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Characterizing soybean vigor and productivity using multiple crop canopy sensor readings

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ABSTRACT

Canopy reflectance has been used in crops, such as corn and wheat, to assess crop status and direct in-season management practices, but less research has focused on using canopy reflectance in soybean research and production. In this study, soybean canopy reflectance measurements were measured at several growth stages throughout the 2015 and 2016 growing seasons using the RapidSCAN CS-45 Handheld Crop Scanner to determine if the normalized difference red edge (NDRE) index could be used to predict relative soybean productivity within a field prior to harvest. The NDRE values were used to calculate the cumulative reflectance of each experimental unit over the season. The cumulative reflectance readings through the R6 growth stage, termed the area under the reflectance progress curve (AURPC), and seed yield of every experimental unit were classified as top 25%, middle 50%, or bottom 25% within each location. Across all locations, bottom AURPC values correctly predicted bottom yield 52.5% of the time, and ranged from 46.7 to 86.2% by location. The probability of incorrectly predicting the bottom yield with a top AURPC value (9.7%) was also lower than incorrectly predicting the top yield with a bottom AURPC value (12.3%). Misclassifications by incorrectly identifying a bottom yield with a top AURPC ranged from 0.0% to 16.7% by location. Additionally, individual NDRE values at R2 were determined to be influenced by seed treatments at seven of the eight locations ($p = 0.10$) and, upon further investigation, found to be correlated to early-season soybean populations ($r^2 = 0.314$).

1. Introduction

Increasing soybean [*Glycine max* (L.) Merrill] yields is one of the primary goals of research involved in soybean production. However, determining the variables that consistently increase soybean yields, or stressors that reduce soybean yields, continues to challenge researchers, agronomists, and producers. The use of crop sensors has emerged as a new technology being used successfully in other cropping systems to monitor and manage agricultural inputs in a site-specific manner (Hatfield et al., 2008; Pinter et al., 2003).

Genetic improvements account for nearly two-thirds of on-farm yield gains (Rincker et al., 2014; Specht et al., 2014). The remaining gain is a result of changes in agronomic practices (Rowntree et al., 2013), including earlier planting dates (Heatherly and Elmore, 2004; Specht et al., 1999), narrower row spacing (Heatherly and Elmore, 2004; Specht et al., 1999; Voldeng et al., 1997), improved weed control (Luedders, 1977; Voldeng et al., 1997), and reduced harvest loss (Specht et al., 1999). Managing soybean diseases and insects is also an important agronomic practice to prevent soybean yield losses (Kandel et al., 2016). To increase soybean yields, growers have increased their use of seed treatments, foliar fungicide and insecticide applications at pod set, and the use of fertilizers (USDA-NASS, 2016). However, yield responses to these inputs are often inconsistent and vary by environment and cultivar (Gaspar et al., 2014; Swoboda and Pedersen, 2009).

Crop canopy sensors have emerged as a technology to evaluate plant characteristics using principles of leaf and canopy reflectance that can eliminate the bias inherent to typical evaluation practices. Reflectance properties in the near infrared (NIR) region (700–1300 nm) of the electromagnetic (EM) spectrum are influenced by leaf density and canopy structure (Kumar and Silva, 1973), while chlorophylls strongly absorb in the blue and red regions of the EM spectrum (Lichtenthaler and Buschmann, 2001). Additionally, absorption in the red edge (RE)

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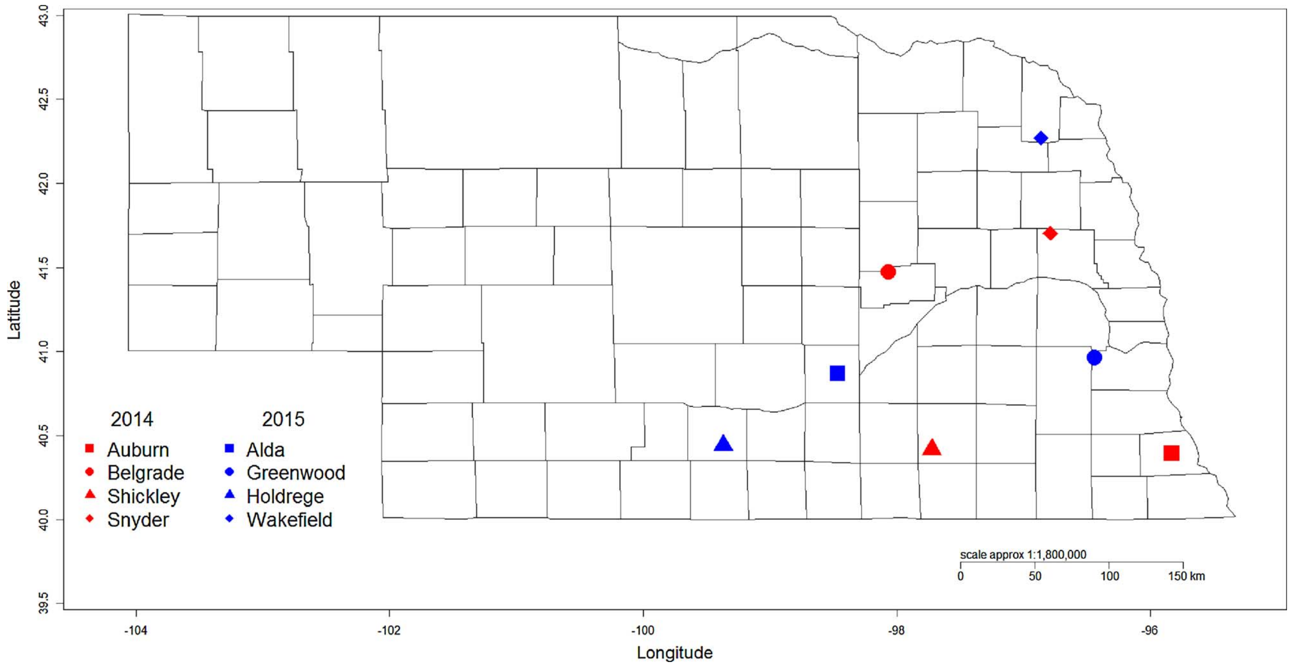

Fig. 1. Field trial locations across eastern Nebraska during 2014 and 2015.

region (680–750 nm) of the spectrum, defined as the inflection point between the red and near infrared regions of the spectrum, is sensitive to changes in chlorophyll content (Gitelson et al., 1996), which is closely related to gross primary productivity of terrestrial plants (Gitelson et al., 2006).

