Exploratory Study to Evaluate the Feasibility of Measuring Leaf Nitrogen Using Silicon-Sensor-Based Near Infrared Spectroscopy for Future Low-Cost Sensor Development

Project No.: **08-HORT10-Slaughter**

Project Leader: David Slaughter
Biological and Agricultural Engineering Department
UC Davis
One Shields Ave.
Davis, CA 95616
(530) 752-5553
dcslaughter@ucdavis.edu

Project Cooperators and Personnel:
Patrick Brown, Plant Sciences, UC Davis
Shrini Upadhyaya, Biological and Ag. Engineering, UC Davis
Carolyn Zhang, Biological and Ag. Engineering, UC Davis

Objectives:

This project initiated a feasibility study for measuring the nitrogen content in almond leaves using rapid optical measurements in the silicon region of the visible (VIS) and near infrared (NIR) spectrum without the need for complex sample preparation steps. The development of a silicon-detector-based VIS-NIR leaf nitrogen measurement technique would facilitate the future development of low-cost sensor technology for plant nitrogen management programs in almond.

The project also explored the feasibility of using multi-spectral techniques to determine leaf nitrogen content and leaf phenology simultaneously. Simultaneous determination of nitrogen and phenology will allow growers to determine the optimum leaf nitrogen content required for full leaf productivity adjusted for the percentage of non-living leaf cell wall material that increases as the leaves mature, which cannot currently be done with other rapid methods. Together these techniques would provide a fully integrated sensing system that could be used at any time on any almond leaf.

Interpretive Summary:

A study of the visible and near infrared spectral properties of almond leaves was conducted at five dates (April, May, June, July, and October) in 2008. A consistent increase in the light absorbance across the spectrum was observed in the April to July period. Rapid optical measurements requiring no sample preparation were developed

that could predict the leaf age (r^2=0.88) with a high degree of accuracy. A model using optical measurements in the near infrared portion of the silicon region was developed that could predict the individual leaf N content on a leaf area basis in the early part of the season (April and May) with a high degree of accuracy (r^2=0.86). Additional research is required to expand the performance of this model to the entire season and to determine the minimum number of wavebands required for robust performance.

Materials and Methods:

Fully exposed, Southwest-facing leaves from non-fruiting branches were collected in an almond (*Prunus dulcis*) orchard in Lost Hills, CA on five dates (April, May, June, July and October) in 2008. This orchard was selected because a four-level nitrogen (N) fertigation trial was being conducted (high N (CAN/UAN) and low N (CAN/UAN)) at this site and a wide range of leaf N concentration was expected. Three trees were randomly selected as the sampling trees at each N level for each N source at the site. The largest (middle) leaf was picked from sampled spurs. Ten leaves were collected for each sampling tree. Leaflets were immediately wrapped in moist paper towels, sealed in plastic zipper closure bags, and transferred into a pre-cooled ice chest. The samples were transported to the laboratory on the UC - Davis campus and stored at 5 ^oC overnight. A total of 400 (80 from each sampling date) leaf samples were used in the study.

In the laboratory, leaf area (model LI-3000, LAMBDA Instruments Corp. Lincoln, NB, USA), fresh weight, and chlorophyll index (model SPAD-502, MINOLTA Camera Co. Ltd. Japan) were determined on each individual whole leaf. All leaves were allowed to equilibrate to room temperature before measurement. The optical transmission and reflectance spectra in the visible and near infrared regions (from 400 to 2,500 nm with 2 nm resolution) were measured using a high-resolution spectrophotometer (model 6500, Foss NIRSystems, Laurel, MD). The central region (~3cm x 1cm, parallel to and including the main vein stem) of each whole leaf was scanned with the leaf top facing the optical detector. Half the leaf samples were scanned using transmission and the remainder using reflectance at four sampling dates (May, June, July and October) to allow comparison of the two methods. Only transmission spectra were collected for the April set.

