In Situ Measurement of Pecan Leaf Nitrogen Concentration using a Chlorophyll Meter and Vis-near Infrared Multispectral Camera

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Abstract. Knowledge of foliar nitrogen (N) concentration is important in pecan [Carya illinoinensis (Wang.) K. Koch] management protocols. Lower cost and/or rapid methods to determine foliar N are desirable and may result in improved management strategies as well as enable precise precision agricultural practices to be deployed in pecan production. This study investigates using a portable chlorophyll meter and Vis-NIR camera to rapidly determine pecan foliar N in situ. Relationships of SPAD values from a chlorophyll meter (Minolta SPAD 502Plus) and vegetative indices calculated from camera image data to foliar N determined by chemical analysis were investigated. SPAD readings were taken monthly from May through October on ‘Pawnee’, ‘Kanza’, and ‘Maramec’ pecan cultivars in Oklahoma in 2010. Images of the same ‘Pawnee’ and ‘Kanza’ trees were collected in September and October of 2010 with a truck-mounted multispectral camera using ambient light. Correlation of foliar N to SPAD values was poor in May for all cultivars but distinct significant linear relationships were found for ‘Maramec’ and ‘Pawnee’ for each of the other months tested with $R^2$ ranging from 0.40 to 0.87. Data from ‘Kanza’ had significant relationships in June and October with $R^2$ of 0.39 and 0.72, respectively. Normalized difference vegetative index (NDVI) and reflectance data extracted from Vis-NIR camera images were significantly correlated to foliar N in both months of the study on ‘Pawnee’ but only in September for ‘Kanza’. The various relationships had $R^2$ between 0.21 and 0.51.

Nitrogen management in pecan orchards is important for tree health, optimizing yield, and managing alternate bearing (Conner and Worley, 2000; Smith et al., 2007; Wood et al., 2004). Overapplication of N wastes resources and can cause environmental harm. Availability of N depends not only on fertilizer application rate, type, and time of application, but also on a variety of cultural practices and environmental conditions, including soil nutrients and properties, weather and climate, irrigation practices, tree condition, orchard floor management, and nut production (McDonald et al., 1991; Soria-Ruiz et al., 2007; Ye et al., 2008). Soil tests and leaf analysis are used to measure N; however, leaf analysis is widely used commercially in pecan to assess N concentration during the growing season because it provides a direct measurement of nutrient status in the tree. The traditional practice for measuring N levels in a pecan orchard consists of harvest picking specific leaves that are then dried and sent to a laboratory for chemical analysis. This process takes considerable time and is typically used to guide the subsequent year’s fertility program rather than adjusting N in the current year. Generally only a subset of the trees in an orchard is tested to reduce cost. A sensor that provides an immediate indication of pecan foliar N in the field would be desirable if it provides adequate performance at a reasonable cost. Such a sensor would also be a key component to enable precision agriculture practices in pecan production.

Much of the N in a leaf is partitioned in chlorophyll; thus, a sensor that measures chlorophyll can often be used to quantify the amount of N in a leaf (Filella et al., 1995). The basis for most optical sensing of N in leaves is based on chlorophyll’s spectral response to light. Chlorophyll absorbs blue and red light ($\lambda = 450$ and 650 nm, respectively) and reflects near infrared (NIR) light ($\lambda$ greater than 750 nm). The intensity of transmitted and/or reflected light at these wavelengths can be used to form empirical relationships, which estimate chlorophyll concentration and, consequently, N concentration in a leaf (Richardson et al., 2002).

A SPAD meter (Konica Minolta, Osaka, Japan) is a handheld device that measures light intensity at wavelengths of 650 and 940 nm transmitted through a 2 X 3-mm area of a leaf clamped in the instrument. The SPAD meter calculates a unitless value between 1 and 100 that has been shown to be positively correlated to leaf chlorophyll concentration. Regression analysis can then be used to predict foliar N from SPAD readings (Markwell et al., 1995). Originally developed for rice, SPAD meters have found use on a variety of field and tree crops for predicting N concentrations (Chang and Robison, 2003; Gianquinto et al., 2004; Neilsen et al., 1995; Perry and Davenport, 2007; Simorte et al., 2001; Reyniers and Vrindts, 2006). NDVI can be calculated from remotely acquired multispectral digital camera image data or from close-proximity sensors. Remote imaging systems generally measure reflected ambient light, whereas some close-proximity sensors collect reflected light that originates from the sensor itself (Jones et al., 2007). In both cases, the intensity of incident light is required to accurately determine relative intensity of the reflected light to control sensor error.

