CHAPTER 2

DIGITAL IMAGE ANALYSIS AND SPECTRAL REFLECTANCE OF SALINITY STRESSED HYBRID BLUEGRASS AND BERMUDAGRASS

ABSTRACT

Because of increasing use of saline water for turfgrass irrigation, monitoring salt stress is necessary to ensure the health of turfgrass. Current methods used to test stress levels in turfgrass can be time-consuming and expensive. This study was conducted to determine if digital image analysis and spectroradiometry could accurately detect salinity stress in turfgrasses and discriminate between degrees of salt stress. Our objective was to assess the correlation between digital images and reflectance, and visual ratings, relative water content (RWC) and leaf osmolality, standard methods used to measure stress in plants. The greenhouse study was conducted in 2005 on hybrid bluegrass [Poa arachnifera (Torr.) x pratensis (L.)] cv. Reveille and bermudagrass [Cynodon dactylon (L.)] cv. Princess 77 at New Mexico State University. Percent chlorosis and hue values obtained from digital image analysis and Normalized Difference Vegetation Index (NDVI) calculated from spectroradiometry readings were moderately to highly correlated with visual ratings, RWC and leaf osmolality in bluegrass. Correlations were similar in bermudagrass, with the exception of a weak correlation between osmolality and NDVI. Both methods

effectively detected salinity stress and may have applications in turfgrass management as rapid and quantitative methods to detect salt stress.

Abbreviations: □DC, direct current; EC, electrical conductivity; NDVI, Normalized Difference Vegetation Index; NIR, near-infrared; PVC, polyvinyl chloride; rpm, revolutions per minute; RWC, relative water content; SAR, sodium adsorption ratio; TDS, total dissolved solids; θ, Soil moisture content; ψ_w, leaf water content.

INTRODUCTION

Water conservation is an important component of turfgrass management. Growing demands on limited water supplies, particularly in arid regions, have led many cities to restrict the use of potable water for non-essential purposes such as landscape and golf course irrigation. As part of this trend, reclaimed water, water which has been treated at a sewage plant after having undergone at least one cycle of (human) use (Harivandi, 2004), is being increasingly used for most non-drinking uses (National Research Council, 1998).

Reclaimed water usually contains high concentrations of salt, and turfgrass irrigated with reclaimed water can become salinity stressed, leading to a reduction in turf quality (Qian and Mecham, 2005). Excess salt ions in reclaimed water decrease osmotic potential in plants, disrupt mineral nutrition, affect the growth or qualitative features of turf, and can be toxic at elevated levels (Tanji, 1990). Even when salt concentrations are comparatively low, long term irrigation with reclaimed water can increase salt loads in the soil, which can be difficult to leach below the root zone (Qian and Mecham, 2005).

Stressed turfgrass recovers slowly and is more susceptible to disease (Beard, 1973). Therefore, turfgrass should be monitored frequently to detect early signs of stress. There are a number of methods used to determine salt- or drought-induced stress levels in plant tissue. These include carbon isotope discrimination (Qian et al., 2004), potassium and sodium determination (Qian et al., 2000), measuring relative water content (RWC) (Netondo et al., 2004) and leaf water potential (ψ_w) (Qian et al., 2001). However, these methods are typically used on relatively small areas of turf. In addition, the narrow width and small surface area of individual turf blades requires that multiple measurements be taken to obtain adequate representation of a moderately sized turf area. Soil characteristics such as soil moisture content (θ), pH, electrical conductivity (EC), total dissolved solids (TDS), and sodium adsorption ratio (SAR) can also be used to estimate potential salinity or drought stress of the turfgrass. However, these data only give an approximate evaluation of salinity stress as they do not measure direct physiological responses. Furthermore, results from laboratory tests are not immediately available and the lag time between sample collection and remedial action can be too long.

