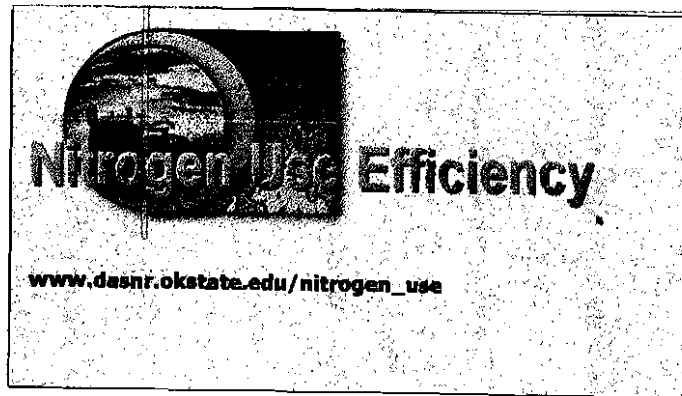


Estimating Vegetation Coverage in Wheat Using Digital Images

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ABSTRACT

No method exists to reliably predict percent vegetation coverage using indirect measures. This study was conducted to evaluate the use of digital image processing techniques applied to digital color, red-green-blue (RGB), images of crop canopies to estimate percent vegetation coverage and biomass. Two field experiments with winter wheat (*Triticum aestivum* L.) "Tonkawa" were planted in October 1996 and 1997 at Perkins, OK on a Teller sandy loam (Udic Argiustoll) and at Tipton, OK on a Tipton silt loam (Pachic Argiustoll). Plot images from winter wheat canopies were taken using a Kodak DC40 Digital Camera (1995)¹ with an image resolution of 756 x 504 pixels. Spectral irradiance readings were taken from wheat canopies in red (671±6 nm) and near infrared (780±6 nm) wavelengths, and normalized difference vegetation index (NDVI) was calculated. Percent vegetation coverage was estimated using image-processing routines in Micrografx Picture Publisher® version 7.0. The digital images were converted from 8-bit RGB tagged image file format (TIFF) files, which were produced by processing the images from the camera with

¹Mention of trade or company name is for information only and does not imply an endorsement by the authors or Oklahoma State University.

Photo Enhancer®, to binary pseudo-color images. Percent of pixels corresponding to the vegetation color was then calculated and used as the percent coverage for each plot. Binary pseudo-color images provided useful estimates of percent vegetation coverage that were highly correlated with wheat canopy NDVI measurements.

INTRODUCTION

Conventional methods of fertilizer application employ soil testing to determine appropriate rates. Soil testing is a good estimator of soil nutrient availability for immobile nutrients, phosphorus (P) and potassium (K). Raun et al. (1998) highlighted that in-season nitrogen (N) deficiencies can now be detected and treated using sensor-based methods. However, past soil testing for in-season treatment has been cumbersome largely because of the time lag required between testing and final fertilizer application. In the last 20 years, newer non-destructive methods of measuring mobile and immobile nutrient availability have been developed.

The normalized difference vegetation index (NDVI) has recently proven to be a reliable estimator of N deficiency in winter wheat (Stone et al., 1996b). On-the-go NDVI measurements can be used for detecting N deficiency and for making in-season topdress N applications. Stone et al. (1997) found that this method could significantly increase nitrogen fertilizer use efficiency.

Individual plants, their shadows, and soil background contribute to spectral measurements made in vegetation canopies. In early experiments with visible and infrared reflectance from wheat canopies, Stanhill et al. (1972) suggested that the difference in crop absorptivity could be accounted for by the differences in biomass and degree of ground cover. Wanjura and Hatfield (1987) pointed out that vegetation indices were affected more by ground cover than by other variables such as fresh and dry biomass or leaf area index. They estimated ground cover by measuring canopy shadow width in different crops. Considering ground cover as an important variable, Huete et al. (1985) measured percent green canopy cover by projecting a 35 mm slide onto a dot grid and counting the dots of light and shaded surface. Later, using the same technique of ground cover estimation, Heilman and Kress (1987) concluded that soil background reflectance had the greatest influence for 50 to 75% ground cover on soils with high reflectance. Low vegetation coverage did not affect soil irradiance significantly whereas soil reflectance was insignificant at high vegetation coverage. Vegetation density and amount of soil included in the sensor view can also affect spectral measurements. In order to evaluate the impact of vegetation coverage on sensor readings Lukina et al. (1997) evaluated percent vegetation coverage at different growth stages and row spacings. Their work demonstrated a high correlation (0.8-0.97) between percent vegetation coverage and NDVI measurements.

