Remote Sensing to Estimate Chlorophyll Concentration in Spinach Using Multi-Spectral Plant Reflectance

C. L. Jones, P. R. Weckler, N. O. Maness, R. Jayasekara, M. L. Stone, D. Chrz

Abstract. *The presence of nitrogen in soil and chlorophyll in plants are directly related; thus, chlorophyll may be used as an indirect indicator of nitrogen levels in fertilizer management systems. This research investigated a non-destructive method of determining chlorophyll content and concentration in field-grown spinach. Biomass was estimated with percent vegetation coverage based on images taken with a multispectral imaging system. Chlorophyll yield was estimated using reflectance-based NDVI from a GreenSeeker ™ hand-held optical sensor and a multispectral imaging system. Strong correlations were found between reflectance-based NDVI and chlorophyll yield (R^2 = 0.92), between NDVI and biomass (R^2 = 0.94), and between biomass and projected plant area (R^2 = 0.96). Combined parameter estimators of chlorophyll concentration had low correlation.*

Keywords. *Chlorophyll, NDVI, Nitrogen application, Precision farming, Spinach, Remote sensing.*

Determination of optimal levels for nitrogen fertilizer application to agricultural crops is not a straightforward process and carries significant uncertainties. Traditionally, pre-plant nitrogen requirements have been estimated by utilizing soil samples and crop-yield levels from previous years. The estimated application rate is then applied uniformly to the field (Sawyer, 1994). Lack of soil homogeneity and difficulty in implementing effective sampling strategies can lead to misapplication of nitrogen. An under-application of nitrogen may diminish crop production, while over-application can lead to negative environmental impacts, including nitrogen leaching and groundwater contamination (Raun, 1998). Fertilizer in excess of plant needs may result in surface runoff and pollution of lakes and streams (Daughtry et al., 2000; Wood et al., 1993). In either case, the economic returns to the producer are reduced. Therefore, a method that would allow real-time, in-field detection of crop nitrogen needs could be useful in crop nitrogen management.

Chlorophyll *a* and *b* and carotenoid concentrations correlate to the photosynthetic potential of a plant and give some indication of the physiological status of the plant (Danks et al., 1983; Gamon and Surfus, 1999; Young and Britton, 1990). Chlorophyll yield (C_{yld}) is defined as the chlorophyll mass per unit area or per plant (kg/ha or kg/plant). Chlorophyll concentration (C_{conc}) is defined as the chlorophyll mass per unit mass of dry plant material (mg/kg). C_{yld} may be used to evaluate the overall photosynthetic capacity or productivity of the plant canopy. C_{conc} may be an indicator of plant physiological status or level of stress (Blackburn, 1998).

Chlorophyll *a* content is mainly determined by nitrogen availability (Moorby and Besford, 1983). Light reflectance by leaves in the visible region of the spectrum depends primarily on the concentration of chlorophylls and carotenoids. A deficiency in nutrients such as nitrogen decreases pigment formation and leaf color, which subsequently increases reflectivity due to reduced radiation absorption.

The normalized difference vegetative index (NDVI) was proposed by Rouse et al. (1974) to separate green vegetation from the soil background and is in common use. Equation 1 gives the NDVI in terms of irradiance from a target:

$$\mathrm{NDVI} = \frac{I_{NIR} - I_{RED}}{I_{NIR} + I_{RED}} \tag{1}$$

where

I_{NIR} = near-infrared irradiance

I_{RED} = red irradiance.

NDVI has a range of −1 to +1, with bare soil surfaces having an NDVI of approximately 0 and heavy vegetative cover having an NDVI of near 1 (Thiam, 1998). Reflected red irradiance (I_{RED}) is strongly diminished through chlorophyll absorption, with peak chlorophyll absorption occurring at 647 nm. Both red and NIR irradiance are strongly influenced by plant cover. Red irradiance (I_{RED}) decreases with plant cover, and NIR irradiance (I_{NIR}) increases. Given these relationships, NDVI from vegetated surfaces is heavily influenced by chlorophyll content of materials in the vegetation. NDVI was introduced as a measure of biomass and is successful because chlorophyll concentration in plants is relatively constant. NDVI has been correlated with such plant properties as leaf area index, fractional vegetation canopy, vegetative condition, biomass, nitrogen content, and ni-

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trogen concentration (Carlson and Ripley, 1997; Sembiring et al., 1998).

