Expanding the Application of Spectral Reflectance Measurement in Turfgrass Systems

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ABSTRACT

Light reflectance from plants can be used as a non-invasive predictor of health and yield for many cropping systems, and has been investigated to a lesser extent with managed turfgrass systems. The frequent agronomic inputs associated with maintaining golf course grasses allow for exceptional stand quality under harsh growing conditions, but often expend resources inefficiently, leading to either stand loss or unnecessary inputs in localized areas. Turfgrass researchers have adopted some basic principles of light reflectance formerly developed for cropping systems, but field radiometric-derived narrow-band algorithms for turfgrass-specific protocols are lacking. Research was conducted to expand the feasibility of using radiometry to detect various turfgrass stressors and improve speed and geographic specificity of turfgrass management. Methods were developed to detect applied turfgrass stress from herbicide five days before visible symptoms developed under normal field growing conditions. Soil volumetric water content was successfully estimated using a water band index of creeping bentgrass canopy reflectance. The spectral reflectance of turfgrass treated with conventional synthetic pigments was characterized and found to erroneously influence plant health interpretation of common vegetation indices because of near infrared interference by such pigments. Finally, reflectance data were used to estimate root zone temperatures and root depth of creeping bentgrass systems using a gradient of wind velocities created with turf fans. Collectively, these studies provide a fundamental understanding of several turfgrass-specific reflectance algorithms and support unique opportunities to detect stresses and more efficiently allocate resources to golf course turf.

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GENERAL AUDIENCE ABSTRACT

Intensively managed turfgrass systems are globally important for aesthetics, recreational uses, and filtering air pollutants. Agronomic advancements have drastically improved our ability to manage golf course grasses under harsh growing conditions, but often rely on excessive use of resources. Refining techniques to rapidly detect stress to turfgrass canopies can lead to improved site-specific management, resulting in reduced use of water and chemicals. This research was focused on advancing our understanding of how light energy reflected from turfgrass canopies, and measured with a radiometer instrument, can be used to indicate various environmental stresses. We explored relationships between light reflectance of turfgrass canopies and several variables that impact normal turfgrass growth. The first objective was to try and detect stress of annual bluegrass with light reflectance before visible symptoms develop, using herbicides with a known response as an example of a stress. We were able to detect herbicide-induced stress of annual bluegrass five days before visible symptoms developed. A second research objective was to quantify the relationship between light reflectance of creeping bentgrass and soil water content that could be used to rapidly assess irrigation needs. Soil water availability was estimated most accurately using a ratio of two wavelengths of near-infrared light reflectance (970 and 900 nm). An alternative method using visible green and red light was less successful at predicting soil water availability, but provided a cheaper solution that can immediately aide turfgrass managers. Synthetic blue-green pigments are often applied to golf course turf to improve the appearance, but there is concern that these products may interfere with plant photosynthesis. A third research objective was to determine how certain synthetic pigments influence normal light absorption and reflectance by creeping bentgrass. The results suggest that absorption of important red and blue photosynthetically active light are not impacted by these products, so photosynthesis should not suffer, but certain pigments may influence reflectance of near infrared and ultraviolet light and introduce errors in the interpretation of plant health data. Finally, the relationships were defined between spectral reflectance of creeping bentgrass putting greens and root zone temperature or root depth, using various wind speeds generated with turf fans. This relationship allowed the development of maps estimating root zone temperatures and root depth across entire putting

surfaces, based on reflectance of few locations. Collectively, these studies improve our ability to detect environmental stressors of intensively managed turfgrasses and allocate resources more efficiently.

DEDICATION

This dissertation, and the years of research that went into it, are dedicated to the three most influential individuals in my life; my wife, Kelley, and my two daughters, Layla and Scarlett. Your love, support, and encouragement provided constant inspiration throughout. You were the reason for pursuing and reaching this pinnacle of my post-graduate education.

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CHAPTER 1. LITERATURE REVIEW

Overview

Technologies to measure plant health are rapidly improving while costs to implement these technologies are decreasing. Rapid, non-destructive remote sampling techniques by capturing light reflectance of plant or canopy surfaces are becoming increasingly popular in agriculture and related fields (Murphy et al. 2014). Plant response to light is commonly utilized to quantify a variety of physiological measurements (Jackson 1986, Gitelson et al. 2005, Blackburn 2007). Rapid measurements to accurately estimate turf physiological responses to external factors are needed to streamline data collection and detect environment, mechanical, and biotic stresses for precision management (Carrow et al. 2010). The underlying objective of the research reported in this dissertation is to expand the applications of spectral reflectance measurement for fine turfgrass systems.

Plant response to light

Energy provided by solar radiation drives growth of plants. Light waves are converted to usable plant energy during photosynthesis through a series of reactions (Hüner and Hopkins 2008). The energy of light is dependent on the frequency of wavelengths, a continuous electromagnetic spectrum of narrow energy bands emitted by the sun or artificial light sources (Gates et al. 1965, Knipling 1970). Most of the light used by plants comes from the visible region of the spectrum, between 400 nm and 700 nm. Visible light is absorbed by chlorophylls and associated pigments in the red (640 – 700 nm) and blue regions (425 – 490 nm), with the remaining light being either transmitted through the outer epidermal layers or reflected back towards the atmosphere. Green (490 – 550 nm) and yellow (550 – 585 nm) light is reflected from plants, providing the visual perception of green, healthy plants. Near infrared (NIR) light is highly reflective in comparison to visible photosynthetically active light because of intercellular structures that scatter light (Gates et al. 1965). Little NIR radiation is absorbed by plants, but is rather transmitted, reflected, or refracted through epidermal and mesophyll cells. Several water absorption bands are present throughout NIR and mid-infrared spectral regions (Jackson 1986, Penuelas et al. 1993, Sims and Gamon 2003).

Direct measurements of solar radiation from different regions have been used to quantify plant health status since the 1960's (Birth and McVey 1968, Knipling 1970). Birth and McVey (1968) used a spectroradiometer to assess the color of actively growing turfgrass with a simple ratio of reflected near infrared (NIR) to visible red (VR) light. This method was successful for estimating the color of turfgrasses because of a full canopy of foliage, but was considered inadequate in many cropping systems because of background noise commonly created from bare soil. The normalized difference vegetation index (NDVI) was created to reduce interference from soil and other variables not directly associated with plant light reflectance, by normalizing reflected red light by reflected NIR (NDVI = (NIR-VR)/(NIR+VR)) (Rouse et al. 1974).

Since its inception, NDVI has been the most widely studied vegetation index across many applications, with many modifications from its original algorithm (Rouse et al. 1974, Jackson 1986, Murphy et al. 2014). Despite the simple ratio index (RVI) being initially described as a means to assess turfgrass color, NDVI has been the most commonly studied vegetation index to estimate visual quality of common turfgrasses (Carrow et al. 2010). Overall turfgrass quality is closely related to NDVI, but is impacted by a variety of factors, many of which have been associated with this index, including color, canopy structure, density, and endogenous pigment concentrations (Bremer et al., 2011, Stiegler et al., 2005, Trenholm et al., 1999, Trenholm et al., 2000). Concentrations of chlorophylls *a* and *b*, lutein, and β–carotene found in creeping bentgrass (CBG, *Agrostis stolonifera* L.) were weakly correlated with NDVI (Stiegler et al. 2005). Previous research has shown that chlorophyll concentrations are negatively impacted by drought stress (DaCosta et al., 2004), and that drought stress also impacts NDVI measurement of CBG (Johnsen et al. 2009).

Vegetation indices to estimate chlorophyll and water availability

Because of the driving role that chlorophyll *a* plays in photosynthesis, it is the most abundant natural pigment and most intensively studied (Gitelson et al. 2005, Davies 2009). As the photosynthetic rate of creeping bentgrass declines, total chlorophyll also declines (Xu et al. 2002). Other pigments that play an important role in photosynthesis and photoprotection are various carotenoids and anthocyanins (Demmig-Adams and Adams 1996, Blackburn 2007). Carotenoids have a dual role of photoreception and photoprotection (Blackburn 2007). Carotenoids are able to dissipate excess solar radiation not needed by chlorophyll, which serves to protect photosynthesis reaction centers (Demmig-Adams and Adams 1996). Photosynthesis is among the most sensitive physiological processes to high temperatures (Crafts-Brandner and Salvucci 2000).

As previously noted, NDVI was shown to be weakly related to endogenous chlorophyll concentrations in creeping bentgrass (Stiegler et al. 2005). Chlorophyll concentrations are linearly related to several important wavelengths of peak chlorophyll fluorescence outside of the visible spectrum, making these wavelengths ideal for distinguishing chlorophyll content from other factors contributing to spectral reflectance, such as water availability (Gitelson et al., 2003). Many vegetation indices derived from narrowband spectral data have been used to estimate chlorophyll concentration in various cropping and non-cropping systems (Gitelson and Merzlyak 1996, Blackburn 1998, Broge 2001, Carter 2002, Haboudane, Miller et al. 2002, Gitelson et al. 2005, Gitelson et al. 2006, Blackburn 2007, Clevers and Gitelson 2013). Alternative narrowband indices have been developed using reflectance of spectra more closely related to chlorophylls *a* and *b* in forest canopies and agronomic crops than NDVI (Blackburn, 2007). While the content and viability of chlorophyll within plant tissue is known to be related to drought (DaCosta et al., 2004), the decline in chlorophyll is most definitely a consequence of water stress and is likely not the best indicator of water availability prior to drought symptom expression.

As many factors can contribute to chlorophyll reductions, spectral indices unrelated to chlorophyll may be more beneficial in monitoring drought stress. Many indices capable of assessing water availability utilize narrowband spectra that overlap with chlorophyll features (Murphy et al., 2014). However, distinct water absorption features independent of pigment concentrations are present across the near infrared region, notably with centers around 970, 1200, 1450, 1950, and 2250 nm (Penuelas et al., 1993, Sims and Gamon, 2003). Absorption bands beyond 1100 nm are more strongly influenced by water content, but radiometers capable of measuring these bands are currently cost prohibitive in many cases. The water band index (WBI = R_{900}/R_{970}) is used as an indicator of plant water status and to monitor drought effects in agronomic crops, desert, and tallgrass prairie ecosystems (Claudio et al., 2006, Murphy et al., 2014, Penuelas et al., 1993, Sims and Gamon, 2003). Because of the unique properties of the water absorption bands beyond photosynthetically active spectra, it appears likely that this index can be used to differentiate turfgrass water stress from other abiotic stresses.

Creeping bentgrass and annual bluegrass culture and management

Creeping bentgrass (*Agrostis stolonifera* L.) and annual bluegrass (*Poa annua* L.) are widely considered highly desirable for golf putting green surfaces, but each are often pushed beyond their zone of adaptation and grown under supraoptimal temperatures (Dernoeden 2012). Both grasses are subject to many environmental and biotic stressors that necessitate intensive chemical and cultural inputs for survival (Stier 2006). Optimal root growth of cool-season grasses occurs between 10 to 18°C, with maximum shoot growth between 15 and 24°C (Beard 1973). Creeping bentgrass subjected to heat stress undergoes many physiological changes (Huang 2003). Air and soil temperatures in excess of 35°C are common during summer months throughout the transition zone and southeastern United States. Root growth of creeping bentgrass and other cool-season grasses is impacted more by high soil temperatures than air temperatures (Beard 1966, Xu et al. 2002). Root growth and root/shoot ratio of ‘L-93’ and ‘Penncross’ creeping bentgrass were reduced after 7 d under high soil/air temperature stress (35/35°C) compared to control (20/20°C) (Xu and Huang 2001). Reducing soil temperatures while maintaining air temperatures (24/35°C) improved root growth and root/shoot ratio of each cultivar to levels comparable to control (20/20°C). ‘L-93’ root growth increased as soil temperatures were lowered to 29°C and 32°C, while air temperature remained the same. Growth of ‘Penncross’ roots increased when soil temperatures cooled to 29°C but not 32°C. Root fresh weight of each cultivar was not impacted by reducing soil temperature to 32°C and 29°C, but increased at 24°C. Xu and Huang (2001) concluded that reducing soil temperature by as little as 3°C can improve potential for creeping bentgrass survival during prolonged periods of high air temperature stress. In addition to heat-related stress, prolonged exposure to shade will reduce creeping bentgrass color and density (Bell and Danneberger 1999).

Increasing air movement is known to positively impact creeping bentgrass survival (Duff and Beard 1966). High output turf fans are commonly used on golf putting greens in areas of limited air circulation or prolonged high temperatures and humidity (Beard, 1998, Stier, 2006).

Canopy and soil temperatures were reduced by as much as 7.2°C and 6.1°C at wind velocities of 1.8 m s^{-1} (Duff and Beard 1966). However, these results were inconsistent with more recent studies (Taylor et al. 1994, Koh et al. 2003, Guertal et al. 2005). Taylor et al. (1994) reported no significant reduction in air temperature 3 cm above the canopy, small decreases in soil and canopy temperatures, and decreased soil moisture when wind velocities were increased from 1.4 to 2.6 m s^{-1}. Guertal et al., (2005) reported a small decrease in mean soil temperatures with air movement alone (<1°C) in one of two years and in increase in root length on 5 of 17 sampling dates.

An alternative, yet increasingly popular strategy to improve aesthetics and potentially alleviate environmental stress is the application of synthetic phthalocyanine-based pigments (McCarty et al. 2013). Such products have been shown to maintain or improve turfgrass quality during periods of supraoptimal temperatures for growth (Lucas and Mudge 1997, Dernoeden 2012). However, the mechanism for improving turf quality with phthalocyanine pigments is not well understood (McCarty et al. 2013). Lucas and Mudge (1997) demonstrated that Pigment Blue 15, a Cu-based phthalocyanine compound, enhanced the overall creeping bentgrass quality and color of creeping bentgrass when added to aluminum tris and mancozeb. However, some Cu-, Zn- and Ti-based phthalocyanine compounds are reported to reduce CO_2 exchange rate, evapotranspiration rate, chlorophyll fluorescence, and light transmission (McCarty et al. 2013, McCarty et al. 2014). Some evidence suggests that a polychlorinated Cu II phthalocyanine compound induced a defense response to the dollar spot pathogen, though the mechanism was unrelated to systemic acquired resistance or induced systemic resistance (Hsiang et al. 2013). Repeat applications of certain synthetic pigments applied as turf paints may reduce light absorption by chlorophyll and subsequent photosynthetic efficiency may decline (Reynolds et al.

2012). Blue and green phthalocyanines, along with other dark pigments, negatively impacted photosynthetically active radiation transmission into the canopy. The authors suggested that turf quality was reduced with prolonged paint coverage by shading foliage from light absorption and reducing photosynthetically active radiation (PAR) transmission. However, chlorinated Cu phthalocyanine and pulverized *Chlorella vulgaris* cells reduced carotenoid degradation in bentgrass exposed to supraoptimal light conditions, but only *C. vulgaris* was able to slow the rate of chlorophyll degradation (Bartley 2012). The application of Green Lawnger turf colorant delayed UV-B degradation of Kentucky bluegrass by limiting reactive oxygen species production and increasing photochemical efficiency (Ervin et al. 2004). The authors suggested the turf colorant blocked harmful UV-B light, but allowed photosynthetically active light to pass into the canopy.

The use of novel chemistries and unique cultural management strategies allow turf managers to grow fine turfgrasses more effectively than ever under adverse growing conditions. Understanding how these approaches impact a stand of turfgrass can help make their uses more efficient. Advancements in our understanding of plant response to light allows researchers to rapidly and non-destructively quantify important characteristics that drive plant growth. Implementation of methods established for precision agriculture and the development of novel approaches using light reflectance are greatly under-explored for characterizing intensively managed turfgrasses. Research presented in this dissertation were designed to improve our ability to rapidly detect various stressors of golf course turf using spectral reflectance measurements.

