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Final Project Report
Establishing the Effectiveness of Active Optical Sensors as a Tool for Soybean Research and Production
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Abstract

Approximately one-third of soybean yield gain is a result of improved agronomic practices, which includes disease and insect management. Treatments containing fungicide, insecticide, biological, and nutrient components were evaluated in Nebraska soybean fields during 2013 through 2015 to determine effects on soybean yield and profitability. The greatest yield (4.83 Mg ha^{-1}, p=0.019) was achieved with a complete seed and pod set treatment, but resulted in the second lowest calculated net return (US$151 ha^{-1}, p=0.019) after accounting for fixed and variable costs at a soybean market price of US$0.367 kg^{-1}. The most profitable treatment was the fungicide seed treatment followed by no pod set treatment (US$241 ha^{-1}, p=0.019). The use of pod set treatments in the absence of significant disease and insect pressure was not profitable in most instances.

Crop canopy reflectance was measured several times throughout the season during 2014 and 2015 to evaluate normalized difference red edge (NDRE) index to predict soybean productivity. The NDRE values were used to calculate a cumulative reflectance value through the R6 growth stage, defined as area under the reflectance progress curve (AURPC). The AURPC values and seed yield were classified as top 25%, middle 50%, or bottom 25% by location. Multinomial regression determined that bottom AURPC values correctly predicted bottom yield 52.5% of the time (p=0.033), but ranged from 46.7 to 86.2% by location. Misclassifications by incorrectly identifying a bottom yield within the top AURPC ranged from 0.0% to 16.7% by location. The AURPC offers a novel method to delineate management zones in soybean production fields.

Soybean canopy reflectance was also evaluated for the relationship between NDRE and soybean response to soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) infection. SCN-resistant and -susceptible varieties were planted in SCN-infested and non-infested sites during 2015 and 2016. Susceptible varieties yielded more than the resistant varieties at the non-infested sites by 245 kg ha^{-1} (p=0.004), and resistant varieties yielded more than the susceptible varieties at the SCN-infested sites by 340 kg ha^{-1} (p=0.0021). Measured NDRE values at R4 and R5 were different between resistant and susceptible varieties, but were not correlated with yield.

Publications

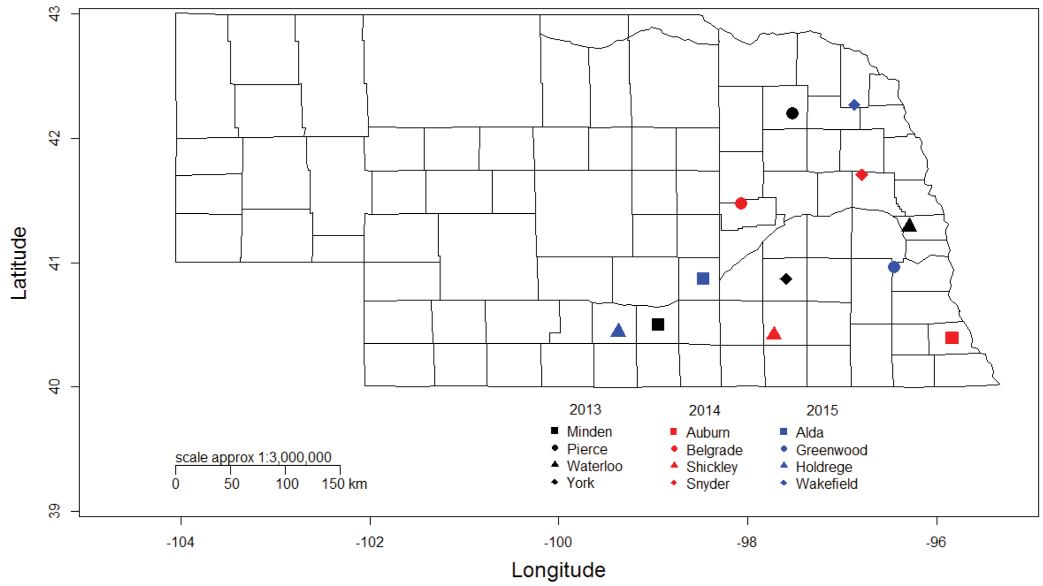
- Miller, J.J., Schepers, J.S., Shapiro, C.A., Arneson, N.J., Eskridge, K.M., Oliveira, M.C., and Giesler, L.J. (2017). Characterizing soybean vigor and productivity using multiple crop canopy sensor readings. Field Crops Research 216:22-31.
- Miller, J.J., Shapiro, C.A., Arneson, N.J., Eskridge, K.M., Oliveira, M.C., and Giesler, L.J. (2017). Agronomic and economic evaluation of common soybean seed and pod set treatments to increase yield and profitability in Nebraska. ***Submitted*** *to Agronomy Journal on July 156, 2017. Manuscript rejected after revision requested with need of greater revision to resubmit.*
- Miller, J.J. (2017). Investigating the use of active crop canopy sensors for soybean management in field research and production. *ETD collection for University of Nebraska - Lincoln*. AAI10272395. http://digitalcommons.unl.edu/dissertations/AAI10272395.