The objective of this study was; to evaluate the use of digital image processing techniques applied to digital color, RGB, images of crop canopies to estimate percent coverage and biomass.

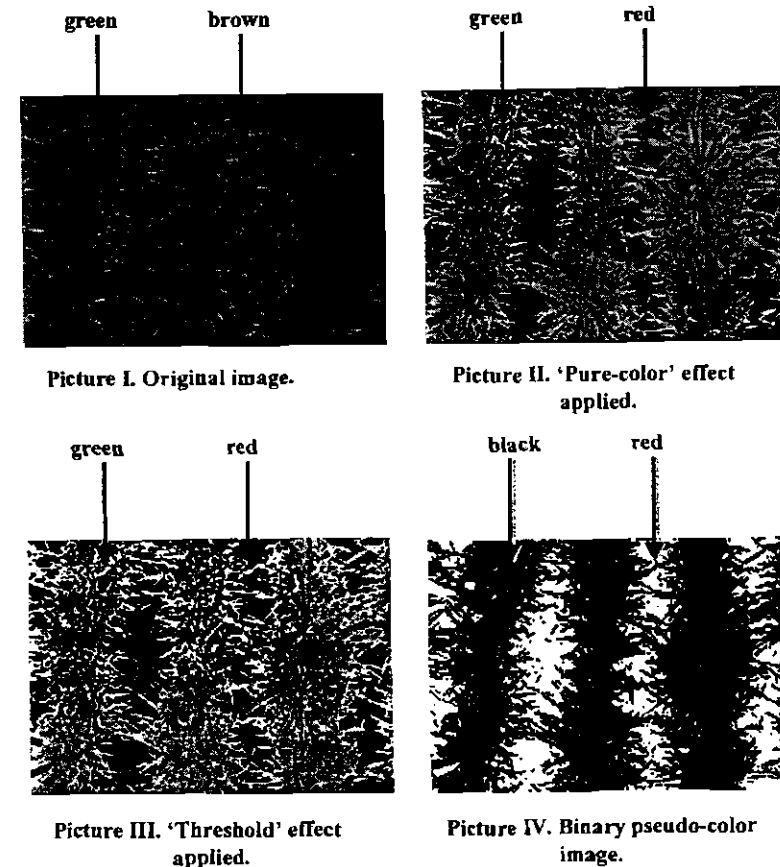


FIGURE 1. Image processing procedure, steps I-IV.

MATERIALS AND METHODS

Winter wheat (*Triticum aestivum* L.) was planted in October 1996 and 1997 at Perkins, OK on a Teller sandy loam (fine-loamy, mixed, thermic Udic Argiustoll) and at Tipton, OK on a Tipton silt loam (fine-loamy, mixed, thermic, Pachic Argiustoll). Four N rates, 0, 56, 112, 168 kg ha⁻¹ as ammonium nitrate were broadcast and incorporated preplant. Seeding rates were 99, 80, 59, and 49 kg ha⁻¹, at row spacings of 15.2, 19.0, 25.4, and 30.5 cm, respectively. Each plot was 2.6 m x 6.1 m. Canopy irradiance measurements were taken from wheat in-situ using red (671±6 nm) and near-infrared (780±6 nm) wavelengths at Feekes growth stages 4 and 5 (Large,

TABLE 1. Method to convert digital images of wheat in vegetative stages to percent vegetation cover.