RESEARCH OBJECTIVES

The objectives of this research were to expand the practical application of spectral reflectance measurement of turfgrasses by 1) developing new methods for early detection of stress on annual bluegrass caused by herbicides using a field radiometer, 2) determining the relationship between spectral reflectance and water stress to creeping bentgrass grown in sandy soils using rapid field estimation with a radiometer, 3) examining how synthetic pigments interfere with light absorption and reflectance properties of creeping bentgrass, and 4) estimating root zone temperatures and root depths of creeping bentgrass using geo-referenced reflectance data.

LITERATURE CITED

Bartley, G. K. 2012. Applicability of Pigment Compounds for Reducing Light Stress in Bentgrass. Plant Sciences, University of Tennessee.

Beard, J. B. 1973. Turfgrass: Science and culture. Englewood Cliffs, NJ, Prentice-Hall.

Beard, J. B. and W.H. Daniel. 1966. Relationship of creeping bentgrass (*Agrostis paulstris* Huds.) root growth to environmental factors in the field. Agron. J. 58: 337-339.

Birth, G. S. and G. R. McVey. 1968. Measuring the color of growing turf with a reflectance spectrophotometer. Agron. J. 60(6): 640-643.

Blackburn, G. A. 1998. Quantifying Chlorophylls and Caroteniods at Leaf and Canopy Scales. Remote Sens. Environ. 66(3): 273-285.

Blackburn, G. A. 2007. Hyperspectral remote sensing of plant pigments. J. Exp. Bot. 58(4): 855-867.

Broge, N. H. 2001. Comparing prediction power and stability of broadband and hyperspectral vegetation indices for estimation of green leaf area index and canopy chlorophyll density. Remote Sens. Environ. 76(2): 156-172.

Carrow, R. N., J.M Krum, I. Flitcroft, and V. Cline. 2010. Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. Precision Agric. 11(2): 115-134.

Carter, G. A. 2002. Optical Properties of Intact Leaves for Estimating Chlorophyll Concentration. J. Environ. Qual. 31(5): 1424.

Clevers, J. G. and A. A. Gitelson. 2013. Remote estimation of crop and grass chlorophyll and nitrogen content using red-edge bands on Sentinel-2 and-3. Int. J. Appl. Earth Observation and Geoinformation 23: 344-351.

Crafts-Brandner, S. J. and M. E. Salvucci. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc. Natl. Acad. Sci. USA 97(24): 13430-13435.

Davies, K. 2009. Annual Plant Reviews, Plant Pigments and their Manipulation, John Wiley & Sons.

Demmig-Adams, B. and W. W. Adams. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci. 1(1): 21-26.

Dernoeden, P. H. 2012. Creeping bentgrass management, 2nd Edition. CRC Press, Boca Raton, FL.

Duff, D. T. and J. B. Beard. 1966. Effects of air movement and syringing on the microclimate of bentgrass turf. Agron. J. 58(5): 495-497.

Gates, D. M., H.J. Keegan, J.C. Schleter, and V.R. Weidner. 1965. Spectral properties of plants. Appl. Optics 4(1): 11-20.

Gitelson, A. A., G.P. Keydan, and M.N. Merzlyak. 2006. Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. Geophysical Research Letters 33(11).

Gitelson, A. A. and M. N. Merzlyak. 1996. Signature analysis of leaf reflectance spectra: algorithm development for remote sensing of chlorophyll. J. Plant Physiol. 148(3): 494-500.

Gitelson, A. A., A. Vina, V. Ciganda, D.C. Rundquist, and T.J. Arkebauer. 2005. Remote estimation of canopy chlorophyll content in crops. Geophysical Research Letters 32(8).

Guertal, E.A., E. van Santen, and D.Y. Han. 2005. Fan and syringe application for cooling bentgrass greens. Crop Sci. 45(1): 245-250.

Haboudane, D., J.R. Miller, N. Tremblay, P.J. Zarco-Tejada, and L. Dextraze. 2002. Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. Remote Sens. Environ. 81(2): 416-426.

Huang, B. 2003. Recent advances in drought and heat stress physiology of turfgrass-a review. Int. Conf. Turf Mngt. Sci. Sports Fields 661.

Hüner, N. P. and W. G. Hopkins. 2008. Introduction to plant physiology, Wiley, New York.

Jackson, R. D. 1986. Remote sensing of biotic and abiotic plant stress. Annu. Rev. Phytopathol. 24(1): 265-287.

Johnsen, A. R., B.P Horgan, B.S. Bulke, and V. Cline. 2009. Evaluation of remote sensing to measure plant stress in creeping bentgrass fairways. Crop Sci. 49(6): 2261-2274.

Knipling, E. B. 1970. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. Remote Sens. Environ. 1(3): 155-159.

Koh, K.J., G.E. Bell, D.L. Martin, and N.R. Walker. 2003. Shade and airflow restriction effects on creeping bentgrass golf greens. Crop Sci. 43(6): 2182-2188.

Lucas, L. T. and L. C. Mudge. 1997. Fungicidal compositions for the enhancement of turf quality. U.S. Patent 5 643 852. Date issued: 03 April.

McCarty, L. B., J.R. Gann, C.E. Wells, T.F. Bruce, and P.D. Gerald. 2013. Physiological responses of creeping bentgrass to pigment-containing products. Agron. J. 105(6): 1797-1802.

McCarty, L. B., J.R. Gann, C.E. Wells, and P.D. Gerald. 2014. Creeping Bentgrass Field Response to Pigment-Containing Products. Agron. J. 106(4): 1533-1539.

Murphy, J. T., C.E. Owensby, J.M. Ham, and P.I. Coyne. 2014. Estimation of Vegetative Characteristics by Remote Sensing. Academic Res. J. Agric. Sci. Res. 2(3): 47-56.

Penuelas, J., I. Filella, C. Biel, L. Serrano, and R. Save. 1993. The reflectance at the 950–970 nm region as an indicator of plant water status. Int. J. Remote Sens. 14(10): 1887-1905.

Reynolds, W.C., G.L. Miller, and T.W. Rufty. 2012. Athletic field paint impacts light spectral quality and turfgrass photosynthesis. Crop Sci. 52(5): 2375-2384.

Rouse Jr, J.W., R.H. Haas, J.A. Schell, and D.W. Deering. 1974. Monitoring vegetation systems in the Great Plains with ERTS. NASA SP 351: 309.

Sims, D. A. and J. A. Gamon. 2003. Estimation of vegetation water content and photosynthetic tissue area from spectral reflectance: a comparison of indices based on liquid water and chlorophyll absorption features. Remote Sens. Environ. 84(4): 526-537.

Stiegler, J., G. Bell, N. Maness, and M. Smith. 2005. Spectral detection of pigment concentrations in creeping bentgrass golf greens. Int. Turf. Soc. Res. J 10: 818-825.

Stier, J. C. 2006. Understanding and managing environmental stresses of turfgrass. XXVII International Horticultural Congress-IHC2006: International Symposium on Horticultural Plants in Urban and Peri-Urban 762.

Taylor, G.R., C.H. Peacock, J.M. DiPaola, and U. Blum. 1994. Influence of wind velocity on temperature, leaf water potential and soil moisture percentage of creeping bentgrass golf greens. 1995 Agronomy abstracts. ASA, Madison, WI: 187.

Xu, Q. and B. Huang. 2001. Morphological and physiological characteristics associated with heat tolerance in creeping bentgrass. Crop Sci. 41(1): 127-133.

Xu, Q., B Huang, and Z. Wang. 2002. Photosynthetic responses of creeping bentgrass to reduced root-zone temperatures at supraoptimal air temperature. J. Am. Soc. Hortic. Sci. 127(5): 754-758.

CHAPTER 2. Detecting herbicide-induced stress of annual bluegrass using hyperspectral radiometry and derivative analysis

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Abstract

Environmental and pathogen-related turfgrass stress is often not apparent until tissue damage leads to visible symptoms. In many cases, the damage may be irreversible and reactive measures are not adequate to save the turf. The ability to detect stress prior to symptom development could enable more proactive management strategies. Hyperspectral radiometric measurements are useful for investigating plant response to visible and near-infrared light at many specific wavelengths. The shape of plotted spectral reflectance data and their derivatives can be used to detect stress by comparing deviations from healthy plant reflectance. Three studies were conducted in Blacksburg, VA to explore narrow-band measurements of annual bluegrass for detection of herbicide-induced stress on golf fairways and greens. Turf was treated with bispyribac-sodium (74.1 g ai ha^{-1}) and amicarbazone (241 g ai ha^{-1}) to elicit a stress response in annual bluegrass. Derivative reflectance data identified a double-peak feature that appeared to be strongly altered by herbicide treatment in as little as two days after treatment. A modification of the previously-reported double-peak index (mDPi) and normalized difference vegetative index

(NDVI) did not consistently detect herbicide treatment effects prior to visible symptom development. A new procedure was developed that utilizes the slope ($Slope_{705}$) across the double-peak feature in derivative reflectance of healthy annual bluegrass. $Slope_{705}$ could consistently discern treatment of amicarbazone two or more DAT and bispyribac-sodium three or more DAT. Results from this experiment provide evidence that physiological responses of annual bluegrass may be detected using narrow-band spectral analyses, as early as five days prior to visible symptom development.

INTRODUCTION

The factor most limiting to managing annual bluegrass (ABG *Poa annua* L.) on golf putting greens is a rapid decline attributed to various biotic and abiotic stresses (Huang 2003). While ABG is often considered a desirable turf for golf putting greens, management requirements are much greater than other species, such as creeping bentgrass (*Agrostis stolonifera* L.) or hybrid dwarf bermudagrass (*Cynodon dactylon* Pers. x *C. transvaalensis* Burtt-Davy) (Beard 1973; Dernoeden 2012). Annual bluegrass is particularly susceptible to anthracnose [*Colletotrichum cereale* Manns *sensu lato* Crouch, Clarke, and Hillman] (J.M Vargas 2003). Management of this disease requires multiple fungicide applications throughout the season. Since the majority of golf putting greens in temperate regions are a heterogeneous mix of annual bluegrass and creeping bentgrass (Dernoeden 2012), the ability to detect stress before symptom development could reduce management inputs and improve playing conditions on most golf courses.

Plant stress from biotic and abiotic factors has been quantified objectively using reflectance data (Knipling 1970). A healthy plant has higher absorbance in the visible red (VR) region and lower absorbance in the near infra-red (NIR) region, compared with a plant under stress (Bell et al. 2000; Fenstermaker-Shaulis et al. 1997; Nutter Jr et al. 1993). The most common approach for utilizing reflectance data with turfgrasses is via vegetative indices derived from wide-band reflectance in the near infrared (NIR) and visible regions of the light spectrum. Indices first developed in production crops such as normalized difference vegetative index (NDVI) and simple ratio vegetation index (RVI) have been evaluated most frequently as indicators of turf health (Bremer et al. 2011a; Trenholm et al. 1999). Normalized difference vegetative index is calculated as [(NIR – VR)/ (NIR + VR)] (Rouse Jr et al. 1974). Ratio vegetative index is calculated as [NIR/VR] (Birth and McVey 1968). The region of sharp change in reflectance of vegetation between VR and NIR is known as the red edge (RE). Numerous indices have been developed to utilize the stable RE region for use with several agronomic cropping systems (Gitelson et al. 2006; Merzlyak et al. 2003; Vogelmann et al. 1993; Zarco-Tejada et al. 2001). For example, herbicide-induced stress of two *Pinus* species was detected prior to symptom development using the ratio of [694 nm/700 nm] (Carter et al. 1996).

Hyperspectral radiometers provide continuous narrow-band light reflectance measurements throughout the visible and NIR regions (Blackburn 2007). These data have been collected under laboratory and field settings at the leaf and canopy scale. Radiometric data is collected in cropping situations using ground-based, airborne, and satellite platforms (Eismann 2012). Vegetation indices derived from hyperspectral data have been used to estimate chlorophyll concentration in various cropping systems and green leaf area index for soybean, corn and wheat (Gitelson et al. 2005; Haboudane et al. 2004; Haboudane et al. 2002). While portable field radiometers are widely utilized for many environmental, forestry, and agronomic purposes, use with highly maintained turfgrasses is mostly unexplored. Hyperspectral radiometry has been evaluated for detection of localized dry spot of creeping bentgrass and to differentiate several common turfgrass and weed species (Hutto et al. 2006; Hutto et al. 2010).

Analysis of derivative spectra has proven to be useful for detecting changes in plant reflectance that may otherwise be undetected (Tsai and Philpot 1998). Derivative analysis of narrow-band reflectance spectra has been used to detect stress on grass, field bean, and winter wheat caused by underground gas leaks (Smith et al. 2004). The most distinguishing feature of derivative reflectance is a double-peak feature found along red edge spectra (Boochs et al. 1990; Clevers et al. 2004). Further exploration of the double-peak feature of derivative reflectance revealed a strong relationship to steady-state chlorophyll fluorescence (Zarco-Tejada et al. 2003). Derivative spectra have not been investigated on intensively managed turfgrasses.

Early detection of a known stress response, such as that exhibited by herbicide injury, may provide a practical method for turfgrass managers to respond prior to symptom development and subsequent death of ABG. The objectives of this research were to a) evaluate spectral derivative reflectance as a means for detecting herbicide injury to ABG and b) explore whether these data can discern herbicide treatment prior to visible symptom development.

MATERIALS AND METHODS

Study area

Replicated field evaluations were conducted on three creeping bentgrass research areas that were naturally infested with annual bluegrass. In 2011 research was conducted at a single putting green site at the Virginia Tech Golf Course, Blacksburg, Virginia, USA, consisting of approximately equal mixture of ABG and creeping bentgrass maintained on a sandy loam root zone. In May 2015, field studies were conducted at two locations: 1) Glade Road Research Facility, Blacksburg VA, on an ‘L93’ creeping bentgrass golf fairway with clay loam root zone, and 2) at the Turfgrass Research Center, Blacksburg VA, on an ‘L93’ creeping bentgrass putting green built to USGA specification with 90/10 sand/peat mixture. Each site was naturally infested with approximately 15% annual bluegrass. Putting greens were mowed five times per week at 4 mm with clippings removed. The fairway was mowed three times per week at 15 mm with clippings returned. Irrigation was applied only to prevent drought stress. Two herbicide treatments, bispyribac-sodium (74.1 g ai ha^{-1}) and amicarbazone (247 g ai ha^{-1}), were applied on October 3, 2011 and on May 15, 2015 using a CO_2-pressurized sprayer calibrated to deliver 408

L ha^{-1} with TTI8003 nozzles. Herbicide treatments were compared to a non-treated control and applied in a randomized complete-block experimental design, with three replications in 2011 and four replications at each location in 2015.

Spectral measurements

Reflectance data were collected using hand-held field radiometers (FieldSpec 3 Pro; Analytical Spectral Devices, Inc., Boulder, CO in 2011 and PSR-1100F; Spectral Evolution, Lawrence, MA in 2015). Each radiometer was fitted with a plant probe with a spot size of 2.5 cm, which was placed in direct contact with ABG colonies. The FieldSpec 3 radiometer collected canopy reflectance from 350 to 2500 nm, with a 1.4 nm sampling bandwidth and a spectral resolution of 3 nm at 700 nm and 10 nm at 1400 nm. The PSR-1100F data were collected across the spectral range of 320 to 1100 nm, with a sampling bandwidth of 1.4 nm and spatial resolution of 3 nm at 600 nm. Ancillary studies were conducted prior to 2015 data collection to ensure uniformity between radiometers (data not shown). Reflectance and derivative spectra were collected from healthy ABG colonies with each radiometer and data were compared for characteristic features.