The first symptoms of salinity stress on turfgrass are wilting and a blue-green appearance followed by an irregular stunting of growth (Beard, 1973). Visual assessment of turfgrass color, leaf firing, and estimates of the number of salt glands on leaves can be used to monitor salinity stress levels (Marcum et al., 1998). However, these assessments are labor intensive, require experience and/or are often expensive.

As a growing number of municipalities require the use of reclaimed water for irrigation of turf, a rapid and nondestructive method to assess salinity stress over large areas is needed. Karcher and Richardson (2003, 2005) have shown that digital image analysis accurately quantified the hue, saturation, and brightness levels of Munsell Plant Tissue color chips corresponding to turf colors, and were able to quantify visual color differences among zoysiagrass and creeping bentgrass receiving different fertility treatments. Trenholm et al. (1999) demonstrated that multispectral radiometry was highly correlated with visual turf quality, shoot density, and shoot tissue injury. Both digital image analysis and spectroradiometry, could potentially be used to detect signs of salinity stress in turf stands and provide a rapid, quantitative, standardized measurement of turf stress.

We conducted a study to determine if digital image analysis and spectroradiometry could be effectively used to detect and quantify salinity stress in turfgrasses. The first objective of this study was to assess the efficacy of digital image analysis and spectroradiometry in detecting and quantifying salinity stress in hybrid bluegrass [*Poa arachnifera* (Torr.) x *pratensis* (L.)] cv. Reveille and bermudagrass [*Cynodon dactylon* (L.)] cv. Princess 77 treated with five salinity levels. To do so, we assessed whether data obtained from digital image analysis and spectroradiometry could be adequately correlated with qualitative visual ratings, RWC and leaf osmolality measurements, three standard methods used to evaluate stress in turfgrass. Hybrid bluegrass and bermudagrass were selected to assess the abilities of the different methods to measure salinity stress in a comparatively salt tolerant species (bermudagrass) (Marcum, 2004) and a more salt sensitive species (hybrid bluegrass)

(Suplick-Ploense et al., 2002). The second objective was to determine if digital image analysis and spectroradiometry could be used to predict the onset of salinity stress before it was readily apparent using standard methods.

MATERIALS AND METHODS

A hydroponics greenhouse study was conducted during the summer of 2005 at the Fabian Garcia Research Center of New Mexico State University in Las Cruces, NM. Washed Reveille and Princess 77 sod was cut into 10 by 10 cm squares and placed on 19.5 by 18.0 cm egg crates (Plaskolite, Columbus, OH), to which four polyvinyl chloride (PVC) pipes 13 cm in length and 3.18 cm in diameter had been attached to create stable stands for the sod (Figure 2-1). Each of the egg crate stands was placed in one of sixteen 28 L plastic containers (SNAP topper, Rubbermaid, Wooster, OH) filled with 23.0 L of a nutrient solution. Two 13 by 14 cm holes were cut into each container lid to ensure the turf was exposed to light (Figure 2-2). The nutrient medium consisted of a 1 g L^{-1} solution of 15-5-15 (N-P-K; including 4% calcium and 2% magnesium) fertilizer (Jack's professional fertilizer Ca-Mg, J.R. Peters, Inc., Allentown, PA) diluted with tap water (EC = 0.6 dS m^{-1}). The nutrient solution in each container was maintained at a constant volume with daily additions of tap water to avoid increasing salinity, and was replaced once a week to regulate pH and maintain nutrient concentrations. The pH of each freshly prepared batch of nutrient solution was measured and if necessary, adjusted to values between 6.5 and

7.5 using NaOH and HCl. Air stones attached to an air pump (Whisper, Tetra, Blacksburg, VA) were used to supply O_2 to the solution. A 1.0 cm air space was maintained between the underside of the egg crate and the surface of the nutrient solution to aerate the sod. The outside of each container was painted black with latex paint, and the covers were painted white to exclude sunlight. Turfgrasses were grown in the containers for 2 months to establish fully before salinity treatments were applied. Grasses were clipped with scissors every three days to maintain a 4.0 cm height. The greenhouse temperature was maintained at 24°C day/18°C night.

