Use of Multispectral Radiometry for Assessment of Rhizoctonia Blight in Creeping Bentgrass

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ABSTRACT

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The ability to identify diseases early and quantify severity accurately is crucial in plant disease assessment and management. This study was conducted to assess changes in the spectral reflectance of sunlight from plots of creeping bentgrass during infection by *Rhizoctonia solani*, the cause of Rhizoctonia blight, and to evaluate multispectral radiometry as a tool to quantify Rhizoctonia blight severity. After inoculation of 6-year-old creeping bentgrass turf with *R. solani* anastomosis group 2-2, reflectance of sunlight from the foliar canopy was measured at light wavelengths of 460 nm (blue) to 810 nm (near infrared [NIR]), at 50-nm

intervals. Visual estimates of disease severity and percentage of canopy reflectance were made daily throughout each of three epidemics of Rhizoctonia blight from the onset of visible symptoms until maximum disease severity was reached. In each experiment, linear regression analysis revealed a significant reduction in the percentage of NIR (760 and 810 nm) reflectance as disease severity increased. However, in the majority of analyses, regression models explained <50% of the variability between components. Multispectral radiometry appears to function best when used to assess differences in disease severity at discrete points in time rather than over an entire epidemic.

Additional keywords: *Agrostis stolonifera* var *palustris*, bentgrass spectral reflectance, turfgrass.

The ability to detect diseases early and quantify severity accurately is fundamental in plant disease assessment and management. Inaccurate and imprecise data may cause costly errors when disease management and policy decisions are based on biased disease evaluations. Multispectral radiometry has greater accuracy (i.e., closeness of an estimate to the actual value) and precision (i.e., degree of reliability) compared to visual estimates of disease severity (13). Nutter et al. (19) reported significant variation among raters when visually assessing dollar spot severity in creeping bentgrass (*Agrostis stolonifera* var. *palustris* Farwell), but percent reflectance data obtained from the same experimental units with a multispectral radiometer exhibited a high degree of precision. It has been suggested that differences in human vision occur because individuals differ in their ability to perceive various wavelengths of light, which can lead to differences in estimates of disease severity (16). Fatigue, lack of concentration, and bias among raters (e.g., overestimation of low levels of disease after assessing high levels and vice versa) all increase the subjective nature of visual assessments of disease severity (16).

Multispectral radiometry provides a nondestructive means of quantifying plant stress, because biotic and abiotic agents modify electromagnetic characteristics of a canopy surface (16,19). For example, the presence of disease may cause changes in pigment concentration and variation in canopy gas exchange, leading to differences in color and temperature that can alter canopy reflectance characteristics (16). In a healthy leaf, reflectance in the visible range (400 to 700 nm) is relatively low (16). Wavelengths in this range are absorbed by photosynthetic pigments, particularly blue (425 to 492 nm) and red (645 to 700 nm) light (8). Near infrared (NIR) radiation (700 to 1,900 nm) is reflected from plant cells deep within the leaf, and high internal scattering within leaf

tissue is responsible for higher NIR reflectance peaks compared to other wavelengths (16). Plant stresses that cause a reduction in chlorophyll content lead to an increase in light reflected in the visible range. Conversely, percent reflectance in NIR wavelengths is reduced as internal leaf structure degenerates. Reflectance in the infrared region also is reduced as healthy leaf area is decreased by diseases and pests (13).

Jackson (10), Hatfield and Pinter (9), and Nilsson (15,16) have reviewed the use of radiometric remote sensing in plant pathology and crop protection. Radiometry has been used to assess the severity of early blight of tomato (*Lycopersicon esculentum*) (12), rust and late leaf spot of peanut (*Arachis hypogaea*) (18), and dollar spot of bentgrass (19). Radiometric assessments of diseases of rice (*Oryza sativa*), corn (*Zea mays*), and sorghum (*Sorghum* spp.) have been conducted in Taiwan (21). Nilsson (14) reported significant correlations between spectral reflectance data and cereal plant growth, green biomass, yield, and symptoms of net blotch in barley (*Hordeum vulgare*), glume blotch in winter wheat (*Triticum aestivum*), and take all in spring wheat. In a recent study, Nilsson and Johnsson (17) reported significant correlations between radiometric assessment of barley stripe disease and subsequent grain yield. Spectral signatures obtained from watermelon (*Citrullus vulgaris*) leaves were significantly different among plants exhibiting symptoms of leaf mosaic virus, downy mildew, or Fusarium wilt (2), suggesting that multispectral radiometry has potential as a diagnostic tool. In turfgrass management, NIR reflectance has been used to map and evaluate drought stress in tall fescue (*Festuca arundinacea*) (6), to detect infection of bentgrass (*Agrostis* spp.) seedlings by *Pythium* spp. (11), and to analyze physical and chemical properties of golf-course soils (5).