Distinct colonies of annual bluegrass were selected from each plot prior to treatment for daily data collection throughout the study period. Reflectance data were collected from ten subsamples in three replications and eight subsamples in four replications in 2011 and 2015, respectively. Collections were made daily between 11:00 am and 1:00 pm prior to herbicide application and on seven subsequent days. Each radiometer was routinely calibrated for reflectance between replications using white $BaSO_4$ calibration panels. Data were not collected on day five in 2015 because of rainfall.

Spectral analysis and optical indices

Raw reflectance data were imported from ViewSpec Pro (Analytical Spectral Devices, Inc., Boulder, CO) in 2011 and DARWin SP (Spectral Evolution, Lawrence, MA) in 2015 into Excel (Microsoft Office Plus Pro 2013) for transformation to ensure calculation uniformity. Subsamples within each plot were averaged for one mean reflectance curve per replication of each treatment per day for each of three sites. First derivatives of mean reflectance spectra were calculated continuously as difference of spectral reflectance every 7 nm using formulae previously described in the FieldSpec 3 Pro User's Manual (Anonymous 2008).

Effects of herbicide application were monitored with canopy reflectance (R) or transformed canopy derivative reflectance (D) (Figure 1). These data were utilized to calculate NDVI, a modified double peak index, and the slope in derivative reflectance over the double peak. The NDVI, calculated as [$(R_{760}-R_{670})/(R_{760}+R_{670})$, where R_x is canopy reflectance at x nm wavelength], was used as a standard comparison because of a strong established relationship with visual turf quality (Bremer et al. 2011b; Carrow et al. 2010). A vegetation index using transformed derivatives that characterized a double-peak feature along the red edge was calculated using a formula modified from Zarco-Tejada et al. (2003) [$mDPi = (D_{705}*D_{730})/D_{715}^2$), where D_x is canopy reflectance at x nm wavelength] (Figure 2a). The rate of change ($Slope_{705}$) from D_{705} to D_{730} was calculated as follows (Figure 2b):

$$b = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sum (x-\bar{x})^2}$$

2.4 Statistical analysis

Daily reflectance of NDVI, mDPi, and $Slope_{705}$ were subjected to analysis of variance for main effects of treatment, location, and their interaction, using SAS PROC GLM (SAS Institute v. 9.3, Cary, NC). Means were separated, when appropriate, using Fisher's protected LSD test at

$P \leq 0.05$. Non-linear regression of daily $Slope_{705}$ was performed for each treatment using the four parameter logistic (4PL) model, with the equation: $y = d + (a - d)/(1 + (x/c)^b)$, where x = time after treatment, y = estimated derivative reflectance, and estimated parameters of minimum asymptote (a), hill slope (b), inflection point (c), and maximum asymptote (d) (Table 1).

RESULTS AND DISCUSSION

Analysis of variance revealed a treatment by location interaction among all three locations, but this interaction was no longer significant when the TRC data were not included in the analysis. Therefore, data from VTGC in 2011 and Glade in 2015 were pooled and presented separately from TRC 2015. Turf at the VTGC and Glade locations was actively growing, with sufficient soil moisture prior to trial initiation, while the TRC location was exhibiting drought stress prior to trial establishment. Average NDVI for all treatments for each site at 0 days after treatment (DAT) were 0.79 (Glade), 0.77 (VTGC), and 0.59 (TRC), indicating that the third location was already under stress at trial initiation. This inherent stress at TRC is presumed to be the cause of the treatment by location interaction.

Visual phytotoxicity and NDVI

Chlorosis of ABG tissue was first observed in plots treated with bispyribac 7 DAT at each location. Amicarbazone injury was less discernible than bispyribac 7 DAT in 2011, and was not visible at either location in 2015. NDVI of ABG, used in this study as a standard objective measurement of turf health (Bremer et al. 2011b), was first impacted by each herbicide 7 DAT at the pooled locations and by bispyribac only at TRC 4 and 6 DAT (Table 2). There were no significant differences in NDVI at 7 DAT for the TRC location and no differences noted prior to 7 DAT at the pooled locations. This method for estimating herbicide injury is congruent with visual observations, but does not provide a method for early detection of herbicide-induced stress.

Spectral reflectance and derivative spectral reflectance

Stress induced by herbicides was indiscernible using raw spectral reflectance (Figure 1). However, examination of first derivative reflectance data exposed a double-peak feature along the red edge (690-730 nm) region throughout the study on healthy, untreated ABG that was lost over time with plants under herbicide-induced stress (Figure 1). This feature was present at Glade and VTGC locations, but not TRC. A similar double-peak of derivative spectra has been described in other cropping systems within the red edge spectra (Boochs et al. 1990; Clevers et al. 2004; Smith et al. 2004; Zarco-Tejada et al. 2004). Zarco-Tejada et al. (2004) concluded this double-peak is closely related to chlorophyll fluorescence and defined a double-peak optical index (DPi = $(D_{688} \times D_{710})/D_{697}^2$). However, the double-peak feature presented in this manuscript occurred at different wavelengths than those reported by the Zarco-Tejada et al. (2004), and therefore the DPi would be insufficient with these data. A modified DPi (mDPi = $(D_{705} \times D_{730})/D_{715}^2$) was calculated using the same parameters as used by Zarco-Tejada et al. (2004), based on spectral location of the double-peak present in this study.

The mDPi was able to discern ABG treated with amicarbazone from healthy, non-treated ABG at 6 and 7 DAT for pooled locations, but not bispyribac (Table 2). There were no significant treatment differences on any date at TRC using mDPi. Based on these data, mDPi is insufficient at detecting herbicide-induced stress prior to symptom development.

Using $Slope_{705}$ allowed detection of herbicide treatment beginning 2 DAT at the pooled locations (Table 2). Treatment with amicarbazone was only detectable at 2 DAT, but both herbicide treatments impacted $Slope_{705}$ on all subsequent days. Herbicide application was undetected using $Slope_{705}$ on any day at the TRC location, where pre-disposed stress likely prevented double-peak feature separation from non-treated turf. Detection of herbicide treatment was possible five days before visible symptom development at two sites over two years when ABG was healthy. The sensitivity of detecting herbicide-induced stress was higher using $Slope_{705}$ than using the mDPi and NDVI methods (Table 1).

The red edge is typically considered the most stable spectral region because of a rapid shift from high absorption of visible red light by chlorophyll to high reflection of near infrared (NIR) light. Near infrared reflectance by plant canopies is associated with increased water content, turgor and cell production leading to internal leaf scattering (Gates et al. 1965; Horler et al. 1983). The red edge position (REP), the inflection point between visible red and NIR light, shifts in response to changes in chlorophyll content and subsequent plant stress (Clevers et al. 2002; Horler et al. 1983; Smith et al. 2004). Using the REP to determine an initial wavelength to calculate slopes of the double-peak feature may alleviate the concern of selecting the proper spectral region to examine, as this appears to deviate among studies.

Treatment with amicarbazone was discernable before bispyribac using both the mDPi and $Slope_{705}$ detection methods. Amicarbazone is a Photosystem II inhibitor, therefore has a stronger impact on chlorophyll production. As Zarco-Tejado (2003) concluded, the double-peak feature is closely related to chlorophyll production. These data suggest that degradation of chlorophyll impacts the double-peak more so than an ALS-inhibiting herbicide, despite faster symptom expression with the latter. The only differences detected using NDVI were in plots treated with bispyribac. This index is less sensitive to chlorophyll content, but broadly predicts overall plant health. This stronger relationship to chlorophyll with mDPi and $Slope_{705}$ could prove useful for other stressors to ABG that are directly related to chlorophyll degradation.

Temporal analysis using nonlinear regression

Daily changes in the double-peak feature within derivative spectra were most accurately quantified using $Slope_{705}$. These data from Glade and VTGC were pooled for nonlinear regression because analysis of variance indicated no location by treatment interaction. Derivative spectra from the TRC did not show a double-peak or have significant treatment effects on any dates for $Slope_{705}$, therefore was not regressed.

The four parameter logistic (4PL) nonlinear regression is designed to model data which follow a symmetrical sigmoidal curve. This model was chosen because of the anticipated response of ABG to herbicide application in order to determine lag time before detection. The 4PL model strongly fit these data ($pr^2 \geq 0.87$). Detection of each herbicide followed a consistent pattern and differences from non-treated ABG were discernible 3 DAT and beyond. The non-treated plots had a steady decline in $Slope_{705}$. No supplemental fertility was applied to these plots throughout data collection. The steady decline may be attributed to declining endogenous chlorophyll in response to inadequate nitrogen. This may be a useful tool to track timing of nitrogen applications to ABG and other intensively managed turfgrasses.

CONCLUSIONS

Data presented in this manuscript document how derivative spectral reflectance may be used for detection of herbicide-induced stress to ABG at least five days prior to visible symptom development. A double-peak feature found in the red edge region of healthy ABG became less distinguishable after stress was induced with two unique herbicides. Both methods tested in this study detected ABG treated with a PSII inhibitor before an ALS inhibitor, further supporting reports by Zarco-Tejada et al. (2003) that the double-peak feature is related to chlorophyll fluorescence. The double-peak feature was present in two of three locations. The location where the double-peak was not evident was under moderate drought stress prior to trial establishment, suggesting that the absence of this feature may indicate early onset of multiple stresses in advance of symptom expression. Our research serves as a proof of concept that this technique is useful for early detection of stress to intensively managed turfgrass systems. Future research will address how this feature is impacted by various environmental and biotic stresses to ABG and other turfgrass species.

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REFERENCES

Anonymous (2004). Velocity SG Herbicide Label. In EPA (Ed.), *EPA Reg. No. 59639-136*.

Anonymous (2008). *ViewSpec Pro User Manual*. ASD., Inc. Boulder, CO, USA.

Anonymous (2012). Xonerate Herbicide Label. In EPA (Ed.), *EPA Reg. No. 59639-136.*

Askew, S., Beam, J., McCall, D., Barker, W., Couch, H., & Chamberlin, J. (2004). Annual bluegrass roughstalk bluegrass, and dollar spot control with bispyribac. *Proceedings of the Northeast Weed Science Society Annual Meeting*, 60: 106.

Beard, J.B. (1973). *Turfgrass: Science and Culture.* Englewood Cliffs, NJ: Prentice-Hall.

Bell, G., Danneberger, T., & McMahon, M. (2000). Spectral irradiance available for turfgrass growth in sun and shade. *Crop Science*, *40*, 189-195.

Birth, G.S., & McVey, G.R. (1968). Measuring the color of growing turf with a reflectance spectrophotometer. *Agronomy Journal*, *60*, 640-643.

Blackburn, G.A. (2007). Hyperspectral remote sensing of plant pigments. *Journal of Experimental Botany, 58,* 855-867.

Boochs, F., Kupfer, G., Dockter, K., & Kühbauch, W. (1990). Shape of the red edge as vitality indicator for plants. *Remote Sensing, 11*, 1741-1753.

Bremer, D.J., Lee, H., Su, K., & Keeley, S.J. (2011a). Relationships between normalized difference vegetation index and visual quality in cool-season turfgrass: I. Variation among species and cultivars. *Crop Science, 51*, 2212-2218.

Bremer, D.J., Lee, H., Su, K., & Keeley, S.J. (2011b). Relationships between normalized difference vegetation index and visual quality in cool-season turfgrass: II. Factors affecting NDVI and its component reflectances. *Crop Science, 51*, 2219-2227.

Carrow, R.N., Krum, J.M., Flitcroft, I., & Cline, V. (2010). Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. *Precision Agriculture, 11,* 115-134.

Carter, G.A., Cibula, W.G., & Miller, R.L. (1996). Narrow-band Reflectance Imagery Compared with ThermalImagery for Early Detection of Plant Stress. *Journal of Plant Physiology, 148,* 515-522.

Clevers, J., De Jong, S., Epema, G., Van Der Meer, F., Bakker, W., Skidmore, A., & Scholte, K. (2002). Derivation of the red edge index using the MERIS standard band setting. *International Journal of Remote Sensing, 23*, 3169-3184.

Clevers, J.G.P.W., Kooistra, L., & Salas, E.A.L. (2004). Study of heavy metal contamination in river floodplains using the red-edge position in spectroscopic data. *International Journal of Remote Sensing, 25*, 3883-3895.

Dernoeden, P.H. (2012). *Creeping bentgrass management, Second Edition*. CRC Press. Boca Raton, FL.

Eismann, M.T. (2012). Hyperspectral remote sensing. In: SPIE Press Monograph, PM210 Bellingham, WA.

Fenstermaker-Shaulis, L., Leskys, A., & Devitt, D. (1997). Utilization of remotely sensed data to map and evaluate turfgrass stress associated with drought. *Journal of Turfgrass Management, 2,* 65-81.

Gates, D.M., Keegan, H.J., Schleter, J.C., & Weidner, V.R. (1965). Spectral properties of plants. *Applied Optics, 4*, 11-20.

Gitelson, A.A., Keydan, G.P., & Merzlyak, M.N. (2006). Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. *Geophysical Research Letters, 33*, Art. No. L11402.

Gitelson, A.A., Vina, A., Ciganda, V., Rundquist, D.C., & Arkebauer, T.J. (2005). Remote estimation of canopy chlorophyll content in crops. *Geophysical Research Letters, 32*, Art. No. L08403.

Haboudane, D., Miller, J.R., Pattey, E., Zarco-Tejada, P.J., & Strachan, I.B. (2004). Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: Modeling and validation in the context of precision agriculture. *Remote Sensing of Environment, 90*, 337-352.

Haboudane, D., Miller, J.R., Tremblay, N., Zarco-Tejada, P.J., & Dextraze, L. (2002). Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. *Remote Sensing of Environment, 81*, 416-426.

Horler, D., Dockray, M., & Barber, J. (1983). The red edge of plant leaf reflectance. *International Journal of Remote Sensing, 4*, 273-288.

Huang, B. (2003). Recent advances in drought and heat stress physiology of turfgrass-a review. In, *I International Conference on Turfgrass Management and Science for Sports Fields 661* (pp. 185-192).

Hutto, K., King, R., Byrd, J., & Shaw, D. (2006). Implementation of hyperspectral radiometry in irrigation management of creeping bentgrass putting greens. *Crop Science, 46*, 1564-1569.

Hutto, K.C., Shaw, D.R., Byrd Jr, J.D., & King, R.L. (2010). Differentiation of turfgrass and common weed species using hyperspectral radiometry. *Weed Science, 54*(2), 335-339.

J.M Vargas, J., A.J. Turgeon (2003). *Poa Annua: Physiology, Culture, and Control of Annual Bluegrass*. John Wiley & Sons, Hoboken, NJ.

Knipling, E.B. (1970). Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. *Remote Sensing of Environment, 1*, 155-159.

Lycan, D.W., & Hart, S.E. (2006). Seasonal effects on annual bluegrass (*Poa annua*) control in creeping bentgrass with Bispyribac-sodium. *Weed Technology, 20*, 722-727.

McCullough, P.E., Hart, S.E., Weisenberger, D., & Reicher, Z.J. (2010). Amicarbazone efficacy on annual bluegrass and safety on cool-season turfgrasses. *Weed Technology, 24*, 461-470.

Merzlyak, M.N., Solovchenko, A.E., & Gitelson, A.A. (2003). Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. *Postharvest Biology and Technology, 27*, 197-211.

