HYPERSPECTRAL REMOTE SENSING FOR DETECTION OF RHIZOCTONIA CROWN AND ROOT ROT IN SUGARBEET

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The soilborne fungus *Rhizoctonia solani* AG-2-2 causes Rhizoctonia crown and root rot (RCRR) of sugarbeet. The pathogen is becoming common and widespread in sugarbeet-growing regions of Minnesota and North Dakota because of increased acreage of soybean, edible bean and corn, which are also infected by *R. solani* AG-2-2 (5, 23). Thus, inoculum of the fungus is building up in soil and contributing to further outbreaks.

Severity of RCRR typically is assessed by a visual rating scale based on the amount of rot on the taproot. This traditional visual rating system, however, is destructive because entire plants are removed from soil. Furthermore, visual disease assessments are subjective in nature and affected by differences between individuals rating roots caused by fatigue, bias, and human error (13).

Remote sensing is an alternative method to non-destructively assess plant diseases rapidly, repeatedly, and over a large area without physical contact with the sampling unit (e.g., sugarbeet foliage) (13). It is based on measuring reflectance of electromagnetic radiation from a subject of interest, primarily in the visible (390-770 nm), near infrared (770-1300 nm), mid infrared (1300-2500 nm), and thermal infrared (2.5-15 µm) ranges (13). Instruments may collect either hyperspectral or multispectral reflectance data. Hyperspectral sensors measure reflectance contiguously as a series of narrow wavelength bands while multispectral sensors measure reflectance at a few wide bands separated by segments where no measurements are taken (17). Hyperspectral and multispectral wavelength bands obtained for plants typically are used to calculated vegetation indices that provide pertinent information (e.g., chlorophyll content) or to correct for background interference from soil or the atmosphere (21).

Remote sensing technology has been applied to the detection of numerous crop diseases, including Cercospora leaf spot (19) and Rhizomania (20) of sugarbeet. Aboveground symptoms of RCRR, including yellowing of foliage and sudden wilting of leaves, would be the basis for remote detection of this disease, but it also may be possible to detect stress in the plants before visible wilting occurs. Reduced photosynthesis rates or water content in sugarbeet plants could produce changes detectable with remote sensing instrumentation, but not the naked eye. Laudien et al. (10, 11) conducted research to determine the potential of remote sensing to detect RCRR, but the authors focused more on the distinction between healthy and unhealthy plants, rather than on the disease. They detected RCRR at the end of the growing season but did not address population of AG 2-2 (IIIB or IV), early-season detection of the disease, or the relationship of reflectance to severity of RCRR. Early detection of RCRR and/or the ability to assess disease severity based on remote sensing will allow assessment of entire fields for disease management.

OBJECTIVES

Our objectives were to 1.) assess the potential for ground-based, hyperspectral remote sensing to detect RCRR and 2.) identify wideband and narrowband vegetation indices optimal for correlation with visual RCRR ratings.

MATERIALS AND METHODS

Field trial. A field trial site (193 x 215 ft) was established at the University of Minnesota, Northwest Research and Outreach Center in Crookston, MN. Prior to planting, the trial was fertilized with 30 lb of nitrogen A^{-1} based on soil fertility tests and cultivated to clear soybean debris. On May 21, 2008, the trial was planted with sugarbeet varieties ‘Van der Have 46531’ and ‘Hilleshog 3035’ (susceptible and partially resistant to RCRR, respectively). A six-row planter sowed seed at a 1-inch depth in the two middle rows at a 1.9-inch spacing, with the other rows at a 2.25-inch spacing. Spacing between seeds was narrower in the middle rows than in the outside rows to ensure adequate plant populations. The insecticide terbufos (1.5 lb product A^{-1} Counter®, BASF, Ludwigshafen, Germany) was applied at planting to control sugarbeet root maggot. There were 16 plots replicated four times in a randomized block design; each plot was 11 x 35 ft. All rows were thinned to 7-inch spacing on June 26.