After scanning, the leaf samples were dried at 50 ^oC for four days and the leaf dry mass measured. The dried leaves were individually ground into a fine powder and sealed in metal foil capsules for N analysis at the UC Davis Stable Isotope facility. Approximately 2 – 3 mg of leaf powder from each leaf was combusted at 1020°C in a reactor. Following combustion, nitrogen was separated and analyzed by an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS). Twenty-three samples were lost during N analysis, resulting in a total of 377 leaf samples for study. Nitrogen concentration was determined for all leaf samples using both unit dry mass (ug/mg) and unit leaf surface area (ug/cm^2) bases.

The optical data (both spectral and SPAD) were analyzed (SAS/STAT software, SAS Institute Inc., Cary NC, USA) to develop models for predicting N concentration. Twenty-fold internal cross validation and the Press statistic were used to determine the correct number of independent spectral factors to use in model development. In addition, the change in the spectral properties with leaf age was investigated. The Savitzky-Golay 2nd derivative pre-processing technique, with 13-point and 21-point convolution windows, was utilized to correct for base-line spectrum shifts due to leaf age.

Results and Discussion:

The SPAD meter is a handheld, portable commercial instrument designed to measure the chlorophyll content of a leaf. Because it can be used in the orchard, is readily available and because chlorophyll meter readings have been shown to be effective as a rapid method to determine the N status of many annual crops (Ziadi et al., 2008), it is worthwhile to assess its potential use in almond as an indirect measure of leaf N. **Table 1** is a summary of SPAD and nitrogen levels at five sampling dates at the Lost Hills almond orchard in 2008. **Figure 1** shows the relationship between SPAD values and chlorophyll A concentration in almond leaves and **Figure 2** shows the range of SPAD and leaf N content for the samples collected in 2008. A general increase in mean SPAD value with leaf age was observed between April and June, with the mean July and October values being similar to the June readings.

Table 1. Summary of SPAD and nitrogen levels at five sampling dates in 2008, Lost Hills.

Date	N	SPAD			N (mg/g)			N (ug/cm2)		
		Mean		CV (%)	Mean		CV (%)	Mean		CV (%)
8-Apr	77	29.3	a	8.7	36.4	a	8.9	224	a	13.6
11-May	75	34.4	b	6.6	32.9	b	10.6	272	b	20.1
18-Jun	73	37.5	c	6.0	27.0	c	15.9	244	a	22.6
23-Jul	70	38.5	c	7.7	26.4	c	12.2	268	b	22.5
20-Oct	82	37.5	c	9.2	18.3	d	15.1	182	c	22.8

Mean values with the same grouping letter are not significantly different at the (α=0.05) level.