Experiment 1

Use of early-season seed and foliar pod set treatments containing fungicide, insecticide, biological, and nutrient components to increase soybean [*Glycine max* (L.) Merr.] yields has increased in Nebraska. Applications are often made prophylactically without pests at economic thresholds, but with anticipation of yield increases and economic returns. Although numerous studies have investigated the use of seed treatments or foliar treatments individually, the literature is sparse regarding the interaction of the two input classes on agronomic characteristics, yield, and economic returns. Economic analyses are becoming more important as market grain prices continue to remain volatile and profit margins become narrow. Analyzing the profitability of treatments, in addition to their yield benefit, will allow for the results of studies to be used to make better recommendations regarding soybean production. Therefore, the objectives of this study were to (i) quantify the effect of seed treatments on early-season populations, yield, and net revenue; (ii) quantify the effect of foliar treatments on disease and insect damage, yield, and net revenue; and (iii) determine if there is an interaction between seed treatments and foliar treatments on soybean yield and net revenue in an irrigated soybean production system.

This study was conducted at four locations each year between 2013 and 2015 in producers' fields across eastern Nebraska for a total of twelve environments (location x year) (**Figure 1**). Experimental plots were planted with a 4-row cone planter (76-cm row spacing) 10.7-m long. A late group II maturity soybean variety was selected each year and used across all locations; NK S28-K1 (Syngenta Seeds, Minneapolis, MN) in 2013, AG2733 (Monsanto Company, St. Louis, MO) in 2014, and Mycogen 5N286R2 (Dow AgroScience, Indianapolis, IN) in 2015. Prior to harvest, the two middle rows of each plot were cut to a uniform length of 9.1-m to eliminate edge effect. The two middle rows were harvested at maturity with an Almaco SPC40 specialized plot combine (Almaco, Nevada, IA) equipped with a two-row head and onboard moisture sensor. All yields were adjusted to 13% grain moisture.

Figure 1. Field trial locations across eastern Nebraska during 2013 through 2015.



The three early-season seed treatments evaluated in this study were: (i) fungicide seed treatment (F-ST: ApronXL at 0.011 mg a.i. seed^{-1}, Maxim 4FS at 0.0037 mg a.i. seed^{-1}, and Vibrance at 0.0011 mg a.i. mg seed^{-1}), (ii) complete seed treatment (C-ST: included F-ST components plus Cruiser at 0.073 mg a.i. seed^{-1}, and urea ammonium nitrate (UAN) at 16.8 kg N ha^{-1} applied at V2 in 2013 and 2014 or QuickRoots in 2015), and (iii) a nontreated control (N-ST). The four foliar treatments evaluated in this study were: (i) fungicide at pod set (F-PD: Stratego YLD), (ii) fungicide + insecticide at pod set (FI-PD: included F-PD plus Leverage 360), (iii) fungicide + insecticide + nutrient at pod set (FIN-PD: included FI-PD plus UAN, N-Rage®, and SoyGrow®), and (iv) a nontreated control (N-PD).

Interactions were observed between early-season seed treatments and foliar pod set treatments for yield and net revenue at all soybean market prices (**Table 1**). The FI-PD treatment yielded more than the F-PD and FIN-PD treatments by 121 and 202 kg ha^{-1}, respectively, when no seed treatment was used. When the F-ST treatment was applied, the FIN-PD treatment yielded more than the F-PD treatment by 139 kg ha^{-1}. There were no differences between the other foliar pod set treatments. The FIN-PD and FI-PD treatments yielded more than the N-PD treatment by 167 and 162 kg ha^{-1}, respectively, when the C-ST treatment was applied. These treatments were also the highest yielding treatments of all twelve treatment combinations evaluated. The difference between the C-ST and FIN-PD treatment combination and the N-ST and N-PD treatment combination was 243 kg ha^{-1}.

Table 1. Effect of three seed treatments by four foliar treatments on soybean yield and net revenue at three soybean market value prices across 11 locations (excludes Waterloo) during 2013 through 2015.

		Net Revenue ($ ha^{-1})†		
Treatment‡	**Yield (kg ha^{-1})**	**$0.367 kg^{-1}**	**$0.441 kg^{-1}**	**$0.514 kg^{-1}**

C-ST				
FIN-PD	4828 a§	151.12 e	507.93 c	862.32 cd
FI-PD	4823 a	193.60 bcd	550.02 abc	904.03 abc
F-PD	4724 abcd	170.90 dce	520.02 c	866.77 cd
N-PD	4662 cdefg	184.95 cde	529.43 c	871.59 cd
F-ST				
FIN-PD	4752 abc	161.47 de	512.66 c	861.48 cd
FI-PD	4681 bcdef	179.86 cde	525.82 c	869.44 cd
F-PD	4613 efg	168.56 cde	509.51 c	848.15 d
N-PD	4709 bcde	240.51 a	588.48 a	934.10 a
N-ST				
FIN-PD	4563 g	104.52 f	441.75 d	776.71 e
FI-PD	4771 ab	225.03 ab	577.59 ab	927.77 ab
F-PD	4646 defg	192.87 bcd	536.23 bc	877.26 bcd
N-PD	4585 fg	207.46 abc	546.29 abc	882.83 abcd

† Net revenue = (GSP * AY) – (VC + FC); GSP = grain sale price (US$ kg^{-1}), AY = actual yield (kg ha^{-1}), VC = variable costs (US$ ha^{-1}), and FC = fixed costs (US$ ha^{-1}).