Near canopy closure (July 10-11), plots of both varieties were inoculated with *R. solani* AG 2-2 IIIB (isolate 87-36-4) at seven rates of inoculum delivered on either infested, ground barley grain (16, 24, 32, 40 or 48 g per row) or corn kernels (one-half or two whole kernels). The fungus was grown on sterilized barley grain for 3 weeks, dried, and ground in a Wiley mill (#3 round-hole screen, 1/8-inch mesh) as described by Ruppel et al. (15). Ground inoculum was applied in a band over the middle four rows of each plot with a Gandy granule applicator (setting number 30, a standard method of inoculation) with two 0.65 mile per hour passes. Inoculum also was grown on whole corn kernels (4). All roots in the center four rows per plot were hand-inoculated by scraping soil from the tap root, placing a *R. solani*-infested corn kernel directly on the root surface (~1 inch below the crown), and re-covering with soil. There were non-inoculated controls for both varieties. Plots were deep-cultivated immediately after inoculation to throw soil into the crown and favor development of disease. To allow each 35-ft plot (six rows wide) to be measured for spectral reflectance as well as disease severity, a flag marked division of the plots into a 20-ft length for collection of spectral data, with the remaining 15-ft length for assessing RCRR.

Spectral analysis. Reflectance data were acquired with a FieldSpec® FR (Analytical Spectral Devices, Inc., Boulder, CO) hand-held spectroradiometer. This hyperspectral spectroradiometer is composed of three separate spectrometers in the same enclosure: Visible and Near Infrared (VNIR) and Shortwave Infrared 1 and 2 (SWIR1 and SWIR2). The instrument has a sampling interval of 1.4 nm for the 350 to 1000 nm region and 2 nm for the 1000 to 2500 nm region. The spectral resolution for the 350 to 1000 nm region is 3 nm and for the 1000 to 2500 nm region is 10 nm. The field of view is 25°. Measurements were taken on clear, sunny days between 1100 and 1500 h Central Standard Time. The instrument was calibrated with a Spectralon white reference panel every 15 minutes while readings were being obtained. The panel reflects close to 100% of all incident radiation, and reflectance values are calculated as a ratio of reflected radiation to incident radiation. Field measurements were taken at 3.5 ft above the sugarbeet canopy at nadir. This allowed for a 50.8 cm-diameter pixel size on the canopy over the center two rows of each plot. Three measurements were taken in the first 20 ft of each plot at roughly 6 ft intervals. Reflectance measurements were made on July 25 and 31, August 7 and 18, and September 3. Measurements from July 31 were unusable and not included in statistical analyses. The four remaining sets of spectral measurements were taken at approximately 2-week intervals. Soil reflectance was measured at varying moisture levels on July 8, 10, and 25 as a background reference.

Disease assessments. Severity of RCRR was measured bi-weekly at, or near, time of spectral reflectance measurements. Tap roots of 10 randomly selected plants were removed from the 15-ft section of each plot. Disease ratings for RCRR on a portion of an inoculated plot are not significantly different from the whole plot (3), and this allowed spectral measurements to be obtained from the undisturbed 20-ft section of each plot. Tap roots were rated for RCRR with a 0 to 7 scale: 0 = clean root with no visible symptoms; 1 = root with superficial, scattered, scurfy, or non-active lesions; 2 = root with shallow rot, dry rot cankers, or active lateral lesions affecting ≤ 5% of the surface; 3 = root with deep dry rot cankers at the crown or with extensive lateral lesions affecting 6 - 25% of the surface, usually with cracks or cankers; 4= root with extensive rot and cankers on 25 - 50% of the upper half with lesions up to 5-mm deep; 5 = root with ≥ 50% of the surface blackened and rot extending into interior tissue; 6 = root surface entirely blackened except for the extreme tip; and 7 = root 100% rotted and foliage dead. Ratings of the 10 beets were averaged for each plot at each assessment date.