Fractional vegetation coverage (%VC) is the fraction of a given area that is covered by vegetation in a two-dimensional view from above. NDVI increases nearly linearly with %VC and then enters an asymptotic condition where large increases in VC bring small increases in NDVI (Carlson and Ripley, 1997). Sembiring et al. (1998) found NDVI to be a better predictor of plant nitrogen content than nitrogen concentration. Studies by Lukina et al. (1999, 2000) also support NDVI as a better predictor of nitrogen content than nitrogen concentration.

Nitrogen content (also known as nitrogen uptake) is the total mass of nitrogen present in the plant vegetation above ground per unit ground surface area (kg/ha). Nitrogen concentration is the unit mass of nitrogen present per unit mass of biomass (mg/kg). Stone et al. (1996) found a high correlation between nitrogen content and plant nitrogen spectral index (PNSI) in winter wheat at several different growth stages. PNSI is inversely related to NDVI. Stone et al. (1996) demonstrated the validity of sensor-based variable-rate technology (sVRT) for nitrogen application through the variable application of nitrogen to winter wheat based on PNSI. The result was an increase in nitrogen efficiency, decreased spatial variation, and increased yield when compared to the standard fixed application rate. Deficiencies in nitrogen may now be detected and corrected using sVRT (Raun et al., 1998).

A sensor that utilizes only sunlight reflected from crop surfaces to calculate NDVI will be sensitive to changes in solar azimuth (Pinter et al., 1990). Cloud cover has been shown to have a minimal effect on ratio-type vegetation indices, such as NDVI, since both the red and near-infrared bands are equally affected (Pinter et al., 1987). Changes in solar angle, however, have been found to significantly affect NDVI readings (Pinter, 1993). Merritt et al. (1994) utilized reflectance calibration to compensate for changes in lighting conditions by estimating the incident red and near-infrared light intensities from a reference surface painted with flat white paint, in addition to the red and near-infrared intensities reflected from the plant vegetation. Identical photo-detector pairs were used to gather both red and near-infrared incident and reflected light intensities. Reflectance values were then placed into the standard NDVI formula in contrast to the more commonly used irradiance-based NDVI introduced by Rouse et al. (1974).

An example of a reflectance-based multispectral sensor is the GreenSeeker hand-held optical sensor unit (NTech Industries, Inc., Ukiah, Cal.). The GreenSeeker uses light-emitting diodes (LEDs) to produce the light from which reflectance is determined. Natural incident light and light from the LEDs are separated electronically, and a voltage signal is produced that is related to the fractions of reflected near-infrared and red light from the LEDs. NDVI is directly computed. The data that are collected with the sensor can be downloaded to a personal computer in a text format that can be accessed by statistical software.

Photo imagery may also be used for estimating biomass. Ter-Mikaelian and Parker (2000) used photo imagery to estimate the biomass of white spruce seedlings by examining a seedling side-view silhouette area. The accuracy of the imaging technique was found comparable to the traditional allometric methods using seedling basal diameter. Adamsen et al. (2000) used color digital camera images to estimate the number of lesquerella flowers in experimental plots. This method produced estimates that were highly correlated with a manual flower count ($r^2 = 0.83$). Lukina et al. (1999) used images from a digital camera to estimate vegetation coverage and a multispectral radiometer to measure NDVI in plots of winter wheat. Vegetation coverage was found to have a strong correlation with NDVI ($r^2 = 0.66$ to 0.96). NDVI, in turn, was found to have a strong correlation with dry biomass ($r^2 = 0.52$) and with nitrogen content ($r^2 = 0.66$). The studies of Ter-Mikaelian and Parker (2000) and Lukina et al. (1999, 2000) support the hypothesis that percent vegetative coverage may be useful in estimating the biomass of a plant canopy.

Total vegetative chlorophyll yield is a product of vegetative chlorophyll concentration and vegetative mass. To date, the ability to non-invasively estimate nitrogen concentration in a plant canopy has not been well demonstrated, although there has been reasonable success in non-invasively estimating the nitrogen content of plant vegetative biomass. The ability to estimate biomass using percent vegetation coverage has also been demonstrated. Since nitrogen content is a product of nitrogen concentration and biomass, and nitrogen content and biomass may be non-destructively estimated, it is reasonable to postulate that nitrogen concentration may be estimated by utilizing estimates of nitrogen content and biomass.

