

TESTING THE NEW 4-BAND SENSOR AND THE
SPECTROMETER FOR EQUIVALENCY
IN WINTER WHEAT

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TESTING THE NEW 4-BAND SENSOR AND THE
SPECTROMETER FOR EQUIVALENCY
IN WINTER WHEAT

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Abbreviations

ADC: analog-to-digital converter
CBYT: Crossing Block Yield Trial
CID: carbon isotope discrimination
CTD: canopy temperature depression
FWHM: full width at half maximum
GNDVI: green normalized difference vegetation index
LCB: Lake Carl Blackwell
LSPYT: Lone Star Preliminary Yield Trial
NDVI: normalized difference vegetation index
NFB sensor: new 4-band sensor
NIR: near infrared radiation
NWI: normalized water index
NWI-1: normalized water index-1
NWI-2: normalized water index-2
NWI-3: normalized water index-3
NWI-4: normalized water index-4
NWI-5: normalized water index-5
PYT: Preliminary Yield Trial
QTL: quantitative trait locus
R: reflectance
RNDVI: red normalized difference vegetation index
SR: simple ratio
SRI: spectral reflectance index
WI: water index

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ABSTRACT

Wheat (*Triticum aestivum* L.) breeding entails a large number of segregating populations that are utilized for selecting high yielding segregates among and within segregating populations. This traditional technique, however, is costly and time-consuming because numerous field evaluations have to be made during several years at multiple locations. One alternative approach to address this issue is to employ the spectral properties of plants. This study was conducted to determine whether the new 4-band (NFB) sensor could replace the Ocean Optics USB4000 spectrometer (Ocean Optics Inc, Dunedin, FL) in collecting spectral reflectance data. Two spectral reflectance indices (SRI) were tested, namely red normalized difference vegetation index (RNDVI) and normalized water index-5 (NWI-5), using both tools during the booting and the grain-filling stages (according to Feekes' scale) in two consecutive years (2008-2009 and 2009-2010) at three locations. A cardboard cone was attached to reduce the surface area of the light-collecting lens in 2009-2010. Results showed that measurements at the grain-filling stage gave better equivalency between the NFB sensor (with and without the cone) and the spectrometer than at the booting stage for both RNDVI and NWI-5 readings. The NFB sensor could be used with and without the cone to replace the spectrometer for taking RNDVI readings at grain-fill. The attachment of the cone improved equivalency

between the sensor and the spectrometer. However, the NFB sensor equipped with the cone could not substitute for the spectrometer in NWI-5 data collection in winter wheat. Furthermore, additional adjustments to the NFB sensor are needed to improve equivalency in taking NWI-5 readings.

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) breeding methods involve numerous segregating populations that are compared and evaluated for selecting high-yielding genotypes among and within segregating populations (Ball and Konzak, 1993). This process requires many crosses for deriving new genotypes that have to be compared with commercial cultivars in diverse environments. Selection of breeding lines for grain yield in advanced nurseries requires repetition to ensure success (Ball and Konzak, 1993). To date, wheat breeding globally has been based mainly on empirical selection criteria (yield *per se*) for yield improvement (Araus *et al.*, 2002). This technique is costly and time-consuming because multiple field evaluations must be made during several years at numerous locations.

Yield has shown low heritability and a high genotype-environment interaction (Slafer and Andrade, 1991; Trethowan *et al.*, 2003). It would be advantageous if grain yield could be predicted before the crop is harvested. The top-performing families could be identified from hundreds of segregating populations in a breeding program prior to harvesting the crop (Royo *et al.*, 2003). An effective breeding strategy requires a better understanding of the factors responsible for development and growth because grain yield

in a given environment is directly and indirectly affected by genetic, physiological, morphological, and environmental components (Richards, 1996).

Spectral properties of plants came into focus as a potential selection tool for grain yield a few years ago (Aparicio *et al.*, 2002; Royo *et al.*, 2003; Babar *et al.*, 2006a). The fundamental principle within canopy spectral reflectance is that specific plant traits are linked with the absorption of specific wavelengths of the spectrum (Reynolds *et al.*, 1999). Spectral reflectance of a crop canopy is related to the total area of leaves and photosynthetic capability in the canopy, pigment concentration, and other physiological factors (Araus *et al.*, 2001). Therefore, the measurements of the spectrum reflected from plants offer information that can be utilized to estimate a great number of parameters (Araus *et al.*, 2001). Furthermore, several researchers have suggested that grain yield can be estimated using spectral reflectance during different crop growth stages (Araus *et al.*, 2001; Aparicio *et al.*, 2002; Babar *et al.*, 2006a,b; Prasad *et al.*, 2007a,b).

The most widely used SRIs are the simple ratio (SR; R_{900} / R_{680}) and normalized difference vegetation index [NDVI; $(R_{900} - R_{680}) / (R_{900} + R_{680})$] (Araus *et al.*, 2002). More importantly, water index (WI; R_{970} / R_{900}) has been demonstrated to predict relative water content, leaf water potential, stomata conductance, and canopy temperature with sufficient water stress (Peñuelas *et al.*, 1993).

NWI-5, which is the most recently introduced index, was used in our study to test wheat canopies. It was suggested based on the previous normal water indices, namely

NWI-1 $[(R_{970} - R_{900}) / (R_{970} + R_{900})]$, NWI-2 $[(R_{970} - R_{850}) / (R_{970} + R_{850})]$, NWI-3 $[(R_{970} - R_{880}) / (R_{970} + R_{880})]$, and NWI-4 $[(R_{970} - R_{920}) / (R_{970} + R_{920})]$. NWI-5 is calculated by using the formula: $NWI-5 = (R_{970} - R_{870}) / (R_{970} + R_{870})$. RNDVI is calculated by using the formula: $RNDVI = (R_{780} - R_{670}) / (R_{780} + R_{670})$. R and the subscripts indicate the light reflectance at the specific wavelengths (in nm). Overall, in this research two avenues were used to measure RNDVI and NWI-5, namely by using either the NFB sensor or the Ocean Optics USB4000 spectrometer. Although the spectrometer is precise, it is very delicate and costly. In addition, handling the spectrometer in the field is cumbersome. On the other hand, the NFB sensor is more affordable and convenient to manage. This sensor collects measurements at only four wavelengths. One of the wavelengths is in the visible portion (670 nm), and the other three are in the near infrared radiation (NIR) portion (780, 870, and 970 nm) of the electromagnetic spectrum. The ultimate objective is to use the NFB sensor to take NWI-5 measurements on breeding materials in the field, but it must be determined if the NFB sensor gives measurements equivalent to the spectrometer.

OBJECTIVES OF THE STUDY

The objectives were (1) to correlate RNDVI and NWI-5 readings from the NFB sensor with those from the Ocean Optics USB4000 spectrometer, (2) to determine if the NFB sensor could replace the spectrometer for measurements in breeders' nurseries, and (3) if necessary, to identify adjustments to the NFB sensor that would improve equivalency between the NFB sensor and the spectrometer.

CHAPTER II

REVIEW OF LITERATURE

Wheat (*Triticum aestivum* L.) is one of the world's largest, most consumed, and most versatile food crops. It is originally from the Fertile Crescent region of the Near East. It has long been a very important crop for humankind, currently with a yearly harvest of more than 620 million tonnes produced in over 40 countries. It represents the staple food for more than 35% of the world population (Williams, 1993). Wheat is superior to most other cereals in terms of nutritive value (Zohary and Hopf, 2000). It plays a significant role in the world economy and stability owing to its massive production and superb ability to be used for making various kinds of foods. Only rice is a close competitor to wheat in terms of a crop for direct human consumption. Wheat is grown on about 220 million hectares globally, nearly half of which is in developing countries (CIMMYT, 1996). It represents more than 25% of the total world cereal grain production, and comprises the main source of calories for more than 1.5 billion people (Reynolds *et al.*, 1999). Thus, continuing wheat improvement is necessary for feeding an ever-increasing world population.

During the first half of the 20th century, world wheat grain yield increased very slowly; however, it has increased by two or three times around the world since 1950

(Calderini and Slafer, 1998). The world demand for wheat increases about 2% per year, and the genetic gains via breeding have slowed considerably (Reynolds *et al.*, 1999). Hence, it will not be possible to feed the whole world in the near future (Sayre *et al.*, 1997). Even though demand for wheat is growing faster than gains in genetic yield potential, investment in conventional breeding by national programs and related organizations is decreasing (Reynolds *et al.*, 1999).

Breeding for desired traits has long been the main focus of the breeders. The traits involved are yield, grain quality, plant architecture, resistance to diseases, lodging, drought, and so forth. On the whole, breeders commonly apply traditional methods for improving the target traits. This approach involves generating massive segregating populations that undergo selection for desired genotypes followed by comparison with commercial cultivars over years in diverse environments. In general, testing needs to be repeated because statistical procedures applied sometimes cannot sufficiently distinguish among genotypes (Bhatti *et al.*, 1991). Usually, multiple genotypes are retained in tests although they should have been discarded, or they are discarded when they should have been retained (Ball and Konzak, 1993). This classical wheat breeding method, therefore, is costly, labor-intensive, and time-consuming. Hence, it is imperative to have an effective and promising way to facilitate plant breeding programs.

Breeders need indirect parameters that can aid their efforts to screen more genotypes within a shorter period of time (Reynolds *et al.*, 1999; Slafer and Satorre, 1999). If desired genotypes could be assessed prior to harvesting, it would save breeders significant work and money because the higher-yielding genotypes could be quickly

identified from an enormous number of entries that comprise the segregates in a breeding program. According to Royo *et al.* (2003) desired high-yielding genotypes can be detected among segregating populations in the field before the crop is harvested. Raun *et al.* (2001) demonstrated that in-season prediction of grain yield potential could be realized by using NDVI in winter wheat. While there is still no complete understanding of the genetic and physiological basis of yield, progress has been made in developing selection technologies that might upgrade the efficiency of empirical breeding (Reynolds *et al.*, 1999). The nature of wheat grain yield is highly complicated because it is influenced by various factors such as physiological, environmental, morphological, and genetic components. With the advancements in plant breeding techniques, there have emerged more efficient, more reliable, and less expensive avenues that better serve breeding purposes (Richards, 1996).

Several tools have been employed for this purpose. From a breeding aspect, the possible contributions of physiological research to plant breeding and its intrinsic restrictions have been broadly evaluated (Jackson *et al.*, 1996). Desirable genotypes have previously been detected by biomass determination in the field via destructive sampling (Regan *et al.*, 1992). This sampling is impractical in large breeding trials due to the high labor demand and the huge sampling errors in discerning genotypic differences (Whan *et al.*, 1991). When canopy temperature depression (CTD) was compared with other potential selection traits measured in the selection environment, including yield, biomass, grain number, and phenological data, CTD demonstrated a greater association with performance in the target environment than the other traits (Reynolds *et al.*, 1999).

Carbon isotope discrimination (CID) was manipulated to advance grain potential in wheat under water deficit environments (Condon *et al.*, 2002, 2004). Yet, CID determination processes are slow and costly. The application of physiological traits as screening tools in plant breeding is yet mainly investigational. At times, the traits are indirectly related to yield (Araus, 1996; Richards, 1996). Meanwhile, wide crossing and the potential application of molecular markers could meet the demands of increasing grain yield to some extent. It is likely to expedite the introduction of beneficial alleles by selecting them in early back-cross generations, if particular markers for yield-improving quantitative trait loci (QTLs) from wheat relatives can be detected from wide-cross progeny (Reynolds *et al.*, 1999).

Within the last twenty years or so, remote sensing techniques and their application to agriculture have received more attention (Maas, 1988; Weigand and Richardson, 1990; Curran and Atkinson, 1998). Remote sensing techniques are convenient for making assessments because they are non-invasive, handy, and less expensive. These techniques can measure the spectra reflected from plant canopies in the visible (400-700 nm), near-infrared (700-1200 nm), and mid-infrared (>1200 nm) portions of the electromagnetic spectrum (Reynolds *et al.*, 1999; Araus *et al.*, 2001). Remote sensing, particularly multispectral visible and infrared reflectance, can supply a non-destructive, immediate, and quantitative evaluation of the plant's ability to intercept radiation and photosynthesize (Ma *et al.*, 1996). Remote sensing in agriculture primarily concentrates on prediction of yield and crop identification. It is utilized in agricultural areas to detect electromagnetic energy that is reflected or emitted from the earth's surface, and has been a crucial tool for evaluating crop production across large areas (Henderson and Badhwar,

1984; Singh *et al.*, 2001). The collected data can be converted and interpreted into a great wealth of parameters that can give guidance to researchers for diagnosing plant health.

Spectral reflectance measured by remote sensing is a promising tool for the evaluation of many physiological traits in crop yield production research, for instance, absorbed radiation, water content, and chlorophyll content. The spectral reflectance measurement by ground-based remote sensing techniques has the potential to provide a non-destructive and accurate assessment of plant biomass via the widely used NDVI (Tucker, 1979; Peñuelas *et al.*, 1993). The application of spectral reflectance into yield production models has improved yield estimates (Clevers *et al.*, 1994; Clevers, 1997). This tool is a good candidate in plant breeding programs for identifying genotypes with better performance (Peñuelas *et al.*, 1997).