Numerous algorithms, or vegetation indices (VIs), have been developed using reflectance measurements in the visible and NIR reflectance bands to estimate biophysical characteristics of vegetation (Hatfield et al., 2004). The normalized difference red edge (NDRE) index, defined in detail in the Materials and Methods section, is a VI that has been used for crop canopy evaluations (Gitelson and Merzlyak, 1994). The RE band penetrates deep into the canopy and is sensitive to crop canopy chlorophyll at higher canopy biomass, overcoming the saturation inherent to the normalized difference vegetation index (NDVI), the most commonly used VI (Li et al., 2014). Eitel et al. (2010) found that using RE reflectance improved the ability to estimate variations in chlorophyll content ($r^2 > 0.73$, RMSE < 1.69) over devices that did not use RE ($r^2 = 0.57$, RMSE $= 2.11$).

Crop canopy sensors have been used for numerous agronomic applications, particularly as a tool in precision agriculture (Pinter et al., 2003). In wheat production (Raun et al., 2005) and corn production (Holland and Schepers, 2010; Solari et al., 2008) algorithms have been developed using vegetation indices to direct in-season nitrogen management based on changes in remotely-sensed chlorophyll content and biomass. Less work has focused on soybean production, most likely because nitrogen management is less important due to the plant's innate ability to fix its own nitrogen (Keyser and Li, 1992). However, research that has utilized crop sensors in soybean has primarily focused on individual components of soybean production, such as detecting weed infestations (Medlin et al., 2000), identifying insect infestations (Board et al., 2007), and detecting stress induced by soybean cyst nematode (SCN) at the field level (Nutter et al., 2002), while some have evaluated the ability to predict soybean yield (Ma et al., 2001; Mourtzinis et al., 2014; Zhang et al., 1999).

Management zones have been used in precision agriculture to efficiently manage agricultural crops. Often, management zones are created from historical yield records, field topography and soil properties, or soil electrical conductivity (Fleming et al., 2000; Schepers et al., 2004). Remote sensing has provided another tool to delineate management zones by providing characteristics of a growing crop during the season (Inman et al., 2008).

The RapidSCAN CS-45 Handheld Crop Sensor (Holland Scientific Inc., Lincoln, NE) is an example of a crop canopy sensor that is being used commercially in the field of agriculture. The RapidSCAN sensor is an active optical sensor that measures crop and soil reflectance at three wavelengths, red (670 nm), RE (730 nm), and NIR (780 nm). Active sensors utilize their own radiation source, thereby eliminating the need for sufficient ambient illumination to collect reflectance readings (Holland et al., 2012). The NDRE index is calculated from the RE and NIR bands to evaluate differences in crop canopy biomass and chlorophyll content (Gitelson et al., 1996). Because of the inherent limitations of the NDVI index, and the capability of this sensor to calculate NDRE, the latter index was examined to determine its utility in a soybean crop.

No studies to date have investigated the ability to use multiple NDRE index values to create management zones in soybeans. Vegetation indices have predominantly been recorded at a single point in the season to evaluate crop canopy characteristics. Therefore, the objectives of this study were to (i) determine if multiple crop canopy sensor readings using NDRE index values over the course of the soybean growing season could be used as an indicator of soybean yield and field productivity, and (ii) determine at what growth stages single readings by a commercially available crop canopy sensor could be used to evaluate physiological responses to soybean inputs in a small-scale research setting using NDRE.

2. Materials and methods

2.1. Experimental site and design

This study was conducted at four field locations each year between 2014 and 2015 across eastern Nebraska for a total of eight different locations (Fig. 1). Sites were selected to represent the major soybean-producing region of Nebraska with no prior knowledge of pest pressure.

The experimental design was an alpha lattice to account for variation inherent in large field experimentation (Barreto et al., 1996). All plots were planted in four complete blocks at 76-cm row spacing. In both years, thirty treatments were arranged in a 5 × 6 alpha-lattice

Table 1
Soybean seed and early season treatments applied during 2014 and 2015 field studies.

Treatment	Treatment Abbreviation	Subset Treatment Abbreviation[a]	Year 2014	Year 2015	Product	Active Ingredient	Growth Stage	Product Rate ($seed^{-1}$)
None	N-ST	N-ST	+	+	–	–	Seed	–
Fungicide	F-ST	F-ST	+	+	Apron XL	mefanoxam	Seed	0.0110 mg a.i.
					Maxim 4FS	fludioxonil	Seed	0.0037 mg a.i.
					Vibrance	sedaxane	Seed	0.0011 mg a.i.
Nitrogen	NIT-ST		+	–	UAN	28 – 0 – 0	V2	16.8 kg N ha^{-1}
Fungicide + Insecticide	FI-ST		–	+	Apron XL	mefanoxam	Seed	0.0110 mg a.i.
					Maxim 4FS	fludioxonil	Seed	0.0037 mg a.i.
					Vibrance	sedaxane	Seed	0.0011 mg a.i.
					Cruiser 5FS	thiamethoxam	Seed	0.0730 mg a.i.
Fungicide + Insecticide + Nitrogen	C-ST	C-ST	+	–	Apron XL	mefanoxam	Seed	0.0110 mg a.i.
					Maxim 4FS	fludioxonil	Seed	0.0037 mg a.i.
					Vibrance	sedaxane	Seed	0.0011 mg a.i.
					Cruiser 5FS	thiamethoxam	Seed	0.0730 mg a.i.
					UAN	28 – 0 – 0	V2	16.8 kg N ha^{-1}
Fungicide + Nitrogen	FN-ST		+	–	Apron XL	mefanoxam	Seed	0.0110 mg a.i.
					Maxim 4FS	fludioxonil	Seed	0.0037 mg a.i.
					Vibrance	sedaxane	Seed	0.0011 mg a.i.
					UAN	28 – 0 – 0	V2	16.8 kg N ha^{-1}
Fungicide + Insecticide + Biological	C-ST	C-ST	–	+	Apron XL	mefanoxam	Seed	0.0110 mg a.i.
					Maxim 4FS	fludioxonil	Seed	0.0037 mg a.i.
					Vibrance	sedaxane	Seed	0.0011 mg a.i.
					Cruiser 5FS	thiamethoxam	Seed	0.0730 mg a.i.
					QuickRoots	*Bacillus amyloliquefaciens* + *Trichoderma virens*	Seed	2.98×10^{-5} ml
Biological	BIOL-ST		–	+	QuickRoots	*Bacillus amyloliquefaciens* + *Trichoderma virens*	Seed	2.98×10^{-5} ml

[a]A subset of seed treatments was used to evaluate the response of crop canopy reflectance to soybean seed treatments. C-ST consisted of fungicide + insecticide + nitrogen in 2014, and fungicide + insecticide + biological in 2015.