Step	Description	Commands	Effect
I	Open the original image.		Figure 1, picture 1
II.a	In the Image Menu chose Effects option	<Image\Effects>	Dialog box is displayed
II.b	In the Image\Effects directory choose Color Saturation option under Color Adjust submenu	<Image\Effects\ Color Adjust\ Color Saturation>	
II.c	In the Color Saturation box (on the right) set the pointer to pure (color), then click Apply button		Original image in Original box will be changed, saturated with pure colors Figure 1, picture 2
II.d	In the Image Effects directory choose Threshold option under Color Adjust submenu	<Image\Effects\ Color Adjust\ Threshold>	
II.e	In the Threshold box, set Breakpoint at 50% in all channels (RGB). Click Apply button		The image in the Original box will change, R, G, and B values greater than 50% are converted to 100%, those less than 50% are converted to 0% The original image will change as shown in the Figure 1, picture 3
II.f	Click OK button		
III.a	In the Mask menu choose Chroma Mask option	<Mask\ Chroma Mask>	
III.b	Select one Color Select button in the dialog box. Point a drop stick cursor on the red part of the image (representing soil) and click the left button of the mouse. Click OK button of the dialog box		Chroma mask appears on the image, soil related compartments of the image are selected
III.c	In the Mask menu choose the Invert Mask option	<Mask\Invert Mask>	Plant related pixels in image are selected
III.d	In the View menu choose Color Palette, or open a Color Palette icon, and choose black color (last chosen color is the active one)	<View\Color Palette>	
III.e	On the toolbar panel, click on the Fill Tools button and choose the Fill the Selected Object with the Active Color option		
III.f	Fill the mask by clicking the left button of the mouse somewhere on the plant related part of the image		Plant related image pixels are colored black
III.g	From the Mask menu choose Remove Mask option	<Mask\ Remove Mask>	Figure 1, picture 4
IV.a	In the Map menu choose the Histogram option	<Map\Histogram>	
IV.b	In the Channel box select the Master channel (Red channel could be used also, but it appeared that Red and Blue channels are switched in 'Micrografx.7')		A histogram of each band (R, G, and B) is computed Soil and plant related pixels are counted. Shadow indicates the percentage of black pixels of the image, which correspond to vegetation and Midtones show the percentage of red pixels, representing soil

1954). Plot images were taken using a Kodak DC40 Digital Camera (Kodak, 1995) with a resolution of 756 x 504 pixels at both locations (Tipton and Perkins), and at the same time and places as spectral irradiance measurements. Percent vegetation coverage was estimated using image-processing routines in Micrografx Picture Publisher® version 7.0 (Micrografx, 1997). The digital images were converted from 8-bit RGB TIFF files, which were produced by processing the images from the camera with Photo Enhancer® (Kodak, 1995), to binary pseudo-color images. Percent of pixels corresponding to the vegetation color was then calculated and used as the percent of coverage for each plot as illustrated in Figure 1. Table 1 summarizes the procedure used for processing the images.

It should be noted that some adjustments of contrast and color balance are required for images taken under different light and soil reflectance conditions. The color of the Udic Argiustoll soil (dark brown 10YR 4/3) at Perkins was lighter than that of the Pachic Argiustoll (very dark grayish brown 10YR 3/2) at Tipton. The Perkins images, taken at Feekes growth stage 5 in 1997, were adjusted for contrast and color balance to improve color separation. Before applying the procedure in Table 1, the contrast (Map\Color Balance ->Joystick\Contrast) was increased (by 5%) and the balance (Map\Color Balance ->Joystick\Balance) of the red channel was shifted towards red (by -10%). Color saturation was then adjusted to maximum (pure): steps I, II.a - II.c in Table 1. The images were then color thresholded in red, green, and blue at a 24% level in the same way as described in steps II.d - II.f in Table 1. A 'chroma mask' was then generated for the combination of red and black portions of the image, and filled with red color. These parts corresponded to soil. The mask was then inverted and filled with a black color and processed as described in steps III.g through IV.b in Table 1.