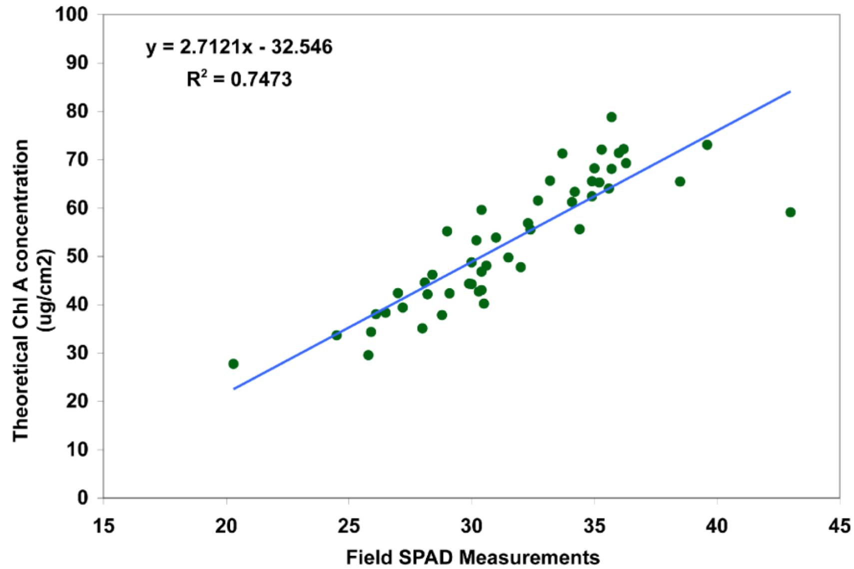
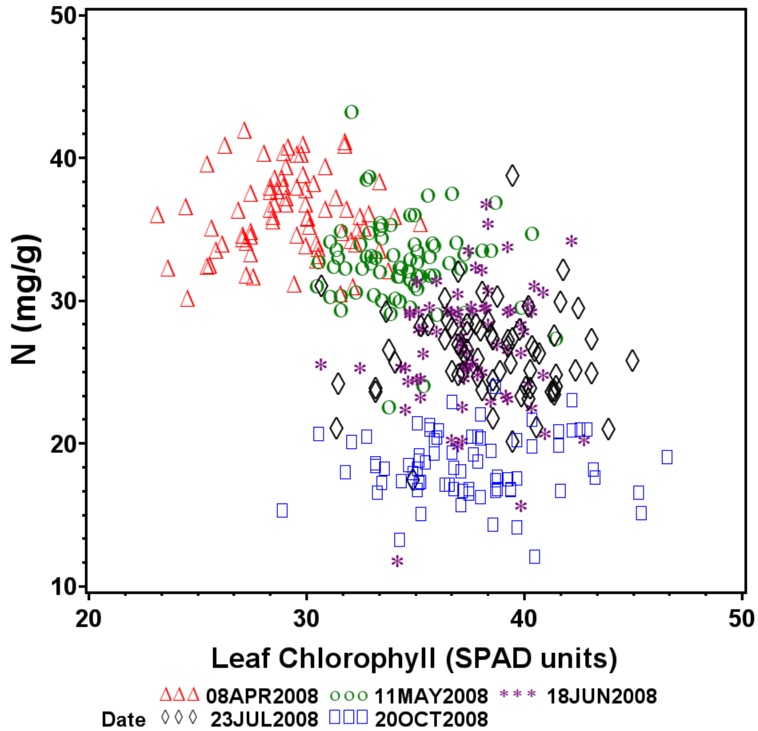
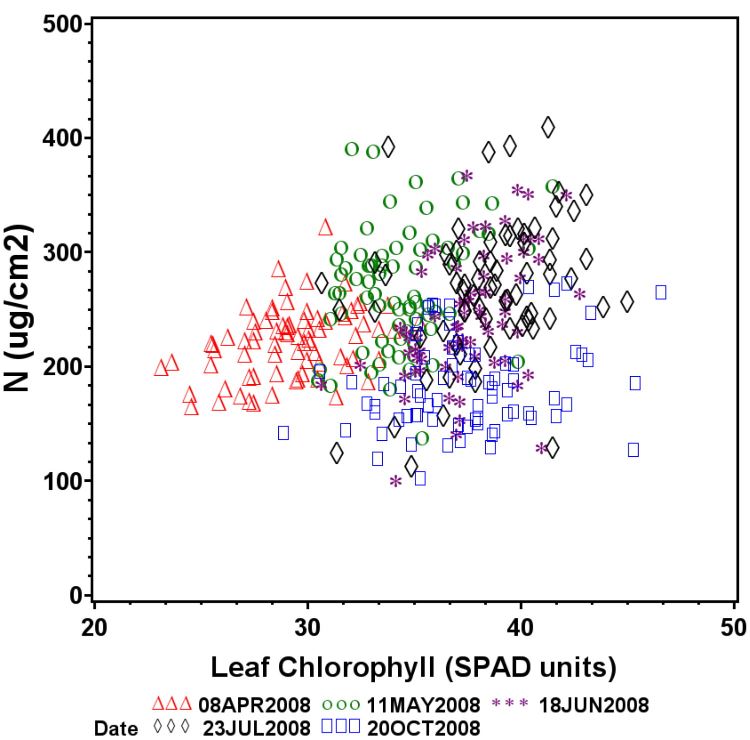


Figure 1. Relationship between SPAD meter values and chlorophyll A level in almond leaves.