Because of the correlation between nitrogen concentration and chlorophyll concentration, this study determines the feasibility of using indirect measurements of chlorophyll yield and biomass to estimate chlorophyll concentration. NDVI was calculated using reflectance data from the GreenSeeker sensor and from the multispectral imaging system. Biomass was estimated using image processing techniques on the digital images acquired with the multispectral imaging system.

Materials and Methods

Studies were conducted in the fall of 2002 and the spring of 2003 at the Oklahoma Vegetable Research Station in Bixby, Oklahoma. These field studies investigated the relationship between NDVI and biomass, chlorophyll yield, and chlorophyll concentration in spinach. The cultivar 'Fidalgo' was planted in the fall studies, while 'San Juan' cultivar spinach was planted in the spring studies. These cultivars were chosen for their typical lush foliage growth in the respective season conditions. Plots were seeded at a rate of 39 seeds per linear meter. While different field areas were used for each study, the soil for all areas was a Severn very fine sandy loam that had been fallowed the previous year. Composite soil sample analyses showed adequate P, K, and micronutrient for spinach and less than 9 kg ha^{-1} of nitrogen (N) present. A prior application of Rowneet common market herbicide offered adequate weed control. The seeds were planted in 12 m eight-row plots with 0.23 m between rows. Each study was arranged in a randomized complete block design with four replications of four fertilizer treatments (16 test plots). Granular 33-0-0 fertilizer was applied immediately after planting. The fertilizer rates were 70, 140, and 210 kg/ha of nitrogen plus control plots that received no fertilizer. Plots in all experiments were irrigated with overhead sprinkler irrigation.

Beginning at the five-leaf stage, multispectral and vegetative coverage data were collected from each of the 16 test plots weekly until the early bolting stage. For the 2002 growing season, five weeks of data were collected. For the 2003 growing season, four weeks of data were collected. A total of 90 samples were taken in 2002 and 64 samples were taken in 2003.

Multispectral Reflectance Sensor

The GreenSeeker hand-held optical sensor unit (NTech Industries, Inc., Ukiah, Cal.) is an active sensor that uses LEDs to produce its own light in the red and NIR bands. The sensor produces a voltage that is related to the reflected NIR and red light. The GreenSeeker was held at 85 cm above the ground using an adjustable shoulder harness. This height provides a 61 cm × 1 cm sensing view at ground level. A sample area indicator frame was placed on a randomly selected area of each test plot. The rectangular frame was constructed of 1.27 cm (3/4 in.) PVC pipe and had an inside dimension of 0.76 m wide × 0.91 m long. Both frame ends had a length of 0.076 m × 0.76 m white sheet metal attached horizontally to the outer edges of the frame. These white strips created an anomaly of reflectance readings in the GreenSeeker sensor data that was later used to isolate the sensor readings that occurred inside the frame. The GreenSeeker sensor was passed over the sample area lengthwise. The sensor was triggered outside of the frame area, passed over the vegetation in the frame, passed over the other end of the frame, and then disengaged. The outer sides of the frame were painted black to minimize interaction with the sensor. Reflectance data from the GreenSeeker sensor were captured in a text file on a hand-held computer. Approximately 2100 data points representing reflectance in the red and NIR wavebands were recorded for each plot. Using equation 1, NDVI was calculated for data points between the anomalies from the marker frame ends and averaged to give an NDVI representative of the biomass inside the marker frame.

Multispectral Imaging System

After the GreenSeeker sensor was passed over the sample area, a multispectral image was acquired of each sample area with the marking frame still in place. The multispectral imaging system included a DuncanTech MS3100 camera (Auburn, Cal.) equipped with a 14 mm focal length lens, a ruggedized field laptop computer, and a PCI-1424 frame grabber (National Instrument Corp, Austin, Tex.). The camera system was configured to collect irradiance data in three narrow optical wavebands (±10 nm) centered at 550, 670, and 780 nm, corresponding to the green, red, and infrared bands, respectively. The multispectral imaging system was mounted to a pole on a platform held 2.29 m above the ground by the forks of a field tractor front-end loader. With the imaging system located at this height, the image view closely matched the size of the marking frame area. The pole projected the camera 2.5 m horizontally to eliminate shadows from the equipment.