SRIs taken by remote sensing techniques are linked with canopy variables which generally determine crop growth. Crop canopies are dynamic entities affected by all manner of management practices, for example, cultivars, soil moisture, seeding rate, and diseases (Rao *et al.*, 1997). Canopy reflectance properties are based mainly on the absorption of light at specific wavelengths associated with plant characteristics (Araus *et al.*, 2002). At the canopy level, spectral reflectance is a combination of vegetation and soil reflectance, and the weighting of either of these two factors depends on external parameters, such as, canopy structure or illumination. Leaf reflectance is mostly low in the visible portion of the spectrum due to absorption by photosynthetic pigments, such as chlorophylls, carotenoids, and anthocyanins. The reflectance level is controlled by

structural discontinuities occurring in the leaf structure. In the near-infrared region, absorption characteristics are not strong. Reflectance values of different variables are primarily related to the absorption characteristics of water and other compounds in the middle infrared portion (Peñuelas and Filella, 1998).

There are many SRIs available for use in the realm of crop production. SR and NDVI are the best known SRIs in remote sensing, not only at ground level, but also at airborne and satellite levels. NDVI is a simple index to measure contrasts in reflectance (Araus *et al.*, 2001). It is the most widely used index that originally was proposed as an approach for evaluating green biomass and now is utilized to indirectly estimate canopy biomass, leaf area index, and light absorption (Tucker, 1979; Gamon *et al.*, 1995; Peñuelas and Filella, 1998; Araus *et al.*, 2001). NDVI is easily affected by solar zenith angles, atmospheric conditions, crop canopy architecture, view angle, and soil background (Jackson and Huete, 1991). Aase and Siddoway (1981) reported that the relationship between NDVI and wheat grain yield deteriorated drastically as wheat ripened. Compared with sensing only once, sensing twice and combining NDVI measurements using a linear model can improve correlation to wheat grain yield (Smith *et al.*, 1995). SR can provide trustworthy information for winter wheat yield prediction under stress conditions (Serrano *et al.*, 2000). SR indices are analytical techniques for differentiating higher-yielding genotypes under dry and irrigated conditions (Gutiérrez *et al.*, 2004). Strong correlations ($R^2 > 0.80$) between SRIs and grain yield and biomass under irrigated and rainfed field conditions were reported in durum wheat genotypes (Aparicio *et al.*, 2002; Royo *et al.*, 2003). Wheat yield potential can be predicted by

taking periodic measurements of spectral reflectance during the growing season (Rudorff and Batista, 1990).

More importantly, by taking advantage of the near-infrared portion of the electromagnetic spectrum, canopy water content can be tested using SRI. As a consequence, WI was proposed to predict leaf water potential, relative water content, and stomata conductance (Peñuelas *et al.*, 1993). Sensitivity of the spectral reflectance at 970 nm appears to be caused by the higher ability of radiation at this wavelength to penetrate into the canopy as compared with other longer water absorption wavelengths. The reflectance at 900 nm is used as a reference band, which is absorbed less strongly by moisture, but tends to change in the same way as 970 nm (Bull, 1991). Wavelengths between 950 nm and 970 nm may be used as predictors of plant water status. WI can measure the plant water status at the leaf and canopy level, and it has proven to be highly correlated with plant water content in several species of crops, grasses, and trees (Peñuelas *et al.*, 1993, 1997). Likewise, NWI-1 and NWI-2 were proposed on the basis of the WI for selecting spring wheat grain yield, and NWI-3 and NWI-4 were proposed for selecting winter wheat grain yield (Babar *et al.*, 2006a; Prasad *et al.*, 2007a). These four normalized water indices (NWI) used 850, 880, 900, and 920 nm as reference bands. Based on NIR wavelengths, these five WIs can be used for predicting grain yield since they have shown strong relationships with grain yield in spring and winter wheat genotypes under various field conditions ($r^2 = 0.15-0.80$) (Babar *et al.*, 2006a,b; Prasad *et al.*, 2007a,b). Among these five WIs, NWI-3 has been recognized as a slightly better index for selecting high-yielding segregates in wheat (Gutiérrez *et al.*, 2010). The WIs

efficiently predicted grain yield variability, and thus they could be a valuable indirect selection tool for wheat breeding for improving grain yield (Babar *et al.*, 2006a; Prasad *et al.*, 2007a). Remote sensing has been shown to be a powerful indirect selection tool with the potential to assist crop breeding programs in selecting superior genotypes, and especially it could benefit the world's wheat production.

CHAPTER III

MATERIALS AND METHODS

Experimental materials

This research was conducted in two consecutive years (2008-2009 and 2009-2010) at three locations. The plots for both years were 10 feet long by 5 feet wide. The soil types for these three experimental locations are listed in Table 2. A cardboard cone was used to adjust the area from which reflected light was collected. This cone was designed with the same height as the four light-emitting lenses and half the diameter (2.25 cm) of the light-collecting lens (at the center of the sensor). The cone was taped on the light-collecting lens of the sensor (Figure 1).

For 2009, spectral data collection was done on the Agronomy Research Farm at Stillwater. There were 22 plots from the Crossing Block Yield Trial (CBYT) and 41 plots from the Lone Star Preliminary Yield Trial (LSPYT). The SRI data were collected on May 21 and May 29, 2009 without using the cone during the grain-filling stage (according to Feekes' scale; Zadoks *et al.*, 1974). The measurements on both dates were performed by following the same procedures. For the measurements on May 21, 2009, a technical problem occurred which led to erroneous data for the CBYT plots. Hence, only data from the LSPYT were obtained on that date. The data obtained from the CBYT and

the LSPYT on May 29, 2009 were used for analysis.

In 2010, 60 plots from the Preliminary Yield Trial (PYT) were measured at Lake Carl Blackwell (LCB) and Perkins, respectively. The measurements were taken at four random spots in each plot with the new 4-band sensor and the spectrometer. Both the NFB sensor and the spectrometer measured approximately the same areas within each plot. The NFB sensor was used twice, with the cone and without the cone, to take measurements at each location. The measurements were carried out at the booting and grain-filling stages (according to Feekes' scale; Zadoks *et al.*, 1974) at each location following the same procedures.

NFB sensor

This instrument was recently developed to obtain spectral reflectance data in wheat breeding materials. The NFB sensor was designed to eliminate the drawbacks of the spectrometer which can only be used with ample sunlight, in ideal weather conditions, and by connecting to a laptop computer. It was fabricated with active illumination similar to the GreenseekerTM sensor. Also, an HP handheld iPAQ was attached to the hand bar for data collection, and came with the Mobile Terminal Emulator software installed. The NFB sensor was used to collect measurements above the wheat canopy at heights of 10, 30, and 50 cm, respectively, and at four spots within each plot. This sensor was purposefully built to obtain the reflectance of four wavelengths. In detail, one (670 nm) was in the visible area and the other three (780, 870, and 970 nm) were in the NIR area of the electromagnetic spectrum. Commonly, several readings were generated for each spot

by pressing the trigger, and these readings were later averaged for one height. By using the four wavelengths, two SRIs were derived, namely RNDVI and NWI-5. These two indices were calculated using the equations, $RNDVI = (R_{780} - R_{670}) / (R_{780} + R_{670})$ and $NWI-5 = (R_{970} - R_{870}) / (R_{970} + R_{870})$.

In this research, we propose NWI-5 as a new water index, which can be calculated using measurements from this NFB sensor. The wavelength of 870 nm was selected because this wavelength was the closest optic that we could find to the wavelength of 880 nm of the NWI-3 within the water absorption range, and NWI-3 has proven to be the better predictor among the other water indices (WI, NWI-1, NWI-2, and NWI-4). Furthermore, RNDVI was selected for the NFB sensor because it is an excellent indicator of wheat biomass, and under certain conditions wheat grain yield (Prasad, 2007a).

Ocean Optics USB4000 spectrometer

Canopy reflectance was taken in the wavelength portion from 293.04 nm to 1732.91 nm at about 0.45 nm intervals of the visible and the NIR region of the electromagnetic spectrum using the Ocean Optics USB4000 spectrometer (Ocean Optics Inc, Dunedin, FL). A two-meter optical fiber (Qp-1000-2-UV/VIS Ocean Optics Inc) with a diameter of 200 nm was fastened to this spectrometer. A laptop computer was attached to the spectrometer that collected the light intensity for each scan. This equipment came with a 16-bit analog-to-digital converter (ADC) resolution and with a full width at half maximum (FWHM) of 1.5 nm in optical resolution. It can take 3648 pixels at a time and each pixel size is 8 μm by 200 μm .

Spectral reflectance collection with the spectrometer

The data were obtained during sunny, cloudless, and windless days, at midday (between 10:30 am and 2:30 pm). The light source for taking measurements was sun light. Four reflectance measurements were taken at four random areas in each plot at the height of 50 cm above the wheat canopy with a field of view of 25° at nadir position. Prior to reflectance collection, an aluminum plate coated with barium sulfate (BaSO₄) that provided maximum irradiance was used to calibrate the spectrometer. The light reflected from the white plate was collected by the spectrometer, and recorded by the laptop computer. The recalibration was performed every 20 minutes. Each measurement was taken by standing and holding the spectrometer above one spot of a plot at a time. Each reflectance measurement of a plot was the average of four readings from the plot. All data collected were later converted into RNDVI and NWI-5.

Spectral data analysis

The SPSS software was used to perform regression analysis for the two SRIs taken by using the NFB sensor with and without the cone at the height of 10, 30, and 50 cm, and the Ocean Optics USB4000 spectrometer in different growth stages. Proc Means was utilized in the SAS software (SAS, 2001) to average several readings produced for each spot when pressing the trigger on the NFB sensor. Microsoft Excel was employed to plot and perform correlation analysis.

CHAPTER IV

RESULTS AND DISCUSSION

Agronomy Research Farm, 2008-2009

NFB sensor without cone at 10 cm

The RNDVI readings of the NFB sensor measured at 10 cm on May 21 and 29, 2009 were significantly correlated to the spectrometer readings ($r^2 = 0.18$ and 0.20 , respectively; $p < 0.01$ for both) (Figures 2 and 3). The NWI-5 readings of the NFB sensor collected at 10 cm on May 21 and 29, 2009 were not significantly correlated to the spectrometer readings ($r^2 = 0.09$ and 0.02 , respectively; $p = 0.06$ and 0.31 , respectively).

NFB sensor without cone at 30 cm

The RNDVI readings from the NFB sensor taken at 30 cm on May 21 and 29, 2009 were significantly correlated to those of the spectrometer ($r^2 = 0.20$ and 0.38 , respectively; $p < 0.01$ for both) (Figures 4 and 5). The NWI-5 readings of the NFB sensor taken at 30 cm on May 21 and 29, 2009 were not significantly correlated to those of the spectrometer ($r^2 = 0.03$ and 0.01 , respectively; $p = 0.25$ and 0.45 , respectively).

NFB sensor without cone at 50 cm

The RNDVI readings of the NFB sensor obtained at 50 cm on May 21 and 29, 2009 were significantly correlated with the spectrometer readings ($r^2 = 0.49$ and 0.33 , respectively; $p < 0.01$ for both) (Figures 6 and 7). The NWI-5 readings of the NFB sensor taken at 50 cm on May 21 and 29, 2009 were not significantly correlated to the spectrometer readings ($r^2 = 0.05$ and 0.02 , respectively; $p = 0.16$ and 0.31 , respectively).

Summary

These data seem to indicate that 30 cm and 50 cm are the best heights for taking RNDVI readings with the NFB sensor without the cone. The RNDVI readings from the sensor are quite well correlated to the spectrometer readings; however, the NWI-5 readings taken with the NFB sensor had no correlation with the spectrometer readings.

LCB, 2009-2010

NFB sensor with cone vs without cone at 10 cm

At booting, both the RNDVI and the NWI-5 readings (Figure 8) of the NFB sensor with the cone were not significantly correlated to the spectrometer readings ($r^2 = 0.01$ and 0.03 , respectively; $p = 0.47$ and 0.18 , respectively). Without the cone on the NFB sensor, neither the RNDVI readings nor the NWI-5 readings (Figure 9) of the NFB sensor were significantly correlated to the spectrometer readings ($r^2 = 0.02$ and 0.01 , respectively; $p = 0.23$ and 0.57 , respectively).

For the grain-filling stage, the RNDVI readings collected using the NFB sensor with the cone were significantly correlated to the spectrometer readings ($r^2 = 0.28$; $p < 0.01$) (Figure 14). However, the NWI-5 readings were not significantly correlated with those of the spectrometer ($r^2 = 0.01$; $p = 0.37$). Without the cone on the NFB sensor, the RNDVI readings of the NFB sensor were significantly correlated to the spectrometer readings ($r^2 = 0.16$; $p = 0.02$) (Figure 15). However, the NWI-5 readings taken with the NFB sensor showed no correlation with the spectrometer readings ($r^2 < 0.01$; $p = 0.83$).

NFB sensor with cone vs without cone at 30 cm

During the booting stage, the RNDVI readings of the NFB sensor with the cone were not significantly correlated to those of the spectrometer ($r^2 = 0.01$; $p = 0.39$), but the NWI-5 readings were significantly correlated to those of the spectrometer ($r^2 = 0.08$; $p = 0.03$) (Figure 10). Without the cone on the NFB sensor, the RNDVI readings of the sensor were significantly correlated to the spectrometer readings ($r^2 = 0.10$; $p = 0.01$), but the NWI-5 readings showed no significant correlation to the spectrometer readings ($r^2 = 0.02$; $p = 0.26$) (Figure 11).