Extremely bright images, taken on a very bright sunny day at Tipton, Feekes growth stage 4, 1998 were treated differently. Soil color at this location was darker than that of Perkins. First images were 'smoothed' (Image-Effects-Photographic-Smooth) by 2 units. Then all steps were executed as described in the Table 1 with the only difference in step II.b, where 'chroma mask' was applied not only to red, but also to white and purple colors.

'Smoothing' can assist with separating bright spots of leaves with that of soil. However, this procedure can misclassify some soil-related pixels as plant pixels.

It was noticed that images taken on a cloudy day were converted with better precision; largely due to the absence of glare. Thus, future images were either taken on a cloudy day or under a shadow, created by black poster boards (81x102cm). Images taken under the shadow were processed with slight differences. For images obtained at Tipton, at Feekes growth stage 5, 1998, the contrast was increased (by 5%) and the balance of the red channel was shifted towards red (by 5%). The 'chroma mask' was generated for red and purple colors (Table 1, step II.b) and then filled with red. The rest of the procedure was unchanged.

Images taken at Perkins, Feekes growth stages 4 and 5, 1998 were adjusted in contrast and balance (by 5 and -5 %, respectively). Colors were thresholded in red,

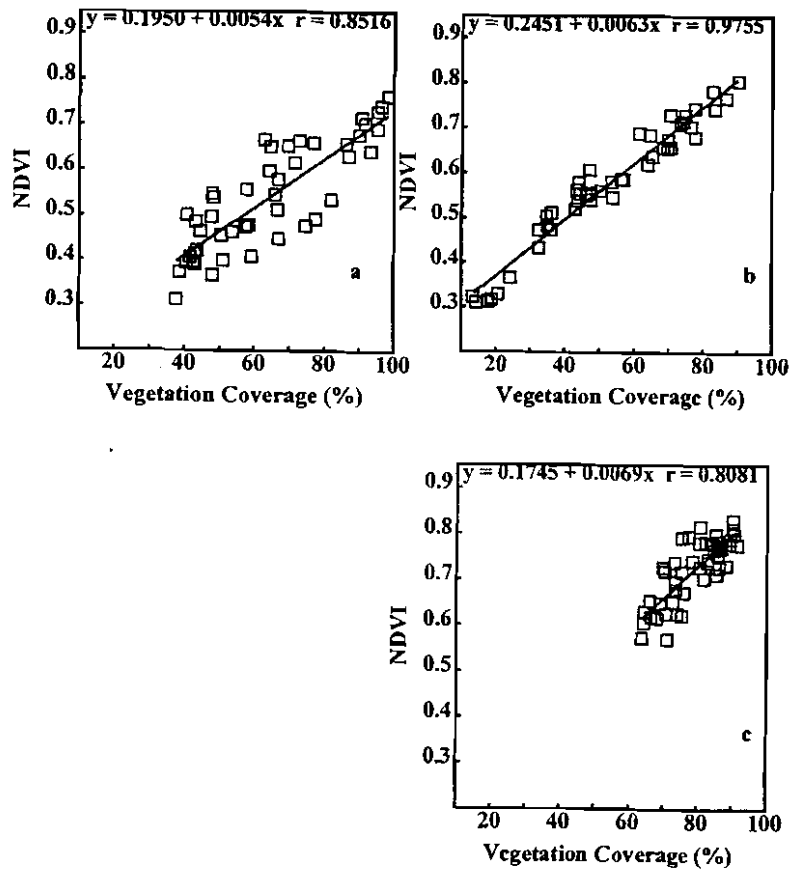


FIGURE 2. Correlation between NDVI measurements and percent vegetation coverage of winter wheat canopies at Perkins, at Feekes 4(a), Feekes 5(b), and Tipton at Feekes 5(c) in 1997.

blue, and green at the 25% level (Table 1, step II.d-II.f). The 'chroma mask' was applied to red and purple colors (Table 1, step II.b). The difference in color adjustment and balance was determined by soil type, specifically color. To expedite the image processing procedure a macro was written for every set of images with the same soil type and brightness.