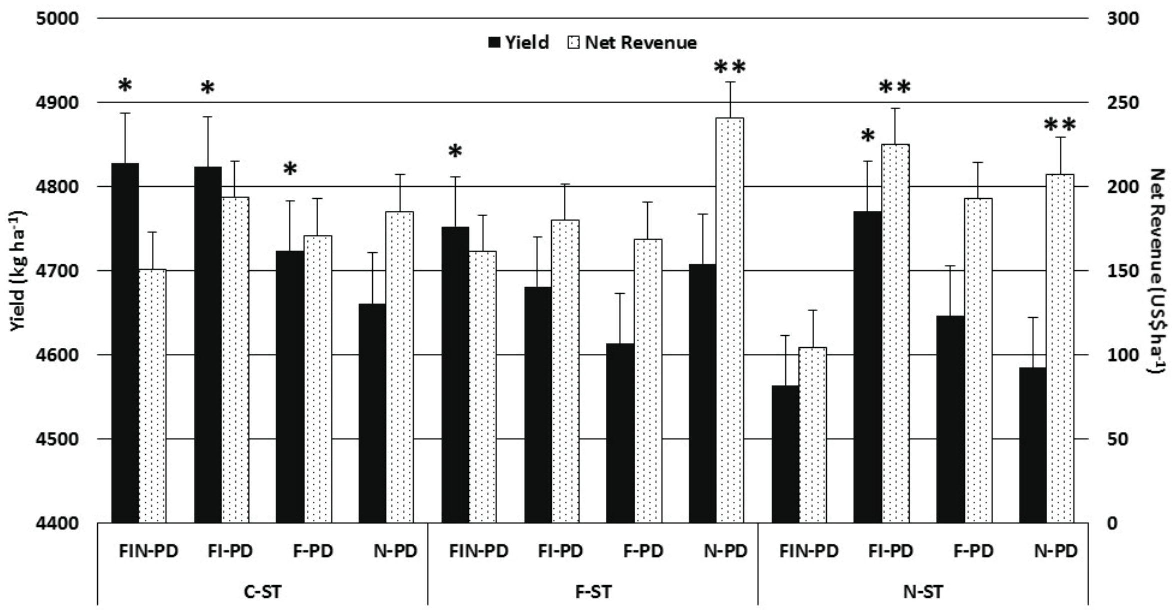
‡ Four foliar treatments listed below each seed treatment. Foliar pod set treatments – FIN-PD (Stratego YLD + Leverage 360 + UAN + N-Rage + Soy Grow); FI-PD (Stratego YLD + Leverage 360); F-PD (Stratego YLD); N-PD (nontreated check). Early-season seed treatments – C-ST (CruiserMaxx Advanced + Vibrance + nitrogen (2013 and 2014) or QuickRoots (2015)); F-ST (ApronMaxx + Vibrance); N-ST (nontreated check).

§ Treatment means within the same column followed by the same letter are not significantly by LSMEANS ($p \leq 0.10$).

At the lowest market grain price (US$0.367 kg^{-1}), the combination of the FIN-PD pod set treatment with both the C-ST and N-ST treatments were the least profitable with a net revenue of US$151 and US$104 ha^{-1}, respectively (**Figure 2**). The high yield of the C-ST x FIN-PD combination treatment was not large enough to compensate for the costs of the chemicals and application. The most profitable treatments at this market grain price were the combinations of the N-PD treatment with both the F-ST and N-ST treatments, and the FI-PD treatment with the N-ST treatment. At higher market grain prices, US$0.441 and US$0.514 ha^{-1}, most treatments were similar in net revenue, but five treatment combinations were significantly lower than the most profitable treatments: the FIN-PD and F-PD treatments with the C-ST treatment, the FIN-PD and F-PD treatments with the F-ST treatment, and the FIN-PD treatment with the N-ST treatment (**Table 1**). In all scenarios evaluated, the use of the FIN-PD treatment resulted in one of the lowest net revenues.