At high levels of biomass, NDVI tends to becomes less sensitive to chlorophyll concentration because reflected red light intensity from leaves asymptotically approaches a minimum. In plant systems where chlorophyll levels are high, vegetative indices (VIs) that include green reflectance may have better performance predicting foliar N than NDVI. Gitelson et al. (1996) compared NDVI to green-NDVI (GNDVI) obtained from satellite images of Norway maple (Acer platanoides L.) and horse chestnut (Aesculus hippocastanum L.) trees and found GNDVI resulted in more accurate predictions of chlorophyll content.
with full canopies. GNDVI is calculated from the intensities of reflected NIR and green light using the formula: \( GNDVI = \frac{(I_{NIR} - I_{green})}{(I_{NIR} + I_{green})} \). Numerous other VIs using red, green, and NIR reflected light have been proposed and evaluated on various plants over the years (Zarco-Tejada et al., 2005).

A SPAD meter and/or a suitable ground-based multispectral camera may have use for assessing the N status of pecan leaves in an orchard. The optical properties of leaves vary among plant species and can change throughout ontogeny and with growth conditions. Foliol N levels on fruiting and vegetative shoots of pecan decrease over the growing season (Diver et al., 1984). Chang and Robison (2003) found that the regression relationships of SPAD readings to foliar N concentration on four species of hardwood tree leaves were different among species and crown position and changed throughout the growing season. In a similar study on citrus, Jifon et al. (2005) found that growth conditions resulted in a variation of leaf thickness and changed the regression relationship of SPAD readings to chlorophyll and N concentration. Similarly, corrections for variation in pecan leaf optical properties may need to be included in a measurement protocol using data from a SPAD meter or a multispectral camera image to measure foliar N.

The efficacy of multispectral cameras and/or portable chlorophyll meters to quantify N in pecan leaves has not been reported. The objectives of these experiments were to evaluate the performance of: 1) a SPAD meter; and 2) a ground based Vis-NIR multispectral camera using ambient light for rapid in situ measurement of foliar N in a pecan orchard during the growing season.

**Materials and Methods**

**Site descriptions.** The study contained trees in two locations in Oklahoma. An orchard near Cleveland, OK, contained 17-year-old ‘Pawnee’ and ‘Kanza’ pecan (Carya illinoensis × Carya tomentosa) on ‘Giles’ seedling rootstocks with 12.2 × 12.2-m diagonal spacing. Soil is a Dennis silt loam (fine, mixed, active, thermic Udic Argudoll). Rainfall in this non-irrigated orchard during the study period (Apr. through Oct. 2010) was ≈640 mm. A 1.8-m wide vegetation-free strip centered on the tree was maintained with selected herbicides, whereas the remainder of the orchard floor was mowed as needed. Trees received commercial management for pests. Fertilizer treatments in the Cleveland, OK, orchard were varied to induce greater variability of foliar N for this study (Table 1). All ‘Pawnee’ trees and one subplot of ‘Kanza’ trees received a base rate of 280 kg ha⁻¹ of diammonium phosphate (18N–20P–0K) applied in a continuous band 1.8 m from the trunk during March. Various subplots also received between 0 and 975 kg ha⁻¹ of urea (46N–0P–0K) hand-broadcast from the trunk to the drip line of the tree (Table 1). One subplot of ‘Kanza’ trees was seeded with ‘Durana’ white clover (Trifolium repens L.) and received no other N supplements. This orchard was used for both the SPAD and Vis-NIR camera studies.

A second orchard at the Cimarron Valley Research Station near Perkins, OK, contained 25-year-old ‘Maramec’ trees on ‘Apache’ seedling rootstocks in a Teller sandy loam soil (fine-loamy, mixed, active, thermic Udic Argiustoll). Trees were diagonally spaced 24.4 × 24.4 m with bermudagrass (Cynodon dactylon (L.) Pers.) groundcover. A 3-m wide vegetation-free area was maintained the entire row length with herbicides. Natural rainfall of 607 mm during the study period (Apr. through Oct. 2010) was supplemented by traveling gun irrigation. Commercial management for pests and fertilization were used. Diammonium phosphate and urea were each applied in a band application at rate of 482 kg ha⁻¹ of material in March of 2010. Ten trees were randomly selected within the orchard for the SPAD study (Table 1).

**Sampling protocol—SPAD meter.** Trees were sampled midmonth from May through September (Perkins orchard) in 2010. SPAD readings (SPAD 502Plus; Konica Minolta) were taken adjacent to the midrib of the leaflet with the adaxial surface of the leaflet facing the light source of the SPAD meter. Individual SPAD readings were averaged for each tree. These same 10 leaflet pairs were collected and combined for elemental analysis. Leaves were dried and ground and then N concentration for each tree was determined using a Leco Truspec N analyzer (St. Joseph, MI).