Salinity Treatment

The salinity treatments were prepared by adding sufficient salt to the nutrient solution to achieve the desired salinity levels (control, 10, 20, 30, and 40 dS m^{-1}). Instant ocean salt (Chloride 19,000 ppm, Sodium 10,622 ppm, Sulfate 2,684 ppm, Magnesium 1,263 ppm, Calcium 374 ppm, and Potassium 478 ppm) (Aquarium Syst., Mentor, OH) was used as the source of salt. The control treatment, which consisted of nutrient solution alone, had a salinity of 2.5 dS m^{-1}. To avoid salinity shock the salinity levels were increased gradually by 4.07 g L^{-1} of salt per day until the final salinity level of each treatment was reached. Data were collected 1 day after final salinity level was reached (Marcum, 1999). Each treatment was replicated four times and the experiment was arranged as a completely randomized design.

Salinity Stress Evaluations

Prior to visual rating, spectroradiometer, and digital image data collection, the turf was clipped with scissors, and the clippings were collected to determine RWC. A portion of the clippings from each plot were re-hydrated overnight, dried at 85C° for 12 hours, and RWC was calculated using the following formula:

RWC (%) = (Fresh weight – Dry weight) / (Turgid weight – Dry weight) * 100 (1)

The remaining clippings were placed in zip lock bags and immediately frozen for later analysis of osmolality. To determine osmolality, clippings were packed in a forensic spin filter (USA Scientific, Inc., Ocala, FL) and centrifuged (Centrifuges 5415D, Eppendorf, New York, NY) at 13.2 revolutions per minute (rpm) for 5 minutes to extract plant sap. Osmolality was measured using a Vapor Pressure Osmometer (Wescore, Inc., Logan, UT). All plots were visually rated for turf quality on a scale of 1 to 9 with 1 corresponding to brown, dead turf and 6 reflecting the minimum acceptable quality. A rating of 9 indicated an optimal quality.

Digital Image Data Collection

The digital images were collected using a web camera (QuickCam Pro4000, Logitech Inc., Fremont, CA) in an enclosed PVC box to provide a controlled light environment (Figure 2-3). The images were collected in the JPEG format at an image size of 1280 × 960 pixels. Camera settings included a shutter speed of 1/100 s and white balance of incandescent mode. The dimensions of the PVC pipe were 15.24 cm in diameter and 35.0 cm in height. The inside of the PVC pipe was painted white with latex paint to enhance maximum light reflectance, and a 0.3 cm thick wooden board covered the top of the pipe. A web camera and two clear 5 watt tungsten halogen light bulbs (OS 64405, Osram Sylvania Inc., Danvers, MA) were placed inside the PVC pipe. All images were downloaded to a PC and cropped to exclude non-turf area for digital image analysis. To quantify percent green cover in the turf plots and to assess percent chlorosis in each picture, hue, saturation, and brightness values were determined using the "Turf Analysis" macro written and described by Karcher and Richardson (2005). Hue describes an angle on a continuous circular scale from 0 to 360° (Adobe Systems, 2002) that determines the position of a color in the color spectrum (Karcher and Richardson, 2003). Hue values between 50° and 150° correspond to the color green. Since there were no bare spots on the turf plots (data not presented), the non-green portion of the digital images were assumed to correspond to leaf chlorosis, a direct indication of salinity injury (Marcum 1999). Percent leaf chlorosis was calculated using the following equation:

Chlorosis (%) = 100 - % green cover (2)

Spectroradiometer Data Collection

The spectroradiometry readings of the turf canopy were taken using a LabSpec Pro (Analytical Spectral Devices Inc., Boulder, CO) under a controlled light environment in an enclosed PVC box similar to the one described above (Figure 2-3).