RESULTS

Binary pseudo-color images obtained using 'Micrografx Picture Publisher® 7' software provided useful estimates of percent vegetation coverage that were highly

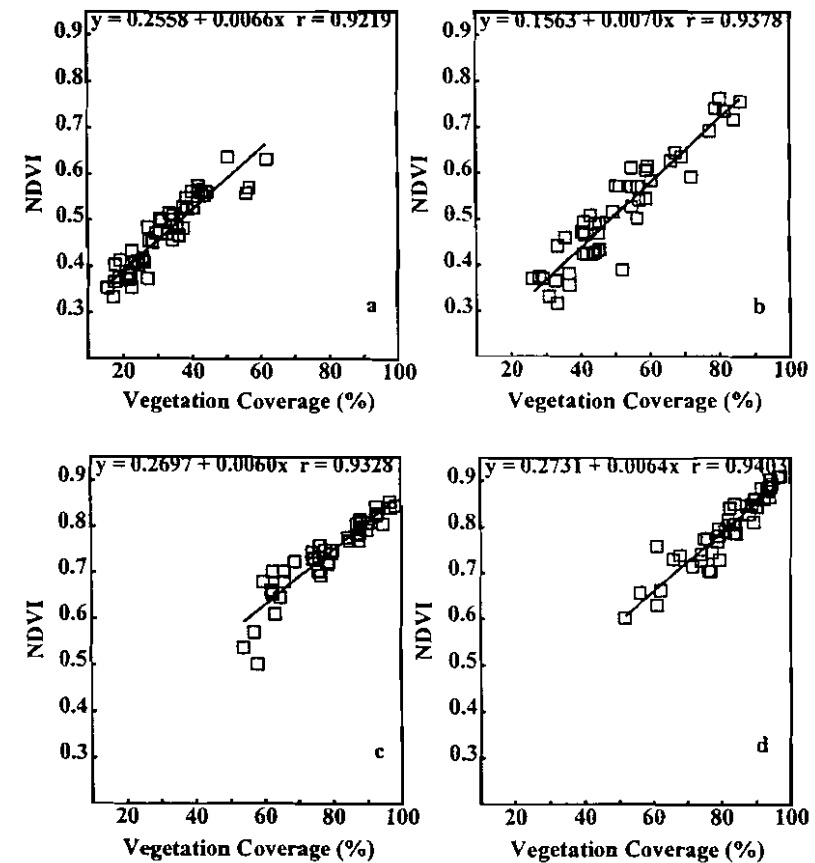


FIGURE 3. Correlation between NDVI measurements and percent vegetation coverage of winter wheat canopies at Perkins, at Feekes 4(a), Feekes 5(b), and Tipton, at Feekes 4(c), and Feekes at 5(d) in 1998.

correlated with NDVI. The Pearson correlation coefficient (r) between NDVI and percent vegetation coverage exceeded 0.80 for Feekes growth stages 4 and 5 at both locations (Figure 2).

At Perkins, wheat canopies ranged between 52-68 and 40-62% vegetation coverage (averaged over N rate) at Feekes growth stages 4 and 5, respectively. At Tipton, vegetation coverage ranged between 72-89 and 69-86% at Feekes growth stages 4 and 5, respectively. A decrease in percent vegetation coverage from Feekes growth stage 4 to 5 at Tipton was due to severe frost damage.

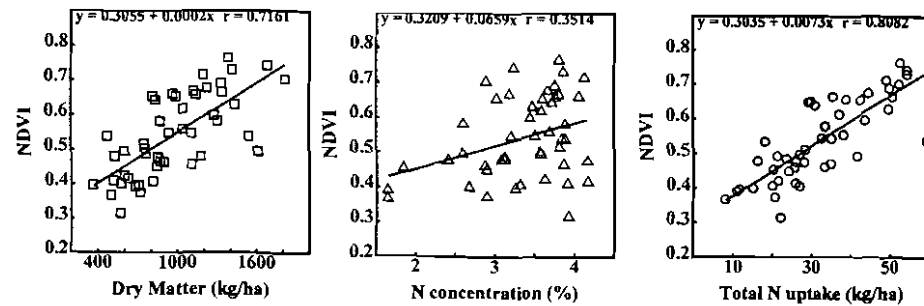


FIGURE 4. Correlation between NDVI and dry matter, N concentration, and total N uptake at Perkins at Feekes 4, 1997.