Data analysis. Average reflectance in the green, red, and near-infrared ranges was calculated to be analogous to standard color infrared (CIR) digital photography bands. Several broadband vegetation indices were calculated from these three reflectance ranges and were correlated with RCRR disease ratings: the normalized difference vegetation index (NDVI, 8), optimized soil adjusted vegetation index (OSAVI, 14), simple ratio vegetation index (SRVI, 22), difference vegetation index (DVI, 9), and green normalized difference vegetation index (GNDVI, 7). Narrowband vegetation indices also were assessed for correlation with RCRR disease ratings to make full use of the high spectral resolution that hyperspectral sensors measure. The narrowband vegetation indices used were the pigment specific simple ratios for chlorophyll *a* and chlorophyll *b* ($PSSR_a$ and $PSSR_b$, 2), red-edge vegetation stress index (RVSI, 12), leaf water index (LWI, 16), photochemical reflectance index (PRI, 6), modified spectral ratio (mSR, 18), and

Table 1. Vegetation indices used in this study and their method of calculation.

Vegetation index	Formula[z]	Reference
GREEN (reflectance in green range)	$R_{548\text{-}563}$	
RED (reflectance in red range)	$R_{668\text{-}683}$	
NIR (reflectance in the near infrared range)	$R_{898\text{-}913}$	
DVI (difference vegetation index)	NIR-RED	9
SRVI (simple ratio vegetation index)	NIR/RED	22
NDVI (normalized difference vegetation index)	(NIR-RED)/(NIR+RED)	8
OSAVI (optimized soil adjusted vegetation index)	(NIR-RED)/(NIR+RED+0.16)	14
GNDVI (green normalized difference vegetation index)	(NIR-GREEN)/(NIR+GREEN)	7
$PSSR_a$ (pigment specific simple ratio, chlorophyll *a*)	R_{800}/R_{680}	2
$PSSR_b$ (pigment specific simple ratio, chlorophyll b)	R_{800}/R_{635}	2
RVSI (red-edge vegetation stress index)	$(R_{714}+R_{752})/2–R_{733}$	12
LWI (leaf water index)	R_{1300}/R_{1450}	16
mSR (modified spectral ratio)	$(R_{750}\text{-}R_{445})/(R_{705}+R_{445})$	18
PRI (photochemical reflectance index)	$(R_{570}\text{-}R_{531})/(R_{570}+R_{531})$	6
NDRE (normalized difference red edge)	$(R_{790}\text{-}R_{720})/(R_{790} + R_{720})$	1

[z] Reflectance at nanometers indicated (548-563, 668-683, and so on).

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normalized difference red edge (NDRE, 1). Equations for these broadband and narrowband vegetation indices are in Table 1. The relationship between disease ratings and each reflectance range or vegetation index was assessed with polynomial regression analysis using Microsoft Excel. Linear, quadratic, cubic, or quartic models were assessed and selected for each reflectance range and vegetation index by visual assessment and comparison of R^2 and *P*-values. Optimal vegetation indices were selected based on R^2 and *P*-values of the selected model for each index.

RESULTS

Significant RCRR developed in all inoculated plots, beginning about 2 weeks after inoculation for heavier treatments (e.g., two corn kernels with the susceptible variety). A wide range of disease ratings were obtained at each assessment date. For the susceptible variety, RCRR ratings ranged from 0.25 to 4.4, 0.6 to 6.75, 0.8 to 7, and 0.9 to 7 on July 25, August 7 and 18, and September 3, 2008, respectively. For the partially resistant variety, RCRR ratings ranged from 0.3 to 3.45, 0.6 to 6.65, 1.1 to 6.45, and 1.2 to 6.9 on July 25, August 7 and 18, and September 3, 2008, respectively. Disease values for RCRR were uniformly high for both varieties when inoculated with one-half or two whole *R. solani*-infested corn kernels at all assessment dates, but when disease was less severe, the partially resistant variety had less RCRR than the susceptible variety (data not shown).