The top of the PVC pipe was covered with a 0.3 cm thick wooden board which contained a small hole through which a fiber optic sensor was inserted and two larger holes through which two clear 5 watt tungsten halogen light bulbs were inserted to illuminate inside the box. The holes were covered with aluminum foil to prevent any sunlight from entering the box. The fiber optic sensor had a 25° angle of view, so that a 7.98 cm diameter of the turfgrass area was measured. In order to reduce noise from the lights, direct current (DC) was used to supply the light source. The spectral reflectance data collected were used to calculate Normalized Difference Vegetation Index (NDVI) using the following equation (Trenholm et al, 1999):

$$\text{NDVI} = (935\ \text{nm} - 661\ \text{nm}) / (935\ \text{nm} + 661\ \text{nm}) \quad (3)$$

Experimental Design and Statistical Analysis

The experimental design was completely randomized with 4 replications. The tank holding the grasses (=salinity treatment) served as the main plot treatment and grass species as the subplot treatments. To test the effect of salinity on the quantitative and qualitative response data collected, percent green cover, hue, NDVI, RWC, osmolality, and visual ratings were subjected to analysis of variance using SAS Proc Mixed (SAS Institute, Inc., 2002) followed by means separation using Fisher's LSD test at the 0.05 probability level. Pearson's correlation coefficients were calculated and examined to determine the degree of association between digital image and spectral data and the other response variables.

RESULTS AND DISCUSSION

The effects of salinity on RWC, osmolality visual rating, percent green cover, hue, and NDVI for both species are shown in Table 2-1. In both species, RWC gradually decreased as salinity increased. RWC dropped in both species from 92% to 79% when salinity increased from 2.5 to 40 dS m^{-1}. Leaf osmolality increased with increasing salinity levels (Table 2-1). Our results support those of Marcum and Murdoch (1990), who observed a decrease in shoot water content in St. Augustinegrass from 82% to 60% when salinity was increased from 0.7 to 30 dS m^{-1}. Our results also confirm Marcum's (1999) findings of increased leaf sap osmolality for several warm season grasses including bermudagrass, when media salinity increased to 600 mM NaCl. The change in osmolality with increasing salinity was more pronounced in hybrid bluegrass than in bermudagrass (Table 2-1). Visual ratings decreased and percent chlorosis increased with increasing salinity for both species (Table 2-1). Hybrid bluegrass quality ratings dropped from a near perfect 8.8 rating under non-saline conditions to an unacceptable 3.8 at 40 dS m^{-1}. Bermudagrass however maintained an acceptable rating of 6.0 or higher at salinity levels as high as 30 dS m^{-1} and dropped only slightly below the minimum acceptable quality threshold of 6.0 at 40 dS m^{-1}. Our results support those of Marcum (1999) who reported that leaf firing, a measure of leaf chlorosis, increased to 40% in bermudagrass when media salinity reached 600 mM NaCl. Qian et al. (2004) also observed an inverse linear relationship between salinity and turf quality as well as increased leaf firing with increasing salinity in Kentucky bluegrass cultivars. Our results differ from those of Suplick-Ploense et al., (2002), who evaluated 9 Kentucky bluegrasses, 5 hybrid bluegrasses (including Reveille), and 3 Texas bluegrasses at salinity levels as high as 9 dS m^{-1}. Leaf firing in Reveille hybrid bluegrass subjected to 5 dS m^{-1} ranged from 33 to 88.3% (Suplick-Ploense et al. 2002). Even at our highest salinity treatment (40 dS m^{-1}), leaf firing was never greater than 72%. Hue decreased in both species as salinity increased (Table 2-1). This was expected due to a greater proportion of yellow colored chlorotic in response to increasing salinity. NDVI, a measure of spectral reflectance, also decreased significantly in both species with increasing salinity (Table 2-1).