In the grain-filling stage, the RNDVI readings collected using the NFB sensor with the cone were significantly correlated with the spectrometer readings ($r^2 = 0.14$; $p < 0.01$) (Figure 16), but the NWI-5 readings were not significantly correlated to those of the spectrometer ($r^2 < 0.01$; $p = 0.63$). Without the cone, neither the RNDVI readings (Figure 17) nor the NWI-5 readings of the NFB sensor were significantly correlated to the spectrometer readings ($r^2 = 0.04$ for both, respectively; $p = 0.11$ and 0.13 , respectively).

NFB sensor with cone vs without cone at 50 cm

At the booting stage, the RNDVI readings of the NFB sensor with the cone were not significantly correlated to the spectrometer readings ($r^2 < 0.01$; $p = 0.80$). However, the NWI-5 readings were significantly correlated to those of the spectrometer ($r^2 = 0.19$; $p < 0.01$) (Figure 12). Without the cone on the NFB sensor, both the RNDVI and the NWI-5 readings (Figure 13) of the sensor were not significantly correlated to the spectrometer readings ($r^2 = 0.04$ and $r^2 < 0.01$, respectively; $p = 0.11$ and 0.89 , respectively).

During the grain-filling stage, the RNDVI readings taken using the sensor with the cone were significantly correlated to the spectrometer readings ($r^2 = 0.23$; $p < 0.01$) (Figure 18), but the NWI-5 readings showed no significant correlation to those of the spectrometer ($r^2 = 0.01$; $p = 0.39$). Without the cone the RNDVI readings of the NFB sensor were significantly correlated with the spectrometer readings ($r^2 = 0.29$; $p < 0.01$) (Figure 19). However, the NWI-5 readings of the NFB sensor were not significantly correlated with the spectrometer readings ($r^2 = 0.01$; $p = 0.42$).

Summary

For the measurements taken at the booting stage, the r values of the NWI-5 readings obtained with the cone were higher than those obtained without the cone at 10, 30, and 50 cm. This demonstrates that the NFB sensor with the cone performed somewhat better in taking the NWI-5 readings than without the cone. At both 30 and 50 cm, the NWI-5 readings of the sensor with the cone were correlated significantly to those of the

spectrometer, and the sensor with the cone worked the best at 50 cm. Therefore, adding the cone on the NFB sensor, which effectively reduces the area from which reflectance is measured, could be an effective means to replace the spectrometer for taking NWI-5 readings. Without the cone on the NFB sensor, the NWI-5 readings were not significantly correlated to the spectrometer readings at the three heights. When comparing the RNDVI readings with and without the cone, the results showed that the correlations had high p values (when $\alpha = 0.05$, $p > 0.05$), except when the data were taken without the cone at 30 cm ($r^2 = 0.10$; $p = 0.01$), indicating that these two approaches for taking RNDVI readings did not work well. Additionally, the results of the RNDVI readings from both years showed that the r values from the year 2009-2010 were much lower than those from the year 2008-2009, and this may be due to the measurement-taking stage difference.

At the grain-filling stage, data indicated that both the RNDVI and the NWI-5 readings taken by the sensor with the cone overall had better equivalency to the readings of the spectrometer than those taken without the cone.

Perkins, 2009-2010

NFB sensor with cone vs without cone at 10 cm

During the booting stage, the RNDVI readings of the NFB sensor with the cone were significantly correlated with the spectrometer readings ($r^2 = 0.13$; $p < 0.01$), and the NWI-5 readings showed no significant correlation to those of the spectrometer ($r^2 = 0.05$; $p = 0.10$). Without the cone on the NFB sensor, neither the RNDVI nor the NWI-5

readings of the sensor were significantly correlated to the spectrometer readings ($r^2 < 0.01$ and $r^2 = 0.01$, respectively; $p = 0.72$ and 0.47 , respectively).

For the grain-filling stage, the RNDVI readings collected using the NFB sensor with the cone were significantly correlated with the spectrometer readings ($r^2 = 0.38$; $p < 0.01$) (Figure 20), and the NWI-5 readings were not significantly correlated to those of the spectrometer ($r^2 < 0.01$; $p = 0.62$). Without the cone the RNDVI readings of the NFB sensor were significantly correlated to the spectrometer readings ($r^2 = 0.31$; $p < 0.01$) (Figure 21). The NWI-5 readings of the NFB sensor showed no significant correlation with the spectrometer readings ($r^2 = 0.02$; $p = 0.25$).

NFB sensor with cone vs without cone at 30 cm

During the booting stage, both the RNDVI and the NWI-5 readings of the NFB sensor with the cone were not significantly correlated to the spectrometer readings ($r^2 = 0.06$ and 0.01 , respectively; $p = 0.06$ and 0.53 , respectively). Without the cone on the NFB sensor, both the RNDVI and NWI-5 readings of the sensor were not significantly correlated with the spectrometer readings ($r^2 < 0.01$ for both, respectively; $p = 0.62$ and 0.90 , respectively).

For the grain-filling stage, the RNDVI readings collected using the NFB sensor with the cone were significantly correlated to the spectrometer readings ($r^2 = 0.52$; $p < 0.01$) (Figure 22), but the NWI-5 readings showed no significant correlation to those of the spectrometer ($r^2 < 0.01$; $p = 0.75$). Without the cone the RNDVI readings of the NFB

sensor were significantly correlated to the spectrometer readings ($r^2 = 0.48$; $p < 0.01$) (Figure 23). The NWI-5 readings of the NFB sensor were not significantly correlated to the spectrometer readings ($r^2 < 0.01$; $p = 0.67$).

NFB sensor with cone vs without cone at 50 cm

At the booting stage, both the RNDVI and the NWI-5 readings of the NFB sensor with the cone were not significantly correlated to those of the spectrometer ($r^2 = 0.04$ and $r^2 < 0.01$, respectively; $p = 0.12$ and 0.92 , respectively). Without the cone on the NFB sensor, both the RNDVI and the NWI-5 readings of the sensor were not significantly correlated to the spectrometer readings ($r^2 = 0.02$ and 0.01 , respectively; $p = 0.31$ and 0.45 , respectively).

During the grain-filling stage, the RNDVI readings collected using the NFB sensor with the cone were significantly correlated to the spectrometer readings ($r^2 = 0.55$; $p < 0.01$) (Figure 24), but the NWI-5 readings were not significantly correlated to those of the spectrometer ($r^2 < 0.01$; $p = 0.95$). Without the cone the RNDVI readings of the NFB sensor were significantly correlated to the readings of the spectrometer ($r^2 = 0.38$; $p < 0.01$) (Figure 25). The NWI-5 readings of the NFB sensor were not significantly correlated to the spectrometer readings ($r^2 = 0.02$; $p = 0.30$).

Summary

During the booting stage, the RNDVI readings obtained with the cone had higher r values than those taken without the cone, and were significantly correlated to the readings

of the spectrometer at 10 cm ($r^2 = 0.13$; $p < 0.01$). This indicates that the RNDVI readings of the sensor with the cone at Perkins gave better equivalency to those of the spectrometer than taking the readings without the cone. Likewise, when equipping the NFB sensor with the cone to collect NWI-5 data, the sensor functioned better than without the cone, except at 50 cm.

At the grain-filling stage, the RNDVI readings measured by both manners (with and without the cone) at 10, 30, and 50 cm were all significantly correlated to the spectrometer readings. When comparing the r values, the RNDVI readings obtained with the cone had relatively higher r values than those obtained without the cone at all the heights. This again indicates that the adjustment of the NFB sensor is quite helpful in improving equivalency. The results indicated that 50 cm was the best height for RNDVI readings collection with the cone. For the NWI-5 readings, the results indicated that both methods gave high p values (when $\alpha = 0.05$, $p > 0.05$), meaning that these two methods did not correlate well with the spectrometer readings.

CHAPTER V

CONCLUSIONS

The feasibility of applying the NFB sensor to replace the spectrometer in winter wheat research was demonstrated in this study. On the basis of the data obtained from the grain-filling stage in the year 2008-2009, the NFB sensor could be used to measure RNDVI at all three heights, but not for measuring NWI-5. The applicability of the NFB sensor to replace the spectrometer for measuring NWI-5 was further researched, and modifications were made in the NFB sensor in the year 2009-2010.

For the year 2009-2010 at LCB, at the booting stage, the results demonstrated that the NFB sensor worked better to collect NWI-5 data with the cone than without the cone at all three heights. This indicated that the cone functioning to reduce the surface area of the light-collecting lens could improve the equivalency of the NWI-5 readings between the sensor and the spectrometer. The 50 cm height performed the best for NWI-5 data collection when the sensor had the cone. Conversely, the NWI-5 data of the sensor without the cone showed that the sensor could not substitute for the spectrometer. Regarding the grain-filling stage, the RNDVI readings with the cone had higher r values at 10 and 30 cm. Thus, using the cone on the sensor gave a more stable and closer equivalency of RNDVI readings to those of the spectrometer. The NWI-5 readings taken

with and without the cone showed that using the cone could improve the equivalency at 10 and 50 cm.

In the year 2009-2010 at Perkins, at the booting stage, the NFB sensor with the cone performed better in taking RNDVI readings than without the cone at all three heights. The 10 cm height was the best for taking RNDVI readings with the cone. The NWI-5 readings collected with the cone showed the cone could improve the equivalency between the NFB sensor and the spectrometer to some extent, but not enough to give reliable equivalency. Taking the grain-filling stage into consideration, the results showed that RNDVI readings taken with the cone had slightly improved the equivalency between the sensor and the spectrometer. The best height for measuring RNDVI with the cone was 50 cm, and without the cone was 30 cm. Nevertheless, the NWI-5 readings obtained with both methods did not correlate well with the spectrometer readings.

In summary, the measurements at the grain-filling stage gave better equivalency than at the booting stage for both RNDVI and NWI-5 readings between the NFB sensor (with and without the cone) and the spectrometer. The NFB sensor could be applied with and without the cone to replace the spectrometer for taking RNDVI readings at the grain-filling stage. The application of the cardboard cone could adequately ameliorate the equivalency between the sensor and the spectrometer. However, the NFB sensor equipped with the cone could not substitute for the spectrometer in NWI-5 data collection in winter wheat. Consequently, additional research is needed to determine the best manner to obtain a reliable NWI-5 reading with the new 4-band sensor.

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APPENDICES

Table 1. Definition of the spectral reflectance indices of this study.

Spectral reflectance indices (SRI)	Formulas†	Functions	References
Water index (WI)	R_{970} / R_{900}	Canopy water status	Peñuelas <i>et al.</i> , 1993
Normalized water index-1 (NWI-1)	$(R_{970} - R_{900}) / (R_{970} + R_{900})$	Canopy water status	Babar <i>et al.</i> , 2006a
Normalized water index-2 (NWI-2)	$(R_{970} - R_{850}) / (R_{970} + R_{850})$	Canopy water status	Babar <i>et al.</i> , 2006a
Normalized water index-3 (NWI-3)	$(R_{970} - R_{880}) / (R_{970} + R_{880})$	Canopy water status	Prasad <i>et al.</i> , 2007a
Normalized water index-4 (NWI-4)	$(R_{970} - R_{920}) / (R_{970} + R_{920})$	Canopy water status	Prasad <i>et al.</i> , 2007a
Normalized water index-5 (NWI-5)	$(R_{970} - R_{870}) / (R_{970} + R_{870})$	Canopy water status	Newly developed
Red normalized difference vegetation index (RNDVI)	$(R_{780} - R_{670}) / (R_{780} + R_{670})$	Canopy photosynthetic area	Raun <i>et al.</i> , 2001
Green normalized difference vegetation index (GNDVI)	$(R_{780} - R_{550}) / (R_{780} + R_{550})$	Canopy photosynthetic area	Aparicio <i>et al.</i> , 2000
Simple ratio (SR)	R_{900} / R_{680}	Canopy photosynthetic area	Gitelson <i>et al.</i> , 1996

† R is the reflectance at a specific wavelength of the light spectrum (in nm).

Table 2. Soil types for the three experimental locations.

Location	Soil types
Agronomy Research Farm at Stillwater	Kirkland silt loam (fine, mixed, thermic Udertic Paleustolls); pH: 6.2-6.5
Lake Carl Blackwell (LCB)	Pulaski fine sandy loam (coarse/loamy, mixed, thermic, Typic, Ustifluent); pH: 6.7-6.9
Perkins	Teller sandy loam (fine, mixed, thermic Udic Argiustolls); pH: 5.1-6.5



Figure 1. The cardboard cone equipped on the NFB sensor in 2009-2010.