Increases in RCRR ratings were associated with increased reflectance in the visible green (495-570 nm) and red (620-750 nm) ranges, decreased reflectance in the near-infrared (770-1300 nm) region, and increased reflectance in the mid-infrared region (1300-2500 nm). Examples of hyperspectral signatures for plots with different RCRR disease severities are shown in Figure 1. Reflectance is plotted along the electromagnetic spectrum from 350 to 2500 nm as a proportion of reflected electromagnetic radiation to incident electromagnetic radiation. Each plot has a different spectral signature based on its canopy reflectance. Variations between these spectral signatures for different plots may be due to RCRR disease. Spectral signatures of plots with maximum RCRR severity (disease rating = 7) are closely associated with the soil line. At the canopy level, spectral responses are influenced by three main factors: the physiological state of the plants, the extent of canopy or leaf cover, and the spectral characteristics of the soil. When sugarbeet plants are dying, the canopy collapses, and canopy reflectance is influenced more by the soil background.

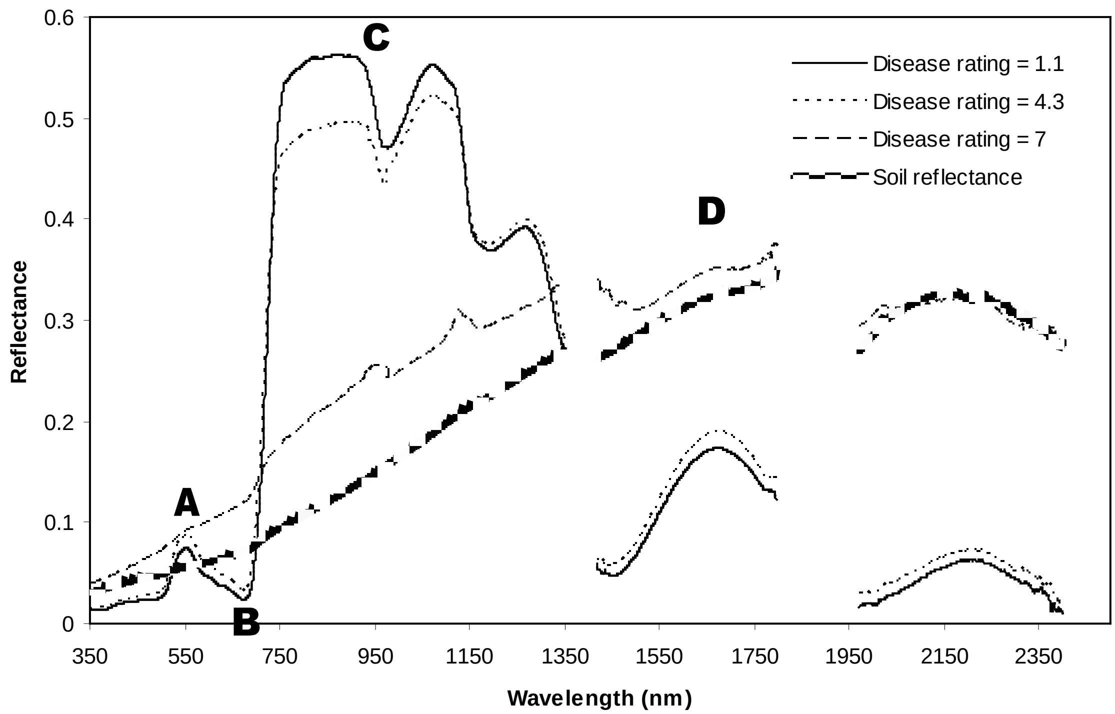

Fig. 1. Examples of spectral signatures for soil background reflectance and sugarbeet canopy reflectance in plots with Rhizoctonia crown and root rot disease ratings of 1.1, 4.3, and 7 for the partially resistant variety (data collected August 18, 2008) where **A.)** is green reflectance (495-570 nm), **B.)** is red reflectance (620-750 nm), **C.)** is near infrared reflectance (770-1300 nm), and **D.)** is mid infrared reflectance (1300-2500 nm).

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Reflectance in the red and near infrared ranges and all vegetation indices assessed yielded statistically significant (P <0.001) polynomial regression models when plotted against disease ratings for RCRR (data not shown). The models for each reflectance range and vegetation index exhibited a similar pattern. Reflectance or vegetation index values tended to stay relatively constant until RCRR disease severity values approached 4.5 for the susceptible variety and 5.5 for the partially resistant variety. Sugarbeet plants with these high RCRR disease ratings have extensive rot and cankers on 25-50% of the root surface, and foliage is beginning to wilt and/or show chlorosis.