Table 2
Soybean foliar treatments applied at pod set (R3 growth stage) in field studies during 2014 and 2015.

Treatment	Treatment Abbreviation	Subset Treatment Abbreviation[a]	Trade Name	Active Ingredient	Product Rate
None	N-PD	N-PD			
Fungicide	F-PD	F-PD	Stratego YLD	prothioconazole	36.8 g a.i. ha^{-1}
				trifloxystrobin	109.7 g a.i. ha^{-1}
Fertility	FERT-PD		UAN	28 – 0 – 0	28 kg N
			N-Rage	23-4-2-0.05Mn	2.8 kg N, 0.48 kg P_2O_5, and 0.24 kg K_2O kg ha^{-1}, and 6.1 g Mn ha^{-1}
			SoyGrow	0.5Mg-0.36Fe-2.6Mn-1.5Zn	7.0 g Mg, 5.6 g Fe, 37.9 g Mn, and 22.4 g Zn ha^{-1}
Fungicide + Fertility	FF-PD		Stratego YLD	prothioconazole	36.8 g a.i. ha^{-1}
				trifloxystrobin	109.7 g a.i. ha^{-1}
			UAN	28 – 0 – 0	28 kg N ha^{-1}
			N-Rage	23-4-2-0.05Mn	2.8 kg N, 0.48 kg P_2O_5, and 0.24 kg K_2O kg ha^{-1}, and 6.1 g Mn ha^{-1}
			SoyGrow	0.5Mg-0.36Fe-2.6Mn-1.5Zn	7.0 g Mg, 5.6 g Fe, 37.9 g Mn, and 22.4 g Zn ha^{-1}
Fungicide + Insecticide	FI-PD	FI-PD	Stratego YLD	prothioconazole	36.8 g a.i. ha^{-1}
				trifloxystrobin	109.7 g a.i. ha^{-1}
			Leverage 360	imidacloprid	36.8 g a.i. ha^{-1}
				β-cyfluthrin	24.5 g a.i. ha^{-1}
Fungicide + Insecticide + Fertility	FIN-PD	FIN-PD	Stratego YLD	prothioconazole	36.8 g a.i. ha^{-1}
				trifloxystrobin	109.7 g a.i. ha^{-1}
			Leverage 360	imidacloprid	36.8 g a.i. ha^{-1}
				β-cyfluthrin	24.5 g a.i. ha^{-1}
			UAN	28 – 0 – 0	28 kg N ha^{-1}
			N-Rage	23-4-2-0.05Mn	2.8 kg N, 0.48 kg P_2O_5, and 0.24 kg K_2O kg ha^{-1}, and 6.1 g Mn ha^{-1}
			SoyGrow	0.5Mg-0.36Fe-2.6Mn-1.5Zn	7.0 g Mg, 5.6 g Fe, 37.9 g Mn, and 22.4 g Zn ha^{-1}

[a] A subset of seed treatments was used to evaluate the response of crop canopy reflectance to soybean seed treatment.

with five incomplete blocks of six plots each in each whole plot block. Treatments consisted of five seed treatments and six foliar treatments per year (Tables 1 and 2). Slurries of all fungicide, insecticide and biological components of seed treatments were applied to the seed with a motorized cement mixer within one week of planting. The nitrogen component of the complete seed treatment in 2014 was applied to the soil surface between the soybean rows with a CO_2-pressurized tractor mounted sprayer using TeeJet fertilizer orifice plates (CP4916-20)

Table 3
Field characteristics and cultural practices of field trials at each location in Nebraska during 2014–2015.

Year	Location	Planting Date	Tillage	Soil Series (Soil Family)	Soil Characteristics[a]				
					CEC	OM	pH	MP3[b]	K
					$cmol_c\ kg^{-1}$	$g\ kg^{-1}$		—$mg kg^{-1}$—	
2014	Auburn	7 May	No-till	Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalfs)	17.0	3.3	6.0	8	232
	Belgrade	28 April	No-till	Hall silt loam (fine-silty, mixed, superactive, mesic Pachic Argiustolls)	15.0	3.0	5.9	54	377
	Shickley	6 May	Conventional	Crete silt loam (fine, smectitic, mesic Pachic Udertic Argiustolls)	8.0	1.5	6.7	11	220
	Snyder	6 May	No-till	Moody/Nora silty clay loam (fine-silty, mixed, superactive, mesic Udic Haplustolls)	23.0	3.9	6.4	7	327
2015	Alda	12 May	Conventional	Hall and Nord silt loam (fine-silty, mixed, superactive, mesic Pachic Argiustolls)	15.2	3.1	6.0	26	480
	Greenwood	29 April	No-till	Tomek silty clay loam (fine, smectitic, mexic Pachic Argiudolls)	15.1	3.6	6.9	40	424
	Holdrege	30 April	Conventional	Kenesaw silt loam (coarse-silty, mixed, superactive, mesic Typic Haplustolls)	20.5	2.4	7.5	94	581
	Wakefield	19 May	Turbo till	Belfore/Nora silty clay loam (fine, smectitic, mesic Udic Haplustolls)	20.6	4.2	6.2	72	423

[a] Soils sampled 0–20 cm at harvest.
[b] MP3, Mehlich 3 phosphorus extraction. Multiply MP3 values by 0.85 to get Bray 1P values.

(Spraying Systems Co., Wheaton, IL) on 38.1-cm spacing. The sprayer was pressurized to 138 kPa to achieve a spray volume of 46.8 L ha^{-1}. All foliar treatments were applied with a CO_2-pressurized tractor mounted sprayer using Turbo TeeJet TTI110015 nozzles (Spraying Systems Co., Wheaton, IL) spaced 50.8-cm apart. The sprayer was pressurized to 276 kPa to achieve a spray volume of 134.7 L ha^{-1}. Foliar treatments were applied when soybeans reached the R3 growth stage (pod set) (Fehr and Caviness, 1977).