One image of each plot was acquired with standard calibrated reflectance targets included in the view. Four 12.5 cm × 12.5 cm reflectance targets (reflectance calibration standard, Labsphere, Inc., New Sutton, N.H.) with nominal reflectances of 10%, 50%, 75%, and 99% were placed in the marker frame area with the plants. The multispectral camera was set to the automatic gain setting. After this image was acquired, the targets were removed and another image was immediately acquired of the same plot with only the vegetation and marking frame in view. Images were saved for further analysis after field data collection. The images were processed using Matlab (MathWorks, Inc., Natick, Mass.) image processing software. Reflectance for each band (red, green, and near-infrared) was obtained using the calibration procedure described by Weckler et al. (2002). The images were converted into reflectance using calibration equations (eq. 2), and then an NDVI image was generated using reflectance of the red and near-infrared bands (eq. 1). The image was binarized, and the %VC was calculated.

$$\rho_{red} = \frac{I_{red}}{I_{red(cal)}}$$

$$\rho_{near\text{-}infrared} = \frac{I_{near\text{-}infrared}}{I_{near\text{-}infrared(cal)}} \quad (2)$$

where

ρ_{red} = red reflectance
$\rho_{near\text{-}infrared}$ = infrared reflectance
I_{red} = reflected red light from the plot
$I_{near\text{-}infrared}$ = reflected near-infrared light from the plot
$I_{red(cal)}$ = reflected red light from the calibration targets
$I_{near\text{-}infrared\ (cal)}$ = reflected near-infrared light from the calibration targets.

Hand-Harvesting for Laboratory Samples

After sensing each plot with the GreenSeeker sensor and imaging with the multispectral imaging system, the plants inside the marker frames were hand-harvested by clipping at ground level. The harvested spinach was placed in plastic bags, labeled and weighed, placed on ice, transported, and refrigerated for processing the following day. In the laboratory, the spinach was washed, freeze-dried, ground with a UDY mill (UDY Corp., Ft. Collins, Colo.) to pass a 1 mm screen, and analyzed for chlorophyll yield according to the spectrophotometric method of Inskeep and Bloom (1985).

Data Processing

The collected data were processed using SAS software (SAS/Stat procedures, release 8.02, SAS Institute, Inc., Cary, N.C.). Analysis of variance using the PROC REG command in SAS was used to determine coefficients of determination for the regressions. The GreenSeeker sensor data were used to calculate NDVI (eq. 1), and the multispectral imaging system provided NDVI information for comparison and validation of the GreenSeeker sensor. The multispectral imaging system provided images that were binarized to determine %VC data. The hand-harvested spinach from the frames provided actual biomass, chlorophyll yield, and chlorophyll concentration data, as determined in the laboratory. NDVI data were correlated to laboratory biomass and chlorophyll data. NDVI was divided by %VC from the images to provide an estimated chlorophyll concentration. This information was correlated to the chlorophyll concentration found in the laboratory analysis. The NDVI calculated from the imaging system reflectance data were used to validate the NDVI calculated using the GreenSeeker data.

RESULTS

Data were fit to both linear and exponential equations, with the exponential equations providing stronger correlation of GreenSeeker NDVI with both biomass and chlorophyll yield. Data from each study showed that NDVI was nearly equally correlated with biomass and chlorophyll yield, with coefficients of determination (R^2) ranging from 0.85 to 0.94 and from 0.88 to 0.92, respectively (table 1). NDVI was exponentially related to biomass and chlorophyll (fig. 1). This relationship concurs with work done by Carlson and Ripley (1997) and Raun et al. (1998). An examination of the vegetative coverage (%VC) calculations from the digital images indicated that there was a strong logarithmic asymptotic correlation between %VC and biomass (R^2 = 0.91 and 0.96) (table 1, figs. 2a and 2b).