Because P-values were comparable for most reflectance ranges and vegetation indices tested, optimal vegetation indices were primarily selected based on R^2 values. The optimized soil adjusted vegetation index (OSAVI) was one of the best wideband vegetation indices assessed in relation to severity of RCRR on both the partially resistant (R^2 = 0.7756, Fig. 2A) and susceptible (R^2 = 0.8602, Fig. 2B) varieties. The modified spectral ratio (mSR) was one of the best narrowband vegetation indices assessed in relationship to RCRR rating on the partially resistant (R^2 = 0.7132, Fig. 3A) and susceptible (R^2 = 0.6991, Fig. 3B) varieties. The pigment specific simple ratio for chlorophyll *a* ($PSSR_a$) also was one of the better narrowband vegetation indices that related to severity of RCRR on the partially resistant (R^2 = 0.7269, Fig. 4A) and susceptible (R^2 = 0.6239, Fig. 4B) varieties. The $PSSR_a$ and mSR indices appeared to allow for earlier detection of RCRR than the OSAVI. Differences were detected in $PSSR_a$ and mSR indices prior to the onset of severe wilting, when reflectance becomes a mixture of soil background and canopy reflectance.

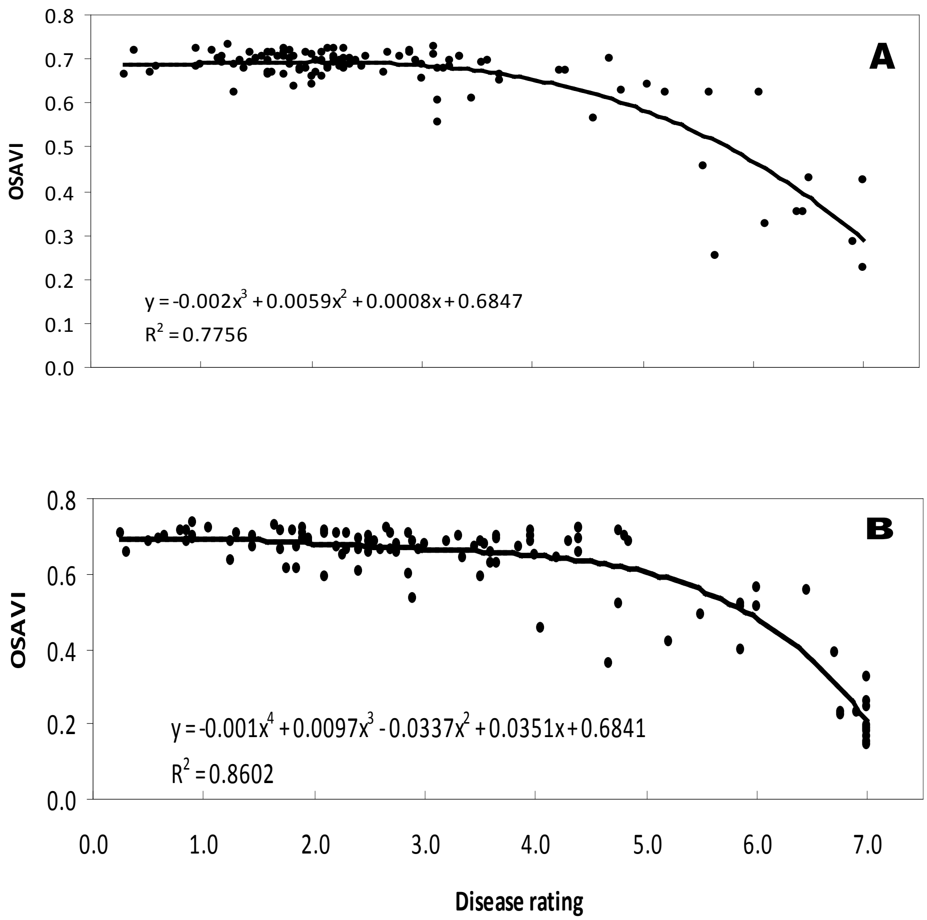

Fig. 2. Optimized soil adjusted vegetation index (OSAVI) values for a **A.)** partially resistant and **B.)** susceptible variety of sugarbeet plotted against ratings for Rhizoctonia crown and root rot (0 – 7 scale, 0 = root healthy, 7 = root completely rotted and foliage dead).