Experimental plots were planted with a 4-row cone planter (76-cm row spacing) 10.7-m long at a rate of 346,000 seeds ha^{-1}. A late maturity group II soybean variety was planted across all locations in each year; Asgrow AG2733 (Monsanto Company, St. Louis, MO) in 2014 and Mycogen 5N286R2 (Dow AgroScience, Indianapolis, IN) in 2015. Field characteristics and cultural practices are reported in Table 3. Plant populations were assessed four weeks after planting and at harvest. Populations were determined by counting the total number of plants in two 3-m sections of row in each plot. Prior to harvest, the two middle rows of each plot were cut to a uniform length of 9.1 m. Plots were harvested with an Almaco plot harvester (Almaco, Nevada, IA) equipped with an onboard moisture sensor at maturity and all yields were adjusted to 13% grain moisture. The center 7.6 m of the two middle rows were harvested for yield to eliminate edge effects.

2.2. Crop canopy reflectance measurements

At regular intervals throughout the season, crop canopy reflectance measurements were recorded according to previously published methods (Mourtzinis et al., 2014) using a RapidSCAN CS-45 Handheld Crop Scanner (Holland Scientific, Lincoln, NE). The sensor was held approximately 1.5-m above the soybean canopy by the evaluator between the middle rows to collect reflectance data from the harvest rows. The evaluator walked between the harvest rows and logged data from the center 7.6-m of every plot. Readings were taken twice during the vegetative growth stages, at approximately V3 and V8 (Fehr and Caviness, 1977), and then at weekly intervals when the soybeans reached the R2 reproductive growth stage. Readings were stopped when soybeans reached full maturity. An average reflectance measurement in the red, RE, and NIR wavebands was recorded during each reading. Reflectance measurements in the NIR and RE wavebands were used to calculate the NDRE index as follows:

$$\mathrm{NDRE} = \frac{\rho_{\mathrm{NIR}} - \rho_{RE}}{\rho_{NIR} + \rho_{RE}}$$

where ρ_{NIR} = reflectance at 780 nm and ρ_{RE} = reflectance at 730 nm.

3. Statistical analyses

3.1. Cumulative reflectance

The NDRE values from all experimental units were plotted by days after planting (DAP) and day of year (DOY) to visualize changes in crop canopy reflectance over the course of the season (Fig. 2). The DOY was selected as the time parameter to evaluate the data based on the similar curves across all locations (R. Development Core Team, 2008).

The area under the disease progress curve (AUDPC), a calculation utilized in plant epidemiology, was used to characterize the cumulative reflectance of each experimental unit after each reading during the course of the growing season (Shaner and Finney, 1977). The calculation was adapted to utilize NDRE values and renamed the area under the reflectance progress curve (AURPC) as follows:

$$AURPC = \sum_{i=1}^{n} \left(\frac{Y_{i+1} + Y_i}{2}\right)(t_{i+1} - t_i)$$

where Y_i = NDRE value at the *ith* observation, t_i = day of the year at the *ith* observation, and n = total number of observations.

3.2. Correlations between cumulative reflectance and yield

Seed yield was statistically analyzed using analysis of covariance computed using the general linear model procedure (PROC GLM) in SAS version 9.4 (S.A.S Institute, 2016) where all treatment factors and locations were considered fixed, whole plot block nested in each location and incomplete block nested in each whole plot block were considered random effects, and cumulative reflectance was considered a covariate. Separate models were run to test AURPC calculated through the R3 and R6 growth stages as separate covariates. Seed yield was analyzed again with the general linear model without the covariate to determine the predicted values of seed yield and residuals of the model explained by the fixed and random effects. A correlation procedure (PROC CORR) was performed by location to determine the relationship between seed yield and the residuals with AURPC at R3 and R6. Seed yield and the residuals were then analyzed using the regression

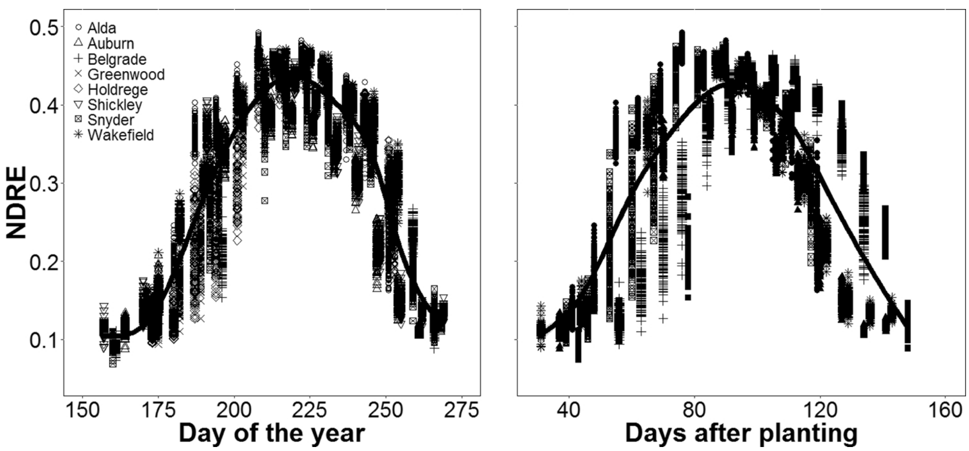

Fig. 2. Normalized difference red edge (NDRE) index values by location plotted by day of year and days after soybean planting in 2014 and 2015. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Average early season temperature and precipitation data of field trials at each location in Nebraska during 2014–2015.