Rhizoctonia blight, caused by *Rhizoctonia solani* Kühn, is a foliar disease that limits the aesthetic and functional value of both cool- and warm-season turfgrasses. In bentgrass, infected leaves first appear water-soaked, then blacken, and finally wither to a light brown color (20). Patches of diseased leaves, varying in size from a few centimeters to a meter in diameter, may coalesce rapidly (12 to 24 h) during periods of extended leaf wetness and

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temperatures between 21 and 32°C (20). Early detection of Rhizoctonia blight is important in limiting disease in high-quality turfgrass swards used for golf putting greens or professional athletic fields. Furthermore, accurate and unbiased assessment of disease severity is a prerequisite for selection of resistant turfgrass cultivars. Experiments were conducted to evaluate multispectral radiometry as an objective method for estimating disease severity by determining which wavelengths are consistently and significantly related to increases in severity of Rhizoctonia blight over time.

MATERIALS AND METHODS

Three experiments were conducted, commencing on 29 May, 27 June, and 15 August 1996, on a 6-year-old stand of creeping bentgrass cv. Penncross at the Georgia Experiment Station, Griffin. The bentgrass was established from seed on a sand-based root-zone mix in accordance with U.S. Golf Association specifications for golf putting greens (7). The turfgrass was maintained at a height of 5 mm by mowing three times per week. Additional maintenance practices were similar to those stipulated for bentgrass golf greens (1).

Turfgrass in six plots (each 1.0 m²) was inoculated with autoclaved grain (an equal mixture by weight of barley, oats, and wheat) infested with *R. solani* anastomosis group 2-2 (isolate Rh 124), as previously described (3). Infested grain was applied by hand to the turf surface in each of the six plots at ≈ 7 g m⁻². Due to the abundance of natural inoculum of *R. solani* in the bentgrass root zone, noninfested, disease-free control plots were not included in the study. Maintenance of such plots would require applications of fungicides, which we found significantly altered the spectral response of the turfgrass canopy (C. Raikes and L. L. Burpee, unpublished data).

One day after inoculation, the turf was mowed twice without collecting foliar clippings. Mowing was first done in a north-south direction and then in an east-west direction to fragment the grain to a size small enough to fall into the turf canopy. The plots then were mowed for a third time, including a collection basket for clippings, to retrieve larger grain pieces that could have interfered

with spectral signatures from the turfgrass surface. Six replications were used in each experiment.

Disease severity was assessed visually each day, with an area quadrat frame (1.0 × 1.0 m) constructed as a wooden frame divided by string into 100 squares (10.0 × 10.0 cm). The quadrat frame was placed on the turf surface in each plot, and the proportion of squares exhibiting turf with visible Rhizoctonia blight symptoms was recorded. Visual assessments were conducted beginning when the first discernible visible symptoms of Rhizoctonia blight (small, roughly circular, light brown patches) were observed and continued each day until 2 to 3 days after maximum disease severity was recorded.

The spectral reflectance of each 1-m² plot was measured daily with a hand-held multispectral radiometer (Cropscan, Inc., Rochester, MN). Each plot was scanned once, a period lasting ≈ 5 s, and 47 readings per channel were made automatically by the radiometer during each scan. Data were obtained within 2 h of solar noon to minimize the influence of solar angle on spectral reflectance from the turf canopy. The multispectral radiometer consisted of eight photodiodes that detected reflectance as a percentage of incoming sunlight in eight discrete wavelength bands. The wavelength bands used were 460 nm (blue) to 810 nm (NIR), at 50-nm intervals. The multispectral radiometer was mounted on top of a telescopic boom and held 2.0 m above the turf canopy, which gave a target area 1.0 m in diameter.