(a)



(b)

Figure 2. Comparison of leaf chlorophyll and leaf nitrogen levels in almond at five sampling dates in 2008. 2a) Comparison to N on a dry mass basis, 2b) Comparison to N on a leaf area basis.

The mean leaf nitrogen level on a dry weight basis decreased with leaf age with significant (α=0.05) differences at all dates except between June and July. When the April through July values were combined there was a limited level of correlation (r=0.6, significant at α=0.01) between the individual leaf SPAD values and leaf N on a mass basis. When the October samples were included in the set, the correlation dropped (r=0.55, significant at α=0.01) due to the change in leaf N in October without a corresponding change in SPAD value. At any single date, however, there was no significant (α=0.05) relationship between individual SPAD values and individual leaf N on a mass basis.

On a leaf area basis, the mean leaf nitrogen content pattern with leaf age was less consistent and with the exception of the April samples, showed a higher level of leaf-to-leaf N variation than on a dry weight basis. At each date, a weak (r < 0.45) but statistically significant relationship between individual leaf SPAD values and leaf N was observed. If the patterns observed in 2008 are indicative of the relationship between leaf chlorophyll and leaf N, then it does not appear that the ability to use an indirect relationship between chlorophyll and leaf N found in annual crops applies well to almond and that SPAD meters are best used to identify leaf stress conditions such as Zn deficiencies that result in leaf chlorosis.

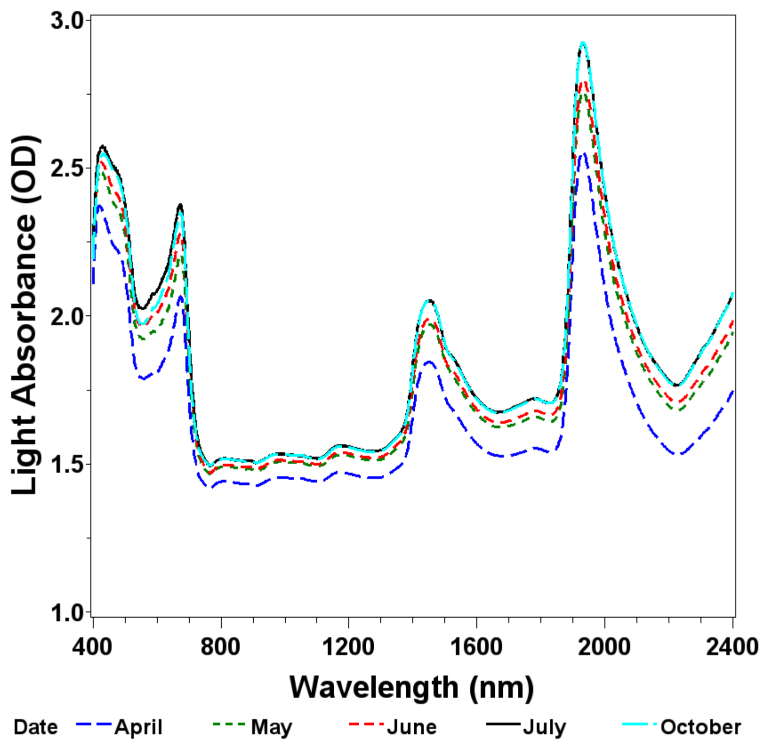


Figure 3. Mean spectral absorbance curves for almond leaves in the visible and near infrared regions at five dates in 2008.