		April				May				
		Max[a]	Min	Average	Departure	Max	Min	Average	Departure	Precipitation[b]
Year	Location	°C				°C				mm
2014	Auburn	18.4	4.1	11.3	−0.8	25.6	11.6	18.6	0.7	78
	Belgrade	16.6	−1.5	7.6	−1.2	22.0	6.3	14.2	−0.4	86
	Shickley	18.4	4.3	11.4	−0.1	24.8	10.3	17.6	0.5	74
	Snyder	16.7	1.9	9.3	−0.2	22.8	9.2	16.0	0.2	24
2015	Alda	13.8	−0.2	6.8	−3.1	22.7	8.7	15.7	−0.3	51
	Greenwood	12.9	−0.4	6.2	−2.4	20.2	9.2	14.7	−0.3	242
	Holdrege	13.8	−0.4	6.7	−3.5	22.4	8.7	15.6	−1.0	107
	Wakefield	14.0	2.0	8.0	−2.4	21.8	10.9	16.3	−0.4	109

[a] Max, average monthly maximum temperature; Min, average monthly minimum temperature; Average, average monthly temperature; Departure; departure of average monthly temperature from normal (1981–2010) average temperature. Data collected from the High Plains Regional Climate Center.
[b] Precip., precipitation totals from one week prior to planting to three weeks after planting. Data collected from the High Plains Regional Climate Center – CLIMOD (climod.unl.edu).

procedure (PROC REG) to determine the fit statistics associated with AURPC at R3 and R6.

3.3. Field productivity classification

The AURPC value calculated through the R3 and R6 growth stage for every plot was classified as either: TOP (top 25% of AURPC values for the given location), MIDDLE (middle 50% of AURPC values for the given location), or BOTTOM (bottom 25% of AURPC values for the given location). The same procedure was performed for the seed yield of every plot. The justification for this procedure was to create four management zones based on the productivity of each field location (Miao et al., 2006). The two middle management zones for AURPC and yield were combined to highlight only the lowest and highest productivity zones in the field. The logistic procedure (PROC LOGISTIC) in SAS version 9.4 was used to perform a multinomial regression analysis on the categorized AURPC and yield values as a combined experiment to determine the probability of predicting the correct yield class from the AURPC class. The same procedure was performed on AURPC values calculated through the R6 growth stage by location.

3.4. Canopy reflectance response to treatments

A subset of treatments was selected to evaluate treatment effects on crop canopy reflectance; three seed treatments [nontreated control (N-ST), fungicide (F-ST), and complete (C-ST)] and four foliar pod set treatments [nontreated control (N-PD), fungicide (F-PD), fungicide + insecticide (FI-PD), and fungicide + insecticide + fertility (FIN-PD)] (Tables 1 and 2). The C-ST treatment included the fungicide + insecticide + nitrogen treatment in 2014 and the fungicide + insecticide + biological treatment in 2015. The NDRE values measures at different growth stages were statistically analyzed using the generalized linear mixed model procedure (PROC GLIMMIX) in SAS Version 9.4 by location and as a combined experiment considering the alpha lattice field design (Barreto et al., 1996). All treatment factors and locations were considered fixed, while whole plot block and incomplete block nested in each whole plot block were considered random effects. Significant differences were determined based on a 0.05 level of significance. The NDRE values for the twelve reflectance measurements for each experimental unit were analyzed as repeated measures with seed treatment, foliar treatment, time, and location evaluated as fixed effects. The NDRE responses to seed treatments were analyzed by location for each reading.

4. Results

Air temperature during April and May (when field studies were planted) was generally cooler than normal (1981–2010). All locations in April and five locations in May deviated below normal recorded temperatures, available through the High Plains Regional Climate Center-CLIMOD, by −0.1 to −3.5 °C (Table 4). Three locations in May 2014 deviated positively from the normal recorded temperatures by 0.2–0.7 °C. Total precipitation measured from one week prior to three weeks after planting was greatest in 2015, and ranged from 50.5 to 241.8 mm across locations in 2015 and from 24.1 to 86.1 mm across locations in 2014. Weather conditions did not deviate from normal where yield responses would be expected from cool, wet environments.

Table 5
Simple and partial Pearson correlation coefficients (R) for the area under the disease progress curve (AURPC) values calculated through the R3 and R6 growth stages with seed yield and residuals.

	AURPC at R3				AURPC at R6			
	Simple R		Partial R		Simple R		Partial R	
Location	**R**	**P > F[a]**	**R**	**P > F**	**R**	**P > F**	**R**	**P > F**
Alda	0.741	****	0.271	**	0.787	****	0.327	****
Auburn	0.277	**	0.050	NS	0.464	****	0.135	NS
Belgrade	0.401	****	0.234	*	0.378	****	0.262	**
Greenwood	−0.184	*	−0.143	+	−0.138	NS	−0.145	+
Holdrege	0.711	****	0.099	NS	0.778	****	0.145	+
Shickley	0.301	***	0.166	+	0.387	****	0.222	*
Snyder	0.555	****	0.224	*	0.711	****	0.336	***
Wakefield	0.264	**	0.214	*	0.280	***	0.221	**

Significance from analysis of covariance: [a]NS = not significant; + = < 0.10; * = < 0.05; ** = < 0.01; *** = < 0.001; **** = < 0.0001.

Table 6
Statistics of linear regression function for seed yield and residuals by area under the reflectance progress curve (AURPC) at the R6 growth stage by location.

Year	Location	Model[1]	Parameter[2]	Estimate	Standard Error	P > F[3]	R^2
2014	Auburn	Yield	a	−319.1	721.8	****	0.2152
			b	165.7	29.1		
		Residual	a	−717.2	484.4	NS	0.0183
			b	29.0	19.5		
	Belgrade	Yield	a	705.7	907.3	****	0.1426
			b	177.3	40.0		
		Residual	a	−1526.1	517.4	**	0.0687
			b	67.3	22.8		
	Shickley	Yield	a	1071.1	909.9	****	0.1494
			b	144.8	31.8		
		Residual	a	−1674.1	676.9	*	0.0493
			b	58.5	23.7		
	Snyder	Yield	a	−1192.5	525.0	****	0.5052
			b	235.6	21.5		
		Residual	a	−1523.6	394.3	***	0.1127
			b	62.4	16.1		
2015	Alda	Yield	a	−11634.0	1031.4	****	0.6193
			b	574.6	38.5		
		Residual	a	−3117.6	770.8	****	0.1067
			b	116.4	28.8		
	Greenwood	Yield	a	5146.1	478.5	NS	0.0191
			b	−31.4	19.3		
		Residual	a	588.8	344.3	+	0.0211
			b	−23.7	13.9		
	Holdrege	Yield	a	1392.9	278.2	****	0.6056
			b	172.3	11.9		
		Residual	a	−390.4	227.9	+	0.0211
			b	16.7	9.7		
	Wakefield	Yield	a	−385.7	1502.6	***	0.0783
			b	190.5	56.2		
		Residual	a	−2866.94	1089.7	**	0.0488
			b	107.3	40.8		

[1] Two models tested for each location defined as: yield = seed yield; residuals = residuals from model accounting for all fixed and random effects.
[2] Parameters for linear regression defined as: a = intercept; b = AURPC at the R6 growth stage.
[3] Significance indicated by: NS = not significant; + = < 0.10; * = < 0.05; ** = < 0.01; *** = < 0.001; **** = < 0.0001.