Figure 2. Yield and net revenue at the lowest soybean market grain price ($0.367 kg^{-1}) for all early-season seed and foliar pod set treatments. Foliar treatments – FIN-PD (Stratego YLD + Leverage 360 + UAN + N-Rage + Soy Grow); FI-PD (Stratego YLD + Leverage 360); F-PD (Stratego YLD); N-PD (nontreated check). Seed treatments – C-ST (CruiserMaxx Advanced + Vibrance + nitrogen (2013 and 2014) or QuickRoots (2015)); F-ST (ApronMaxx + Vibrance); N-

ST (nontreated check). * Denotes significantly highest yields at p=0.10; ** Denotes significantly highest net revenue for US$0.367 kg-1 at p=0.10.



Conclusion

The C-ST treatment increased early-season populations at six of the twelve locations and harvest populations at five. Although disease severity was low at all locations, foliar pod set treatments that contained a fungicide component did reduce brown spot severity. Early-season seed treatment and foliar pod set treatment combinations influenced yield and economic returns under three soybean market value scenarios. The treatment combination with the most inputs, C-ST x FIN-PD, resulted in the highest yield; however, when the costs of treatments were taken into consideration, the highest yielding treatment was not the most profitable at any market grain price evaluated. Because of the low disease and insect pest pressure, the results of this study should be used to determine the economic benefit of using early-season seed treatments and foliar pod set treatments under these conditions. The economic analysis concluded that the fungicide seed treatment with no pod set treatment (F-ST and N-PD) resulted in the greatest net revenue at all soybean market values. Therefore, this study suggests that the use of a fungicide seed treatment can be used to reduce early-season soybean mortality and increase yields in Nebraska soybean fields. However, the use of fungicides and insecticides to control foliar diseases and insects should be determined using an ET-based IPM approach. Although yield increases were observed, accounting for the costs of treatments indicated that no foliar treatment increased net revenue over the control. Fungicides and insecticides should be used when ETs are met to reduce off-target effects, decrease the risk of resistance build-up, and as shown in this study, ensure the greatest chance of an economic return.

Experiment 2

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) 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 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 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).

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.

This study was conducted at the locations of experiment 1 during 2014 and 2015. 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, 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:

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

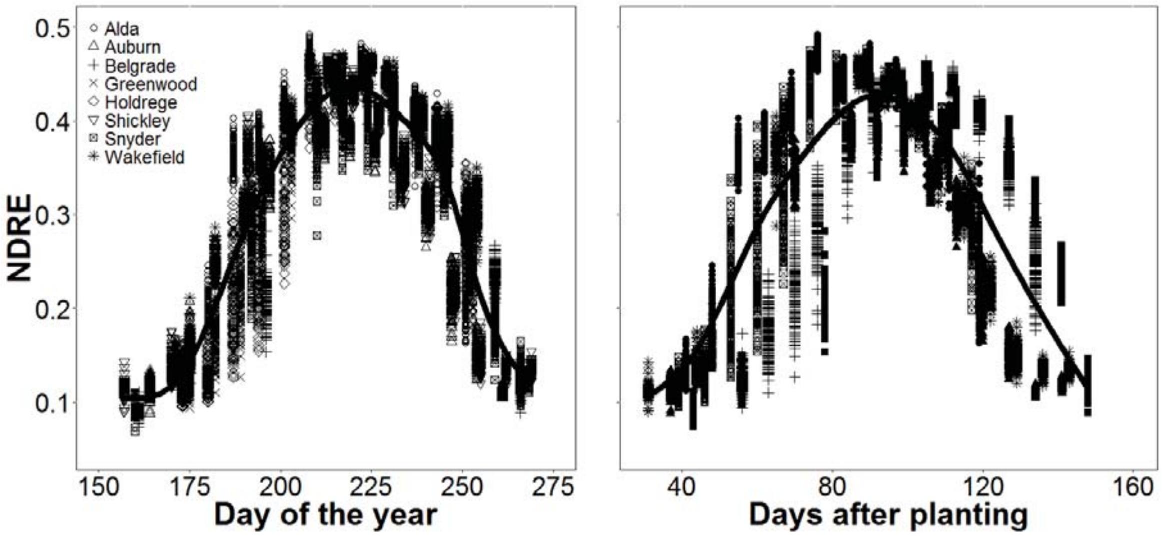
where ρ_{NIR} = reflectance at 780 nm and ρ_{RE} = reflectance at 730 nm.

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 (**Figure 3**). The DOY was selected as the time parameter to evaluate the data based on the similar curves across all locations. 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 *i*th observation, t_i = day of the year at the *i*th observation, and n = total number of observations.

Figure 3. Normalized difference red edge (NDRE) index values by location plotted by day of year and days after soybean planting in 2014 and 2015.



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.

The AURPC values were 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 2**). 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.

Table 2. 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†	**Bottom‡ (SE)**	**P>F§**	**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)	****

† 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.

‡ 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.

§ Significance indicated by: NS = not significant; + = <0.10; * = <0.05; ** = <0.01; *** = <0.001; **** = <0.0001

The analysis by location of AURPC at R6 revealed that the Greenwood location was poorly classified using this method (**Table 3**). 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).

Table 3. 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†	**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	0.500 (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)

† 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.