Percent reflectance at wavelengths between 550 and 700 nm increased as salinity levels increased (Figure 2-4). In contrast, percent reflectance decreased with increasing salinity at wavelengths between 700 and 1300 nm (Figure 2-4). Percent reflectance between 1400 and 1950 nm also increased as salinity increased. Carter (1993) observed notable responses in leaf infrared reflectance in stressed plants, but only when the stress was great enough to cause severe leaf dehydration. Carter (1993) further suggested that spectral reflectance ranges of 535 – 640 nm and 684 – 750 nm are most likely to indicate plant stress, and that photography or digital analysis within these ranges may improve the capability to detect plant stress. Percent reflectance below 450 nm and above 1900 nm was noisy and may have been caused by the Tungsten halogen light bulbs, indicating that these ranges of reflectance may be difficult to use for salt stress determination.

The six methods we compared varied in their abilities to detect differences in salt sensitivity between the salt-tolerant bermudagrass and salt sensitive hybrid bluegrass. RWC did not differ significantly between the two species at any given salinity level, suggesting that both species maintained RWC similarly under same salinity level (Table 2-1). Osmolality was higher in bermudagrass than in bluegrass at all but one treatment level (40 dS m^{-1}), however the differences were only significant at 20 dS m^{-1}. Percent chlorosis was significantly lower for bermudagrass than bluegrass at 10 and 30 dS m^{-1}, which indicates bermudagrass's superior salt tolerance. Because hue and NDVI both relate to color, we expected these values to also reflect species differences in salt sensitivity. However, hue values only separated the 2 species at 30 and 40 dS m^{-1} (Table 2-1) and NDVI did not differ significantly between the 2 species at any salinity treatment level. This suggests that hue values may require calibration for each species being monitored in order to better detect salt stress, whereas NDVI may not be suitable to compare salinity stress between species.

Percent green cover, hue and NDVI were strongly correlated with RWC, osmolality, and visual ratings in hybrid bluegrass, with highest r values (r = 0.91-0.98) with visual ratings (Table 2-2). In bermudagrass, correlations were similar to bluegrass, with the exception of osmolality, which correlated weakly with NDVI ($r = -0.49$, $p = 0.027$) (Table 2-3). It was not surprising that visual rating, percent green cover, hue, and NDVI correlated strongly with one another (Table 2-2 and 2-3), as they are all measures of different aspects of turf appearance such as color, cover, and density, which all relate directly to turf health. Among those factors, percent green cover is a primary component of turfgrass quality (Marcum and Pessarakli, 2006). Hue obtained from digital image analysis has been used to quantify turfgrass color (Karcher and Richardson, 2003) and cover (Richardson et al., 2001). NDVI has also been shown to correlate strongly with visual ratings for turf color and moderately with cover in tall fescue and bentgrass (Bell et al., 2002). In comparison, RWC and osmolality only provide single physiological measures of plant stress which may not immediately reflect a change in turfgrass appearance.

Our second objective was to determine if digital image analysis and spectroradiometry were effective methods of quickly detecting early signs of salinity stress, potentially before they were visible by standard methods. Of the 6 methods we used to detect salinity stress, visual rating, percent green cover, and NDVI were most effective at detecting early signs of stress in hybrid bluegrass, as evidenced by values significantly different from controls (2.5 dS m^{-1}) at the lowest salt treatment (10 dS m^{-1}) (Table 2-1). Hue and RWC values differed significantly from controls at 20 dS m^{-1}, whereas salt levels of 30 dS m^{-1} were needed before osmolality values differed significantly from control values. RWC, percent green cover, hue, and NDVI were more sensitive to changes in salinity than visual rating and osmolality in bermudagrass (Table 2-1). To determine if any single method is most effective at detecting early signs of salt stress, further studies would be needed using a greater number of salinity concentrations between control levels and the lowest treatment levels that produced significant effects and exposing grasses to salinity treatments for more than 24 hours.

CONCLUSIONS

Our data suggest that digital image analysis and spectral radiometry have potential applications in turfgrass management by providing rapid, inexpensive, and quantitative tools to monitor salt stress. Further research is needed to assess salt stress under different durations of exposure to given salt levels to determine whether these methods could detect critical stress thresholds during which time remedial actions could be taken. In addition, more research is needed to evaluate the ability of spectral radiometry and digital image analysis to distinguish salt stress from other forms of stress, such as drought stress or heat stress.