Table 7
Probability of classifying yield into three management zones based on area under the disease progress curve (AURPC) calculations through the R3 and R6 growth stage.

	AURPC at R3						AURPC at R6					
Yield Class[a]	Bottom[b](SE)	P > F[c]	Middle (SE)	P > F	Top (SE)	P > F	Bottom (SE)	P > F	Middle (SE)	P > F	Top (SE)	P > F
Bottom	0.5127 (0.033)	****	0.1726 (0.017)	*	0.1441 (0.023)	****	0.5245 (0.033)	****	0.1767 (0.017)	+	0.1229 (0.021)	****
Middle	0.3729 (0.031)	*	0.5904 (0.022)	****	0.4407 (0.032)	NS	0.3771 (0.032)	****	0.5925 (0.022)	****	0.4322 (0.032)	NS
Top	0.1144 (0.021)	****	0.237 (0.019)	*	0.4153 (0.032)	****	0.09746 (0.019)	****	0.2308 (0.019)	+	0.4449 (0.032)	****

[a] Yield classification defined as: Bottom = lowest 25% of yield by location; Middle = middle 50% of yield by location; Top = top 25% of yield by location.
[b] AURPC classification defined as: Bottom = lowest 25% of AURPC by location; Middle = middle 50% of AURPC by location; Top = top 25% of AURPC by location.
[c] Significance indicated by: NS = not significant; + = < 0.10; * = < 0.05; ** = < 0.01; *** = < 0.001; **** = < 0.0001.

Table 8
Probability of classifying yield into three management zones based on area under the disease progress curve (AURPC) calculations through the R6 growth stage by location in 2014 and 2015.

	2014											
	Auburn			Belgrade			Shickley			Snyder		
Yield Class[a]	Bottom[b] (SE)	Middle (SE)	Top (SE)	Bottom (SE)	Middle (SE)	Top (SE)	Bottom (SE)	Middle (SE)	Top (SE)	Bottom (SE)	Middle (SE)	Top (SE)
Bottom	0.500 (0.091)	0.200 (0.052)	0.100 (0.055)	0.533 (0.091)	0.150 (0.046)	0.167 (0.068)	0.467 (0.091)	0.183 (0.050)	0.167 (0.068)	0.633 (0.088)	0.167 (0.048)	0.033 (0.033)
Middle	0.433 (0.090)	0.567 (0.064)	0.433 (0.090)	0.433 (0.090)	0.500 (0.065)	0.567 (0.090)	0.367 (0.088)	0.583 (0.064)	0.467 (0.091)	0.333 (0.086)	0.683 (0.060)	0.300 (0.084)
Top	0.067 (0.046)	0.233 (0.055)	0.467 (0.091)	0.033 (0.033)	0.350 (0.062)	0.267 (0.081)	0.167 (0.068)	0.233 (0.055)	0.367 (0.088)	0.033 (0.033)	0.150 (0.046)	0.667 (0.086)
2015												
	Alda			Greenwood			Holdrege			Wakefield		
Yield Class	Bottom (SE)	Middle (SE)	Top (SE)	Bottom (SE)	Middle (SE)	Top (SE)	Bottom (SE)	Middle (SE)	Top (SE)	Bottom (SE)	Middle (SE)	Top (SE)
Bottom	0.689 (0.087)	0.131 (0.043)	0.034 (0.034)	0.241 (0.079)	0.250 (0.056)	0.310 (0.086)	0.862 (0.064)	0.049 (0.028)	0.034 (0.034)	0.276 (0.083)	0.288 (0.059)	0.138 (0.064)
Middle	0.310 (0.086)	0.738 (0.056)	0.241 (0.079)	0.379 (0.090)	0.467 (0.064)	0.621 (0.090)	0.138 (0.064)	0.721 (0.057)	0.448 (0.092)	0.621 (0.090)	0.475 (0.065)	0.379 (0.090)
Top	0.000 (0.0000)	0.131 (0.043)	0.724 (0.083)	0.379 (0.090)	0.283 (0.058)	0.069 (0.047)	0.000 (0.000)	0.230 (0.054)	0.517 (0.093)	0.104 (0.057)	0.237 (0.055)	0.483 (0.093)

[a] Yield classification defined as: Bottom = lowest 25% of yield by location; Middle = middle 50% of yield by location; Top = top 25% of yield by location.
[b] AURPC classification defined as: Bottom = lowest 25% of AURPC by location; Middle = middle 50% of AURPC by location; Top = top 25% of AURPC by location.

Table 9
Growth stage and location where significant differences in individual normalized difference red edge (NDRE) index values were detected for early season seed treatments.

		Growth Stage			
Year	Location	Early Vegetative	Late Vegetative	R2	R3
2014	Auburn	NS[a]	*	****	**
	Belgrade	NS	NS	+	NS
	Shickley	NS	+	**	NS
	Snyder	NS	*	**	**
2015	Alda	NS	NS	+	NS
	Greenwood	***	**	**	**
	Holdrege	**	**	**	**
	Wakefield	NS	NS	NS	NS

[a] NS = no significant difference; + = < 0.10; * = < 0.05; ** = < 0.01; *** = < 0.001; **** = < 0.0001.

4.1. Correlation between cumulative reflectance and yield

Analyzing seed yield using the analysis of covariance with reflectance as a covariate indicated that cumulative reflectance values were significantly associated with yield at the R3 growth stage ($F = 40.01$, $p < 0.0001$) and the R6 growth stage ($F = 70.58$, $p < 0.0001$) combined over locations after accounting for the fixed treatment effects and random effects. The reflectance values were significantly correlated with yield ($p < 0.01$) for all locations except Greenwood (Table 5). Residuals obtained from the general linear model without the reflectance values as covariates were less correlated with yield and only significant ($p < 0.05$) at five of the eight locations using AURPC at R6 and four of the eight locations using AURPC at R3 (Table 5).