‡ 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.

Gaining a better understanding of soybean canopy reflectance will help researchers and growers use crop sensing technology to help further soybean research and production. 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 Handheld Crop Scanner 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.

Experiment 3

Soybean cyst nematode (SCN: *Heterodera glycines* Ichinohe) is an important pathogen of soybean [*Glycine max* (L.) Merrill] occurring globally wherever soybean is grown (Mitchum, 2016). It is consistently the most yield limiting pathogen of soybean in the United States, causing an estimated yield loss of 3.3 MMT in 2009 (Koenning and Wrather, 2010). Above-ground symptoms of SCN infection are often inconspicuous or absent altogether (Wang et al., 2003). The severity of symptoms is typically associated with the level of infestation, but soybean yield losses of up to 30% have been verified without the expression of detectable above-ground symptoms (Noel, 1992; Noel and Edwards, 1996; Wang et al., 2003; Young, 1996). Above-ground symptoms include general chlorosis and stunting that occurs in circular patches on the field (Niblack and Riggs, 2015). When visible symptoms are present, yield losses of 90% have been observed (Sinclair, 1982).

The use of SCN-resistant soybean varieties in combination with rotations to non-host crops is the most effective management strategy for controlling SCN (Niblack, 2005; Tylka, 2008). The genetic basis for host resistance remains narrow because of the reliance on PI 88788 and Peking (PI 548402) resistance sources in soybean breeding programs (Concibido et al., 2004). Due to the prevalence of cultivars with resistance derived from PI 88788, an increasing number of nematode populations across all soybean-producing areas have evolved resistance to this source. In Illinois, 70% of SCN populations were virulent to PI 88788 in 2005, up from 35% in 1991 (Niblack et al., 2008). A survey of 118 populations in Nebraska found that 46% of the populations were virulent on PI 88788 and 29.7% of the populations were virulent on Peking (Broderick, 2016). The objective of this study was to determine if the RapidSCAN sensor, used in experiment 2, could be used to detect differences in crop canopy reflectance relative to yield responses due to SCN infection using multiple resistant and susceptible soybean varieties.

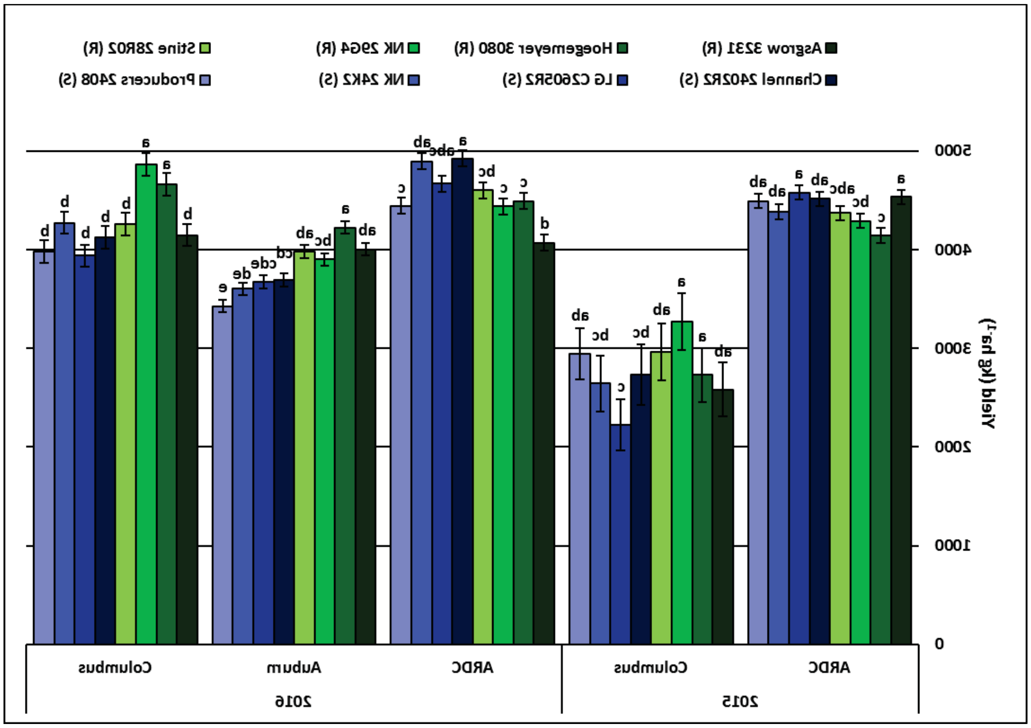
This study was conducted at one soybean cyst nematode (SCN)-infested site in 2014 near Columbus, NE and two SCN-infested sites in 2015 near Columbus and Auburn, NE. A non-infested site was also planted near Mead, NE at the Agricultural Research and Development Center (ARDC) each year to test SCN-resistant soybean variety yields in the absence of SCN for a total of five environments. Infested sites were selected based on preliminary composite soil samples from the location that were identified as having a minimum of 500 SCN eggs/100 cm3. The experimental design was a randomized complete block with eight replications per location. Treatments consisted of eight soybean varieties, four rated as SCN-susceptible and four rated as SCN-resistant with PI 88788 resistance. The four susceptible varieties were: Channel 2402R2 (Maturity group (MG) 2.4; Monsanto, St. Louis, MO), LG C2605R2 (MG 2.6; LG Seeds, Elmwood, IL), NK 24K2 (MG 2.4; Syngenta, Greensboro, NC), and Producers 2408 (MG 2.4; Producers Hybrids, Battle Creek, NE). The four resistant varieties were: Asgrow 3231 (MG 3.2; Monsanto, St. Louis, MO), Hoegemeyer 3080 (MG 3.0; Hoegemeyer Hybrids, Hooper, NE), NK 29G4 (MG 2.9; Syngenta, Greensboro, NC), and Stine 28R02 (MG 2.8; Stine Seed, Adel, IA).