Nutter Jr, F., Gleason, M., Jenco, J., & Christians, N. (1993). Assessing the accuracy, intra-rater repeatability, and inter-rater reliability of disease assessment systems. *Phytopathology, 83*, 806-812.

Rouse Jr, J.W., Haas, R., Schell, J., & Deering, D. (1974). Monitoring vegetation systems in the Great Plains with ERTS. *NASA SP-351*, 309.

Shimizu, T., Nakayama, I., Nagayama, K., Miyazawa, T., & Nezu, Y. (2002). Acetolactate synthase inhibitors. *Herbicide classes in development* (pp. 1-41): Springer Publishing, New York, NY, USA.

Smith, K., Steven, M., & Colls, J. (2004). Use of hyperspectral derivative ratios in the red-edge region to identify plant stress responses to gas leaks. *Remote Sensing of Environment, 92*, 207-217.

Trenholm, L., Carrow, R., & Duncan, R. (1999). Relationship of multispectral radiometry data to qualitative data in turfgrass research. *Crop Science*, *39*, 763-769.

Tsai, F., & Philpot, W. (1998). Derivative analysis of hyperspectral data. *Remote Sensing of Environment, 66*, 41-51.

Vogelmann, J.E., Rock, B.N., & Moss, D.M. (1993). Red edge spectral measurements from sugar maple leaves. *International Journal of Remote Sensing, 14*, 1563-1575.

Zarco-Tejada, P.J., Miller, J., Morales, A., Berjón, A., & Agüera, J. (2004). Hyperspectral indices and model simulation for chlorophyll estimation in open-canopy tree crops. *Remote Sensing of Environment, 90*, 463-476.

Zarco-Tejada, P.J., Miller, J.R., Noland, T.L., Mohammed, G.H., & Sampson, P.H. (2001). Scaling-up and model inversion methods with narrowband optical indices for chlorophyll content estimation in closed forest canopies with hyperspectral data. *Geoscience and Remote Sensing, IEEE Transactions on, 39*, 1491-1507.

Zarco-Tejada, P.J., Pushnik, J., Dobrowski, S., & Ustin, S. (2003). Steady-state chlorophyll a fluorescence detection from canopy derivative reflectance and double-peak red-edge effects. *Remote Sensing of Environment, 84*, 283-294.

CHAPTER 2: Tables and Figures

Table 1. Estimated parameter values for derivative spectra of three herbicide treatments on annual bluegrass, using the four parameter logistic nonlinear regression model.

	Estimated parameter				
Treatment	*a*	*B*	*c*	*d*	*Pseudo* R^2
Amicarbazone	-0.002807751	4.439199	3.167156	-0.009370773	0.9418
Bispyribac-sodium	-0.001997761	4.532394	3.11065	-0.009431405	0.9596
Non-treated	-0.000698932	0.8243771	59.28303	-0.01592888	0.868

Table 2. Analysis of variance of the daily (DAT) reflectance collected from herbicide-treated annual bluegrass (ABG), where $Slope_{705}$ = rate of change in first derivative reflectance over the spectral range of 705-730 nm, mDPi = modified Double Peak Index of derivative reflectance $(D_{705}*D_{730})/D_{715}^2$, and NDVI = normalized difference vegetation index of canopy reflectance $(R_{760}-R_{670})/(R_{760}+R_{670})$. Data from the Glade Road Research Facility (Glade) and the Virginia Tech Golf Course (VTGC) were pooled, but data from the Virginia Tech Turfgrass Research Center (TRC) were analyzed separately. Significance levels (*P*) indicate the difference between non-treated ABG and ABG treated with amicarbazone ([a]), bispyribac ([b]), or both ([c]), and are listed for each day after treatment unless insignificant (NS).

	Glade & VTGC			TRC (pre-stressed)		
DAT	**$Slope_{705}$**	**mDPi**	**NDVI**	**$Slope_{705}$**	**mDPi**	**NDVI**
0	NS	NS	NS	NS	NS	NS
1	NS	NS	NS	NS	NS	NS
2	0.023[a]	NS	NS	NS	NS	NS
3	0.058[c]	NS	NS	NS	NS	NS
4	0.007[c]	NS	NS	NS	NS	0.002[b]
6	0.024[c]	0.010[a]	NS	NS	NS	0.007[b]
7	0.053[c]	0.0001[a]	0.054[c]	NS	NS	NS

Figure 1. Canopy reflectance (gray line) and derivative reflectance (black line) collected from healthy, nontreated (solid) and bispyribac-treated (broken) annual bluegrass using a field radiometer over a spectral range of 300-1100 nm. Derivative spectra scaled up (*100).

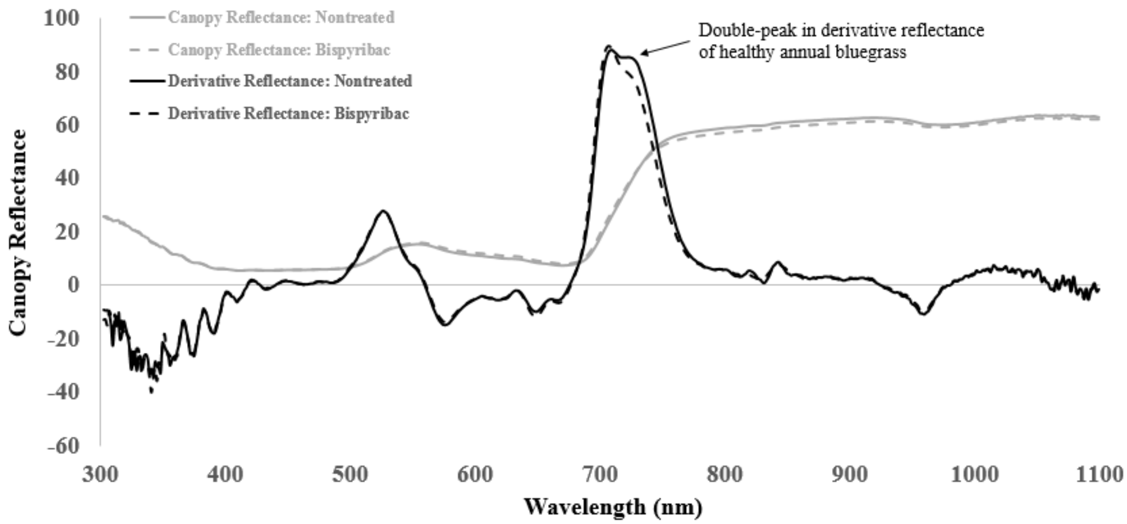


Figure 2. Double-peak spectral feature of derivative reflectance collected from healthy (solid line) and herbicide-stressed (broken line) annual bluegrass using a field radiometer in the 705-730 nm spectral range. A modified double-peak index (mDPi) was calculated using derivative reflectance bands 705, 715, and 730 nm (a) and slope of derivative spectra between 705 and 730 nm (b).

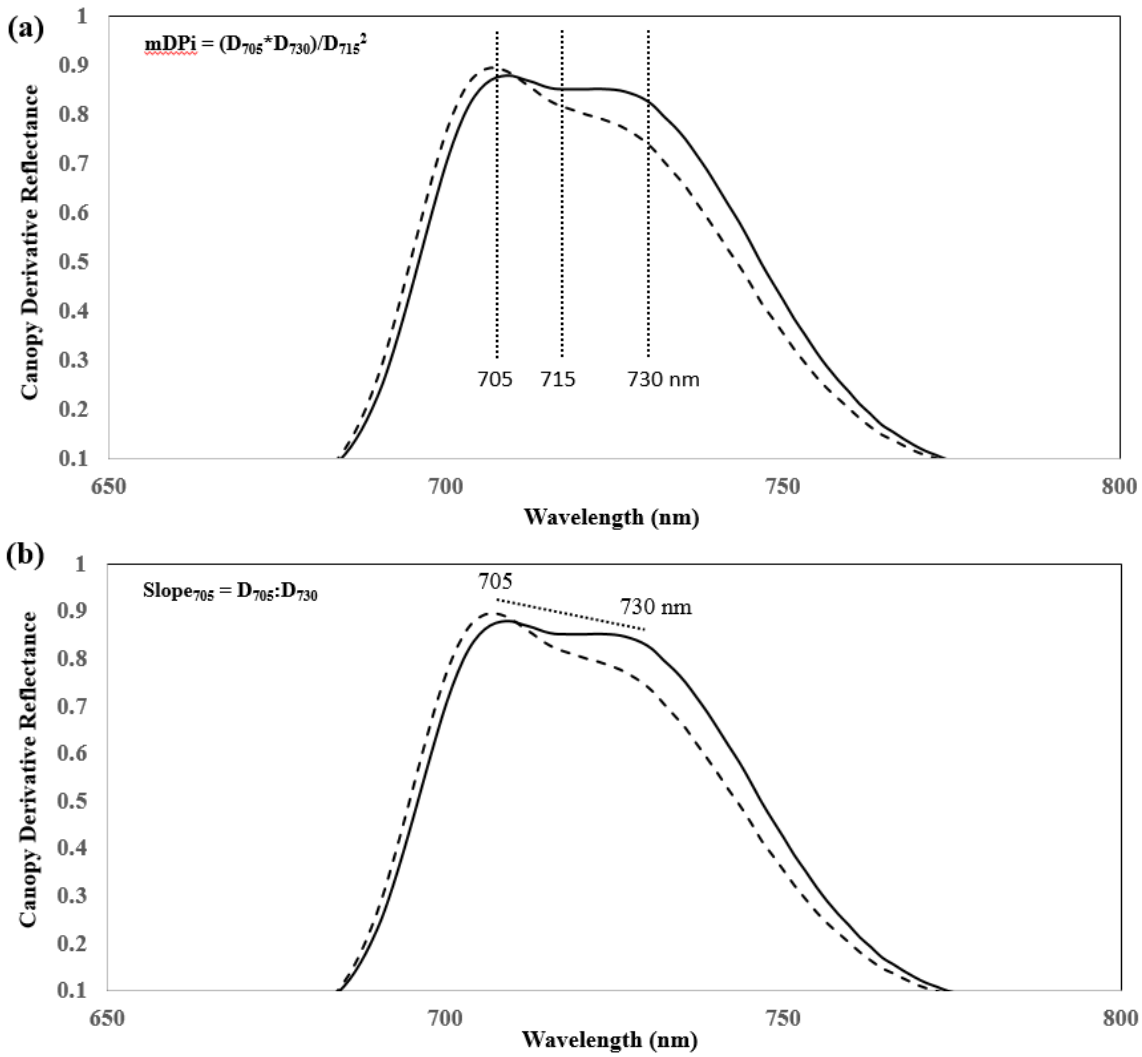
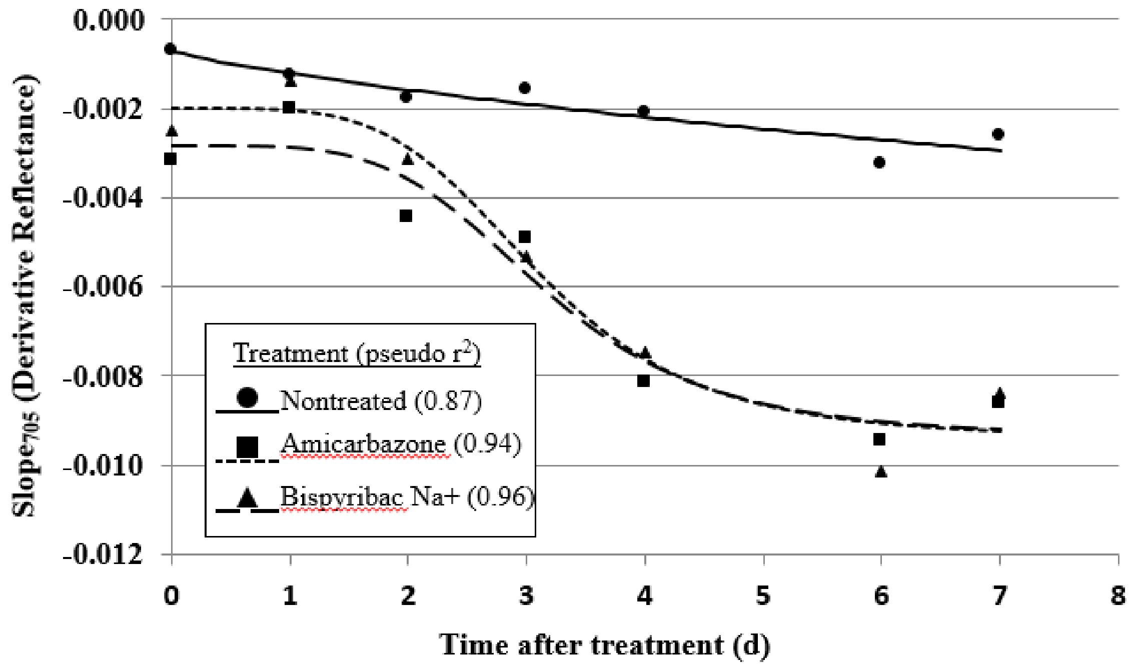


Figure 3. Herbicide treatment effect on first derivative spectral reflectance of annual bluegrass over time using slope between 705 and 730 nm.



CHAPTER 3. Enhanced soil moisture assessment using narrowband reflectance vegetation indices in creeping bentgrass[1]

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Abbreviations: NDVI, normalized difference vegetation index; RVI, simple ratio vegetation index; WBI, water band index

ABSTRACT

The suitability of maintained turfgrasses are measured by aesthetic appearance and ability to withstand a variety of stresses instead of yield. Historically, researchers have quantified the acceptance of a turfgrass by its visual quality. Rater inconsistencies and bias necessitate the use of vegetation indices (VI) as an objective alternative measurement. The normalized difference vegetation index (NDVI) is commonly used in agricultural research and related fields because of a strong relationship to many plant health characteristics. Relationships have been established between NDVI of turfgrass canopies and important factors that impact stand health, including

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both soil moisture content and leaf chlorophyll content. While moisture stress and chlorophyll content contribute to the overall health of grasses, NDVI is unable to differentiate between variables. Numerous VI have been established in many cropping systems because of demonstrated relationships to specific variables of interest. The water band index (WBI) has been useful for predicting water availability in cropping and grassland systems, but has not been explored with turfgrass systems. The primary objective of this study was to compare the relationships of sixteen established VI to tissue chlorophyll content and soil water content of creeping bentgrass. Eight to twelve week old ‘L-93’ creeping bentgrass was maintained under greenhouse conditions with a soil profile built to USGA specifications to test irrigation and nitrogen fertility effects on spectral and measured responses of water content and chlorophyll. All VI were moderately to strongly correlated to visual turf quality (r = 0.46 - 0.86) and total chlorophyll content (r = 0.49 - 0.85). The water band index was most closely related to soil volumetric water content of all indices tested. Only WBI ($r \geq 0.80$) and the green/red ratio index (GRI, $r \geq 0.50$) were significantly related to soil volumetric water content in each of two trials. Normalized difference vegetative index was weakly related to soil water content in only one trial (r = 0.49). Non-linear regression was used to show that WBI can be useful for estimating a decline in soil water content as it drops below field capacity and creeping bentgrass approaches the permanent wilting point.