NDVI was highly correlated with chlorophyll yield, and %VC was highly correlated with biomass. To estimate chlorophyll concentration, the chlorophyll yield estimator (NDVI) was divided by the biomass estimator (%VC) (eq. 3). Equation 4 shows the dimensional equivalent of equation 3:

Table 1. Sensor and camera coefficients of determination (R^2) in spinach chlorophyll studies

Study	Biomass vs. NDVI	Chlorophyll Yield vs. NDVI	%Vegetative Coverage vs. Biomass	NDVI / %VC vs. Chlorophyll Concentration
Fall 2002[a]	0.94	0.92	0.91	0.03
Spring 2003[b]	0.85	0.88	0.96	0.25

[a] Fall 2002 sample size, n = 90, $p < 0.05$.
[b] Spring 2003 sample size, n = 64, $p < 0.05$.

$$\frac{\text{NDVI}}{\%\text{ VC}} = \frac{\text{chlorophyll yield estimation}}{\text{biomass estimate}} = \text{chlorophyll concentration estimate} \quad (3)$$

$$\frac{[\text{mg chlorophyll}]}{[\text{kg spinach biomass}]} = [\text{mg/kg chlorophyll conc.}] \quad (4)$$

This estimate of chlorophyll concentration was poorly correlated to laboratory chlorophyll concentration measurements (R^2 = 0.03 and 0.25, figs. 3a and 3b). However, when

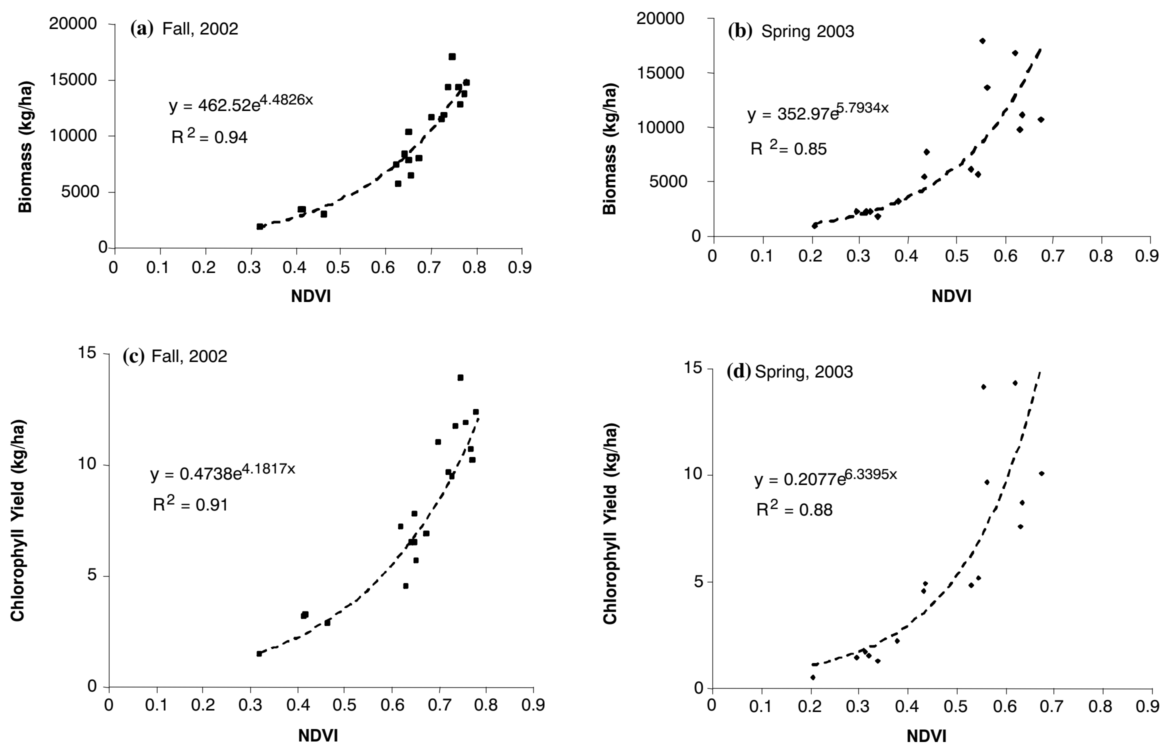

Figure 1. NDVI comparison to biomass and chlorophyll yield: (a) fall 2002 biomass vs. NDVI, (b) spring 2003 biomass vs. NDVI, (c) fall 2002 chlorophyll vs. NDVI and (d) spring 2003 chlorophyll vs. NDVI.