Soil samples were collected from every plot immediately after planting and within one week of harvest to obtain initial and final SCN egg population densities, respectively. Ten 15 – 20 cm deep soil cores were collected from each plot and mixed in a bucket to obtain a composite sample. Samples were placed in a plastic bag and stored in a cooler maintained at 4°C until they were processed. All soil samples were processed in a laboratory according to previously published methods (Pérez-Hernández and Giesler, 2014). Reproduction factor (Rf) was calculated from each experimental unit as follows:

$$\mathrm{Rf} = \frac{\text{Final SCN population} + 40}{\text{Initial SCN population} + 40}$$

Crop canopy reflectance measurements were recorded at the R2 (full bloom), R4 (full pod), and R5 (beginning seed) growth stages (Fehr and Caviness, 1977), according to previously published methods (Mourtzinis et al., 2014) using a RapidSCAN CS-45 Handheld Crop Scanner. The sensor was held approximately 1.5 m above the soybean canopy by the evaluator between the two middle rows. The evaluator walked between the harvest rows and logged data from the center 4.5 m of every plot. 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.

Figure 4. Yield† of SCN-resistant (R) and -susceptible (S) soybean varieties by location during 2015 and 2016. Treatment means within the same location followed by the same letter are not significantly different by LSMEANS (p≤0.10). Error bars represent standard error within each location. † Harvest yield adjusted to 13% moisture.



The non-infested ARDC sites in 2015 and 2016, and the SCN-infested Columbus site in 2016 all yielded above the state average, producing 4,400 kg ha^{-1}, 4,600 kg ha^{-1}, and 4,300 kg ha^{-1}, respectively. The SCN-infested Columbus site in 2015 and Auburn site in 2016 yielded below the state average, producing 2,800 kg ha^{-1} and 3,800 kg ha^{-1}, respectively. There was a significant interaction between variety and location (p<0.10) for all response variables in the complete ANOVA analyzing variety, location, and variety x location (**Table 4**). Variety significantly influenced Rf values and seed yield. Seed yield means for each variety differed by location (**Figure 4**) and contrasts revealed that, as a group, resistant varieties yielded 340 kg ha-1 more than the susceptible varieties in SCN-infested sites (p=0.0041), and susceptible varieties yielded 245 kg ha-1 more than resistant varieties in the non-infested sites (p=0.0004) (**Table 5**).

Table 4. Significance (*P* values) of fixed effects and contrasts between soybean cyst nematode (SCN)- resistant (R) and -susceptible (S) varieties for normalized difference red edge (NDRE) index values at the R2, R4, and R5 growth stages, reproduction factor (Rf) † values and seed yield during 2015 and 2016.

		NDRE				
		R2	**R4**	**R5**	**Rf Value†**	**Yield (kg ha^{-1})**
Treatment	**df**			***P* > F**		
Variety	7	0.6221	0.4033	0.8005	<0.0001	0.0503
Location	4	<0.0001	<0.0001	<0.0001	NA‡	<0.0001
Variety x Location	28	0.0616	0.0020	0.0005	NA	0.0065

Contrasts R vs S						
Infested Site	1	0.3772	0.3755	0.1872	<0.0001	0.0021
Non-infested Site	1	0.0396	0.0468	0.0096	NA	0.0004

† Reproduction factor calculated by (final SCN population density + 40) / (initial SCN population density + 40)

‡ Rf values only calculated from SCN-infested locations

Table 5. Means for normalized difference red edge (NDRE) index values at the R4 and R5 growth stage and yield by variety and averaged across resistant (R) and susceptible (S) varieties within soybean cyst nematode (SCN)-infested and non-infested locations during 2015 and 2016.