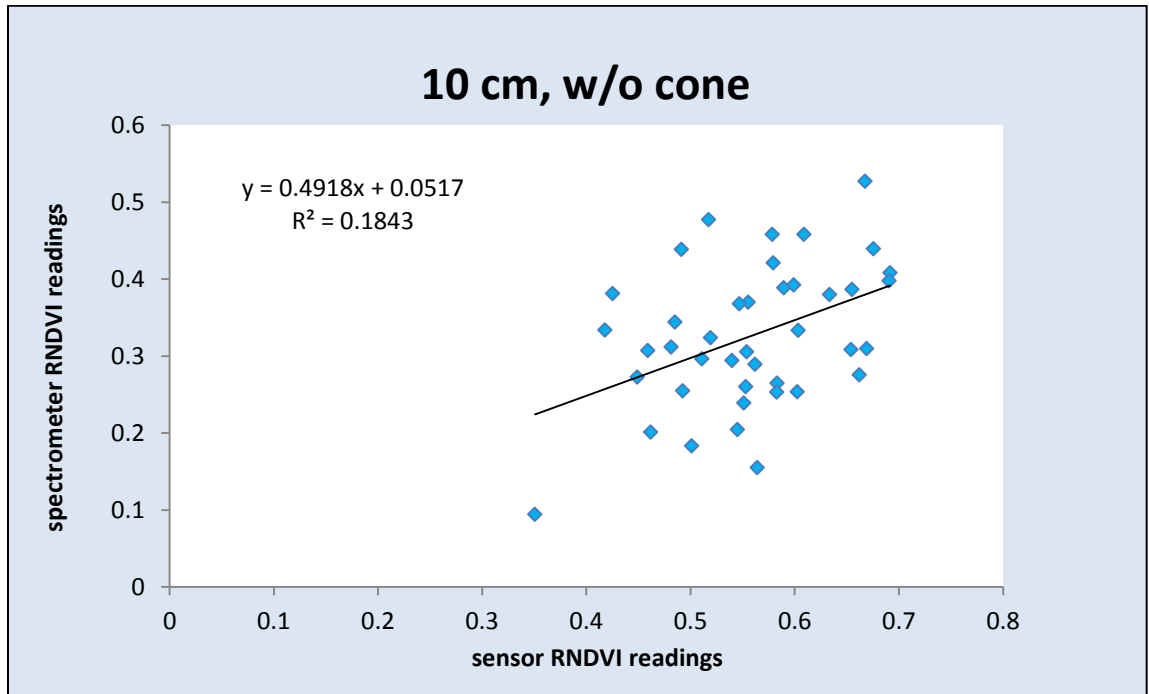


Figure 2. Relationship between RNDVI readings measured by the NFB sensor without the cone at 10 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 21, 2009, Stillwater, OK.

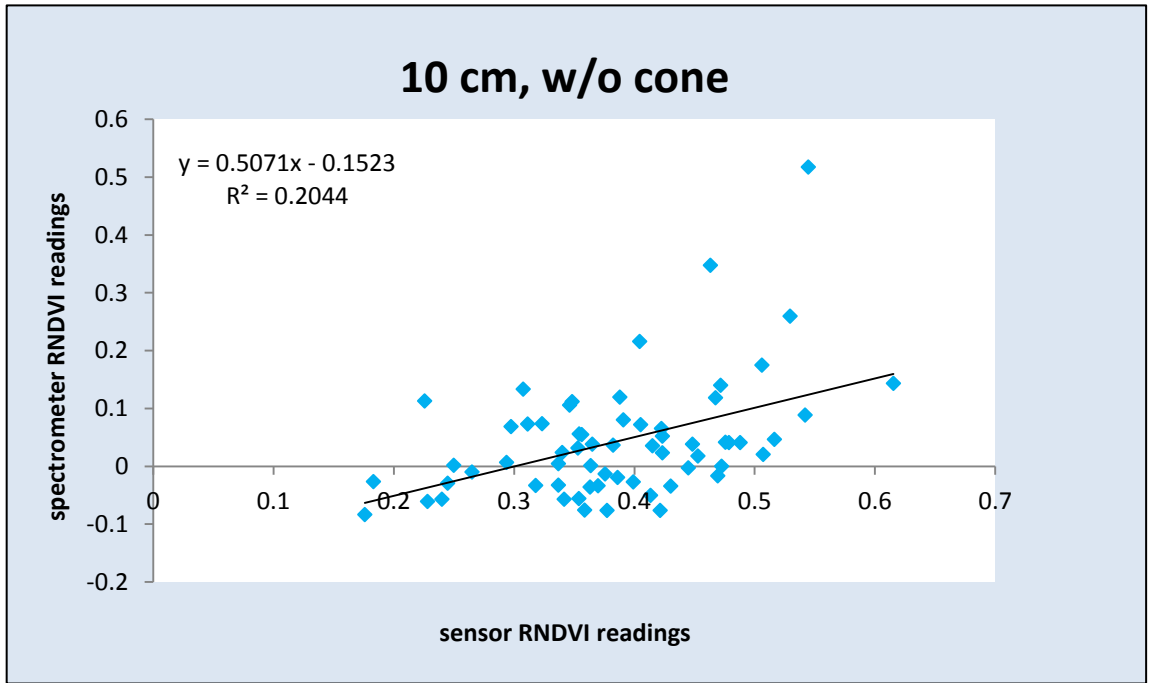


Figure 3. Relationship between RNDVI readings measured by the NFB sensor without the cone at 10 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 29, 2009, Stillwater, OK.

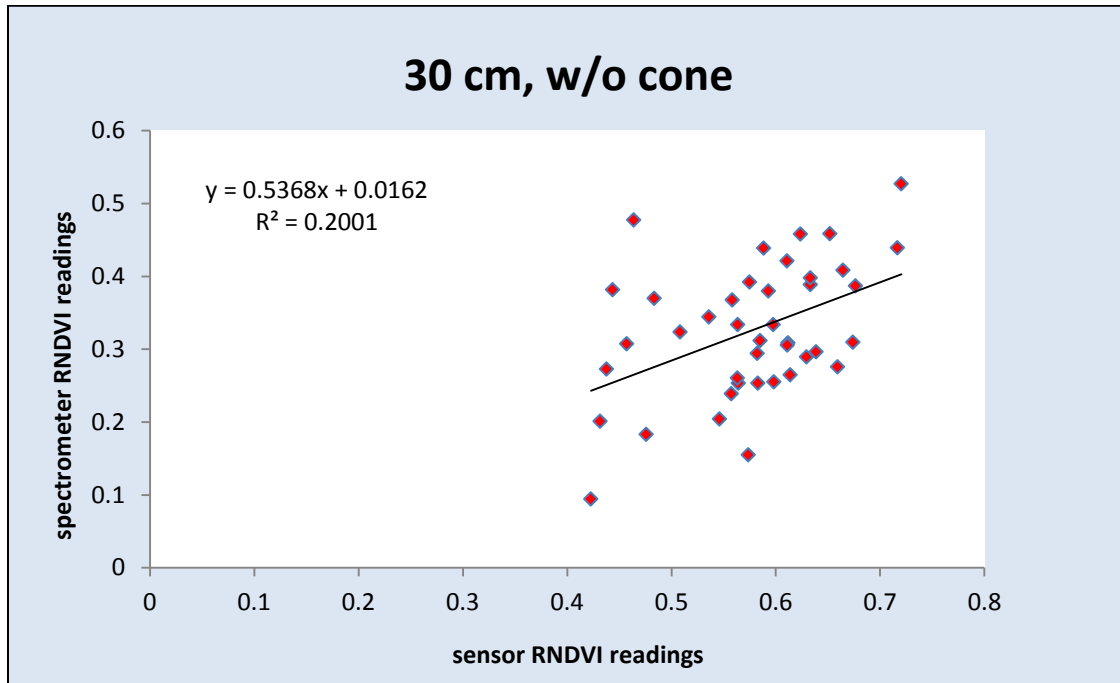


Figure 4. Relationship between RNDVI readings measured by the NFB sensor without the cone at 30 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 21, 2009, Stillwater, OK.

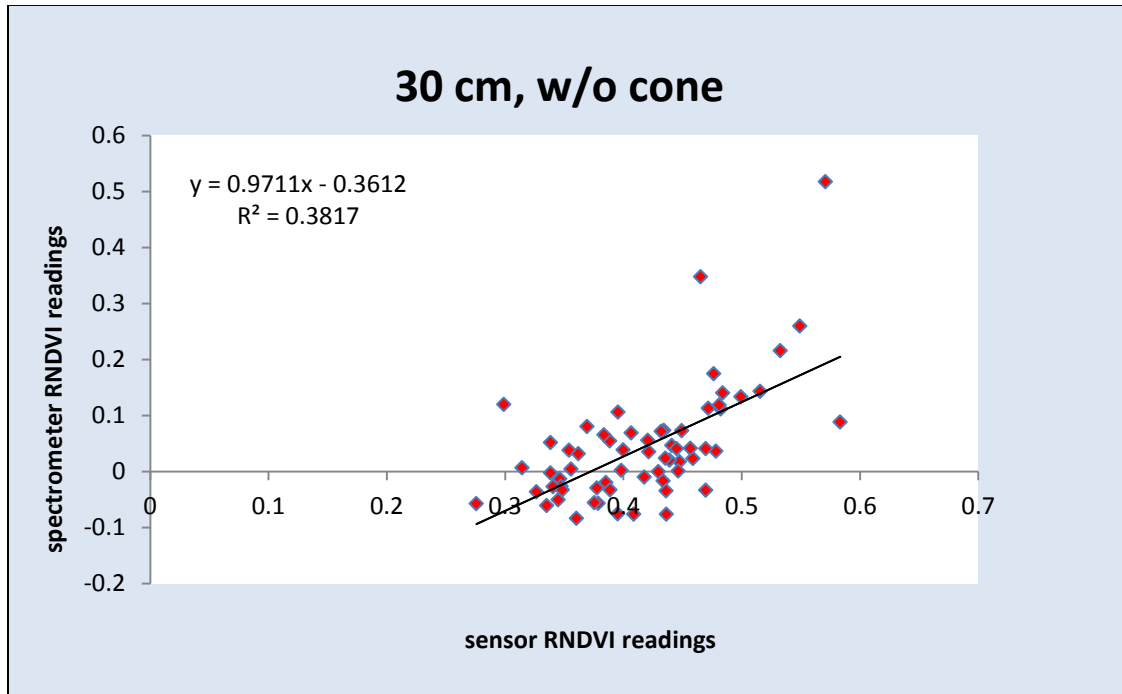


Figure 5. Relationship between RNDVI readings measured by the NFB sensor without the cone at 30 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 29, 2009, Stillwater, OK.

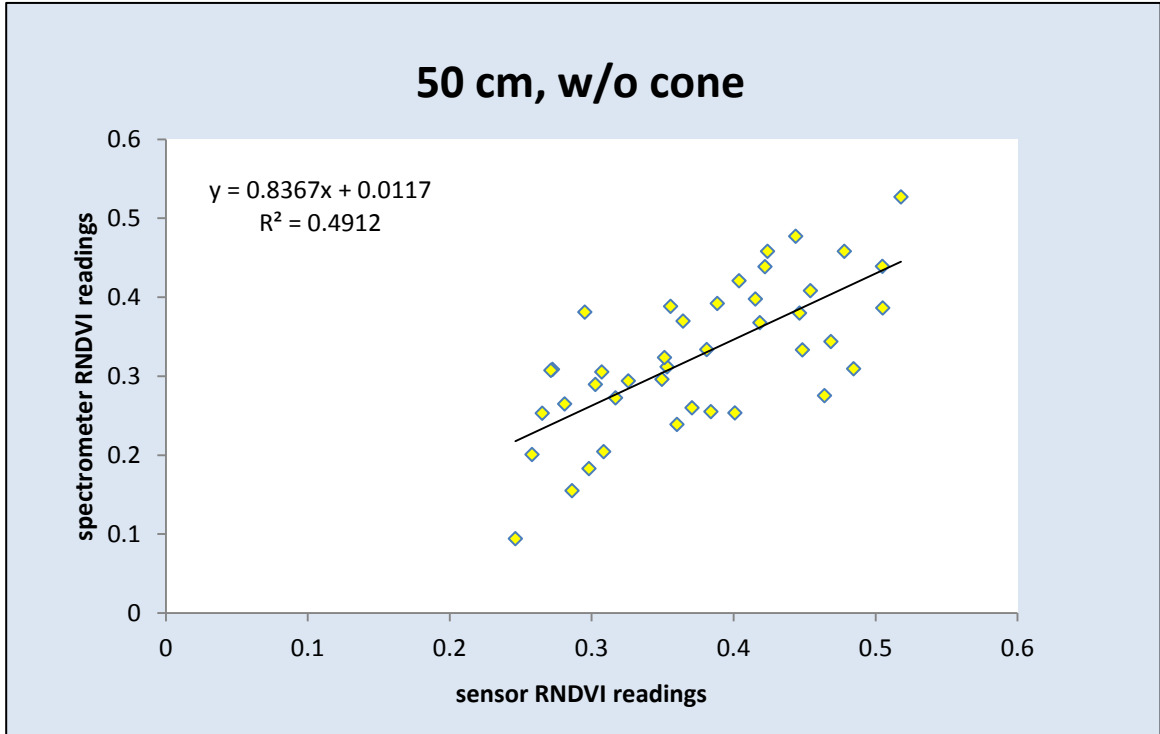


Figure 6. Relationship between RNDVI readings measured by the NFB sensor without the cone at 50 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 21, 2009, Stillwater, OK.