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TABLES AND FIGURES

Table 2-1. Least square differences between species and treatments for relative water content (RWC), osmolality, visual rating, percent green cover, hue, and Normalized Degree Vegetation Index (NDVI).

	Treatments (dS m^{-1})														
Species	2.5			10			20			30			40		
	RWC (%)														
Hybrid bluegrass	92.06	a †	A‡	88.77	a	AB	84.79	a	BC	83.86	a	C	78.63	a	D
Bermudagrass	92.06	a	A	90.29	a	AB	85.07	a	C	86.68	a	BC	78.52	a	C
	Osmolality (mmol kg^{-1})														
Hybrid bluegrass	766	a	C	902	a	BC	930	b	BC	1250	a	B	2158.25	a	A
Bermudagrass	1098	a	B	1153	a	B	1476	a	AB	1634	a	A	1713.50	a	A
	Visual rating¶														
Hybrid bluegrass	8.8	a	A	7.0	a	B	6.5	a	B	5.3	a	C	3.8	b	D
Bermudagrass	7.5	b	A	7.5	a	A	6.8	a	AB	6.0	a	BC	5.8	a	C
	Chlorosis (%)														
Hybrid bluegrass	17.54	a	D	33.07	a	C	42.38	a	C	54.67	a	B	72.45	a	A
Bermudagrass	15.93	a	C	27.21	b	C	32.71	a	B	36.56	b	B	63.44	a	A
	Hue (Degree)														
Hybrid bluegrass	84.85	a	A	78.20	a	A	63.84	a	B	53.42	b	C	39.98	b	D
Bermudagrass	77.89	a	A	79.66	a	A	67.60	a	B	65.86	a	B	49.89	a	C
	NDVI														
Hybrid bluegrass	0.89	a	A	0.86	a	B	0.85	a	BC	0.82	a	C	0.77	a	D
Bermudagrass	0.87	a	A	0.87	a	A	0.84	a	B	0.83	a	B	0.78	a	C

†a, b: Within each column and variable, means sharing a letter are not significantly different from one another (Fisher's LSD, α=0.05)

‡A, B, C, and D: Within each row, means sharing a letter are not significantly different from one another (Fisher's LSD, α=0.05)

¶ Visual ratings were taken on a scale from 1 to 9 with 1 = worst, 9 = best and 6 = minimum acceptable quality

Table 2-2. Pearson correlation coefficients for hue, relative water content (RWC), osmolality, visual rating, percent green cover, and NDVI of hybrid bluegrass.

Parameter	Osmolality		Visual rating		Percent green cover		Hue		NDVI	
RWC	-0.68	**	0.82	***	0.83	***	0.79	***	0.81	***
Osmolality			-0.81	***	-0.79	***	-0.78	***	-0.83	***
Visual rating					0.95	***	0.91	***	0.91	***
Percent green cover							0.98	***	0.97	***
Hue									0.96	***

* ** *** Correlations significant at the 0.05, 0.01, and <0.0001 level of probability.

Table 2-3. Pearson correlation coefficients for hue, relative water content (RWC), osmolality, visual rating, percent green cover, and NDVI of bermudagrass.

Parameter	Osmolality		Visual rating		Percent green cover		Hue		NDVI	
RWC	-0.61	**	0.71	**	0.76	***	0.78	***	0.74	**
Osmolality			-0.61	**	-0.54	*	-0.52	*	-0.49	*
Visual rating					0.84	***	0.84	***	0.88	***
Percent green cover							0.99	***	0.97	***
Hue									0.96	***

* ** *** Correlations significant at the 0.05, 0.01, and <0.0001 level of probability.

Figure 2-1. Turfgrass sod on egg crate with stands.

Figure 2-2. Containers with turfgrass plots.

Figure 2-3. Spectroradiometer set on top of turfgrass plots.

Figure 2-4. Spectral reflectance of salinity stressed Texas bluegrass and bermudagrass.