The mean light absorbance curves for almond leaves at each of the five sampling dates are shown in **Figure 3**. The predominant peaks observed are due to light absorbance by water and chlorophyll. The data in **Figure 3** was obtained by transmission measurements on individual leaves where the absorbance is equal to the negative logarithm of the transmission value at each wavelength. The absorbance curves derived from the reflectance measurements (not shown) have a similar appearance. The absorbance curves in **Figure 3** show a consistent increase in optical absorbance across the visible and near infrared region with leaf age between April and July. These changes in absorbance are most likely due to changes in the leaf structure at the cellular level as the leaf develops. The change in optical absorbance is most dramatic between the April and May sampling dates, which is consistent with the rate of change in leaf phenology at that time compared to the rate later in the season. The July and October spectra are very similar with small differences in the visible region. Multivariate linear regression models can be developed using information from the silicon region (400 to 1100nm) to predict leaf age from the spectra. For example, a model using five wavebands in the infrared between 740 and 850 nm can predict leaf age ($r^2 = 0.86$) between April and July with a standard error of 15 days. If three visible wavebands associated with chlorophyll absorbance are matched with two bands in the infrared the performance improves ($r^2 = 0.88$) and the standard error drops slightly to about 13 days. When the October data is included, the coefficients of determination (r^2) remain high due to the increased range of values, but the standard error increases to about 25 days due to the difficulty in distinguishing the July and October spectra.

Full spectrum optical absorbance models (using partial least squares regression) were developed to predict leaf N on both a dry weight and leaf area basis. Models based solely upon infrared wavebands as well as models using both visible and infrared wavebands were investigated. Second derivative spectral preprocessing (based upon the Savitzky-Golay method) was utilized to reduce the baseline shift observed in the spectra due to leaf age. When predicting individual leaf N on a dry weight basis, it was possible to develop models using wavebands in the near infrared portion of the silicon region with performance levels comparable or superior to models using either infrared absorbance above the silicon region (i.e., between 1100 and 2500 nm) or models that combined visible and infrared wavebands. An NIR model using 8 Partial Least Squares (PLS) factors based upon the 700 to 1050 nm portion of the silicon region was able to predict individual leaf N with a standard error of cross-validation (RMSECV) of 3 mg/g N (r^2=0.8), **Figure 4**. However a pattern, similar to that observed for the SPAD meter, was observed when using NIR models to predict the leaf N on a dry weight basis showing good performance across the season, but poor performance at any one sampling date. The RMSECV was about half the standard deviation of the leaf N values for the season, but it was similar in magnitude to the standard deviation of the leaf N values at any one date. This appears to indicate that the NIR model is ineffective in predicting the leaf N level on a dry weight basis at any individual date. All models for leaf N on a dry weight basis, irrespective of the portion of the spectrum utilized or the spectral preprocessing methods employed showed this same pattern of good performance on a season level, but poor performance on a daily level.

Better success was obtained in developing a NIR model to predict leaf N on a leaf area basis at the early part of the season. An eight PLS factor NIR model based upon the second derivative spectrum in the 700 to 1100 nm silicon region was developed that showed good predictive ability (r^2=0.86) for leaf N on an area basis for samples collected in April and May, **Figure 5**.