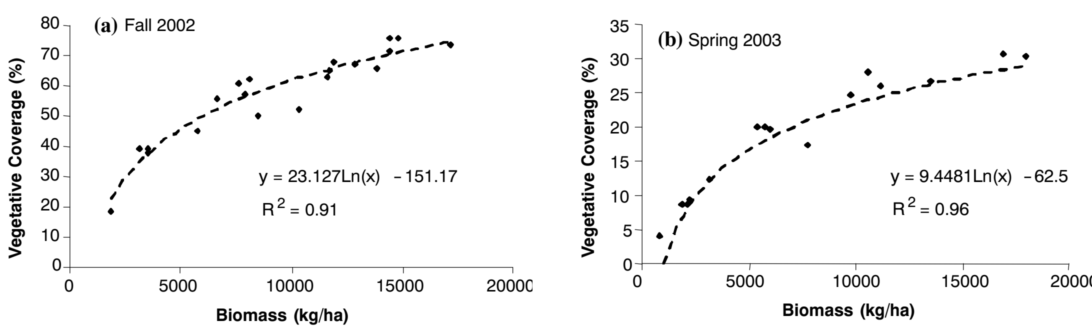

Figure 2. Comparison of vegetative coverage to actual biomass: (a) fall 2002 and (b) spring 2003.

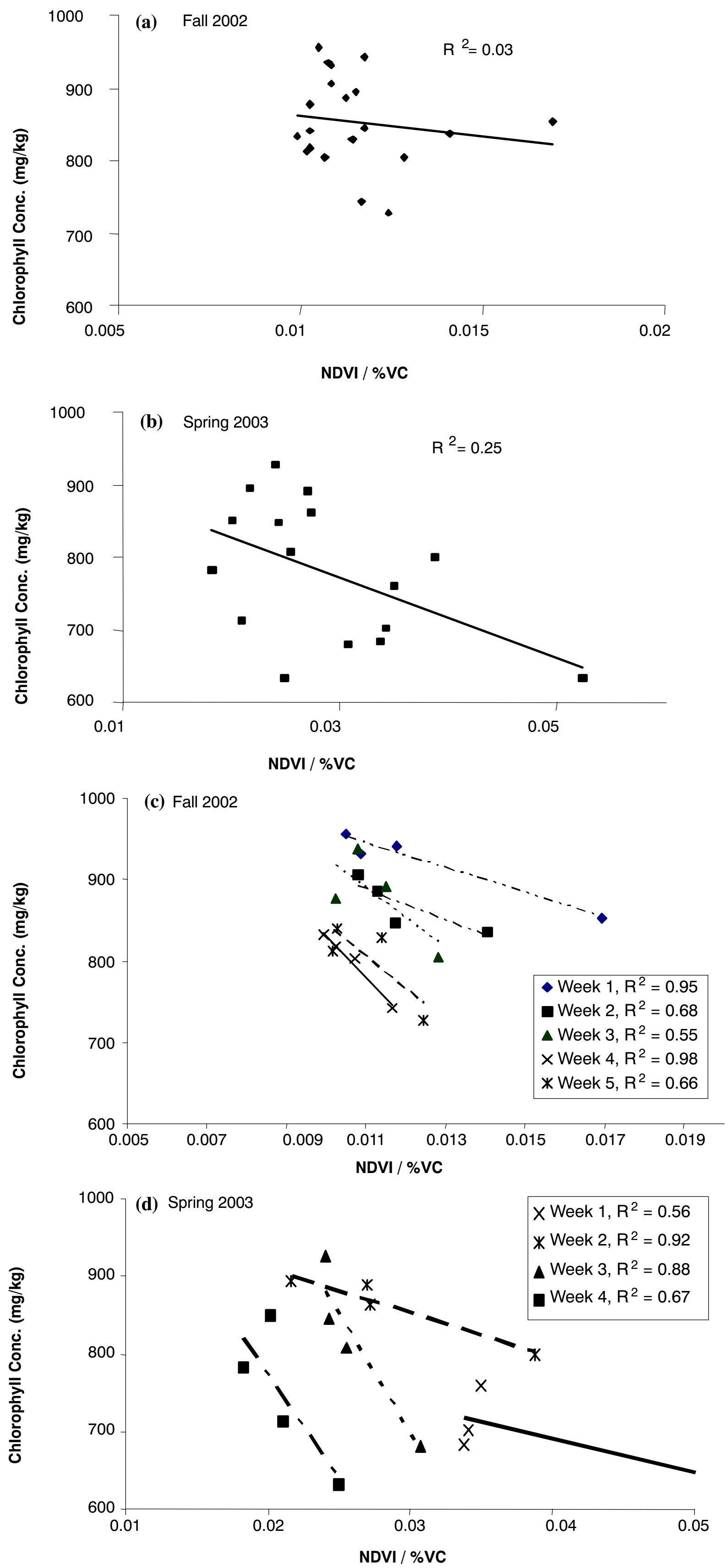

Figure 3. NDVI / %vegetative coverage vs. chlorophyll concentration for two seasons and by weekly maturity: (a) fall 2002 season analysis, (b) spring 2003 season analysis, (c) fall 2002 weekly analysis, and (d) spring 2003 weekly analysis. Each point represents the average of four replications of each fertility treatment.