INTRODUCTION

Water conservation for intensively managed turfgrasses is a critical priority, as water quality and availability are widely recognized as increasing global concerns. Golf courses alone used an estimated 775 trillion gallons of water in the Unites States in 2005 (Throssell et al., 2009). Conservation strategies employed by golf course superintendents and their staff resulted in an estimated water savings of 21.8% by 2013 (Anonymous, 2015). Although these savings are significant, further reductions are needed to offset a growing global water demand. While some facilities chose to reduce total irrigated acreage, many were able to conserve water through other practices, such as hand watering, maintaining a drier soil profile, using moisture sensors, and scheduling irrigation based on evapotranspiration (ET) rates. ET-based irrigation provides a more precise prescription in terms of frequency and amount required for a general area from weather station data, but does not provide sufficient spatial resolution for site-specific water needs (Feldhake et al., 1983). The use of hand-held moisture meters has become increasingly prevalent on golf greens to rapidly assess site-specific irrigation needs, but is time consuming and root zone moisture content can change dramatically throughout the day (Gatlin, 2011).

Precision turfgrass water management using time-domain reflectometry (TDR) and spectral reflectance mapping was proposed as an alternative method to improve irrigation efficiency (Krum et al., 2010). The normalized difference vegetation index (NDVI = $(R_{NIR} - R_{red})/(R_{NIR} + R_{red})$), a reflectance index of relative plant health that has also been associated with soil moisture (Jiang et al., 2009, Johnsen et al., 2009), was used by the authors to relate turfgrass stress to soil moisture. Krum et al. (2010) suggested that volumetric water content of soils was more accurately estimated using TDR than NDVI. Jiang et al. (2009) demonstrated a moderate, yet significant ($P = 0.001$), correlation between NDVI and both soil water content ($r = 0.22 – 0.30$)

and leaf relative water content (r = 0.39 – 0.72). Similarly, Johnsen et al. (2009) established a relationship between NDVI and volumetric water content of soil (r = 0.28 – 0.64) as well as turf quality (r = 0.42 – 0.71), indicating that factors beyond water availability were impacting overall turf quality.

Normalized difference vegetative index is a measurement of red and near infrared light reflectance, and has become the most commonly used vegetation index for agronomic crops and maintained turfgrass (Carrow et al., 2010, Murphy et al., 2014, Rouse et al., 1974). Several factors that contribute to overall turf quality have been associated with this index, including color, canopy structure, density, and chlorophyll concentrations (Bremer et al., 2011, Stiegler et al., 2005, Trenholm et al., 1999, Trenholm et al., 2000). Previous research has shown that chlorophyll concentrations are negatively impacted by drought stress (DaCosta et al., 2004). As grasses experience water stress, chlorophyll and related pigments begin to lose functionality (Hüner and Hopkins, 2008). Concentrations of various endogenous pigments of creeping bentgrass (*Agrostis stolonifera* L.), including chlorophylls *a* and *b*, lutein, and β–carotene, are known to be weakly related to NDVI (Stiegler et al., 2005).

Alternative narrowband indices have been developed in forest canopies and agronomic crops using reflectance of spectra more closely related to chlorophylls *a* and *b* than NDVI (Blackburn, 2007). Chlorophyll concentrations are linearly related to several wavelengths of peak chlorophyll fluorescence, making these wavelengths ideal for distinguishing chlorophyll content from other factors that contribute to spectral reflectance, such as water availability (Gitelson et al., 2003). While the content and viability of chlorophyll within plant tissue is known to be related to drought (DaCosta et al., 2004), the decline in chlorophyll is most definitely a consequence of water stress and is likely not the best indicator of water availability prior to drought symptom expression.

Many indices capable of assessing water availability utilize narrowband spectra that overlap with chlorophyll features (Murphy et al., 2014). However, distinct water absorption features independent of pigment concentrations are present across the near infrared region, notably with centers around 970, 1200, 1450, 1950, and 2250 nm (Penuelas et al., 1993, Sims and Gamon, 2003). The water band index (WBI = R_{900}/R_{970}) is used as an indicator of plant water status and to monitor drought effects in agronomic crops, desert, and tallgrass prairie ecosystems (Claudio et al., 2006, Murphy et al., 2014, Penuelas et al., 1993, Sims and Gamon, 2003). Because of the unique properties of the water absorption bands beyond photosynthetically active spectra, it is highly probable that this index can be used to differentiate turfgrass water stress from other abiotic stresses.

Optimizing data efficiency through proper sensor selection will lend itself to precision-guided irrigation when combined with geographic information systems applications. It is becoming increasingly feasible to rapidly collect time-sensitive data across large areas, such as golf courses, with the commercialization of unmanned automated systems, commonly referred to as drones. These systems are able to capture large data sets with mounted sensors. However, improper interpretation of these data may lead to mismanagement of water and other resources. Therefore, the objectives of this research are to 1) explore various broadband and narrowband indices that are best used to detect soil and leaf tissue moisture content in turfgrass systems and 2) define the relationship between indices and soil moisture content to be used as a predictor of future water stress.

MATERIALS & METHODS

Greenhouse experiments were conducted at the Glade Road Research Facility on Virginia Tech's campus in Blacksburg, VA in December 2015 and February 2016. Research was conducted on 12- to 16-week-old 'L-93' creeping bentgrass grown from seed under greenhouse conditions in D40H Deepot Conetainers (Stuewe & Sons, Tangent, OR) filled with a 90:10 sand/peat root-zone mix. The turf was mowed twice per week at 10 mm with scissors, watered daily, and fertilized weekly with of 7.3 kg N ha^{-1} (46-0-0). Weekly N was withheld from Conetainers two and three weeks prior to treatment applications in studies one and two, respectively.

Treatments of irrigation and fertility were arranged as a 2 by 2 factorial in a randomized complete block design over five replications. Conetainers were either irrigated with 1 cm 48 hr^{-1} to remain above field capacity or not irrigated. Fertility treatments included no additional fertility and 24.4 kg N ha^{-1} (46-0-0). All treatments were irrigated deeply until the root zone was fully saturated and excess moisture drained from Conetainer bottoms prior to treatment. Nitrogen fertilizer was mixed with water and applied uniformly over all fertility treatments using a CO_2 pressurized spraying system, calibrated to deliver 813 L ha^{-1} through TTI11004 flat fan nozzles. All Conetainers were then irrigated overhead with an additional 2 mm water to move N from leaves to prevent burn.

Spectral Reflectance Measurements

Spectral reflectance data were collected using a handheld portable field radiometer (PSR-1100F, Spectral Evolution, MA), fitted with a plant probe measuring a spot size of 2.5 cm directly from the canopy surface. This radiometer measured 512 unique spectra across a range of

320 to 1100 nm at a 1.4 nm sampling bandwidth and 3 nm at 700 nm spectral resolution. Reflectance sensors were calibrated prior to collection of each replication using a $BaSO_4$ white reference calibration panel by placing the plant probe directly on the panel surface.

Vegetation indices were derived from spectral data using formulas presented in Table 1. The table is separated into two categories: those that could be obtained using a typical three- to five-band multispectral sensor and those requiring reflectance measurements from distinct small bandwidth spectra. Several commercially available multispectral sensors are equipped to measure reflectance from blue, green, red, near infrared, and the red edge regions. Calculations in this manuscript were used as comparisons to filters being utilized in a Crop Circle ACS-470 (Holland Scientific, Inc., Lincoln, NE) multispectral radiometer, which included red (670 nm), near infrared (760 nm), red edge (730 nm), and green (550 nm) spectra. Wavelength modifications were made for NDVI, RVI, GNDVI, RGI, and CHLOR1 to fit these filters (Table 1), without compromising original integrity of principles described in the literature (Birth and McVey, 1968, Gamon and Surfus, 1999, Gitelson and Merzlyak, 1994, Rouse et al., 1974). The photochemical efficiency index (PEI) was derived using spectra related to chlorophyll fluorescence (Schmidt et al., 1999, Zhang and Schmidt, 2000). All other indices were calculated using previously described equations (Gitelson and Merzlyak, 1994, Gitelson et al., 1998, Lobell and Asner, 2003, Penuelas et al., 1993, Thenkabail et al., 2011).

Leaf Pigment Content

Leaf pigment concentrations were determined from harvested plots 11 d after trial initiation, in accordance with previously described wet lab procedures (Lichtenthaler, 1987, Zhang et al., 2005). Pigments were extracted from 50 mg of fresh leaf tissue in 5 ml pure acetone in total darkness for approximately 72 hr. Spectral absorbance at 470 nm, 645 nm, and 662 nm, was measured using a laboratory spectroradiometer (Multiskan GO, Thermo Fisher Scientific, Inc., Waltham, MA). Chlorophyll content was derived using formulas defined by Lichtenthaler (1987) as follows:

Chl *a* = (11.24 × absorbance at 661.6 nm) – (2.04 × absorbance at 644.8 nm)

Chl *b* = (20.13 × absorbance at 644.8 nm) – (4.19 × absorbance at 661.6 nm)

Chl *a*+*b* = (7.05 × absorbance at 661.6 nm) + (18.09 × absorbance at 644.8 nm)

Carotenoids (xanthophylls and β-carotene) =

(1000 × absorbance at 470 nm) – (1.90 × Chl a – 63.14 × Chl b) ÷ 214

Remaining foliage was used to obtain tissue water content (TWC) by collecting fresh weight at harvest and dry weight after 72 hr in a drying oven. Tissue water content was calculated as (1 – dry weight / fresh weight). To minimize the impact of tissue moisture on chlorophyll content, fresh weights were adjusted using the following formula and expressed as $\mu g\ g^{-1}$ dry weight: $Chl_x\ \mu g\ fw\ ml^{-1}/(1-TWC)*10$

Data Collection

All data were collected 11 d after trial initiation but prior to tissue harvest for chlorophyll extraction and leaf water content. Soil volumetric water content was collected from each plot using a Field Scout TDR-300 fitted with 3.8 cm turf rods inserted into the root zone (Spectrum Technologies, Inc., Plainfield, IL). Spectral reflectance measurements were collected prior to soil moisture measurements to prevent potential canopy distortion from physical disruption by the TDR-300. Visual turf quality was assessed using a 1 to 9 scale, where 1 = dead turf, 6 = minimally acceptable, and 9 = lush, dense vegetation. Visual turf color was assessed using a similar scale, where 1 = extreme chlorosis and 9 = dark green vegetation. A homogenized mixture of 50 mg leaf tissue was used for pigment extraction and tissue water content was calculated using the remaining tissue.

Statistical Analysis

The eighteen spectral reflectance indices, four pigment concentrations, soil water content, tissue water content, visual turf quality, and visual turf color were subjected to ANOVA with sums of squares partitioned to reflect fertility, irrigation, and trial effects and their interactions using SAS PROC GLM (SAS Institute v. 9.3, Cary, NC). Means were separated using Fisher's protected LSD test at $P \leq 0.05$ when appropriate. Pearson correlation coefficients were calculated to assess the relationships among response variables using PROC CORR of SAS. Non-linear regression of the water band index and soil water content was performed for non-irrigated treatments using the exponential model.

RESULTS & DISCUSSION

Vegetation Indices Correlation

Since all measured responses except the WBI had significant trial interactions, Pearson correlation coefficients were conducted separately by trial to examine the relationships among all indices tested. Six representative indices are compared against all other indices in Table 2. These six indices, NDVI, RVI, PRI, $NDVI^{705}$, GRI, and WBI, were chosen because they are the most common index used by plant scientist (NDVI, PRI), have been proposed to better apply to turfgrass systems (RVI), allow for exclusion of water-related effects on chlorophyll estimation ($NDVI^{705}$), or allow for exclusion of chlorophyll-related effects on water content estimation

(WBI, GRI) (Carrow et al., 2010; Garbulsky et al., 2011; Gitelson and Merzlyak, 1994; McCurdy et al., 2014; Penuelas et al., 1993).

Correlations of NDVI, RVI, and PRI with all other indices ranged from 0.49 to 0.99 and were significant ($P < 0.05$) in each trial. The relationships between $NDVI^{705}$ and RGI, GRI, and WBI were significant on one of two trials. $NDVI^{705}$ was strongly related ($r = 0.92 – 1.00$) to all other indices derived to estimate chlorophyll in both trials, including CHLOR1, CHLOR2, PRI, and PEI. There was also a strong relationship between $NDVI^{705}$ and NDVI3, RVI3, and GNDVI for each trial ($r = 0.98 – 0.99$). Because of these strong relationships, $NDVI^{705}$ is used to represent all chlorophyll-related indices for the remainder of this manuscript.

There were no significant relationships between WBI and NDVI3, RVI3, GNDVI, CHLOR1, CHLOR2, or SGI for either trial. The relationships of WBI to $NDVI^{705}$ and PEI were significant in Trial 2 ($r = 0.46 - 0.48$) but not Trial 1. Similarly, GRI was not related to NDVI3, RVI3, GNDVI, CHLOR1, CHLOR2, NDVI705, SGI, or PEI in Trial 1. Each of these indices except SGI was related to GRI in Trial 2. However, these indices are less closely related to GRI ($r = 0.55 - 0.65$) than all other indices ($r = 0.79 - 0.96$). SGI and GRI were not related in either trial. The strongest correlation to WBI was either GRI or RGI for each trial. RGI is the inverse of GRI and typically has a negative correlation with most indices.

Of the three variations of NDVI and RVI using traditional multispectral bands, the correlation was always strongest to WBI or GRI using red edge (730 nm) and red (670 nm), followed by near infrared (760 nm) and red. NDVI or RVI derived using near infrared and red edge were not significantly related to WBI and related to GRI in Trial 2 only, but less closely than other derivations. Conversely, near infrared and red edge formulas were consistently most closely related to $NDVI^{705}$, followed by near infrared and red, and least related using red edge and red spectra. This is an important consideration when using multispectral radiometry to build relationships to turf quality, as the wavelengths selected may be more closely associated with chlorophyll content or water availability, which can impact interpretation of data.

Measurement Correlation

Selected vegetation indices described above were compared against turf quality, turf color, total chlorophyll, tissue water content, and soil water content using Pearson correlations (Table 3). Each index was related to visual turf quality in Trials 1 & 2 (r = 0.46 – 0.86, P < 0.05) and can be used as an acceptable estimate of overall turf health. Ratio vegetative index was more closely related to turf quality than NDVI in each trial. This is congruent with reports that RVI is a more acceptable index than NDVI on fully established turfgrass canopies (McCurdy et al., 2014). The original intention of NDVI was to overcome background noise caused by soil that is present in an open canopy of agricultural crops. Open canopies within a sward of turfgrass contribute to reduced turf quality and are, therefore, an important consideration when choosing a vegetation index to represent visual estimations of turf quality. Other selected indices are secondary predictors of overall turf quality through association with different quality-influencing variables. The index most closely associated with chlorophyll absorption bands is $NDVI^{705}$ (Gitelson and Merzlyak, 1994). Photochemical reflectance index is associated with photosynthetic activity and CO_2 flux in many plant species and ecosystems (Garbulsky et al., 2011). Water absorption bands are defined using the WBI (Penuelas et al., 1993).

Chlorophylls *a* and *b*, total chlorophyll, and total carotenoid concentrations were all strongly correlated to each other (r = 0.98 – 0.99, P < 0.0001) (data not shown), therefore total chlorophyll is used to represent all pigments in Table 3. The relationship between chlorophyll concentration and turf color was significant in each trial ($r = 0.60 – 0.66$), but not as closely related as some indices. All indices were related to total chlorophyll ($r = 0.49 – 0.85$), with RVI, PRI, and WBI consistently having the strongest correlations within each trial. Normalized difference vegetative index, RVI, $NDVI^{705}$, and PRI were correlated to turf color ($r = 0.51 – 0.97$). The relationships between turf color and WBI were not significant for either trial. GRI was related to turf color in Trial 2 ($r = 0.59$), but the relationship in Trial 1 was insignificant. Visual turf color had the strongest correlation to $NDVI^{705}$ in each trial, considerably higher than wet laboratory quantification of pigments. The primary influence of leaf tissue greenness is most strongly influenced by leaf pigment concentrations (Hüner and Hopkins, 2008). It is worth noting that spectral reflectance measurements were a better indicator of turf color than direct measurement with these data.