Regression analysis on seed yield was significant ($p < 0.0001$) for AURPC at R6 for all locations except for Greenwood, with the highest correlation at Alda ($R^2 = 0.6193$) (Table 6). Regression analysis on residuals demonstrated the relationship of the variance that was not accounted for by the treatment and random effects with AURPC at R6. Two locations (Alda and Snyder) were significant at the 0.001 level and three locations (Belgrade, Shickley, and Wakefield) were significant at the 0.05 level. The largest correlation between residuals and AURPC at R6 was at Snyder ($R^2 = 0.1127$).

4.2. Field productivity classification

The AURPC values were further used to predict soybean yield by using a classification scheme, whereby AURPC and yield values were classified as top 25%, middle 50%, or bottom 25% within a given location. This approach was used to determine if management zones could be established to identify the top and bottom producing areas of a production soybean field prior to harvest. A combined analysis and analysis by location were performed to determine the probability of a given yield class being associated with a given AURPC class. In the combined experiment, using AURPC at R6 resulted in slightly higher probabilities of predicting the bottom yield with the bottom AURPC and the top yield with the top AURPC than using AURPC at R3 by 0.012 and 0.030, respectively (Table 7). The AURPC at R6 also resulted in a slightly lower probability of incorrectly identifying the top yield with the bottom AURPC and the bottom yield with the top AURPC by 0.017 and 0.021, respectively.

The analysis by location of AURPC at R6 revealed that the Greenwood location was poorly classified using this method (Table 8). Greenwood also had the lowest correlation using the linear model. Wakefield had a low probability of correctly classifying the low yield class (0.276). Other locations correctly predicted the bottom yield with the bottom AURPC with probabilities ranging from 0.4667 (Shickley) to 0.8621 (Holdrege). Predicting the top yield class with the top AURPC was more variable as probabilities ranged from 0.2667 (Belgrade) to 0.7241 (Alda). The probability of an opposite classification, top yield with bottom AURPC or bottom yield with top AURPC, was also low among all locations excluding Greenwood. The probability of incorrectly classifying the bottom yield with the top AURPC ranged from 0.1667 (Belgrade and Shickley) to 0.3333 (Snyder). Alternatively, the probability of incorrectly classifying the top yield with the bottom AURPC ranged from 0.000 (Alda and Holdrege) to 0.1667 (Shickley).

4.3. Treatment effect on individual crop canopy reflectance readings

Individual reflectance measurements were evaluated based on the response to a subset of the seed and foliar treatments used in the complete experiment. A repeated measures analysis indicated a significant 3-way interaction between seed treatment x location x time influencing NDRE index values ($p = 0.0004$), but no influence of foliar treatments on NDRE reflectance. The NDRE responses to seed

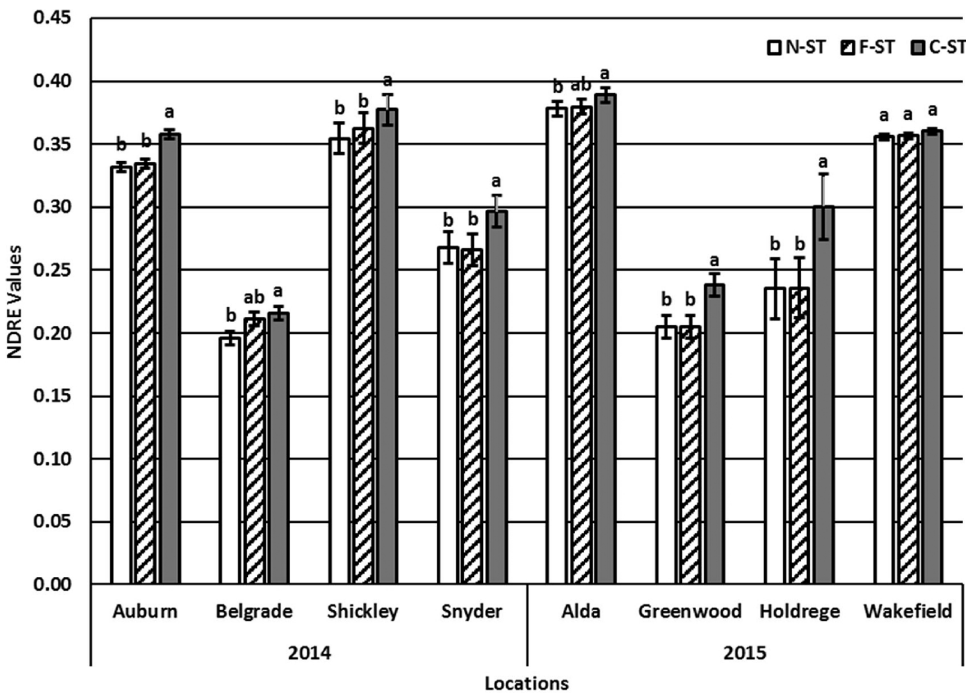

Fig. 3. Differences in normalized difference red edge (NDRE) index values by early season seed treatment at the R2 growth stage at each location. Different letters denote significant differences within locations at p = 0.05 (except Belgrade, p = 0.09). Seed treatments – CST [CruiserMaxx Advanced + Vibrance + nitrogen (2014) or CruiserMaxx Advanced + Vibrance + QuickRoots (2015)]; FST (ApronMaxx + Vibrance); NST (nontreated check). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 10
Pearson's linear correlation coefficients between normalized difference red edge (NDRE) index values and early soybean populations for each location and three evaluation times.

