. These results were similar to those reported by Trenholm et al. (1999b) in a study conducted on seashore paspalum and bermudagrass. In comparison, NDVI, Stress1, and Stress2 produced the strongest relationship with the N concentration in 2003, while yielding comparably weak results during 2002 (Table 2). Similar limitations in the consistency for NDVI predictions of N concentration have been reported by Bronson et al. (2005) in cotton grown under varying N rates. The strength of a remote sensing system will be judged by its reliability throughout the growing season. Basing management decisions on NDVI would require recalibration of the model for each sampling date against a well-fertilized control to ensure reliable results (Bronson et al., 2005). While this might be possible in turfgrass management systems, it may not always be practical.

Analysis of the reflectance data by PLS regression canopy reflectance data in 2002 and 2003 yielded better predictive tissue N concentration results based on maximum r^2 and minimum SEP values than were observed for the vegetation indices (Table 3). These results are supported by those of Hansen and Schjoerring (2003) who reported an improvement in prediction of N concentration through PLS regression when compared to NDVI in winter wheat (*Triticum aestivum* L.). Similarly, Bronson et al. (2005) compared PLS to NDVI in predicting the N concentration of cotton and reported an improvement using PLS regression. The results for the PLS regression in 2003 indicate a slightly weaker relationship between the actual and predicted N concentration in the tissue than was observed during 2002 (r^2 = 0.71 vs. 0.95) (Table 3, Fig. 3). This may be explained by reduced uniformity in plot quality that resulted from localized dry spots that were present in several of the plots for a limited amount of time in 2003.

Table 3. Partial Least-Squares (PLS) regression statistics for estimation of N concentration, chlorophyll concentration, biomass production, and visual quality for creeping bentgrass in Ames, IA, during 2002 and 2003. Results include data from five sampling dates in 2002 (N = 60) and four sampling dates in 2003 (N = 48).

Calibration	No. of factors†	Regression coefficient (r^2)	SEP‡
		2002	
N concentration, g kg^{-1}	8	0.95	1.51
Biomass production, g m^2 d^{-1}	5	0.56	0.80
Chlorophyll concentration, mg kg^{-1}	6	0.12	66.59
Visual quality	3	0.76	0.71
		2003	
N concentration, g kg^{-1}	4	0.76	2.85
Biomass production, g m^2 d^{-1}	3	0.64	0.66
Chlorophyll concentration, mg kg^{-1}	2	0.02	98.72
Visual quality	2	0.65	0.90

† The number of factors necessary to achieve a minimum global standard error of prediction for the final partial least-squares regression model.
‡ Standard error prediction (SEP), the average difference between the actual values and predicted values of samples not used to develop the equation.

In comparison to the other vegetation indices evaluated in this study, PLS regression yielded a stronger relationship between the actual and predicted N concentration across all dates in 2002 and 2003, indicating the potential benefit in using it to develop models for future remote sensing systems.

Results of PLS regression did not indicate a relationship between the actual and the predicted chlorophyll concentration in the plant tissue using reflectance data in 2002 or 2003 (Table 3). These results were not expected considering the broad range in chlorophyll concentrations that were observed in the tissue during 2002 and 2003 (Table 1). We hypothesize the N deficiency symptoms that resulted from the N treatments, which resulted in the presence of a thin stand and necrotic tissue, masked the influence of chlorophyll concentration by significantly altering the spectral patterns of the plants when analyzing reflectance across a large number of wavelengths.

The results from this study indicate the potential for using PLS regression in the development of models for predicting the N status of creeping bentgrass. In comparison to other spectral analysis methods such as NDVI, PLS was able to accurately predict the N concentration of the tissue during both growing seasons while the results for NDVI were variable between years. While the results of this study are promising, more research is needed to determine if PLS regression

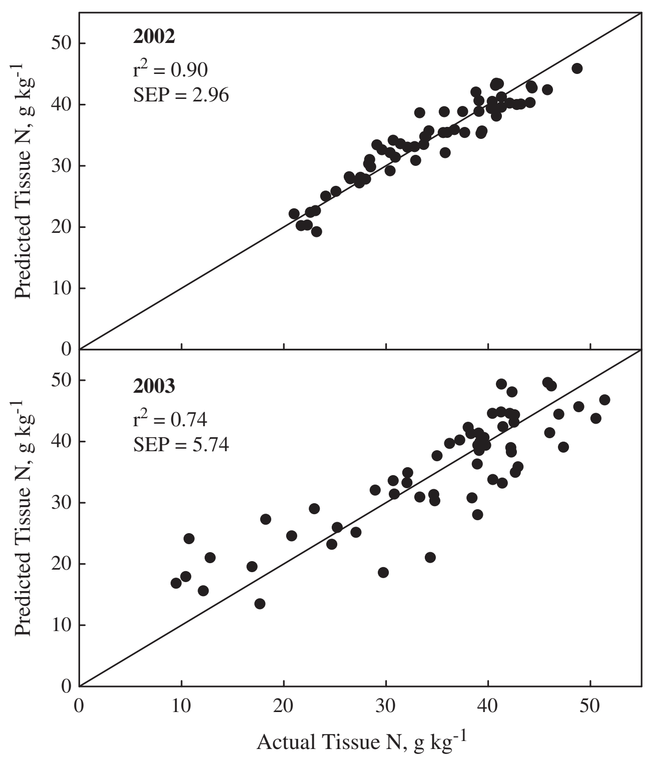

Fig. 3. Predicted versus actual tissue N concentration for all data collected in 2002 and 2003 from creeping bentgrass (*Agrostis stolonifera* L.) obtained using partial least-squares regression to relate spectral reflectance data in the visible/near-infrared wavelength range to the reference tissue N concentration values. The graphed line represents a 1:1 relationship. SEP, standard error of prediction.

models are able to distinguish N deficient turfgrass plots from those subjected to other plant stresses. Successful development of a remote sensing system capable of accurately identifying nutrient deficiencies may make it possible to base fertilizer applications on analysis of canopy reflectance data without calibrating against traditional plant analysis results, which in turn would allow for immediate correction of nutrient deficiencies.

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