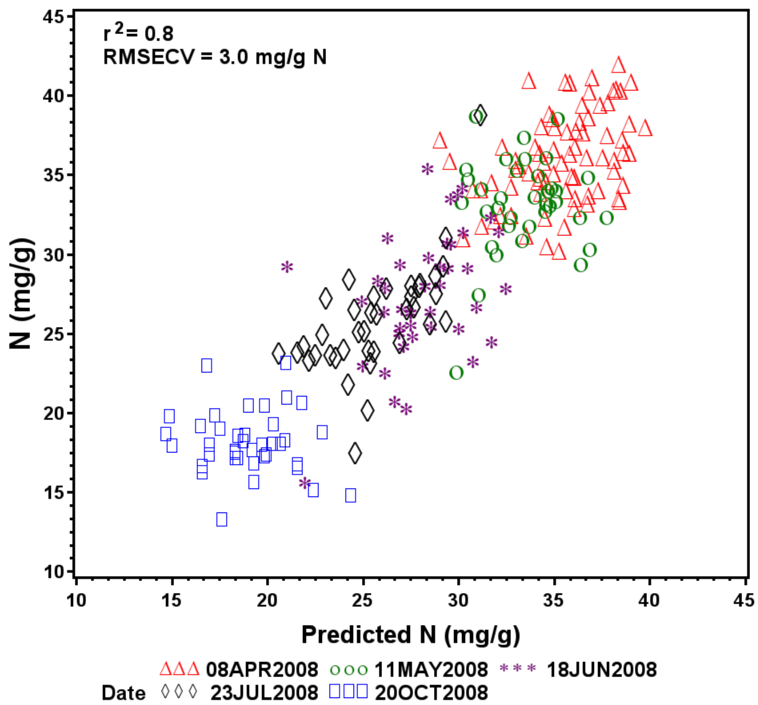


Figure 4. Performance of an 8 PLS factor NIR model developed to predict the individual leaf N level on a dry weight basis in almond.

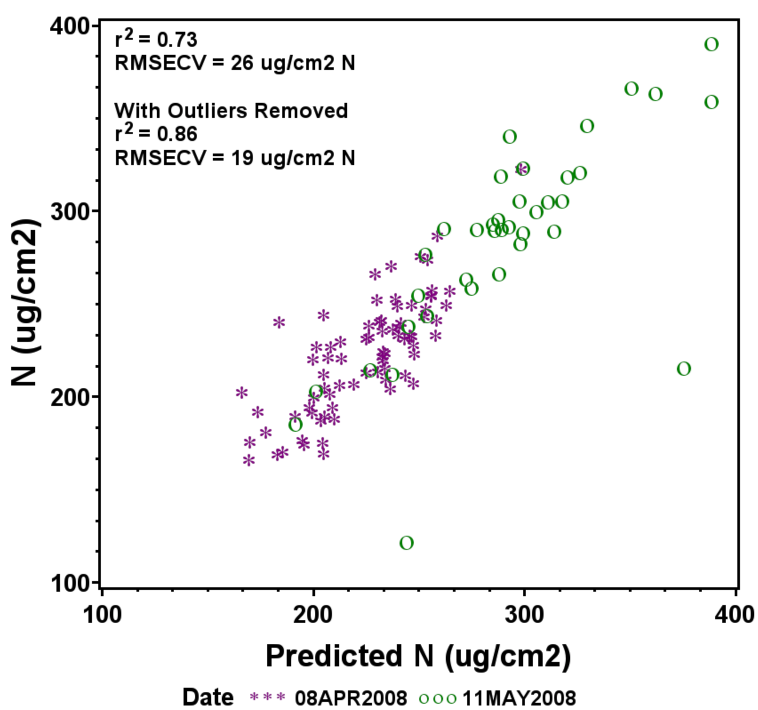


Figure 5. Performance of an 8 PLS factor NIR model developed to predict the individual leaf N level on a dry weight basis in almond

The ratio of the standard deviation of leaf N to the standard error for this model was 2.9, which is better than that obtained for the dry weight based model. In addition, the model showed good performance at each of the two sampling dates with ratios of leaf N standard deviation to model standard error of 1.5 and 3 for April and May, respectively. The higher ratio in May was due to the wider range of leaf N observed in the samples. Model performance degraded when attempts were made to predict leaf N values in June, July and October. Similar performance was obtained using a 3 PLS factor NIR model based upon the second derivative spectrum in the 1600 to 2400 nm region. Additional study is required to better understand the limitations of this model and to develop techniques that have similar performance across the season.

Acknowledgement:

The authors would like to acknowledge the assistance of Saiful Muhammad, Safirah Baikey, and Kameron Chun of the University of California, Davis in conducting this study.

References:

Ziadi, N., Brassard, M., Belanger, G., Claessens, A., Tremblay, N., Cambouris, A.N., Nolin, M.C., Parent, L-E. 2008. Chlorophyll measurements and nitrogen nutrition index for the evaluation of corn nitrogen status. Agronomy J. 100(5):1264-1273