**Table 1.** Cultivar and nitrogen treatments for pecan trees in the study.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Number of trees</th>
<th>Base rate (kg ha⁻¹ N)</th>
<th>Urea (kg ha⁻¹ N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pawnee</td>
<td>5</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Pawnee</td>
<td>5</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td>Pawnee</td>
<td>5</td>
<td>50</td>
<td>112</td>
</tr>
<tr>
<td>Pawnee</td>
<td>5</td>
<td>50</td>
<td>224</td>
</tr>
<tr>
<td>Kanza</td>
<td>5</td>
<td>50</td>
<td>448</td>
</tr>
<tr>
<td>Kanza</td>
<td>5</td>
<td>50</td>
<td>112</td>
</tr>
<tr>
<td>Kanza</td>
<td>5</td>
<td>50</td>
<td>‘Durana’ (0 N)</td>
</tr>
<tr>
<td>Maramec</td>
<td>10</td>
<td>87</td>
<td>222</td>
</tr>
</tbody>
</table>

\( N = \text{nitrogen.} \)
MS3100, Auburn, CA) was used to obtain images of the same trees measured with the SPAD meter in the Cleveland, OK, orchard. Images were acquired on 15 Sept. and 13 Oct. 2010, the same day that the SPAD meter measurements were taken. The camera images were 1392 × 1040 pixels by eight bits (256 intensity levels) in three optical wavebands centered at 550, 670, and 780 nm (± 10 nm) through a 14-mm focal length lens. The camera was mounted on a truck at a height ≈2.5 m above ground level.

A reflectance target containing two panels with diffuse reflectance of 10% and 99% was mounted on a tripod, 1.8 m above ground level, facing the camera to provide a reference of solar irradiance in each image (Model SRT-SP-050; Labsphere, Inc., North Sutton, NH). It was located under the drip line of the tree so it was approximately the same distance from the camera as the near edge of the tree canopy. An image containing a pecan tree and the reflectance target was composed by positioning the truck so that the sun was directly behind the camera (Fig. 1). Distance from the camera to the trunk of each tree was between 6 and 10 m. Rows in the orchard were oriented east to west. Images were taken midmorning on cloud-free days to ensure that the sun directly illuminated a significant portion of the tree and the reflectance target. Images were saved to a portable computer, which was also used to set gain and exposure of the camera. Gain and exposure time for each waveband were adjusted such that light from the 10% and 99% reflectance targets resulted in intensities in the linear range of the camera response and did not saturate the camera sensor. Target image intensities were 40 and 220 (out of 255) for the 10% and 99% reflectance targets, respectively. Camera gain and exposure time were set once at the start of each sampling date and remained constant for ≈1.5 h while 40 trees in the sample were imaged.

Images were processed using Matlab (MathWorks, Inc., Natick, MA) software. The location of the reflectance target was first selected from the image to obtain a reference intensity of red, green, and NIR ambient light. A rectangle was then selected manually, roughly including the canopy area of each tree and excluding other objects, trees, and grass in the background from the tree image (Fig. 1). Image pixels outside the rectangle were cropped and not included in further analysis. Irradiance data for each pixel were then extracted from the red, green, and NIR cropped images. The intensity of each pixel in the three cropped images was linearly normalized to a 0 to 255 scale using intensity values obtained from the 10% and 99% reflectance targets in the same image. This step allowed images of different trees to be compared with each other.

Leaves are highly reflective to NIR radiation, whereas branches, trunks, and other materials generally are not. Pixels from the normalized NIR image with intensities within a threshold window were classified as leaf pixels (LPs). Various threshold windows, with low NIR intensities ranging from 25 to 200 in increments of 25, were evaluated to classify LP. Threshold window spans were defined in two ways. One method set the high value 50 units above the lower limit and the other used 255 as the high-intensity limit for all threshold windows. A VI was calculated for each LP by using the appropriate individual pixel intensities of the NIR, green, and red images for each threshold window. VI for each tree was the mean of the VI calculated for LP ed classified in each image. The optimum threshold window for classifying LP was determined by regression analysis.

Statistical analysis. Data were analyzed using SAS MIXED (SAS Institute Inc., Cary, NC) using repeated measures. Regressions using least squares analysis techniques tested for significant main effects and interactions of SPAD, VI, cultivar, and sampling date to foliar N concentration. Linear, quadratic, and cubic relationships of SPAD...
and VI data to foliar N concentration were investigated.

Results and Discussion

Foliar nitrogen. Foliar N concentration ranged from 1.8% to 3.8% of dry leaf mass across all samples (Fig. 2). Nitrogen levels fell with time ($P < 0.0001$) during this study following expected seasonal variations.