Water band index and GRI were correlated to soil water content in each trial. The relationship was stronger between WBI ($r = 0.80 – 0.81$, $P < 0.0001$) than GRI ($r = 0.50 – 0.73$, $P < 0.05$). Volumetric soil water content was not significantly correlated with RVI, $NDVI^{705}$, or PRI, and NDVI was significantly correlated with volumetric soil water content in Trial 1 only ($r = 0.49$, $P < 0.05$). There were no significant irrigation main effects or trial by irrigation effects ($P < 0.05$) of WBI or GRI (Table 4). Since both WBI and GRI were strong predictors of SWC in these experiments, the lack of detectable irrigation effect from these reflectance indices can likely be attributed to the strong trial and fertility influence on SWC in these trials (Table 4). The probability of an irrigation main effect from WBI was $P = 0.0683$ and GRI was $P = 0.0565$, both of which were considerably higher than that of SWC ($P = 0.2857$) and other indices. Because WBI and GRI exhibited the highest probability of irrigation main effects ($P < 0.1$) and exhibited strong correlations with volumetric soil water content, t-tests for mean separation were performed for these indices (Table 5). An irrigation by fertility interaction was observed, thus WBI mean separations were reported separately for irrigation and fertility treatments. Irrigated plots had a higher WBI than non-irrigated plots in both fertilized and non-fertilized treatments, though the magnitude was greater in fertilized plots. For discussion and visualization, WBI values were modified in Table 5 using the transformation (1-WBI)*100. In non-fertilized plots, WBI decreased from 3.96 when irrigated to 1.42 in non-irrigated plots. Plots that received fertilizer had a WBI of 5.99 when irrigated, compared to 1.75 in non-irrigated plots. GRI was significantly impacted by irrigation, regardless of trial or fertility, and was therefore pooled. Mean GRI for irrigated plots was 2.93 compared to 2.35 in non-irrigated plots.

These data suggest that the water band index is a useful tool to estimate water stress for creeping bentgrass grown in a root zone mixture built to USGA specifications. This is congruent with the literature, where the WBI was successful at discerning differences in soil moisture content in several agronomic crops and non-cropping ecosystems. Soil moisture after 11 d in non-irrigated Conetainers was below the permanent wilting point for some plots of creeping bentgrass used for this study (data not shown). Clear moisture stress was evident by this time, as indicated by the decline in visual turf quality and color. Plots under the most extreme drought stress had lower than 4% soil water content. These plots typically had a negative WBI when adjusted using the formula (1-WBI)*100 or values below 1.0 using non-transformed WBI.

Non-linear regression of WBI and SWC was performed for all irrigated and non-irrigated plots using the exponential equation, with no convergence in irrigated plots (data not shown). The curve fit was highly significant to data from non-irrigated plots, where WBI accounted for a large portion of the variability in the model ($r = 0.88$, $P < 0.0001$) (Figure 2). According to this model, WBI values remained relatively steady while soil moisture content was adequate (>6%). As soil moisture declined to stress-inducible levels, the WBI also declined.

CONCLUSIONS

Golf course superintendents and other turfgrass managers are faced with maximizing water use efficiency because of increased global demand of the resource. Conservation efforts to mitigate water use are essential for the viability of the turfgrass industry. This research demonstrates how the water band index can be used as an indicator of soil moisture stress in creeping bentgrass grown in a sand-based root zone. Regression analysis of these data suggest that WBI values decrease dramatically as soil moisture approaches the permanent wilting point of creeping bentgrass grown in high sand root zones. Future research will address this relationship of WBI to water holding capacity of various soil types, as physical characteristics of soil vary tremendously. Use of the WBI may provide a useful tool for rapidly estimating irrigation deficits.

Data presented in this manuscript were collected from creeping bentgrass grown under greenhouse conditions, using a hyperspectral radiometer with a small field-of-view. Evidence is available in the literature that water bands are detectable from reflectance data collected at the canopy scale of agronomic and non-agronomic crops in remote sensing applications. Future work should address how remote sensing approaches can be applied to fine turfgrass systems, such as golf courses and athletic field complexes. The use of unmanned automated systems are being heavily investigated for practical applications in turfgrass management because of the capacity for rapid data collection over large areas using spectral sensors. Sensors designed to capture data from the most optimal bands will benefit prediction efforts. This research demonstrates the ability to differentiate water stress from chlorophyll concentration to guide allocation of resources. A primary advantage of using this index over alternative indices is that validation measurements could be minimal or even eliminated because of the unique spectral properties of the water band. In this study, the WBI was the only measured response that had no trial interactions (Table 4).

While the water band index was most closely related to soil water content, alternative indices that are more practical may still be useful. Several indices that can be derived using data collected from a traditional multispectral radiometer showed potential for predicting water availability, independent of chlorophyll content. The green-red ratio index was most closely related to WBI and to soil water content. Because of inconsistencies between trials, more work is needed before this is considered a viable alternative to WBI. The NDVI and RVI derivations that utilized red edge (730 nm) and red (670 nm) reflectance were more closely related to water content than those utilizing near infrared (760 nm) and red edge. Careful consideration should be made when making generalizations about soil moisture content predictions with NDVI and RVI, and direct measurement of volumetric water content should be conducted for validation.

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LITERATURE CITED

Anonymous. 2015. Golf Course Environmental Profile, Phase II, Volume I. 2014 Water use and conservation practices on U.S. golf courses. Golf Course Superintendents Association of America, Lawrence, KS.

Birth, G.S. and G.R. McVey. 1968. Measuring the color of growing turf with a reflectance spectrophotometer. Agron. J. 60: 640-643.

Blackburn, G.A. 2007. Hyperspectral remote sensing of plant pigments. J. Exp. Bot. 58: 855-867.

Bremer, D.J., H. Lee, K. Su and S.J. Keeley. 2011. Relationships between normalized difference vegetation index and visual quality in cool-season turfgrass: II. Factors affecting NDVI and its component reflectances. Crop Sci. 51: 2219-2227.

Carrow, R.N., J.M. Krum, I. Flitcroft and V. Cline. 2010. Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. Precision Agriculture 11: 115-134.

Claudio, H.C., Y. Cheng, D.A. Fuentes, J.A. Gamon, H. Luo, W. Oechel, et al. 2006. Monitoring drought effects on vegetation water content and fluxes in chaparral with the 970 nm water band index. Remote Sens. Environ. 103: 304-311.

DaCosta, M., Z. Wang and B. Huang. 2004. Physiological adaptation of Kentucky bluegrass to localized soil drying. Crop Sci. 44: 1307-1314.

Feldhake, C., R. Danielson and J. Butler. 1983. Turfgrass evapotranspiration. I. Factors influencing rate in urban environments. Agron. J. 75: 824-830.

Gamon, J. and J. Surfus. 1999. Assessing leaf pigment content and activity with a reflectometer. New Phytol. 143: 105-117.

Garbulsky, M.F., J. Peñuelas, J. Gamon, Y. Inoue and I. Filella. 2011. The photochemical reflectance index (PRI) and the remote sensing of leaf, canopy and ecosystem radiation use efficiencies: a review and meta-analysis. Remote Sens. Environ. 115: 281-297.

Gatlin, R. 2011. A giant leap forward: How soil moisture meters are changing the game of putting green irrigation. USGA Green Section Record 49(38).

Gitelson, A. and M.N. Merzlyak. 1994. Spectral reflectance changes associated with autumn senescence of Aesculus hippocastanum L. and Acer platanoides L. leaves. Spectral features and relation to chlorophyll estimation. J. Plant Physiol. 143: 286-292.

Gitelson, A.A., C. Buschmann and H.K. Lichtenthaler. 1998. Leaf chlorophyll fluorescence corrected for re-absorption by means of absorption and reflectance measurements. J. Plant Physiol. 152: 283-296.

Gitelson, A.A., Y. Gritz and M.N. Merzlyak. 2003. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. J. Plant Physiol. 160: 271-282.

Hüner, N.P. and W.G. Hopkins. 2008. Introduction to plant physiology. Wiley, New York, NY, USA.

Jiang, Y., H. Liu and V. Cline. 2009. Correlations of leaf relative water content, canopy temperature, and spectral reflectance in perennial ryegrass under water deficit conditions. HortScience 44: 459-462.

Johnsen, A.R., B.P. Horgan, B.S. Hulke and V. Cline. 2009. Evaluation of remote sensing to measure plant stress in Creeping Bentgrass (L.) fairways. Crop Sci. 49: 2261-2274.

Krum, J., R. Carrow and K. Karnok. 2010. Spatial mapping of complex turfgrass sites: Site-specific management units and protocols. Crop Sci. 50: 301-315.

Lichtenthaler, H.K. 1987. [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 350-382.

Lobell, D.B. and G.P. Asner. 2003. Comparison of Earth Observing-1 ALI and Landsat ETM+ for crop identification and yield prediction in Mexico. Geoscience and Remote Sensing, IEEE Transactions on 41: 1277-1282.

Murphy, J.T., C.E. Owensby, J.M. Ham and P.I. Coyne. 2014. Estimation of Vegetative Characteristics by Remote Sensing. Academic Research Journal of Agricultural Science and Research 2: 47-56.

Penuelas, J., I. Filella, C. Biel, L. Serrano and R. Save. 1993. The reflectance at the 950–970 nm region as an indicator of plant water status. International journal of remote sensing 14: 1887-1905.

Rouse Jr, J.W., R. Haas, J. Schell and D. Deering. 1974. Monitoring vegetation systems in the Great Plains with ERTS. NASA SP-351: 309.

Schmidt, R., X. Zhang and D. Chalmers. 1999. Response of photosynthesis and superoxide dismutase to silica applied to creeping bentgrass grown under two fertility levels. J. Plant Nutr. 22: 1763-1773.

Sims, D.A. and J.A. Gamon. 2003. Estimation of vegetation water content and photosynthetic tissue area from spectral reflectance: a comparison of indices based on liquid water and chlorophyll absorption features. Remote Sens. Environ. 84: 526-537.

Stiegler, J., G. Bell, N. Maness and M. Smith. 2005. Spectral detection of pigment concentrations in creeping bentgrass golf greens. Int. Turf. Soc. Res. J 10: 818-825.

Thenkabail, P.S., J.G. Lyon and A. Huete. 2011. Hyperspectral remote sensing of vegetation, CRC Press, Boca Raton, FL, USA.

Throssell, C.S., G.T. Lyman, M.E. Johnson, G.A. Stacey and C.D. Brown. 2009. Golf course environmental profile measures water use, source, cost, quality, management and conservation strategies. Appl. Turf. Sci. 6(1).

Trenholm, L., R. Carrow and R. Duncan. 1999. Relationship of multispectral radiometry data to qualitative data in turfgrass research. Crop Sci. 39: 763-769.

Trenholm, L., M. Schlossberg, G. Lee, W. Parks and S. Geer. 2000. An evaluation of multi-spectral responses on selected turfgrass species. Int. J. Remote Sens. 21: 709-721.

Zhang, X., E. Ervin and R. Schmidt. 2005. The role of leaf pigment and antioxidant levels in UV-B resistance of dark-and light-green Kentucky bluegrass cultivars. J. Am. Soc. Hortic. Sci. 130: 836-841.

Zhang, X. and R. Schmidt. 2000. Application of trinexapac-ethyl and propiconazole enhances superoxide dismutase and photochemical activity in creeping bentgrass (*Agrostis stoloniferous* var. *palustris*). J. Am. Soc. Hortic. Sci. 125: 47-51.

Chapter 3: Tables and Figures

Table 1. Listing of vegetation indices investigated for correlation to soil water content, tissue water content, total chlorophyll content, turf quality, and turf color of creeping bentgrass grown under greenhouse conditions. Indices in **bold** are used throughout manuscript to represent all other indices.

Acronym	Name	Formula used	Citation
NDVI	**Normalized difference vegetation index**	**$(R_{760} – R_{670})/(R_{760} + R_{670})$**	**†Rouse et al. 1974**
NDVI2	NDVI using red edge & red	$(R_{730} - R_{670})/(R_{730} + R_{670})$	†Gitelson & Merzlyak 1994
NDVI3	NDVI using NIR & red edge	$(R_{760} - R_{730})(R_{760} + R_{730})$	†Gitelson & Merzlyak 1994
RVI	**Simple ratio vegetation index**	**R_{760}/R_{670}**	**†Birth & McVey 1968**
RVI2	RVI using red edge & red	R_{730}/R_{670}	†Gitelson & Merzlyak 1994
RVI3	RVI using NIR & red edge	R_{760}/R_{730}	†Gitelson & Merzlyak 1994
GNDVI	Green NDVI	$(R_{760} - R_{550})/(R_{760} + R_{550})$	†Gitelson & Merzlyak 1998
RGI	Red to green ratio index	R_{670}/R_{550}	†Gamon & Surfus 1999
GRI	**‡Green to red ratio index**	**R_{550}/R_{670}**	**†Gamon & Surfus 1999**
CHLOR1	Chlorophyll index	$(R_{760}/R_{730}) – 1$	†Gitelson et al. 2003
NDVI 705	NDVI at 705 nm	$(R_{750} - R_{705})/(R_{750} + R_{705})$	Gitelson & Merzlyak 1994
SGI	Sum green index	Mean of $(R_{500-600})$	Lobell & Asner 2003
PRI	**Photochemical reflectance index**	**$(R_{531} - R_{570})/(R_{531} + R_{570})$**	**Gamon & Surfus 1997**
PEI	Photochemical efficiency index	R_{735}/R_{700}	§Schmidt et al. 1999
CHLOR2	Chlorophyll index	R_{790}/R_{715}	Barnes et al. 2002
WBI	**Water band index**	**R_{900}/R_{970}**	**Penuelas et al. 1993**

†Modified based on principles described by authors using spectra equivalent to filters used on ACS-470 multispectral radiometer (NIR = 760, RE = 730, R = 670 nm, G = 550 nm).

‡Modified from Gamon & Surfus (1999) to eliminate confusion of negative correlation to most tested vegetation indices.

§Derived from wavelengths of chlorophyll fluorescence spectra discussed by Schmidt et al. (1999).

Table 2. Pearson correlation coefficients (*r*) between select vegetation indices and all tested indices derived from spectral reflectance of creeping bentgrass grown under greenhouse conditions in Blacksburg, VA.

	NDVI		**RVI**		**PRI**		**NDVI705**		**GRI**		**WBI**	
Index	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
NDVI	---	---	0.96†	0.93	0.88	0.95	0.81	0.91	0.8	0.87	0.68	0.57
NDVI2	0.98	0.99	0.93	0.91	0.8	0.93	0.68	0.87	0.9	0.91	0.73	0.61
NDVI3	0.73	0.85	0.79	0.93	0.87	0.92	0.98	0.99	NS	0.56	NS	NS
RVI	0.96	0.93	---	---	0.92	0.96	0.87	0.96	0.73	0.79	0.62	0.64
RVI2	0.96	0.95	0.97	0.99	0.84	0.94	0.74	0.92	0.85	0.88	0.66	0.69
RVI3	0.73	0.84	0.79	0.93	0.87	0.92	0.98	0.99	NS	0.55	NS	NS
GNDVI	0.75	0.85	0.82	0.94	0.89	0.9	0.99	0.99	NS	0.56	NS	NS
RGI	-0.79	-0.9	-0.68	-0.74	-0.49	-0.78	NS	-0.65	-0.98	-0.96	-0.81	-0.64
GRI	0.8	0.87	0.73	0.79	0.52	0.79	NS	0.65	---	---	0.76	0.72
CHLOR1	0.76	0.85	0.82	0.93	0.89	0.93	0.99	0.99	NS	0.56	NS	NS
NDVI705	0.81	0.91	0.87	0.96	0.92	0.96	---	---	NS	0.65	NS	0.46
SGI	-0.51	-0.63	-0.63	-0.77	-0.69	-0.7	-0.82	-0.84	NS	NS	NS	NS
PRI	0.88	0.95	0.92	0.96	---	---	0.92	0.96	0.52	0.79	0.57	0.54
PEI	0.86	0.9	0.92	0.97	0.95	0.95	0.99	1	NS	0.65	NS	0.48
CHLOR2	0.73	0.84	0.79	0.93	0.87	0.92	0.98	0.99	NS	0.55	NS	NS
WBI	0.68	0.57	0.62	0.64	0.57	0.54	NS	0.46	0.76	0.72	---	---

†All *r*-values significant ($P < 0.05$), unless noted NS as not significant.