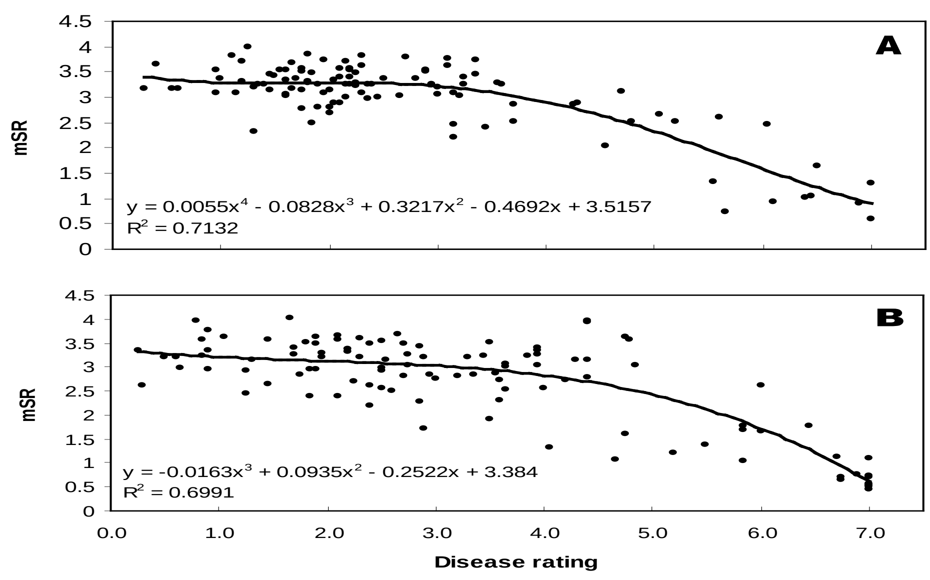

Fig. 3. Modified spectral ratio (mSR) values for a **A.)** partially resistant and **B.)** susceptible variety of sugarbeet plotted against ratings for Rhizoctonia crown and root rot (0 – 7 scale, 0 = root healthy, 7 = root completely rotted and foliage dead).