Ratios and indices, derived by combining values of percent reflectance in discrete wavelengths, were calculated to assess their usefulness as indicators of disease severity. Various ratios were tested, because different waveband ratios and indices correlate highly with green leaf area, biomass, and stress, as well as other morphological characteristics of vegetative canopies (15). Ratios were derived from the values of NIR reflectance (x) and visible light reflectance (y), i.e., x/y , and indices were derived from the normalized ratios of NIR and visible light reflectance according to the formula $(x - y)/(x + y)$ (Table 1). By combining data from various spectral bands, multispectral radiometric data could be reduced to a single number.

Linear regression analyses were used to quantify the relationship between visual assessments of disease severity (x) and spec-

TABLE 1. Statistics from linear regressions of disease severity versus reflectance values from creeping bentgrass inoculated with *Rhizoctonia solani*

Wavelength, ratio or index (nm)	Experiment 1		Experiment 2		Experiment 3	
	Slope coefficient ^a	r ²	Slope coefficient ^a	r ²	Slope coefficient ^a	r ²
460	0.002**	0.14	-0.008***	0.30	-0.005***	0.25
510	0.003**	0.14	-0.015***	0.55	-0.003*	0.06
560	-0.001	0.01	-0.023***	0.46	-0.010**	0.10
610	0.005**	0.13	-0.020***	0.32	-0.012***	0.18
660	0.006**	0.21	-0.013***	0.40	-0.005**	0.11
710	0.007***	0.22	-0.019***	0.33	-0.013***	0.24
760	-0.055***	0.49	-0.097***	0.51	-0.042***	0.27
810	-0.061***	0.55	-0.091***	0.42	-0.050***	0.25
810/710	-0.013***	0.56	0.006*	0.12	0.005*	0.08
810/660	-0.024***	0.39	0.013**	0.18	0.002	0.03
810/610	-0.010***	0.50	0.004*	0.10	0.003*	0.06
760/710	-0.012***	0.45	0.004	0.06	0.003**	0.11
760/660	-0.022***	0.38	0.009*	0.12	0.003	0.01
760/610	-0.009***	0.50	0.002	0.02	<0.001*	0.07
810/560	-0.006***	0.32	0.004*	0.12	-0.002	0.05
810/510	-0.019***	0.46	0.016***	0.43	0.006	0.01
810/460	-0.023***	0.53	0.007	0.05	0.001	0.03
760/560	-0.005***	0.33	0.002	0.05	-0.001	0.01
760/510	-0.017***	0.45	0.012*	0.30	0.007	0.03
760/460	-0.020***	0.51	0.002	0.09	<0.001*	0.08
810/710 ^b	-0.001***	0.48	<0.001*	0.12	<0.001	0.09
810/660	-0.001***	0.42	<0.001**	0.18	<0.001**	0.04
810/610	-0.001***	0.51	<0.001	0.09	<0.001*	0.06
760/710	-0.001***	0.47	<0.001	0.05	<0.001**	0.12
760/660	-0.001***	0.42	<0.001*	0.11	<0.001	0.01
760/610	-0.001***	0.51	<0.001	0.02	<0.001	0.07

^a Values followed by *, **, and *** are significant at $P < 0.05$, < 0.01 , and < 0.001 , respectively.

^b Wavelengths separated by a colon represent indices normalized by the formula $(x - y)/(x + y)$, where x = the first wavelength and y = the second wavelength.

tral data (y). In addition, values of area under reflectance curves (AURC) for each wavelength and index were regressed against values of area under disease progress curves (AUDPC). Values of AURC and AUDPC were calculated from the formula

$$\sum_{i=1}^{n-1} [(y_i + y_{i+1})/2](t_{i+1} - t_i)$$

where n is the number of assessment times, $i = 1, 2, 3, \dots, n-1$, y_i is the amount of reflectance or disease at the i th rating, and t_i is the time at the i th rating (4). Standardized residuals were plotted for all regression analyses to ensure model variance was not correlated with the independent variable (x). Regression analyses were conducted with data collected from the first day of visible symptoms until maximum disease severity was reached in each experiment, i.e., one regression model was produced for each wavelength, ratio, and index. Analyses were performed using Statistical Analysis Software (SAS Institute, Inc., Cary, NC) procedures.