SPAD meter. SPAD values varied from 26.2 to 52.3 with the lowest readings occurring in May. Foliar N was generally positively correlated to SPAD readings, but the specific relationships varied across the three cultivars and growing season (Fig. 3). There was a significant cultivar-by-month interaction ($P < 0.0001$); thus, equations predicting foliar N from SPAD meter readings are reported for each cultivar by month (Table 2). No cultivar exhibited significant relationships between SPAD and foliar N in May, the first month of the study. Significant linear models were found for ‘Maramec’ and ‘Pawnee’ trees in all subsequent months. For ‘Kanza’, the only significant models found were in June and October. Correlation analysis shows that from 39% to 87% of the variation of leaf N was predicted by SPAD readings where significant relationships were found (Table 2; Fig. 3). Higher-order models did not result in improved correlations. Slopes of the regression equations generally decreased as the season progressed indicating that SPAD meter response to foliar N concentration was also declining with ontogeny.

Researchers evaluating SPAD meter use on other plant species have found patterns similar to the present study reflecting seasonal changes in foliar N and leaf optical properties. Chang and Robison (2003) found that SPAD readings of sweetgum (Liquidambar styraciflua L.) tree leaves were positively correlated with foliar N; however, the regression relationships varied with sampling date and location in the crown. Seasonal physiological changes and environmental factors resulted in variation of leaf optical properties, which in turn affect response data from the SPAD meter. They improved...
correlation of the regression relationships by adding a term for foliar moisture content or by predicting N content on a leaf area basis instead of concentration by leaf mass. By dividing SPAD response by specific leaf weight (ratio of leaf dry weight to leaf area), Peng et al. (1993) were able to increase the coefficient of determination (R²) of the regression equations from 0.49 to 0.93 on five rice (Oryza sativa L.) cultivars. This correction essentially accounts for variations in leaf thickness among the samples. Adding these or similar measures to the SPAD sampling protocol increases the duration and effort required to gather data, thus negating some of the advantages inherent to the SPAD sampling protocol.

**Vis-near infrared multispectral camera.** The distribution of the intensity of NIR LPs, after normalization to a 0 to 255 scale, was approximately log normal with peak values ranging from 35 to 81 for all trees in the study. The distributions of pixel intensity in the red and green images were both maximum at zero intensity; however, green images were more reflective than red (Fig. 4). The peak of the NIR histogram occurred at higher intensities for 'Kanza' than 'Pawnee' but there was no significant temporal difference (Table 3).

At low NIR intensity thresholds, some of the reflected light was from branches and other non-leaf areas in the images. As the threshold level was raised to 150, most of the non-leaf reflections were removed from the images (Fig. 5). Raising the threshold level reduced the number of pixels remaining in the image for VI calculations. On several images in this study no NIR pixels had intensity values above 200. Correlation coefficients of foliar N to NDVI and GNDVI obtained from linear regression were maximized when the NIR threshold window for classification of leaf pixels ranged from 150 to 200 (data not shown). Linear models relating foliar N to NDVI and GNDVI were found for 'Pawnee' trees in both months of the study, although the correlation coefficients were generally low (Table 4). No simple regression models were identified for 'Kanza' however. The mean intensity of leaf pixels in the red image when using a threshold window of 150 to 200 was 9.9 ± 0.38 SE. This low value suggests that NDVI may be saturating and green reactivity may provide additional predictive power to the model (Gitelson et al., 1996). The corresponding intensity of green pixels was higher at 29.8 ± 0.56 SE. Regression models using GNDVI did not have significantly better performance however (Table 4).

Multiple linear regression modeling using NIR, green, and red reflected light intensity in addition to NDVI and GNDVI as predictor variables for foliar N did not produce models superior to simple linear regressions. Relationships that included the mean intensity of reflected green light for LP and NDVI improved performance slightly and produced significantly better models for 'Kanza' in September and 'Pawnee' in both months of the study (Table 4). Correlations for these relationships were weak with maximum R² of 0.51.

### Conclusion

These experiments indicate that SPAD meters and in-field Vis-NIR cameras have potential to provide useful indications of pecan leaf N concentration for some cultivars of pecan. The change in instrument response resulting from seasonal and cultivar variation necessitates obtaining calibration equations for each pecan cultivar at selected growth stages. The correlation of SPAD and/or Vis-NIR camera image data to leaf N concentration was generally low. Improvements are needed before the tested protocols can replace traditional foliar N analysis. These protocols may however provide a useful alternative to chemical analysis in cases in which rapid and/or relative foliar N measurements are required.

### Literature Cited