		Late Vegetative[a]			R2			R3		
Year	Location	R	R^2	P < |r|	R	R^2	P < |r|	R	R^2	P < |r|
2014	Auburn	0.2879	0.0829	0.0472	0.3661	0.1340	0.0105	0.2168	0.0470	0.1388
	Belgrade	−0.1000	0.0100	0.4994	0.0176	0.0003	0.9056	0.1780	0.0317	0.2263
	Shickley	0.5145	0.2647	0.0002	0.5310	0.2820	0.0001	0.3919	0.1536	0.0059
	Snyder	0.4797	0.2301	0.0006	0.3420	0.1170	0.0173	0.4025	0.1620	0.0046
2015	Alda	0.2019	0.0408	0.1688	0.1825	0.0333	0.2143	0.1341	0.0180	0.3635
	Greenwood	0.4647	0.2159	0.0009	0.5601	0.3137	< 0.0001	0.4863	0.2365	0.0005
	Holdrege	0.4835	0.2338	0.0006	0.4988	0.2488	0.0004	0.3565	0.1271	0.0139
	Wakefield	0.1459	0.0213	0.3279	−0.0502	0.0025	0.7376	−0.1686	0.0284	0.2572

[a] Three evaluation times were late vegetative (between V5 and V8 growth stages), the R2 (late flowering) growth stage, and the R3 (beginning pod set) growth stage.

treatments were evaluated by location at each evaluation time. Seed treatments influenced NDRE values at R2 at five locations ($p < 0.01$) and two locations ($p < 0.10$) (Table 9). Means comparison analysis at R2 indicated that at the five locations with a *p*-value less than 0.01, the C-ST treatment resulted in a greater NDRE value than both F-ST and N-ST. The NDRE values of the C-ST treatment were greater than the N-ST at the two locations, Belgrade and Alda, with a *p*-value less than 0.10 (Fig. 3).

Pearson's simple linear correlation coefficients between NDRE values and early soybean populations were calculated for each location and three evaluation times, early vegetative, R2, and R3 growth stages. All locations that had a significant seed treatment effect at the R2 growth stage ($p < 0.05$) (Table 9) had a significant correlation between NDRE reflectance and soybean population ($p = 0.05$) (Table 10). All correlations (r^2) were less than 0.3 except for the evaluation at R2 at the Greenwood location that had a coefficient of 0.3137. Therefore, we can conclude that the influence of soybean population on NDRE reflectance is present, but accounts for a small portion of the variability associated with reflectance.

5. Discussion

This study used a common calculation associated with plant pathology to provide more information about the season-long growth of soybean. The area under the disease progress curve (AUDPC) is used in epidemiology to quantitatively measure disease severity over time. This calculation was exploited to quantitatively measure accumulated reflectance over time, thereby providing information on the biomass and chlorophyll status of the soybean plots over the course of the season. Individual NDRE measurements were also evaluated to determine if seed and pod set treatments influenced soybean canopy reflectance at various times through the season.

The AURPC value calculated through the R3 and R6 growth stages were selected to determine their association with seed yield. A positive correlation was found between seed yield and both AURPC values at seven of the eight locations. However, this correlation was influenced by the fixed treatment effects and random effects accounted for by the experimental design, so residuals were obtained to determine the amount of variation in yield not associated with these effects. The correlation between the residuals and AURPC values was much less, but still significant ($p < 0.05$) for AURPC at R3 and R6 at four and five of the eight locations, respectively.

Creation of management zones was also used to determine the association between seed yield and AURPC. Characterizing the productivity of the field as low or high could be useful to producers for investigating potential problem areas in the field and making appropriate management decisions. A classification scheme identifying the yield and AURPC within a location as the top 25%, middle 50%, or bottom 25% was created. Probabilities were calculated for correctly identifying a yield class with an AURPC class, and conversely, incorrectly identifying a yield class with the opposite AURPC class. The results of this analysis were positive with high probabilities of correct classifications and low probabilities of incorrect classifications. This method should be investigated further to develop a process that could enable growers to identify low and high producing areas of their soybean fields prior to harvest.

From this study, it was found that the use of a crop canopy sensor could differentiate between seed treatments by using NDRE. Investigating the source of the variation revealed that the NDRE reading was partially correlated to soybean population, data that is commonly collected manually in small-plot soybean research. Determining soybean population involves evaluators, potentially several, counting individual soybean plants over a given distance and recording the number of plants. The process is time consuming and often biased based on the evaluator. The use of active crop canopy sensors provides more information than stand counts alone can provide on the influence of seed treatments on soybean growth and development, and should be evaluated as an alternative method to stand counts to save time and remove evaluator bias from the process.

No associations between NDRE and foliar treatments were found in this study. However, it should be noted that this study was investigating the use of foliar fungicide and insecticide products as part of a planned pod set (R3) application, and very little disease or insect pressure was present. Therefore, to evaluate the effectiveness of detecting foliar treatment effects on soybean growth and development, studies should be performed under controlled disease and insect pressures where responses to treatment are expected. This study indicates that the use of foliar treatments in the absence of heavy disease and insect pressure did not influence soybean canopy reflectance.

6. Conclusion

Gaining a better understanding of soybean canopy reflectance will help researchers and growers use crop sensing technology to help further soybean research and production. Although numerous vegetation indices are available for research, the NDRE index was used in this experiment because it can be calculated using the RapidSCAN CS-45 Handheld Crop Scanner, a commercially-available optical sensor. The use of the NDRE index provides the ability to use a vegetation index that can be used at higher canopy biomass and an active sensor eliminates the limitations inherent to passive sensors, especially regarding changes in intermittent cloud cover and timing of sensor readings. It also offers an alternative to make crop evaluations in an unbiased manner that is inherent to many data collection methods.

The RapidSCAN CS-45 was used to evaluate soybean canopy reflectance in a study evaluating the use of seed and foliar treatments to increase yield in Nebraska. The NDRE vegetation index was used for its relation to crop canopy biomass and chlorophyll content. Cumulative reflectance was calculated to provide a quantitative measure of reflectance over the growing season and named the area under the reflectance progress curve (AURPC). Using AURPC calculated through the R3 and R6 growth stages revealed a correlation between the reflectance values and seed yield. A novel classification method was used to identify the high and low producing soybean plots. The high probability of correctly classifying yield (same AURPC and yield class) and the low probability of incorrectly classifying yield (opposite AURPC and yield class) indicates that this method could be used to delineate management zones based on the potential productivity of a production soybean field that may require management prior to harvest. Additionally, individual NDRE readings at R2 were influenced by seed treatments and, upon further investigation, were correlated to early-season soybean populations. Further research is needed to validate the classification process for identifying management zones in production soybean fields and the ability to use the RapidSCAN sensor to evaluate physiological responses to soybean seed treatments. The methods proposed in this paper should be evaluated further using aerial or satellite based sensors equipped with RE and NIR wavebands to determine if the spatial resolution is adequate to create field level management zone maps using NDRE as the selected vegetation index.

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