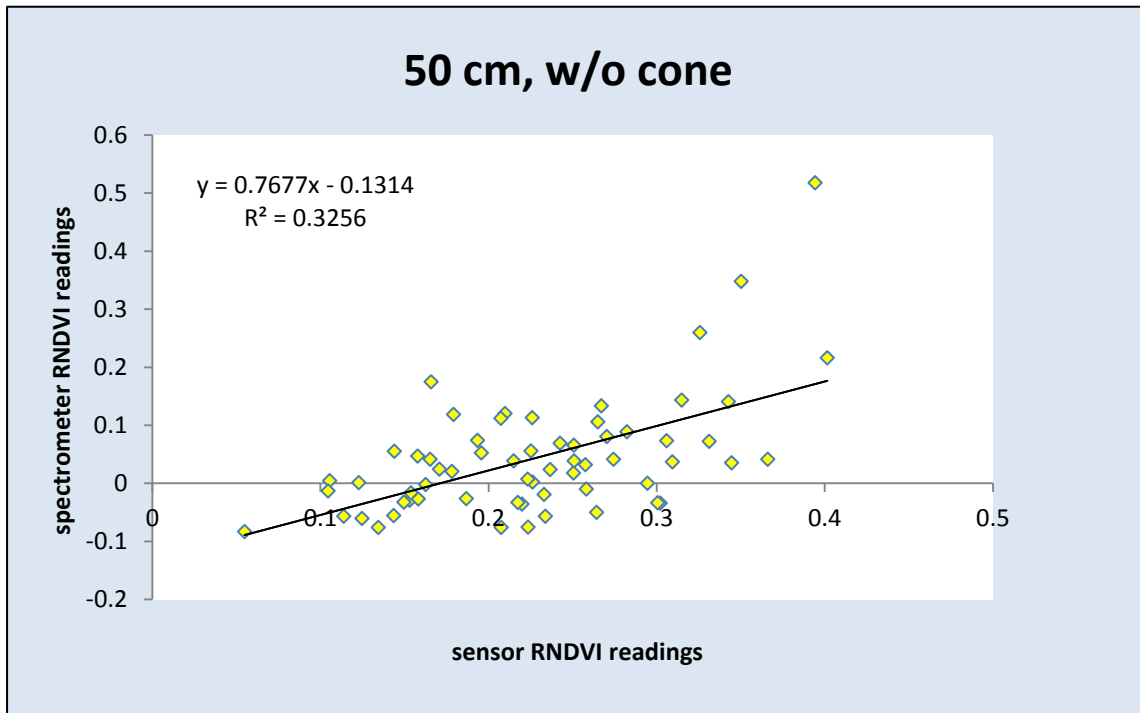


Figure 7. Relationship between RNDVI readings measured by the NFB sensor without the cone at 50 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 29, 2009, Stillwater, OK.

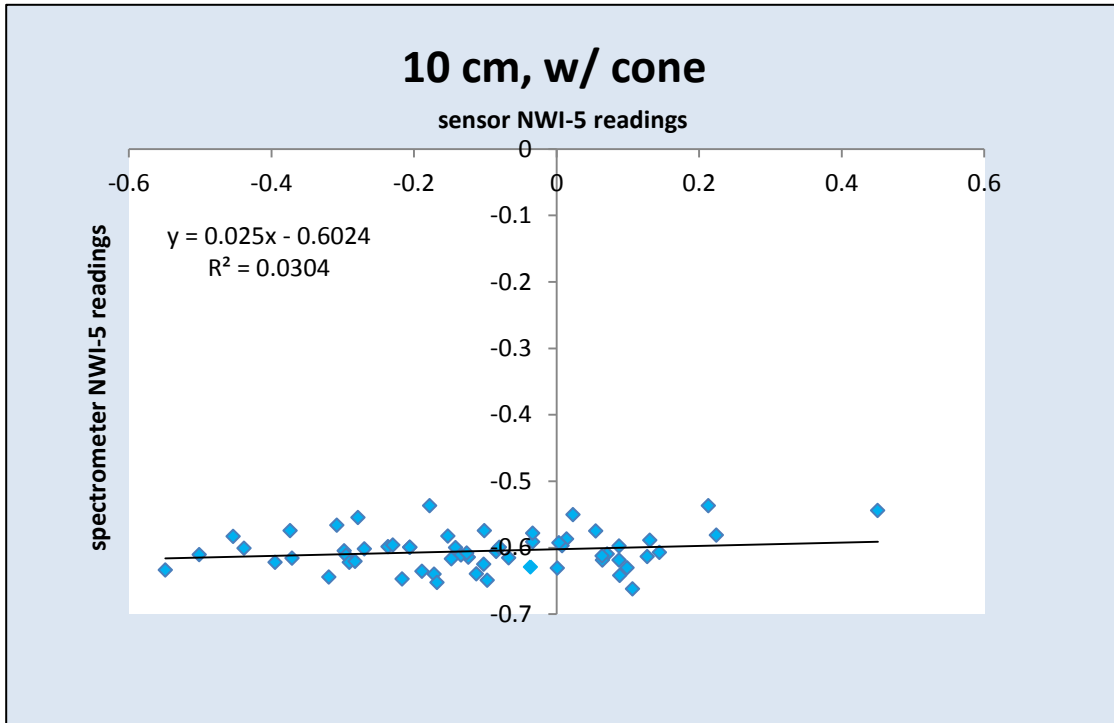


Figure 8. Relationship between NWI-5 readings measured by the NFB sensor with the cone at 10 cm and those measured by the spectrometer in winter wheat at the booting stage, April 27, 2010, LCB, OK.

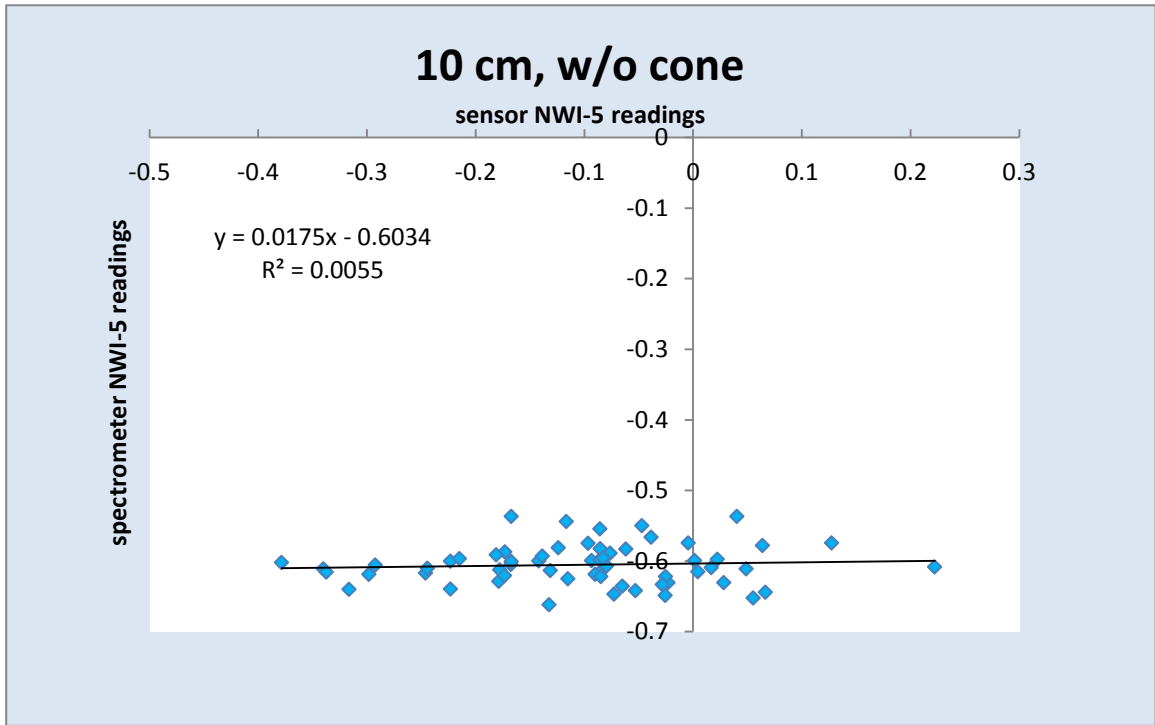


Figure 9. Relationship between NWI-5 readings measured by the NFB sensor without the cone at 10 cm and those measured by the spectrometer in winter wheat at the booting stage, April 27, 2010, LCB, OK.

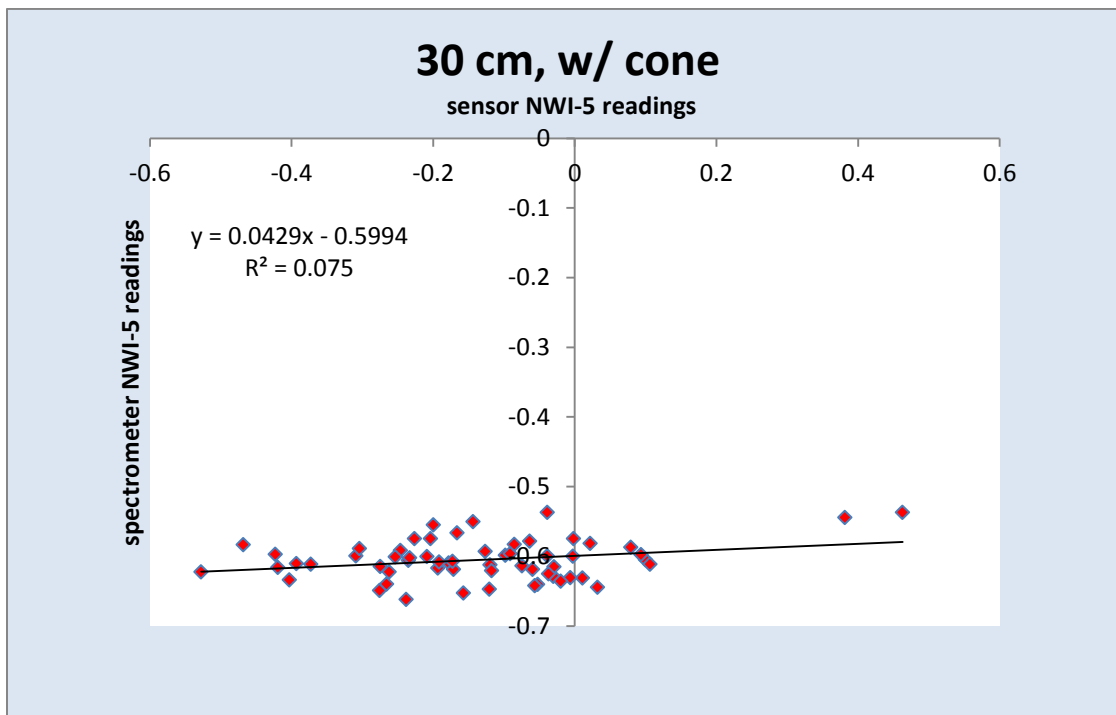


Figure 10. Relationship between NWI-5 readings measured by the NFB sensor with the cone at 30 cm and those measured by the spectrometer in winter wheat at the booting stage, April 27, 2010, LCB, OK.

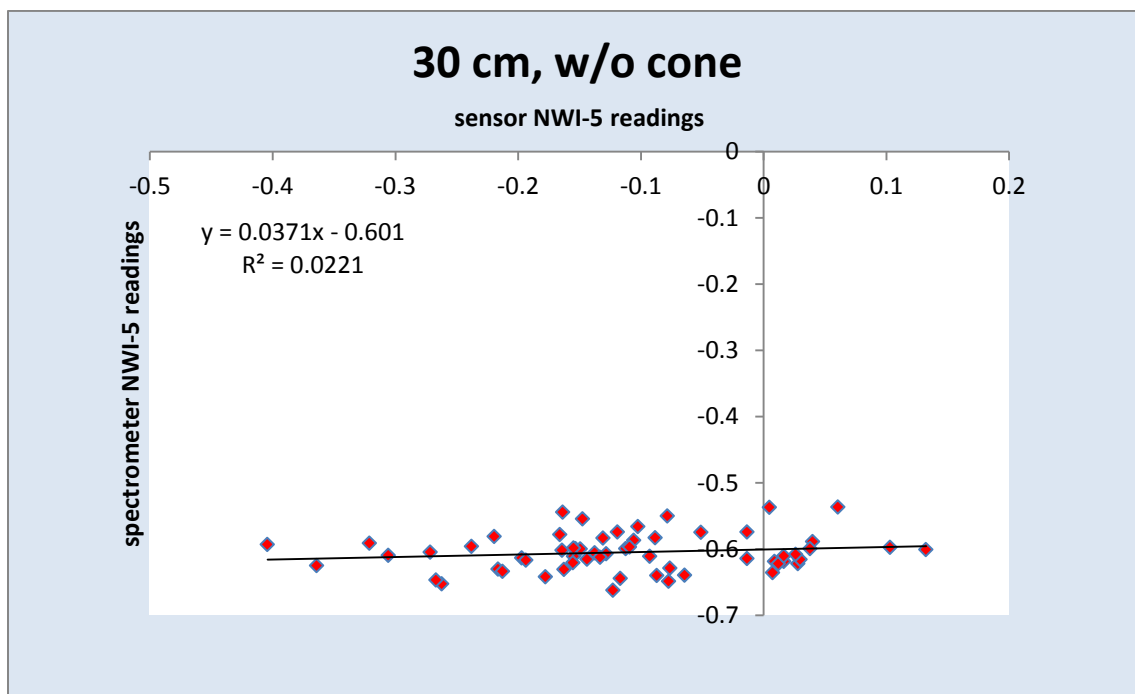


Figure 11. Relationship between NWI-5 readings measured by the NFB sensor without the cone at 30 cm and those measured by the spectrometer in winter wheat at the booting stage, April 27, 2010, LCB, OK.