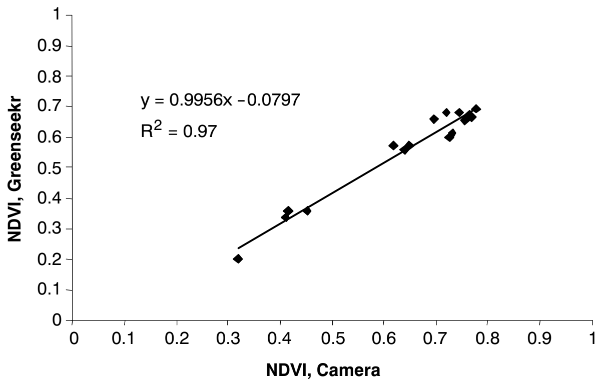

Figure 4. NDVI from sensor vs. NDVI from multispectral camera.

the data were analyzed by maturity, better correlation was exhibited (figs. 3c and 3d). Sample size for each week was 16 instead of 90 and 64 for the 2002 and 2003 seasons, respectively, reducing the statistical significance of the comparisons. NDVI results from the hand-held sensor correlated well with NDVI calculated from image data acquired by the multispectral camera ($R^2 = 0.97$, $n = 35$, $p < 0.001$) (fig. 4). This confirms the performance of the GreenSeeker sensor in the row crop environment and with the specific test crop (spinach).

Conclusion and Discussions

The NDVI data acquired using the GreenSeeker sensor and the multispectral imaging system were sensitive to changes in plant biomass and plant chlorophyll yield in row crop spinach. Correlations of NDVI to biomass were approximately the same as the correlation of NDVI to chlorophyll yield. Prior to this research, this sensing technology had not been used on row crop leafy plants such as spinach.

This study reaffirmed the correlation between vegetative coverage and dry biomass found by Lukina et al. (1999, 2000) and Ter-Mikaelian and Parker (2000). High correlation (R^2 = 0.91 to 0.96) was observed between vegetative coverage of the spinach as measured with digital imagery and biomass as measured in the laboratory. The findings of Lukina et al. (1999, 2000) and Sembiring et al. (1998) were also supported regarding NDVI producing a stronger estimate of chlorophyll yield than of chlorophyll concentration. These correlations indicate the appropriate use of the sensing equipment to estimate biomass and chlorophyll yield in row crop spinach.

Chlorophyll concentration estimates were calculated by dividing chlorophyll yield estimates (NDVI) by biomass estimates (%VC). When these chlorophyll concentration estimates were compared to laboratory chlorophyll concentration data, weak correlation was found (R^2 = 0.03 to 0.25). Stronger correlation with chlorophyll concentration was found with NDVI divided by %VC data by considering weekly maturity (R^2 = 0.55 to 0.98). Partially responsible for the better correlation could be the smaller sample size of 16 samples for each week compared to sample sizes of 90 and 64 for the entire data sets for 2002 and 2003, respectively. The improvement in correlation may be noteworthy, and a more exhaustive study focusing primarily on multispectral response to maturity may provide stronger statistical data when based on weekly resolution. Weekly data analysis from this study provides impetus for a more specific and exhaustive maturity study.

Each growing season demonstrated a different response relationship between sensor response and plot characteristics even though the trends were similar. For this information to be used in a variable-rate fertilizer application and management program, a set of calibration plots to establish seasonal response should be used so that temporal and climactic variation between seasons is normalized. GreenSeeker sensor NDVI information combined with the %VC information from the digital image provides a strong estimate of biomass and chlorophyll yield of various fertilizer treatment calibration plots, which can be used to prescribe fertilizer application rates for production plots. This information is critical for extension to automatic variable-rate technology of fertilizer application for row crop leafy vegetables.

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