	NDRE at R4 Growth Stage		**NDRE R5 Growth Stage**		**Yield (kg ha^{-1})**	
	Infested	**Non-infested**	**Infested**	**Non-infested**	**Infested**	**Non-infested**
Asgrow 3231 (R)	0.3769a†	0.4107a	0.4150a	0.4139bc	3606b	4303c
Hoegemeyer 3080 (R)	0.3608a	0.4137a	0.4050a	0.4190abc	4094b	4318c
NK 29G4 (R)	0.3702a	0.3918b	0.4028a	0.4095c	4034a	4366c
Stine 28R02 (R)	0.3635a	0.4187a	0.4036a	0.4202abc	3735ab	4485bc
Channel 2402R2 (S)	0.3712a	0.4185a	0.4019a	0.4236ab	3563b	4719a
LG C2605R2 (S)	0.3711a	0.4150a	0.4128a	0.4229ab	3413b	4625ab
NK 24K2 (S)	0.3619a	0.4152a	0.4047a	0.4287a	3639b	4642ab
Producers 2408 (S)	0.3678a	0.4168a	0.4086a	0.4219ab	3491b	4468bc
p-value	0.9454	0.0139	0.8523	0.1270	0.0337	0.0121
SE	0.010	0.005	0.007	0.005	164.9	97.1983
Resistant	0.3679a	0.4087b	0.4065a	0.4156b	3865a	4368b
Susceptible	0.3681a	0.4164a	0.4070a	0.4243a	3525b	4613a
p-value	0.9790	0.0541	0.9326	0.0091	0.0041	0.0004
SE	0.005	0.003	0.003	0.002	82.6	50.9

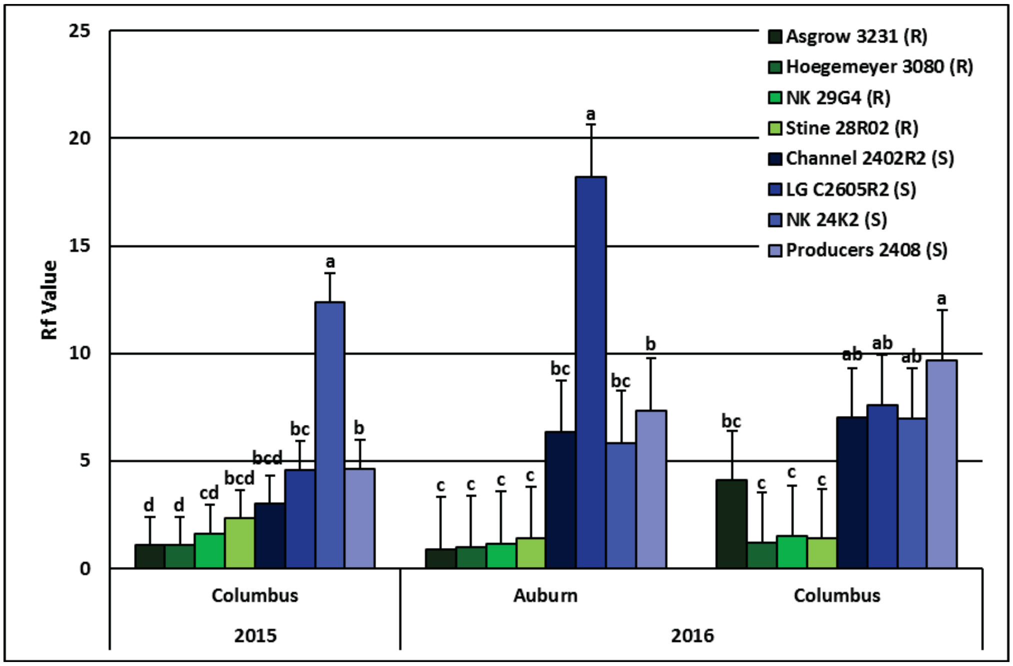
† Treatment means within the infested or non-infested location for each response variable followed by the same letter are not significantly different by LSMEANS ($p \leq 0.10$).

Mean NDRE values measured at the R4 and R5 growth stage were analyzed to detect differences in canopy reflectance between resistant and susceptible varieties during peak water use. The NDRE values at R4 and R5 were not significantly different between varieties in the SCN-infested sites. At the R4 growth stage, varieties significantly influenced NDRE in the non-infested sites (p=0.0139) primarily because of a low value observed for the variety NK 29G4. The NDRE values at R4 were the similar for all other varieties, and the NDRE value at R5 was not significantly different between varieties in the non-infested sites (p=0.1270). Contrasting resistant and susceptible varieties in the non-infested sites did show differences in NDRE at both R4 (p=0.0541) and R5 (p=0.0091) (**Table 5**). Susceptible varieties had higher NDRE values at both times by an average of 0.0077 at R4 and 0.0087 at R5. The NDRE values at R4 and R5 were not significantly different between resistant varieties.

The Rf value was higher for the susceptible varieties than the resistant varieties in the three SCN-infested sites (p<0.0001). Susceptible varieties averaged an Rf of 5.2 at Columbus in 2015 and 9.4 and 7.8 in Auburn and Columbus in 2016, respectively (**Figure 5**), while resistant varieties averaged 1.6 at Columbus in 2015 and 1.1 and 2.1 in Auburn and Columbus in 2016, respectively. The highest Rf value for a resistant variety was 4.1, observed in Asgrow 3231 at the

Columbus site in 2016. This illustrates the variability in efficacy of the resistance source PI 88788, especially in the presence of different SCN populations.