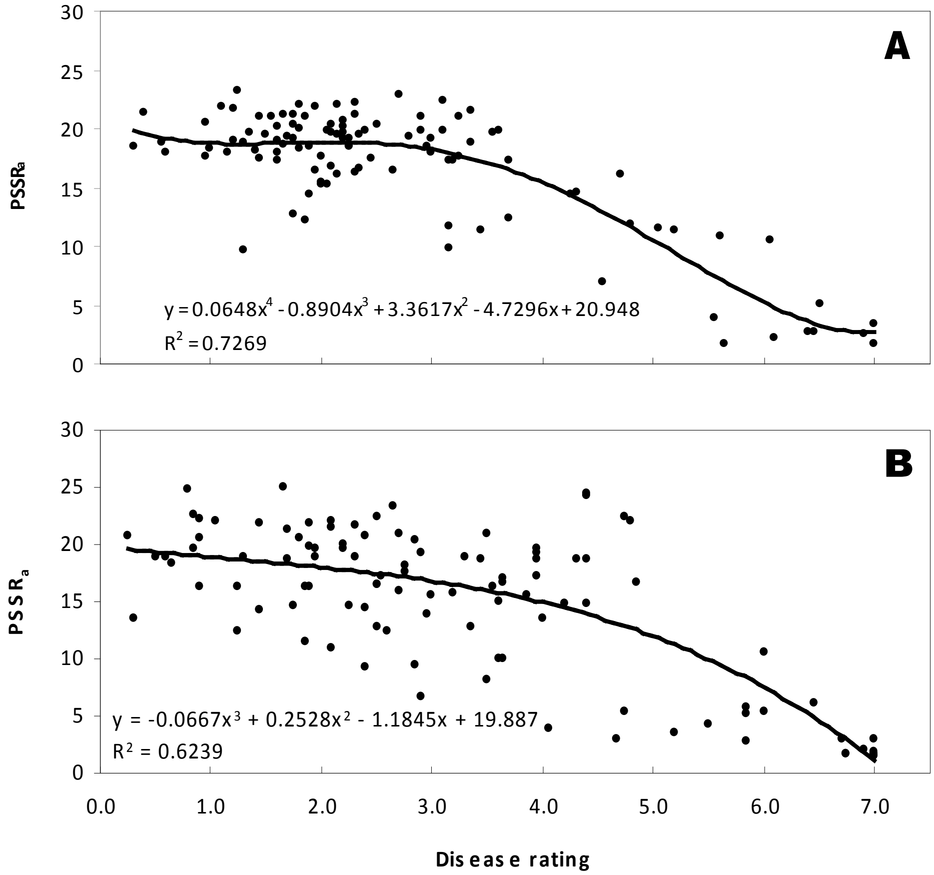

Fig. 4. Pigment specific simple ratio ($PSSR_a$, chlorophyll a) values for a **A.)** partially resistant and **B.)** susceptible variety of sugarbeet plotted against ratings for Rhizoctonia crown and root rot (0 – 7 scale, 0 = root healthy, 7 = root completely rotted and foliage dead).

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DISCUSSION

Leaf pigments strongly absorb visible light, and so reflectance in this region is low overall. Chlorophyll absorbs violet-blue and red light for photosynthesis. Green light is not absorbed for photosynthesis, hence most plants appear green. The changes observed in these visible bands of light may indicate reduced chlorophyll content in the sugarbeet foliage. Although visible wilting begins as spectral changes become evident, the canopy still is full. Thus, soil background does not appear to be playing a role in the early changes in reflectance in the visible regions, and reduced chlorophyll content in the sugarbeet canopy is highly probable. Near-infrared light is not absorbed by leaf pigments, so reflectance is high in this region and depends on internal leaf structure. The changes observed in the near-infrared region may be due to wilting in the sugarbeet canopy. Finally, the mid-infrared reflectance region is associated with water content. The changes observed in this region may be due to water stressed sugarbeet foliage.

The wideband (OSAVI) and both narrowband (mSR and $PSSR_a$) indices (all of which are associated with chlorophyll content) correlated with severity of RCRR. However, other vegetation indices tested that were associated with other variables (e.g., water content, data not shown) had similar patterns when plotted against RCRR disease severity values. Thus, it is unclear if changes in the vegetation indices observed with higher disease severity ratings are associated with an actual reduction in chlorophyll content or are simply associated with increased soil background reflectance. The close association between reflectance in plots with high disease severity and the soil reflectance line may indicate that changes in all of the vegetation indices were based more on increased soil reflectance due to wilting leaves rather than any particular biophysical variable such as chlorophyll or water content. Thus, further research is planned to assess the effect of RCRR infections on chlorophyll content in sugarbeet leaves.

Because reflectance responses coincided with the onset of visible aboveground RCRR symptoms (e.g., wilting), it appears that the disease cannot be detected remotely until sugarbeet roots reach disease ratings of at least 4, when they are 25 to 50% rotted. At this point, the disease has progressed too far for remedial management measures (e.g., fungicide applications) to be taken. Remote sensing of severe RCRR infections, however, may have other applications. Infection centers of RCRR could be mapped to identify areas with potentially high *R. solani* inoculum for future growing seasons. This would allow growers to employ precision agriculture technology to apply protectant fungicides in areas of fields prone to RCRR, based on earlier detection of "hot spots". Partially resistant varieties also could be selected for planting in these areas. Furthermore, remote sensing of RCRR "hot spots" would allow for rapid assessment of fields for crop insurance purposes.

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