RESULTS

The incubation periods of isolate Rh 124 were 9, 3, and 6 days in experiments 1, 2, and 3, respectively (Fig. 1). Severity of Rhizoctonia blight peaked ≈ 9 days after inoculation in experiment 2 and ≈ 20 days after inoculation in experiments 1 and 3.

Regression statistics were similar for relationships between AURC and AUDPC and between reflectance values and disease severity. Therefore, statistics that describe the latter group of relationships are presented. Slope coefficients from linear regressions of reflectance values and disease severity were significant for most of the individual wavelength bands tested in each experiment (Table 1). In contrast, slope coefficients of regressions based on reflectance ratios and indices were statistically significant in experiment 1 but not in experiments 2 and 3. Slope coefficients were highly significant ($P < 0.001$), and coefficients of determination (r^2) were highest for regressions between NIR reflectance wavebands (760 and 810 nm) and disease severity. As disease severity increased from initial symptom development to maximum disease severity, a significant decrease in reflectance was detected at 760 nm (Fig. 2) and 810 nm (Fig. 3).

DISCUSSION

Reflectance of sunlight from a creeping bentgrass canopy was significantly altered during the postsymptomatic stages of Rhizoc-

Fig. 1. Disease progress curves for epidemics of Rhizoctonia blight of creeping bentgrass beginning 29 May (experiment 1), 27 June (experiment 2), and 15 August (experiment 3) 1996.

tonia blight. NIR reflectance declined in each experiment. Overall, NIR radiation is strongly reflected by green vegetation. Discontinuity between air spaces and plant cell walls leading to high internal light scattering coupled with reduced absorption (10) causes increased NIR reflectance. This scattering occurs deep within the leaf tissue, and the percentage of sunlight reflected in the NIR region may provide information on the physiological condition of a leaf or plant under stress (13). The reduction of NIR reflectance noted in each experiment was most likely a result of a reduction in healthy green leaf area caused by *R. solani* infection (17). A similar reduction in NIR reflectance caused by a pathogen-induced

Fig. 2. Percent reflectance of light at a wavelength of 760 nm from creeping bentgrass inoculated with *Rhizoctonia solani* in A, experiment 1, B, experiment 2, and C, experiment 3, from initial symptom development to maximum disease severity.

limitation in healthy green leaf area has been reported in other pathosystems (15,17,18).

Our results demonstrate that multispectral radiometry exhibits potential as a method for quantitative assessment of *Rhizoctonia* blight on creeping bentgrass. However, in two of the three experiments, <50% of the variability was explained by linear regressions of disease severity and percent reflectance. Other researchers have reported a stronger relationship between these components (17–19). However, in these cases, data were analyzed for individual assessment dates, rather than over an entire epidemic, thereby reducing environmental or plant variability that may significantly affect reflectance. In our study, variability in reflectance related to

exposure of the soil surface due to disease-induced thinning of the turfgrass canopy over time probably functioned in limiting r^2 values. Based on our findings, the multispectral radiometer appears to function best when used to assess differences in disease severity at discrete points in time rather than over an entire epidemic.

Reduction in NIR reflectance is one of the earliest symptoms of plant stress, and this reduction often can be detected before visible disease symptoms become apparent (17). Further studies to investigate the potential of multispectral radiometry as a tool to detect spectral changes during pathogen incubation periods would be particularly useful in deploying control procedures (e.g., fungicide applications) in a timely fashion.

Multispectral radiometry can provide an indirect measure of plant stress, but its use has been limited in differentiating specific stress-causing agents (16), e.g., pathogen infection versus drought stress. Future work with multispectral radiometry might incorporate the use of midinfrared wavelengths ($\approx 1,000$ to $2,500$ nm), because reflectance of sunlight in the longest wavelength bands tested in this study (NIR 760 and 810 nm) appeared to be the most sensitive in quantifying severity of *Rhizoctonia* blight.

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Fig. 3. Percent reflectance of light at a wavelength of 810 nm from creeping bentgrass inoculated with *Rhizoctonia solani* in **A**, experiment 1, **B**, experiment 2, and **C**, experiment 3, from initial symptom development to maximum disease severity.