Figure 5. Reproduction factors (Rf)[†] of SCN-resistant (R) and -susceptible (S) soybean varieties in SCN-infested sited during 2015 and 2016. Treatment means within the same location followed by the same letter are not significantly different by LSMEANS (p≤0.10). Error bars represent standard error within each location. [†] Reproduction factor calculated by (final SCN population density + 40) / (initial SCN population density + 40).



The general linear model revealed that neither the NDRE value at R4 (F=0.03, p=0.8735) or R5 (F=0.91, p=0.3426), nor the initial SCN density (F=0.01, p=0.9431) accounted for variation in yield after accounting for variation attributed to the fixed and random effects. An initial correlation analysis indicated a significant relationship between seed yield and NDRE values at R4 (r=0.310, p<0.0001), NDRE values at R5 (r=0.534, p<0.0001), and initial SCN density (r= -0.432, p<0.0001). However, it was determined in Chapter 4 that NDRE values can differ significantly between locations. Subsequently running the correlation analysis by location revealed that there was only a significant correlation (r=0.342, p=0.0057) between NDRE at R5 and yield at the Columbus location in 2016, and initial SCN density was not correlated with yield at any location (**Table 6**).

Table 6. Pearson correlation coefficients (R) for the normalized difference red edge (NDRE) index at the R5 growth stage and log-transformed initial soybean cyst nematode (SCN) densities with seed yield by location.

		NDRE at R5		SCN Density	
Year	**Location**	**R**	**P>F[†]**	**R**	**P>F**
			Yield		

2015	Columbus	0.125	NS	-0.151	NS
2016	Auburn	0.101	NS	0.272	NS
	Columbus	0.342	**	0.083	NS

† NS = not significant; + = <0.10; * = <0.05; ** = <0.01; *** = <0.001; **** = <0.0001

Soybean breeding programs are vital to improving soybean yield potential, which includes improving resistance to diseases such as SCN. Most SCN-resistant varieties currently available derive resistance from the same source, PI 88788. However, SCN populations across the United States are evolving resistance to this source and breeding efforts will need to focus on increasing the diversity of resistance sources in the future. This study examined the ability to use the RapidSCAN active crop canopy sensor to evaluate the physiological response of SCN-resistant and -susceptible soybean varieties to SCN infection. The NDRE vegetation index was used for its relation to crop canopy biomass and chlorophyll content, which is known to be related to the overall health of the plant. The yield of susceptible varieties was higher in the non-infested sites and the yield of resistant varieties was higher in SCN-infested sites in this study, indicating the impact SCN resistance can have on soybean yield when SCN is present. The NDRE value at R5 and initial SCN density were analyzed to determine if they were correlated with seed yield. The NDRE at R5 was only correlated with seed yield at one location and initial SCN density was not correlated with seed yield at any location. Therefore, the use of crop canopy sensors to measure NDRE does not appear to be a useful method for screening soybean varieties for their yield response to SCN infection. Alternative regions of the electromagnetic spectrum, including the thermal wavebands, should be evaluated to determine if they account for more of the yield variation caused by SCN infection in resistant and susceptible varieties. Other methods, including chlorophyll fluorescence, should also be considered as they have been useful in detecting *Heterodera* spp. in other crops (Schmitz et al., 2006). Under conditions where water is limited in August during pod elongation and pod fill (R3 – R6 growth stages), the use of NDRE may prove to be more beneficial in screening varieties when physiological responses to SCN infection would be greater.

General Conclusions

Although crop canopy sensors have been primarily used in grass crops, such as corn and wheat, the results of Experiment 2 show that soybean canopy reflectance provides useful information on the yield potential and physiological status of the soybean plant under low stress environments. Using the NDRE index allows researchers to use reflectance values later in the season when crop canopy biomass is larger, and the use of cumulative reflectance is an alternative to using single point reflectance readings. Because Experiments 1 and 2 were conducted in environments of low disease and insect pressure, more research is needed to evaluate the relationship between cumulative reflectance and soybean physiology in different environments. Measuring crop canopy reflectance of soybeans exposed to different levels of disease and insect pressure and nutrient and water stress will provide more information on how crop canopy reflectance changes under varying conditions. The sensor was not effective at detecting differences in the response of varieties to SCN infection. Alternative wavebands and sensing techniques should continue to be explored to determine if other characteristics of the soybean canopy can be detected using remote sensing. Additionally, remote sensing alternatives, such as aerial- and satellite-based sensors equipped with the red edge (RE) and near infrared (NIR) wavebands, should be evaluated to determine if they have the spatial resolution to calculate AURPC to create field-level management zones. With continued research and investigation, crop canopy reflectance could provide a valuable tool to improve soybean yield and productivity with site-specific management.

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