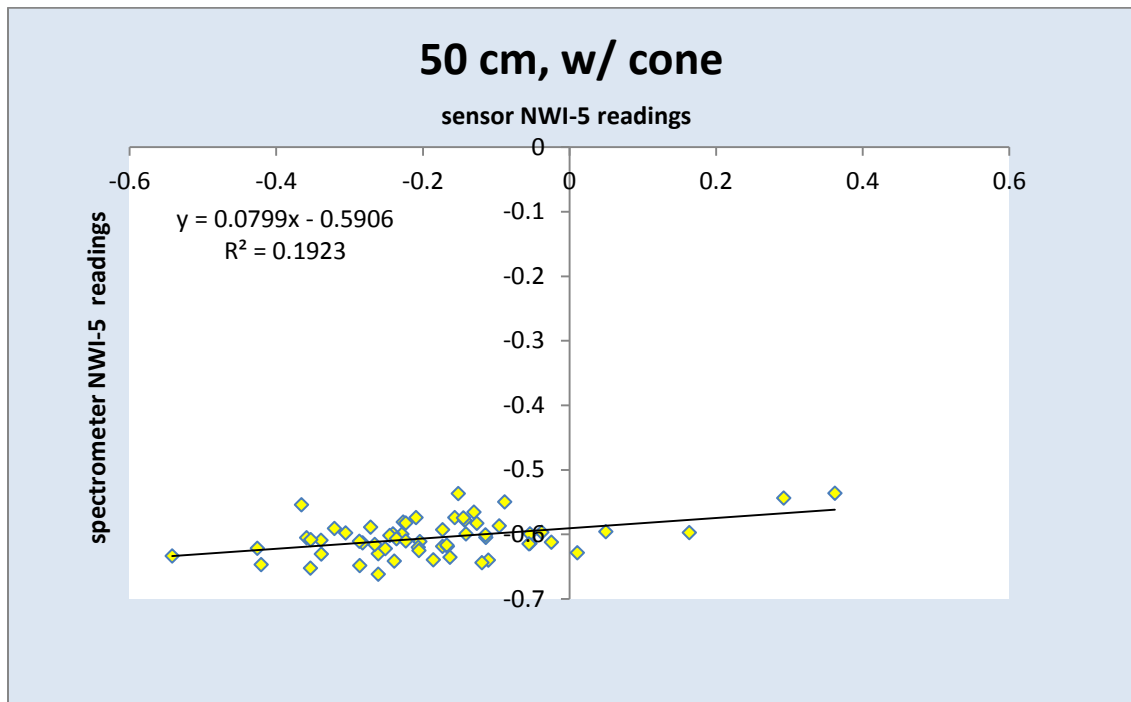


Figure 12. Relationship between NWI-5 readings measured by the NFB sensor with the cone at 50 cm and those measured by the spectrometer in winter wheat at the booting stage, April 27, 2010, LCB, OK.

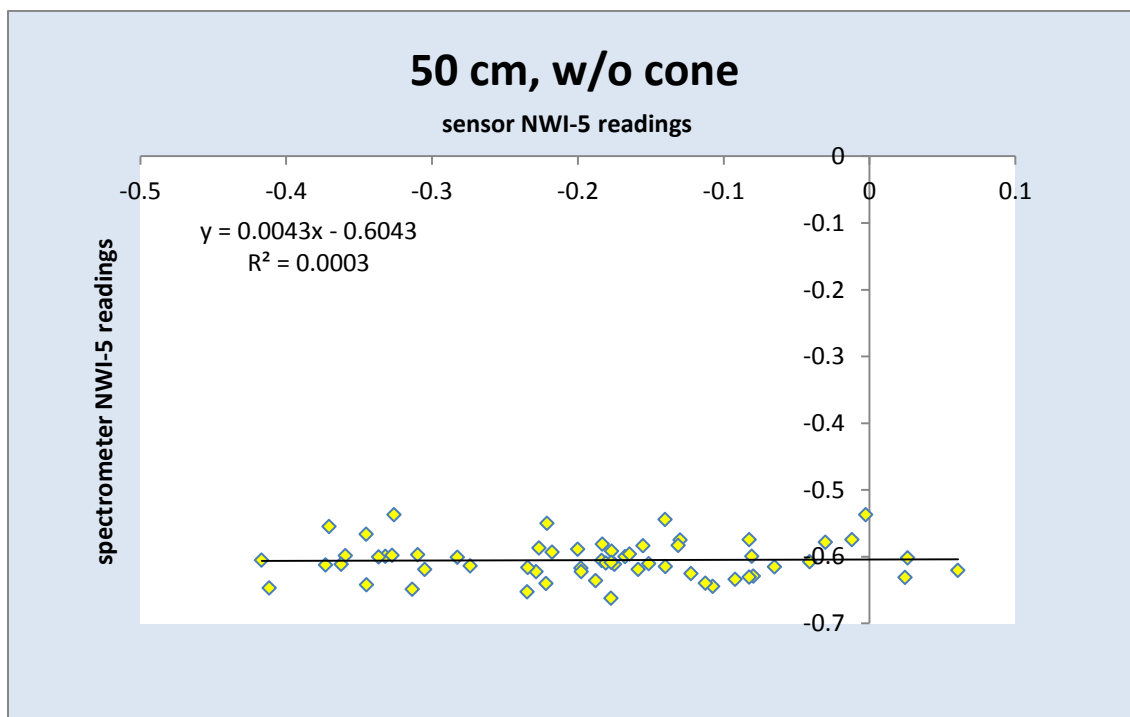


Figure 13. Relationship between NWI-5 readings measured by the NFB sensor without the cone at 50 cm and those measured by the spectrometer in winter wheat at the booting stage, April 27, 2010, LCB, OK.

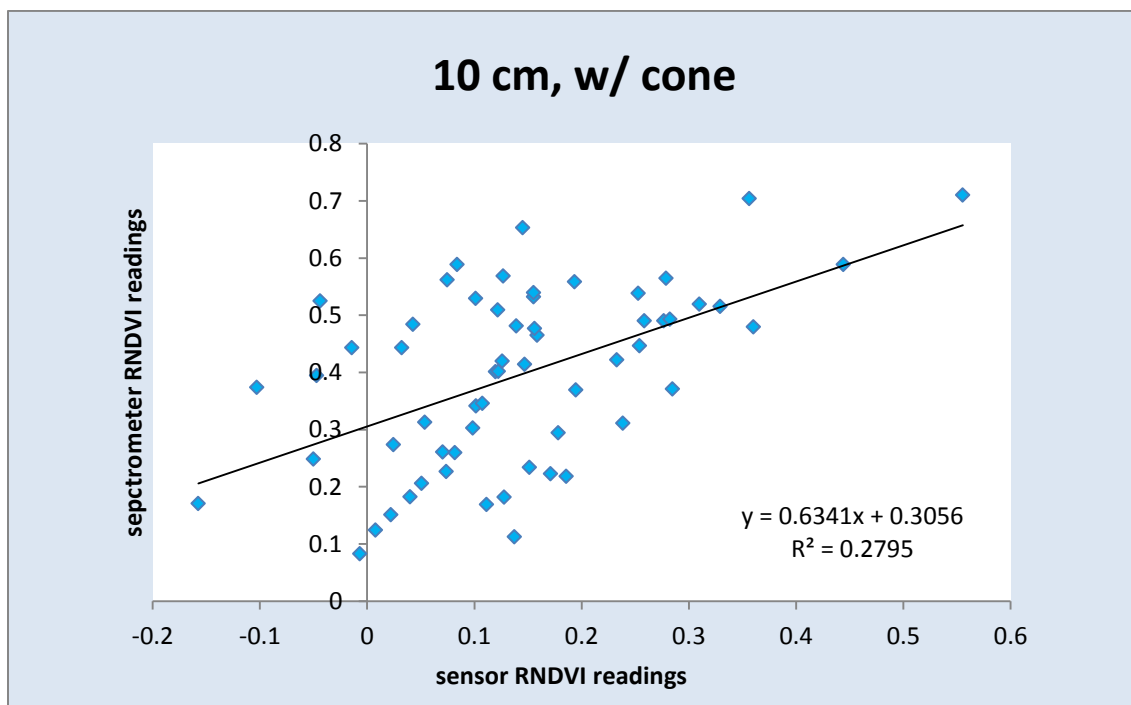


Figure 14. Relationship between RNDVI readings measured by the NFB sensor with the cone at 10 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, LCB, OK.

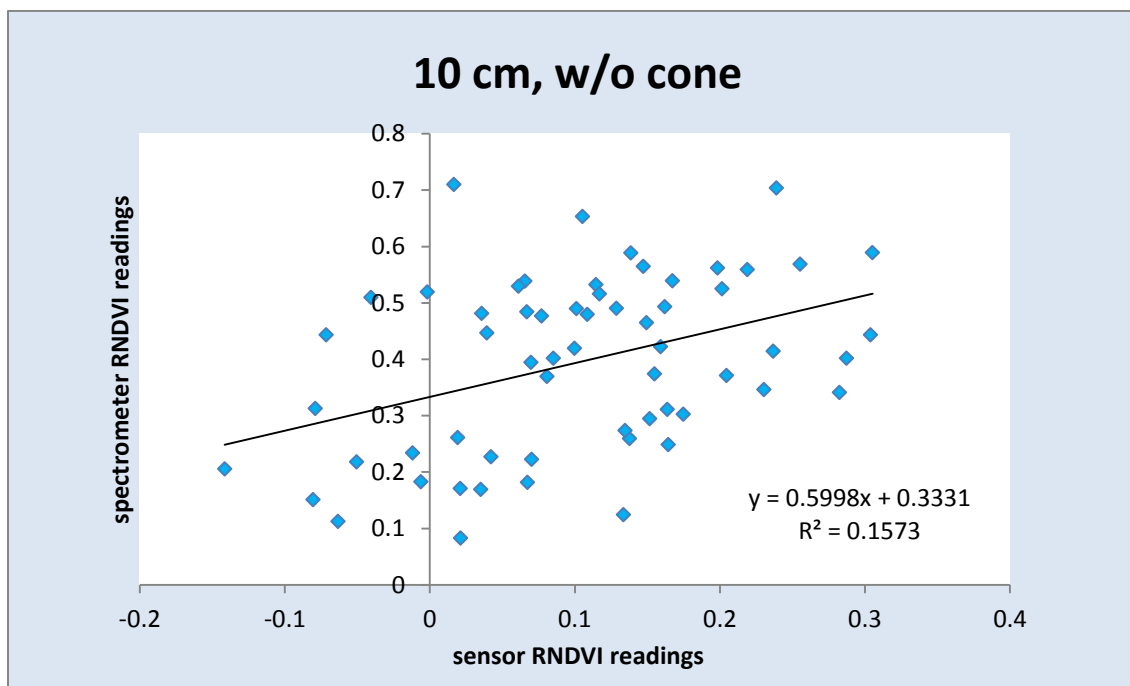


Figure 15. Relationship between RNDVI readings measured by the NFB sensor without the cone at 10 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, LCB, OK.

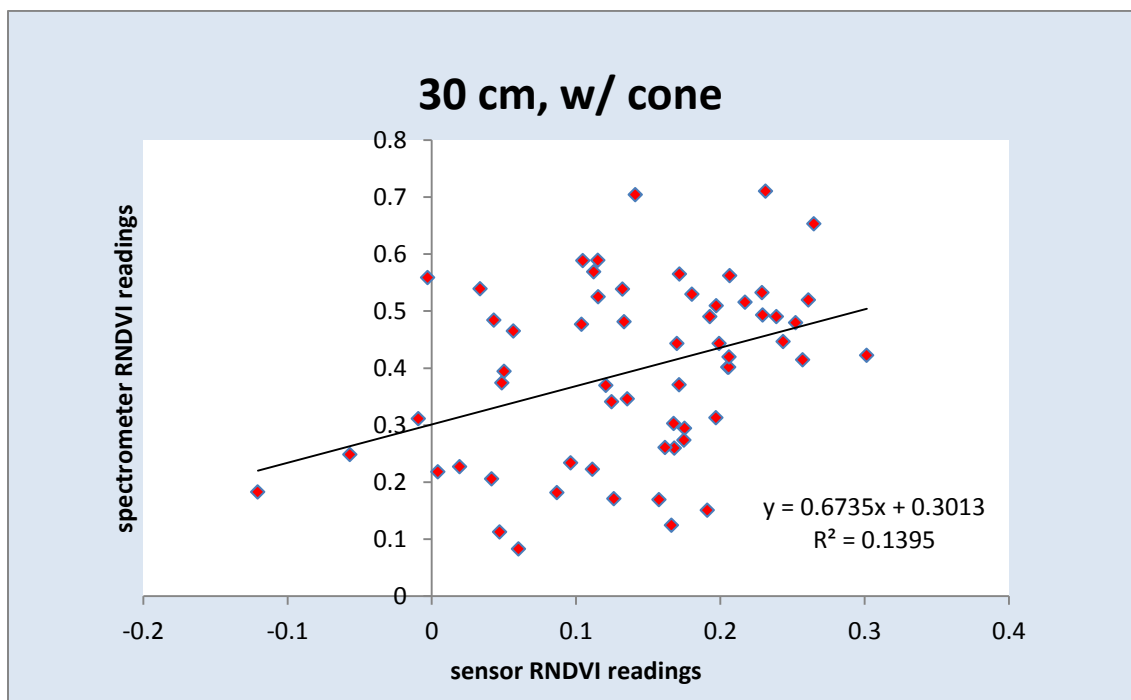


Figure 16. Relationship between RNDVI readings measured by the NFB sensor with the cone at 30 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, LCB, OK.