Table 3. Pearson correlation coefficients (*r*) between turf quality, turf color, total chlorophyll content, tissue water content, soil water content, and vegetation indices derived from spectral reflectance of creeping bentgrass grown under greenhouse conditions in Blacksburg, VA.

	Turf Quality†		**Turf Color‡**		**Total Chlorophyll**		**Tissue Water Content§**		**Soil Water Content¶**	
Index	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
NDVI	**0.77*****	0.53*	0.52*	**0.85*****	0.63**	0.65**	0.54*	NS	0.49*	NS
RVI	**0.81*****	0.62**	0.61**	**0.91*****	**0.69*****	**0.81*****	0.59**	NS	NS	NS
NDVI $_{705}$	**0.78*****	0.46*	**0.81*****	**0.97*****	0.59**	**0.67****	0.50*	NS	NS	NS
PRI	**0.85*****	0.53*	**0.76*****	**0.93*****	**0.76*****	**0.68*****	**0.66****	NS	NS	NS
GRI	0.49*	**0.67****	NS	0.59**	0.49*	**0.67****	NS	NS	**0.73*****	0.50*
WBI	**0.72*****	**0.86*****	NS	NS	**0.69*****	**0.85*****	0.52*	**0.82*****	**0.80*****	**0.81*****

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. NS denotes not significant at *P* = 0.05.

†Based on a 1 to 9 scale, with 1 = dead, 6 = minimally acceptable, and 9 = lush, dense turf.

‡Based on a 1 to 9 scale, where 1 = chlorotic and 9 = dark green.

§Water content of leaf tissue as a ratio of dry weight to fresh weight.

¶Soil water content calculated using time-domain reflectometry TDR.

Table 4. Analysis of variance for visual estimations of quality and color, spectral reflectance, soil moisture, tissue moisture, and chlorophyll concentrations obtained from creeping bentgrass grown under greenhouse conditions at the Glade Road Research Facility in Blacksburg, VA.

Source	**Turf Quality**†	**Turf Color**‡	**Total chlorophyll**	**Tissue water content**§	**Soil water content**¶	**NDVI**	**RVI**	**ND_{705}**	**PRI**	**GRVI**	**WBI**
Irrigation	---	---	---	---	---	---	---	---	---	0.057	0.068
Fertility	0.075	---	0.079	---	---	---	---	---	---	---	---
Irr. x Fert.	---	---	---	---	---	---	---	---	---	---	0.061
Trial x Fert.	---	0.003	---	<.001	---	0.004	0.005	<.001	0.001	0.024	---
Trial x Irr.	0.002		0.003	0.010	<.001	---	---	---	---	---	---
Trial x Fert. x Irr.	---	0.005	---	0.009	<.001	---	---	0.006	0.020	---	---

†Based on a 1 to 9 scale, with 1 = dead, 6 = minimally acceptable, and 9 = lush, dense turf.
‡Based on a 1 to 9 scale, where 1 = chlorotic and 9 = dark green.
§Water content of leaf tissue as a ratio of dry weight to fresh weight.
¶Soil water content calculated using time-domain reflectometry (TDR).

Figure 1. Treatment means for the effect of irrigation on the water band index (WBI = R_{900}/R_{970} *100) and the green/red ratio index (GRI = R_{550}/R_{670}) pooled over creeping bentgrass trials conducted under greenhouse conditions in Blacksburg, VA.

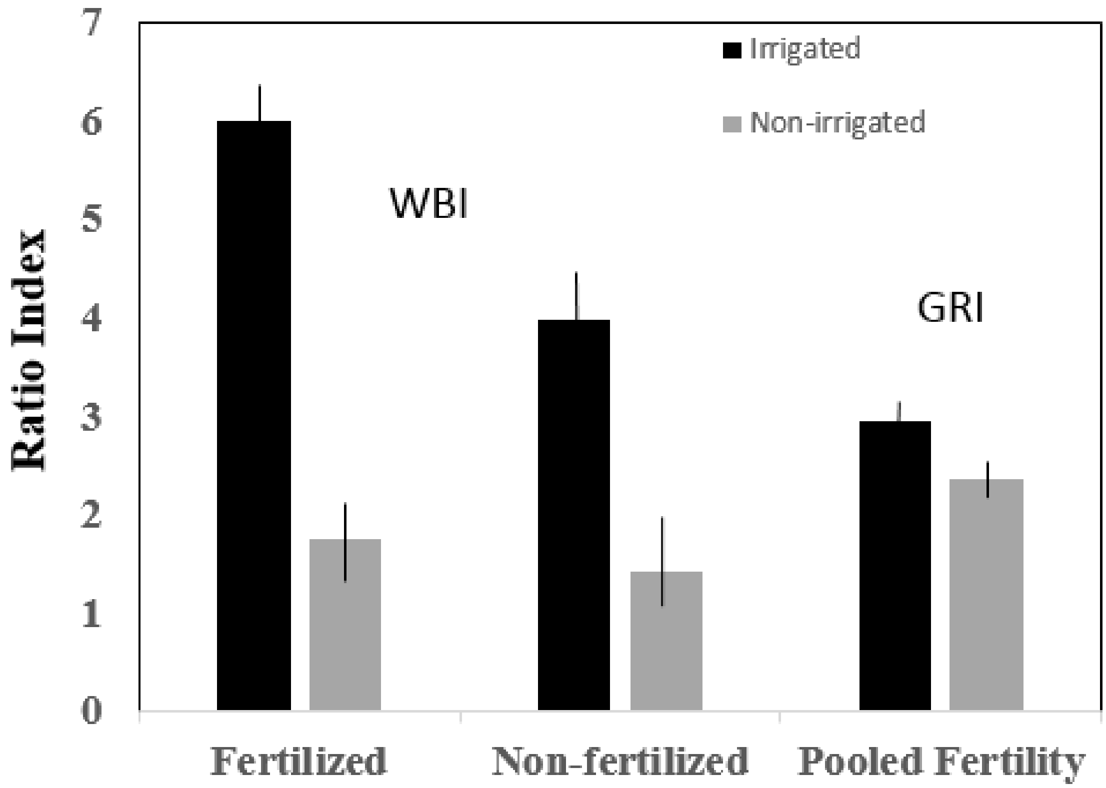
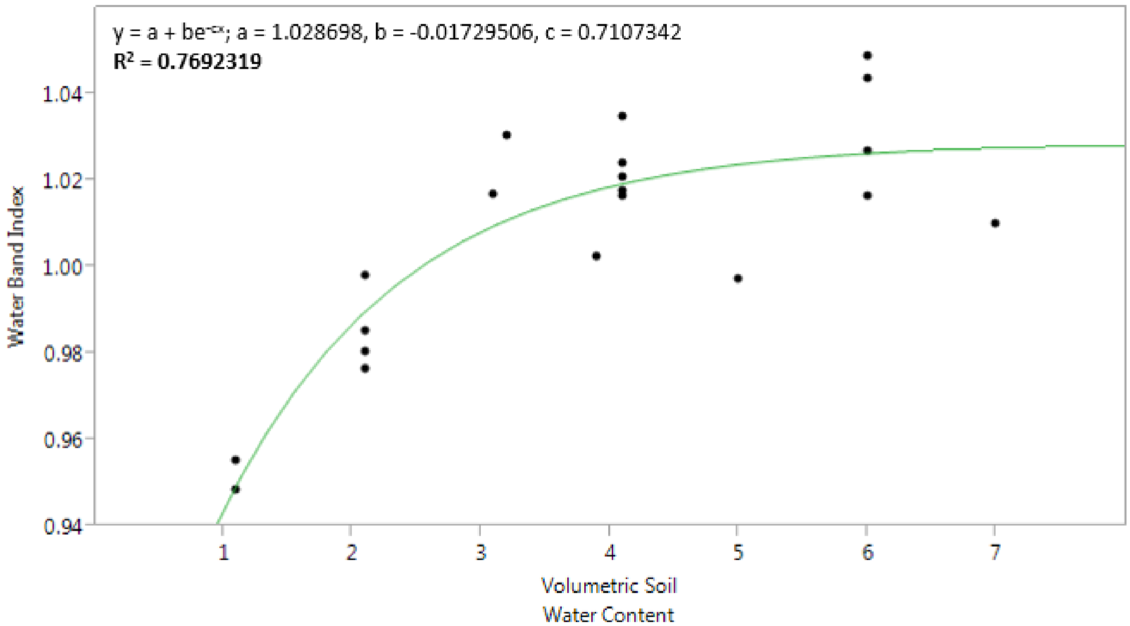


Figure 2. Three-parameter exponential nonlinear regression of water band index and soil water content from non-irrigated creeping bentgrass grown under greenhouse conditions in Blacksburg, VA.



CHAPTER 4. Influence of synthetic phthalocyanine pigments on light reflectance of creeping bentgrass[2]

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KEYWORDS: phthalocyanine, pigment, reflectance, bentgrass, NDVI, radiometry

ABSTRACT

The use of synthetic pigment-containing products on golf playing surfaces has increased dramatically by golf course superintendents to provide added green color and improve stress tolerance. Most turf colorants are synthesized from various phthalocyanine pigments, which share visible spectral properties with green plant tissue. Vegetation indices, such as normalized difference (NDVI) and simple ratio (RVI), are commonly used by researchers to quantify plant health or turf quality. Research reports have indicated that turf canopy reflectance is sometimes positively and sometimes negatively impacted by synthetic pigments. The specific spectral wavelength utilized for vegetation indices varies by sensor type, which may explain these inconsistencies. A greater understanding of light absorption characteristics of synthetic pigments is needed. Therefore, the research objectives were to determine the spectral properties of synthetic phthalocyanine pigment-containing products alone and to quantify their influence on light reflected from treated creeping bentgrass canopies. Narrow bandwidth (1.5 nm) reflectance

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was collected from across the visible and near infrared regions of the electromagnetic spectrum using a handheld field radiometer (PSR-1100, Spectral Evolution, Inc., Lawrence, MA) to develop spectral signatures of pigmented products alone, healthy turf, and healthy turf treated with pigmented products. Synthetic pigment-containing products had a strong influence on light reflectance of near infrared light, but did not impact photosynthetically active spectra. Some products tested impacted UV-A radiation. Endogenous chlorophyll concentrations 7 days after treatment were not altered by products tested. Spectral characteristics of NDVI and similar reflectance measurements of creeping bentgrass quality utilizing near infrared light may be negatively impacted when the turf is treated with phthalocyanine pigment-containing products.

INTRODUCTION

Synthetic pigments are commonly applied to creeping bentgrass putting greens and fairways with the intent to alleviate stress and improve overall aesthetic appearances (McCarty et al. 2014). This strategy is one of many aimed to maintain or improve turfgrass quality during periods of temperatures supraoptimal for growth (Dernoeden 2012). The summer bentgrass decline complex is a result of the breakdown of several physiological plant responses due to prolonged high temperature stress (Carrow 1996, Huang 2003). During prolonged periods of supraoptimal temperature stress, photosynthetic activity is reduced as respiration rises (Xu and Huang 2000). Reduction in endogenous chlorophyll concentrations is directly related to reduced photosynthetic rate of creeping bentgrass (Xu et al. 2002). In addition to heat-related stress, prolonged exposure to shade will reduce creeping bentgrass color and density (Bell and Danneberger 1999). The authors reported a reduction in chlorophyll and related natural pigments in bentgrass grown in perpetual shade.

The role of synthetic pigments in improving turf quality is not well understood (McCarty et al. 2013). Several fungicides, including combinations with synthetic pigments, are reported to improve overall health of creeping bentgrass during heat stress (Lucas and Mudge 1997, Latin 2011, Dernoeden 2012). Lucas and Mudge (1997) demonstrated that the addition of Pigment Blue 15, a Cu-based phthalocyanine compound, to aluminum tris and mancozeb enhanced overall creeping bentgrass quality and color. However, some Cu- Zn- and Ti-based phthalocyanine compounds are reported to reduce CO_2 exchange rate, evapotranspiration rate, chlorophyll fluorescence, and light transmission (McCarty et al. 2013, McCarty et al. 2014). Some evidence suggests that a polychlorinated Cu II phthalocyanine compound induced a defense response to the dollar spot pathogen, though the mechanism was unrelated to systemic acquired resistance or induced systemic resistance (Hsiang et al. 2013).

A primary concern with repeated, sequential applications of synthetic pigments is the potential lessening of light absorption by chlorophyll and subsequent photosynthetic efficiency decline (Reynolds et al. 2012). Turf paints, applied as thicker coats to athletic fields and dormant bermudagrass fairways, vary as to their impact on total canopy photosynthesis (Reynolds et al. 2013). Blue and green phthalocyanines were among the darkest pigments tested and had the greatest negative impact on photosynthetically active radiation (PAR) transmission into the canopy. The authors suggested that prolonged covering with these synthetic paints will reduce turf quality by shading and a reduction in PAR transmission. Chlorinated Cu phthalocyanine and pulverized *Chlorella vulgaris* cells reduced carotenoid degradation in bentgrass exposed to supraoptimal light conditions, but only *C. vulgaris* was able to slow the rate of chlorophyll degradation (Bartley 2012). The authors suggested that these pigmented compounds may protect plants from excess photosynthetically active light. The application of Green Lawnger turf colorant delayed UV-B degradation of Kentucky bluegrass by limiting reactive oxygen species production and increasing photochemical efficiency (Ervin et al. 2004). The authors suggested the turf colorant blocked harmful UV-B light, but allowed photosynthetically active light to pass into the canopy.

Available literature on the positive or negative impacts of synthetic pigments is inconsistent. Experiments described in this manuscript were designed to address questions concerning light absorption and reflectance of commercially available pigments that are applied to actively growing creeping bentgrass using a field radiometer. Analyses of spectral reflectance of synthetic pigments were carried out in the laboratory, while the impact of various pigments on actively growing bentgrass were conducted under greenhouse and field conditions.

MATERIALS AND METHODS

Spectroradiometer measurements

A hand-held field radiometer (PSR-1100F; Spectral Evolution, Lawrence, MA) was fitted with a plant probe with a spot size of 2.5 cm to record spectral data. Data were collected by placing directly over the canopy so the entire field of view measured creeping bentgrass. The PSR-1100F measured 512 unique spectra over range of 320 to 1100 nm at a 1.4 nm sampling bandwidth and 3 nm spectral resolution. A $BaSo_4$ calibration panel was used for white reference calibration before each replication by placing the plant probe directly in contact with the surface.