In the second year of the experiment, the range of vegetation coverage was 66-90% and 67-93% at Tipton, at Feekes growth stages 4 and 5, respectively, and 26-34% at Perkins, Feekes growth stage 5. The Pearson correlation coefficient between percent vegetation coverage and NDVI was higher than 0.92 at both locations and growth stages (Figure 3). The Pearson correlation coefficients between NDVI and biomass ranged from 0.35 to 0.80, and correlation coefficients between NDVI and N concentration in dry biomass ranged from 0.12 to 0.45 for data obtained in 1997. Higher r -values were observed between NDVI and total N uptake, ranging between 0.47 and 0.83. Stone et al. (1996a) reported that NDVI measurements depend on two factors, N concentration and biomass. Assuming that a change in N concentration or biomass affects NDVI, total N uptake should be a better predictor of NDVI since it takes into account variations in both (N concentration and biomass) factors. This is also consistent with results illustrated in Figure 4 where correlation was improved between NDVI and total N uptake as compared to NDVI versus N concentration and/or biomass alone. The Pearson correlation coefficients between percent vegetation coverage and biomass ranged from 0.33 to 0.81. As was expected, high r -values were observed between percent vegetation coverage and total N uptake, which ranged from 0.42 to 0.82.

CONCLUSIONS

Digital image processing techniques provided good prediction of percent vegetation coverage. In addition, high correlation was observed between percent vegetation coverage and NDVI. The method delineated here could help determine critical percent vegetation coverage needed for precise calibration of spectral indices for topdress N application.

This method was affected by high light reflectance and thus required the use of shadowing. This was especially true on light colored soils, thus requiring location specific contrast and balance adjustment. A single procedure could not be found that would handle the range of soil color and brightness found in the images used in the study. Images from Perkins and Tipton locations had to be adjusted differently for the brightness and color due to differences in soil type. Similar methodology may be useful to separate dead vegetation from fresh plant tissue.

ACKNOWLEDGMENTS

Appreciation is extended to the Oklahoma Soil Fertility team for technical assistance of J. Dennis, S. Phillips, W. Thomason, J. LaRuffa, C. Woolfolk, H. Lees, H. Sembiring, S. Taylor, S. Norton, D. Drury, K. Wynn, C. Lively, D. Cossey, T. Johnston, and J. Tallman.

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Rhizobium and Phosphorus Influence on Lentil Seed Protein and Lipid

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ABSTRACT

Root nodulation by rhizobial bacteria and P fertilization may affect seed protein and lipid composition in plants by altering nitrogen (N) and phosphorus (P) nutrition or by eliciting metabolic responses by the host plant. This study was conducted to determine the effects of rhizobium and P fertilization on seed protein and lipid contents and yield of lentil (*Lens culinaris* Medik). Lentil was grown to maturity in a greenhouse with P levels of 0 (low) and 50 (high) mg kg⁻¹ soil with or without inoculation with *Rhizobium* bacteria. At the low level of P, protein and lipid concentrations and protein contents were significantly higher in inoculated than in uninoculated plants. Seed dry weight and protein concentrations and contents were higher in inoculated than in uninoculated plants at the high level of P. Seed protein/lipid (Pro/L) concentration ratios varied between inoculated and uninoculated plants at both P levels, and was related to the intensity of root nodulation. Lipid and protein contents were highly correlated with P content in lentil seeds. Seed lipid and protein contents were lower at the high level of P in uninoculated than inoculated plants. The data indicate different patterns of seed P accumulation and different relationships between seed P content and protein