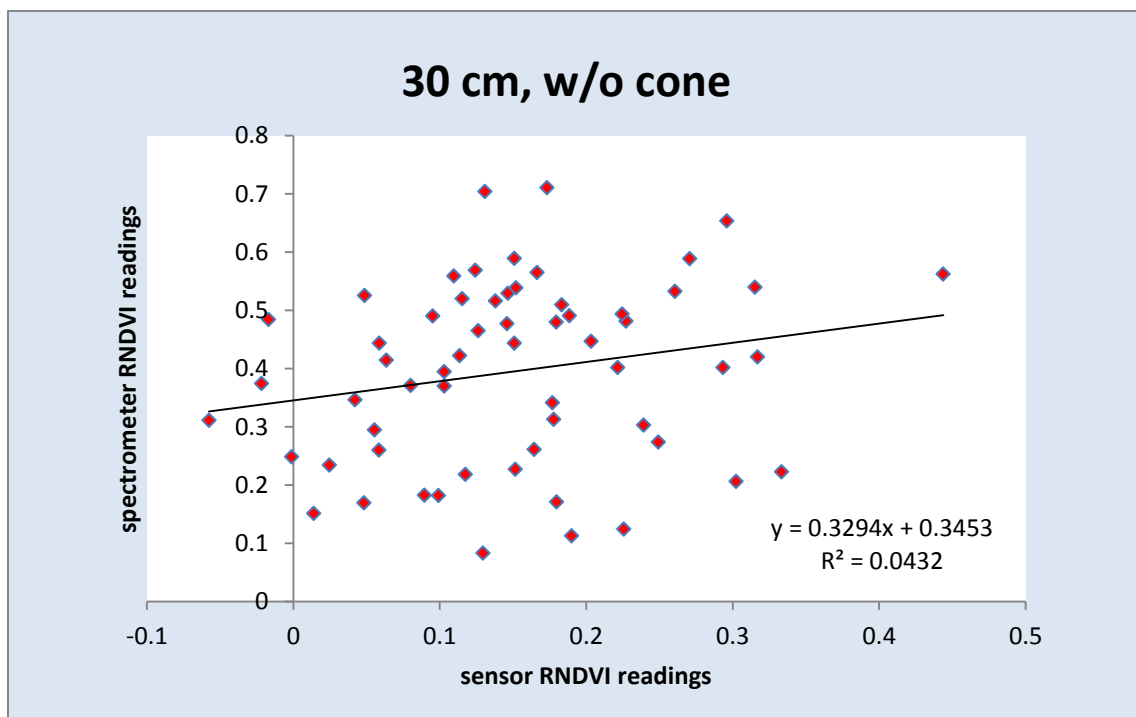


Figure 17. Relationship between RNDVI readings measured by the NFB sensor without the cone at 30 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, LCB, OK.

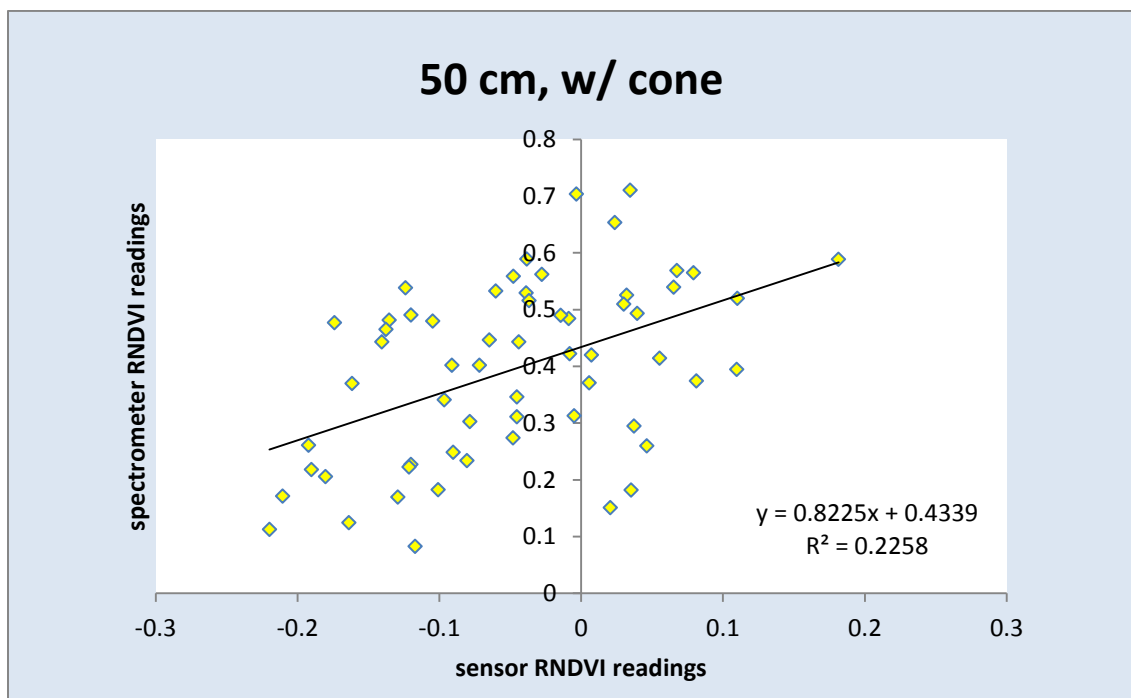


Figure 18. Relationship between RNDVI readings measured by the NFB sensor with the cone at 50 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, LCB, OK.

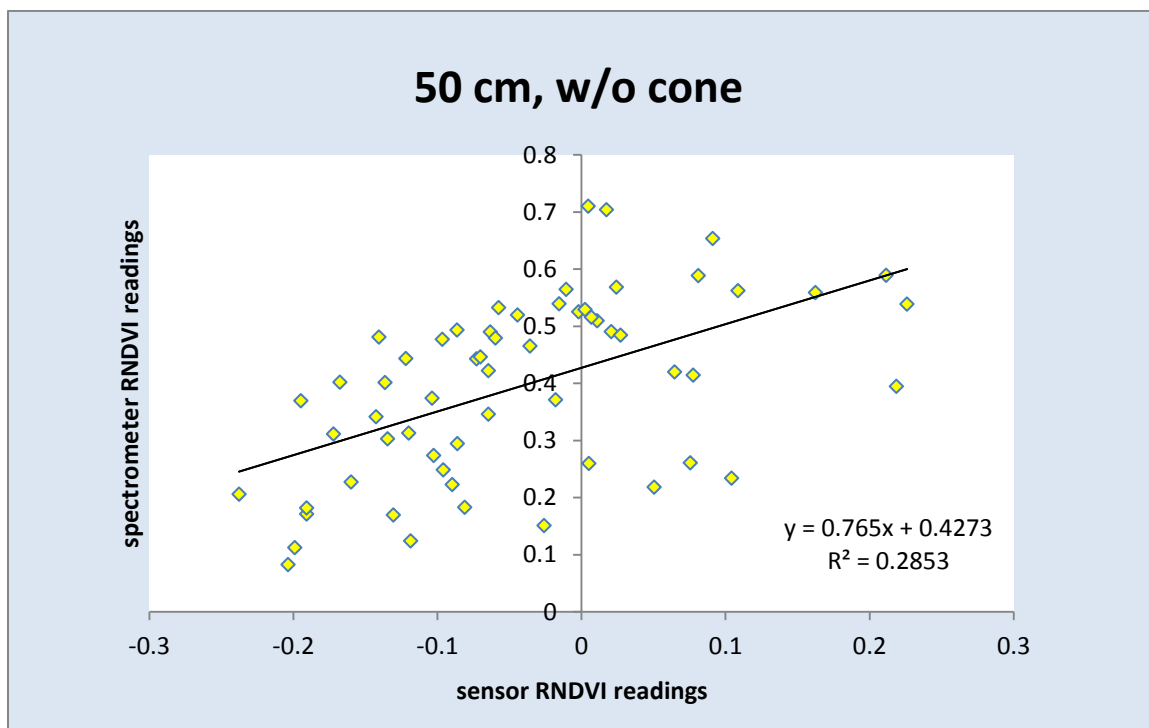


Figure 19. Relationship between RNDVI readings measured by the NFB sensor without the cone at 50 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, LCB, OK.

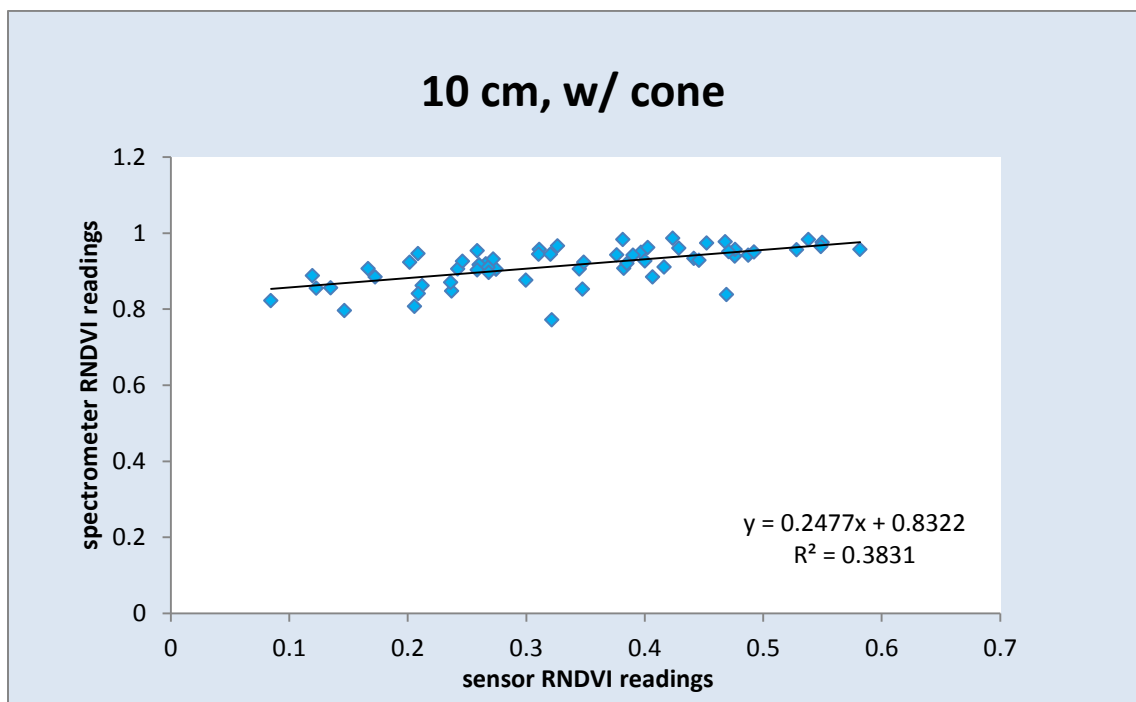


Figure 20. Relationship between RNDVI readings measured by the NFB sensor with the cone at 10 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, Perkins, OK.

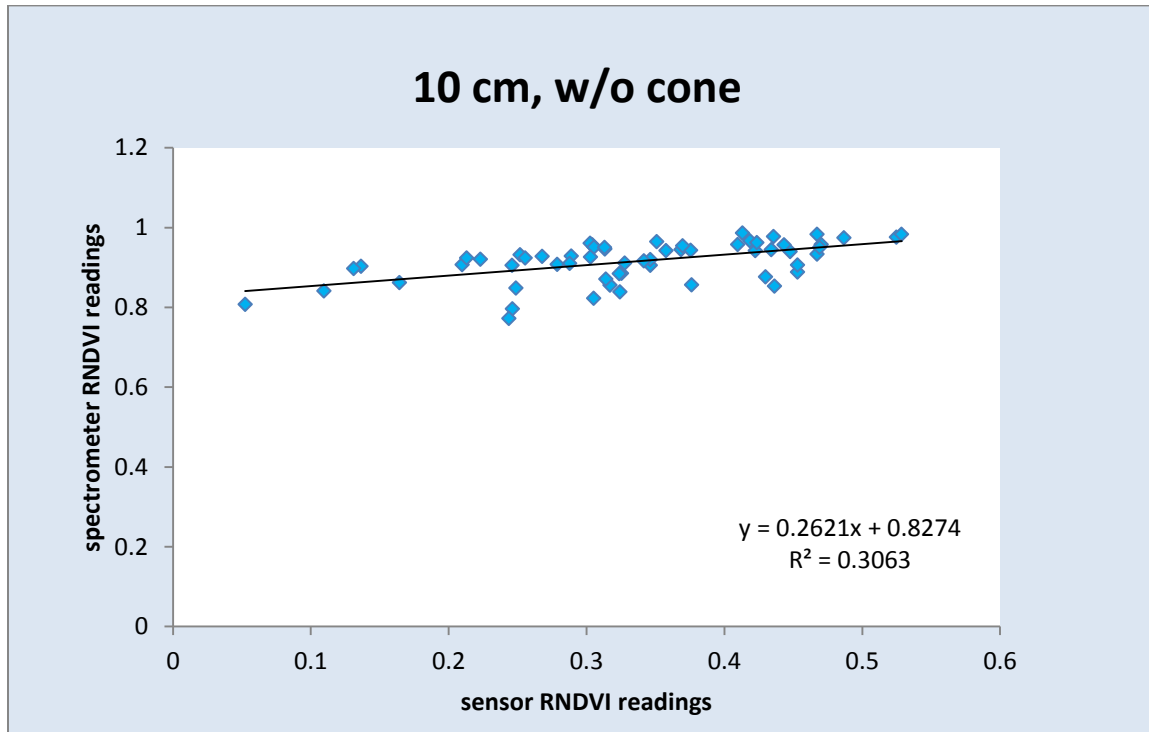


Figure 21. Relationship between RNDVI readings measured by the NFB sensor without the cone at 10 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, Perkins, OK.

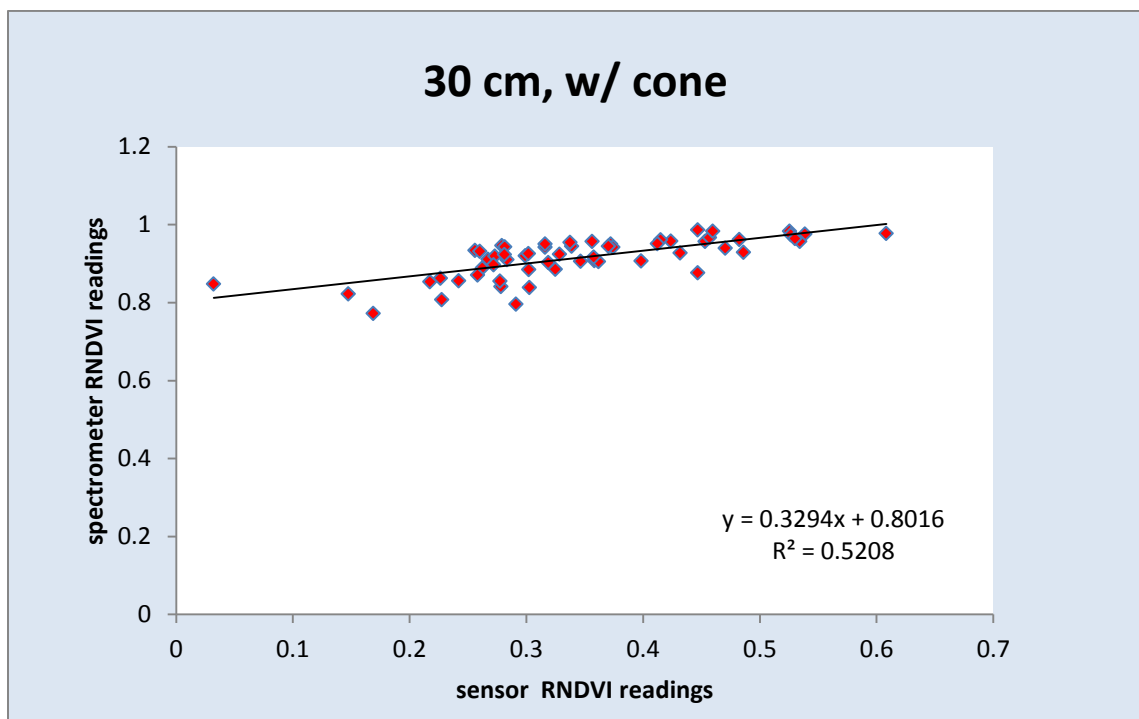


Figure 22. Relationship between RNDVI readings measured by the NFB sensor with the cone at 30 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, Perkins, OK.

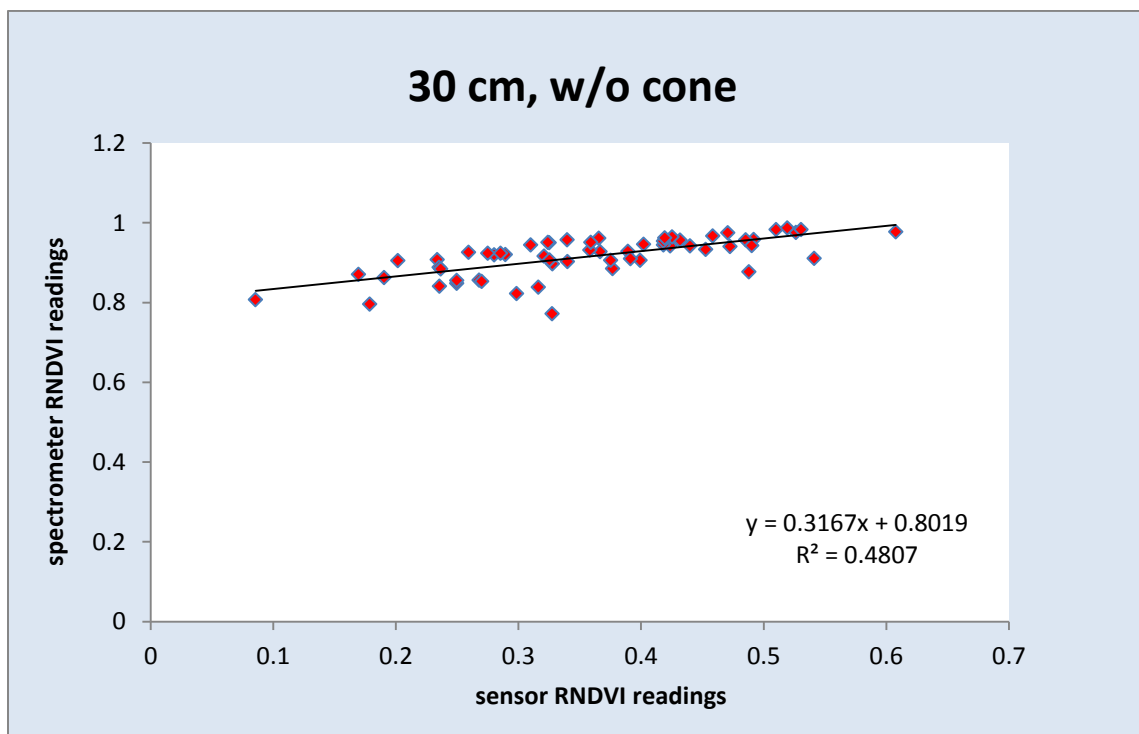


Figure 23. Relationship between RNDVI readings measured by the NFB sensor without the cone at 30 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, Perkins, OK.

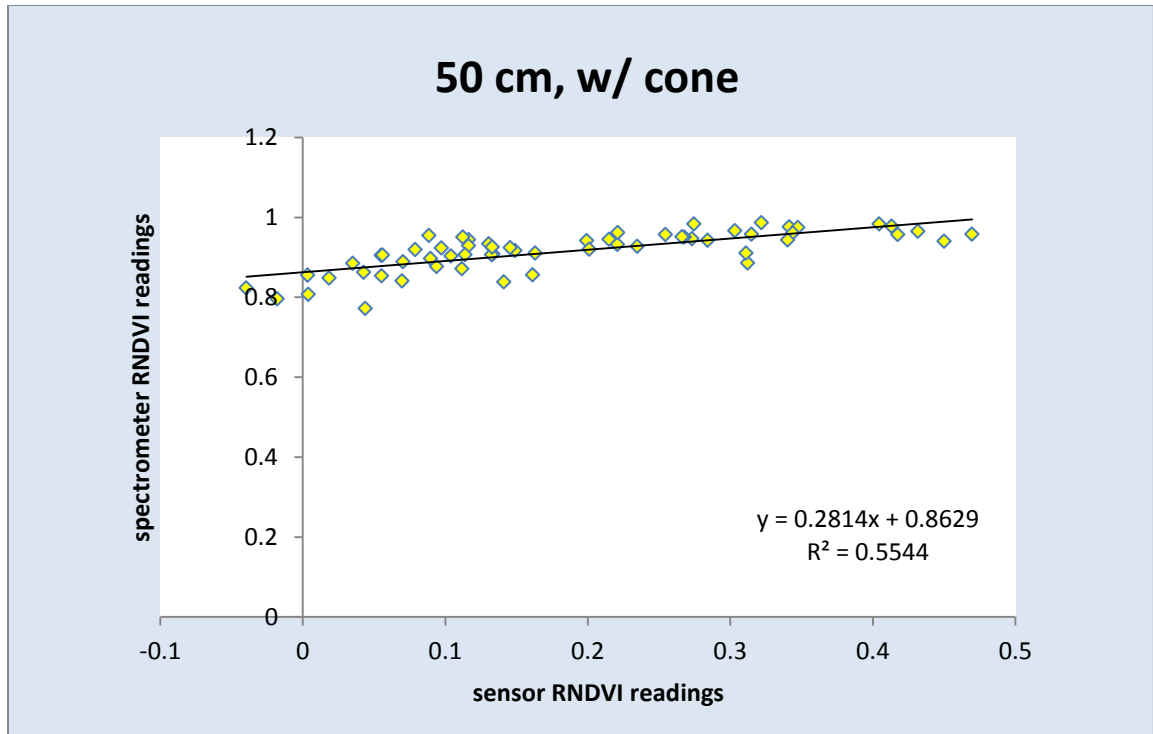


Figure 24. Relationship between RNDVI readings measured by the NFB sensor with the cone at 50 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, Perkins, OK.

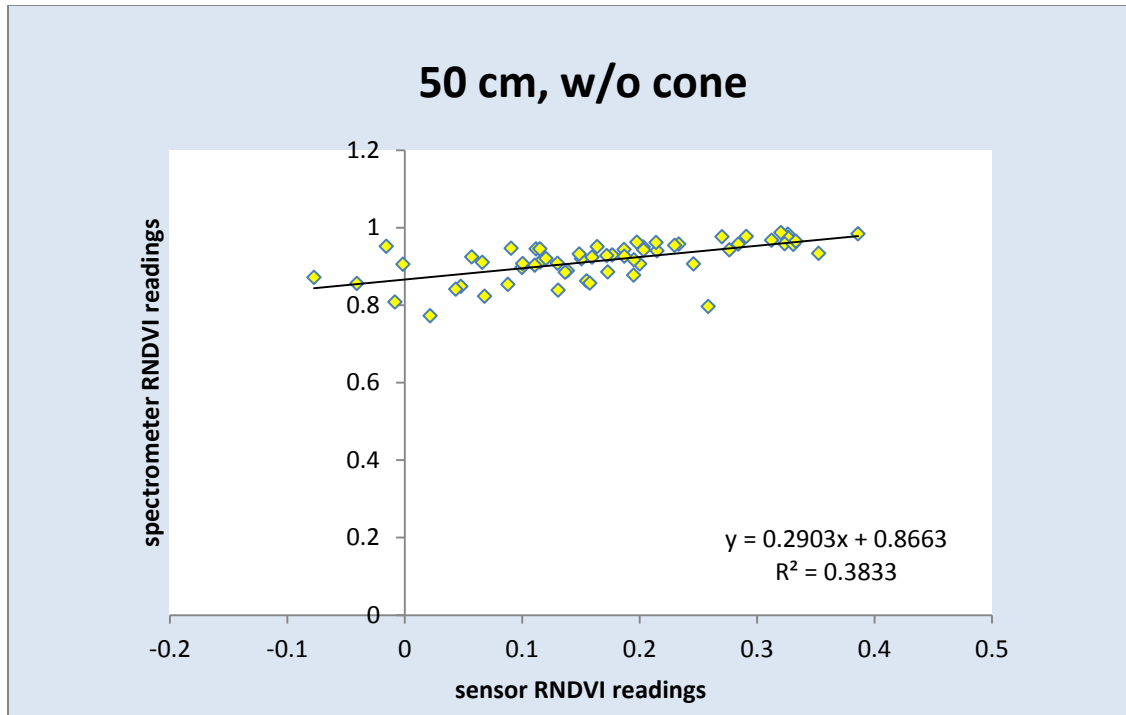


Figure 25. Relationship between RNDVI readings measured by the NFB sensor without the cone at 50 cm and those measured by the spectrometer in winter wheat at the grain-filling stage, May 28, 2010, Perkins, OK.

VITA

Zhiyong Wang

Candidate for the Degree of

Master of Science

Thesis: TESTING THE NEW 4-BAND SENSOR AND THE SPECTROMETER FOR
EQUIVALENCY IN WINTER WHEAT

Major Field: Plant and Soil Sciences

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Education: Received the Bachelor of Science degree in Agricultural Sciences at Anhui Agricultural University, Hefei, China in 2005. Completed the requirements for the Master of Science degree in Plant and Soil Sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2010.

Experience: Employed by the Department of Plant and Soil Sciences at Oklahoma State University as a graduate research assistant, 2008-present.

Professional Memberships:

American Society of Agronomy
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Name: Zhiyong Wang

Date of Degree: December, 2010

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: TESTING THE NEW 4-BAND SENSOR AND THE SPECTROMETER
FOR EQUIVALENCY IN WINTER WHEAT

Pages in Study: 63

Candidate for the Degree of Master of Science

Major Field: Plant and Soil Sciences

Scope and Method of Study:

The feasibility of using the new 4-band (NFB) sensor to replace the spectrometer in winter wheat research was demonstrated in this study. The two SRIs (RNDVI and NWI-5) were measured by using the NFB sensor and the spectrometer to test the equivalency between both tools. The study was to determine if the newly developed NFB sensor could substitute for the spectrometer in the field.

Findings and Conclusions:

The measurements at the grain-filling stage gave better equivalency between the NFB sensor (with and without the cone) and the spectrometer than at the booting stage in winter wheat for both RNDVI and NWI-5 readings. The NFB sensor could be applied with and without the cone to replace the spectrometer for taking RNDVI readings at the grain-filling stage. The application of the cardboard cone to reduce the surface area of the light-collecting lens could adequately ameliorate the equivalency between the sensor and the spectrometer. However, the NFB sensor equipped with the cone could not substitute for the spectrometer in NWI-5 data collection in winter wheat. As a consequence, additional adjustments to the NFB sensor are needed to improve equivalency in taking NWI-5 readings.

ADVISER'S APPROVAL: Dr. Arthur R. Klatt