Synthetic pigment reflectance

Six synthetic pigments (Table 1.) were investigated under a laboratory setting to determine spectral properties in the absence of plant tissue. Petri plates were filled with 15 ml of water agar (17 g L^{-1}) amended with five Cu-based phthalocyanine pigments and one TiO_2 ZnO pigment, which also contains a phthalocyanine pigment. Treatments were compared against a water agar control to investigate uniformity of spectral properties among phthalocyanine-containing products. Treatments included Foursome (0.127 L ha^{-1} Quali-Pro, Pasadena, TX), GreenPig (0.127 L ha^{-1}, Grigg Bros., Albion, ID), Appear (1.27 L ha^{-1}, Syngenta Professional Products, Greensboro, NC), Interface (1.27 L ha^{-1}, Bayer Environmental Science, Research Triangle Park, NC), Civitas Pre-M1xed (2.70 L ha^{-1}, Suncor Energy, Mississauga, Ontario, Canada), and TurfScreen (0.79 L ha^{-1}, TurfMax LLC., Erdenheim, PA). TurfScreen is a combination of ZnO and TiO_2 white pigments, and a Cu-based phthalocyanine pigment. Each treatment was replicated five times and arranged in a randomized complete block design. Granulated agar was added to deionized water and autoclaved at 121°C for 20 min. Products were added to agar suspension when liquid was cool enough to touch and placed on a stir-plate for 5 min before adding to petri plates. Product-amended agar plates were allowed to solidify and stored at 4°C for 24 hrs.

Field and greenhouse studies

Field and greenhouse studies were conducted at the Glade Road Research Facility in Blacksburg, VA. Field sites consisted of a 12-yr-old ‘L-93’ creeping bentgrass (*Agrostis stolonifera* L.) fairway established on a clay loam soil and a two-yr-old ‘007’ creeping bentgrass putting green built to U.S. Golf Association specifications (Staff 2004). The fairway and green were mowed three times per week at 15 mm and five times per week at 3.5 mm, respectively, with each irrigated to prevent drought stress. ‘L-93’ creeping bentgrass was grown from seed under greenhouse conditions for 12 wks in D40H Deepot Conetainers filled with a 90:10 sand/peat root-zone mix. Conetainer turf was clipped twice per week at 13 mm with scissors and watered daily. Two greenhouse studies were performed in December 2014 and a third in March 2015. Six treatments were compared against a non-treated control to determine the influence of pigmented turf products on creeping bentgrass. Treatments consisted of Foursome (0.127 L ha^{-1}), GreenPig (0.127 L ha^{-1}), Appear (1.27 L ha^{-1}), Interface (1.27 L ha^{-1}), Civitas Pre-M1xed (2.70 L ha^{-1}), and TurfScreen (0.79 L ha^{-1}). Treatments were applied in a spray volume dilution of 813 L ha^{-1} using a CO_2 pressurized sprayer equipped with TTI 11004 flat fan nozzles. Treatments were arranged over four replications in a randomized complete block design. Spectral reflectance was collecting with a field radiometer 24 hr after application.

Endogenous chlorophyll and carotenoid content was measured seven days after application from the fairway location and one greenhouse experiment for comparison to canopy reflectance-derived chlorophyll indices. Chlorophyll was extracted from plant tissue in acetone in accordance with procedures described by Lichtenthaler (1987). Briefly, 5 ml of 100% acetone were added to 50 mg of fresh tissue weight and then dark-incubated at 4°C for 48 hr. Absorbance was measured at 645, 662, and 470 nm using a spectrophotometer (Thermo MultiSkan GO, Thermo Fisher Scientific, Waltham, MA). Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoids were calculated as follows:

Chl *a* = (11.24 × absorbance at 661.6 nm) – (2.04 × absorbance at 644.8 nm)

Chl *b* = (20.13 × absorbance at 644.8 nm) – (4.19 × absorbance at 661.6 nm)

Chl *a+b* = (7.05 × absorbance at 661.6 nm) + (18.09 × absorbance at 644.8 nm)

Carotenoids (xanthophylls and β-carotene) =

(1000 × absorbance at 470 nm) – (1.90 × Chl *a* – 63.14 × Chl *b*) ÷ 214

Vegetation indices (VI) with established relationships to chlorophyll content, photochemical efficiency, and visual turf quality were derived from spectral reflectance data (Gamon et al. 1997, Gitelson and Merzlyak 2004, Zarco-Tejada et al. 2004, Bremer et al. 2011). Two chlorophyll indices were calculated using different spectral regions that appear to be impacted differently by synthetic phthalocyanine pigments. One reported chlorophyll index utilizes NIR and red edge reflectance (Chl VI1 = R_{790}/R_{715}, where R_x = reflectance at $_x$ wavelength in nm) (Gitelson and Merzlyak 1997). A second is the chlorophyll curvature index (Chl VI2 = $(R_{675}*R_{690})/R_{683}^2$), previously described by Zarco-Tejada et al. (2004), which utilizes visible red light reflectance along the base of the red edge where chlorophyll is less impacted by extraneous factors in the NIR and along the red edge. Photochemical efficiency is commonly estimated using the photochemical reflectance index (PRI = $(R_{531}-R_{570})/(R_{531}+R_{570})$) (Gamon et al. 1992, Garbulsky et al. 2011).

Statistical analysis

Spectral and chlorophyll data were subjected to ANOVA to determine treatment and trial effects, and their interaction using SAS PROC GLM (SAS Institute v. 9.3, Cary, NC). Means were separated using Fisher's protected LSD test at $P \leq 0.05$, when appropriate. Data were pooled over five experiments when no treatment by trial interaction existed.

RESULTS & DISCUSSION

Synthetic pigment reflectance

Light reflectance of synthetic pigment-containing products was examined under laboratory conditions for UV-A, visible light, and near infrared spectra. Mean spectra of Foursome, GreenPig, Appear, Interface, and Civitas Pre-M1xed had similar reflectance characteristics, and were therefore pooled (Fig. 1). Spectral characteristics of TurfScreen varied considerably from other pigmented products and are shown separately. Dark colors absorb most visible light waves (400 – 700 nm), while light colors strongly reflect light. As previously stated, TurfScreen contains both natural white pigments (TiO_2 and ZnO) and green phthalocyanine pigments. All other products tested contain only Cu-based phthalocyanine pigments. The dark green pigments used for these studies absorbed essentially all light throughout the visible region, excluding a peak of reflectance in the cyan region (490 – 520 nm) (Figure 1). This spectral region corresponds to the blue-green coloration that is visible on treated turfgrass swards. Photosynthetically active radiation comes from light emitted within the visible region, most notably in blue (400 – 500 nm) and red (600 – 690 nm) spectra. TurfScreen, however, strongly reflected light across the entire visible spectrum.

There is concern that application of synthetic pigments may interfere with normal plant photosynthesis by absorbing useful light (Reynolds et al. 2013). Based on these data using amended agar, phthalocyanine pigments will essentially compete with natural plant pigments for photosynthetically active light absorption.

While UV-B light is more harmful to plant tissue, UV-A can still cause damage (Hüner and Hopkins 2008). The radiometer used in this research is incapable of collecting reflectance within the UV-B spectra (280 – 315 nm) effectively. Light reflectance from UV-A spectra (315 – 400 nm) in agar amended with Cu-based pigments averaged 3.9%, with a range of 3.0% (GreenPig) and 5.7% (Appear) (data not shown). Reflectance of UV-A light was 25.9% and 31.4% for non-amended agar and TurfScreen-amended agar, respectively. In the absence of plant material, Cu-based phthalocyanine pigments may act as a photoprotector of UV-A radiation by absorbing UV energy. This is consistent with previously suggested reports (Ervin et al. 2004). Alternatively, TurfScreen reflects UV-A light away, and therefore could also serve as a photoprotector by re-directing UV light away from a plant canopy.

Actively growing plants have a sharp increase in reflectance along the region between visible and near infrared (NIR) light (690 – 730 nm), known as the red edge. NIR light reflectance plateaus beyond a red edge shoulder between 730 and 760 nm (Jackson 1986). Many field radiometers used for NDVI calculations use information obtained within this region. NIR light did not reflect from phthalocyanine-amended agar until further into the NIR region (800 nm and beyond, Fig. 1). Phthalocyanines used in this study absorb NIR light in the absence of plants, a critical consideration when using NDVI in the presence of a pigmented turfgrass canopy. Reflectance of TurfScreen within the NIR region is comparable to unamended agar.

It is worth noting that a decline in NIR reflectance of unamended agar near 970 nm is consistent with a water band feature associated with moisture availability in plants (Penuelas et al. 1993). This feature was present in agar amended with the label rate of each phthalocyanine pigment, but not in agar amended with TurfScreen. This feature was also lost in ancillary studies with higher rates of Foursome (3x and 10x tested rate) (data not shown). This information is important, as both highly reflective and absorptive materials can influence this water band spectral region. Using the water band feature as an indicator of drought stress when higher concentrations of pigments are used may provide inconsistent results.

Canopy reflectance and chlorophyll concentration

The spectral response to all phthalocyanine pigments applied to creeping bentgrass was comparable to nontreated plants, with a unique but consistent decline in the red edge shoulder position (Fig. 2). There was a significant trial effect for all spectral regions (Table 2), likely attributed to diverse growing conditions across putting green, fairway, and greenhouse microclimates. Treatment with all tested phthalocyanines resulted in a decrease in canopy reflectance at 760 nm, the average red edge, and the slope across spectra of 760 – 900 nm (Table 3). The spectral response of each treatment was a normal plateau immediately following the shoulder, but then a second increase in reflectance around 800 nm (Fig. 2). This spectral location is consistent with the sharp increase in reflectance of phthalocyanine-amended agar previously described (Fig. 1). The red edge shoulder of 760 nm, defined here as the reflectance point where typical NIR plateau begins, was significantly higher in nontreated plots (52%) than all other treatments ($\leq$ 49.2%, $P < 0.0001$). The calculated slope across the NIR region from the red edge shoulder to the point where influence of the tested pigments is less apparent (R_{760} - R_{900}) was lowest for nontreated plots (P < 0.0001). While R_{760} and the slope of R_{760} to R_{900} were both significantly reduced is this experiment, the slope may be more consistent than reflectance at 760 nm alone. Ambient NIR light reflectance can be inconsistent, as influenced by many extraneous factors, such as light availability to the sensor, canopy structure and angulation of leaves (Carrow et al. 2010). Normalizing across multiple data points can minimize this inconsistency (Jackson 1986).

Most visible light is absorbed by healthy plants to drive photosynthesis, with a portion of unused green light being reflected away from the canopy. Near infrared light is strongly reflected from the canopy when plants are healthy, with a sharp increase in reflectance between the visible and near infrared region of the spectrum (Blackburn 2007). Reflectance typically plateaus across the NIR region following what is commonly referred to as the red edge shoulder, typically between 730 and 760 nm. A distinct water band is often present within the near infrared region between 950 and 970 nm (Penuelas et al. 1993). The research being presented aims to address how synthetic pigments impact normal canopy reflectance of turfgrass stands. Canopy reflectance of nontreated creeping bentgrass used in these studies followed the typical response characteristics described (Fig. 2). The red edge shoulder where canopy reflectance begins to plateau occurs around 760 nm. The water band feature is present at 970 nm.

Average reflectance across the red edge before the shoulder plateau (690 – 730 nm) was highest in nontreated plots (P = 0.0016) (Table 3). Red reflectance from nontreated plots compared favorably with all phthalocyanines except GreenPig (P = 0.0333, Table 2). There were no differences in green or blue spectral reflectance among treatments (Table 2). UV-A light was impacted by treatments at P = 0.0501. UV-A reflectance of nontreated creeping bentgrass was the same as that of Appear, Interface, and TurfScreen. However, UV-A reflectance was lower in plots treated with Foursome, GreenPig, and Civitas Pre-M1xed. Mean separation of canopy reflectance at 760 nm was lowest in plots treated with Civitas Pre-M1xed, which was significantly lower than all other phthalocyanines except GreenPig (Table 3). Civitas Pre-M1xed contains a petroleum-derived spray oil, which is reported to persist on leaf blades (Kreuser and Rossi 2014). Repeat application at high rates (comparable to that tested here) caused significant injury to the bentgrass canopy. These spray oils can alter stomatal openings on leaf surfaces, which likely contributes to reports of phytotoxicity (Hodgkinson 2002). The persistent oil and altered cuticle wax may alter normal leaf reflectance properties, therefore causing the most dramatic change in the red edge shoulder position and UV-A spectra.

GreenPig and Foursome, the two stand-alone pigments used for aesthetics and as spray pattern indicators, compared favorably with each other in all spectral regions. However, the NIR reflectance slope of plots treated with GreenPig was higher than in those treated with Foursome. Higher slopes in the red edge region of interest indicate a greater pigment effect. Since phthalocyanines are not regulated by the Environmental Protection Agency like pesticides, the formulation and inert additives are proprietary and not listed on labels. Differences in formulation between these pigment-containing products may contribute to altered spectral properties.

Phthalocyanines are additives to the fungicides Appear and Interface. Reflectance at 760 nm was closest to nontreated creeping bentgrass with Appear (49.2%), and was significantly higher than all treatments except Interface and TurfScreen (Table 3). Appear-amended agar was lighter in color than other phthalocyanine pigments pooled, though overall spectral characteristics were similar (data not shown). The NIR reflectance slope was significantly higher in plots treated with Interface than with Appear, indicating a stronger pigment effect.

TurfScreen, which reflected most light across all spectra in water agar (Fig. 1) because of TiO_2 and ZnO white pigments, had lower reflectance at 760 nm than the nontreated, but more than Civitas Pre-M1xed and GreenPig. The NIR slope of TurfScreen treated plots compared favorably with those of Foursome and Interface. TurfScreen did not influence UV-A or photosynthetically active (visible light) spectral reflectance compared with the nontreated. TurfScreen contains pigments that both absorb (phthalocyanine) and reflect (TiO_2 and ZnO) light energy. It is possible that the phthalocyanine pigment absorption is more heavily influencing light characteristics than the white pigments within the NIR region when applied to actively growing turfgrass.

Chlorophyll *a*, chlorophyll *b*, total chlorophyll content, and total carotenoids (xanthophyll and β–carotene) of creeping bentgrass were not impacted ($P = 0.4268$) seven days after the application of phthalocyanines (Table 4). Pigment-containing products had no effect on the second chlorophyll index, CHL VI2, which utilizes the curvature of the red to red edge reflectance as a measurement of chlorophyll concentration ($P = 0.8721$). However, CHL VI1 estimated a lower relative chlorophyll concentration in plots treated with Civitas Pre-M1xed than all other treatments and the nontreated ($P = 0.0094$, mean separation not shown). As previously mentioned, this index utilizes light reflectance from the NIR and red edge regions, both of which were negatively impacted by Civitas Pre-M1xed, potentially because of oil persistence on the leaves. While chlorophyll content was typically unaffected by treatment with phthalocyanines, the photosynthetic efficiency, measured as PRI, was impacted by treatment ($P = 0.0081$). This index is strongly related to the de-epoxidation of the xanthophyll cycle, which ultimately impacts the chlorophyll:carotenoid ratio (Garbulsky et al. 2011). PRI values decrease as photochemical efficiency increases (Gamon et al. 1992). Therefore, photosynthetic efficiency was negatively impacted by each phthalocyanine treatment (Table 3).

CONCLUSIONS

In summary, these data suggest that light reflectance of healthy creeping bentgrass is not impacted by phthalocyanines applied at normal label rates in photosynthetically active spectra because of high plant absorption characteristics, but the impact is greater in regions of higher plant reflectance, primarily the red edge shoulder and NIR region. Reynolds et al. (2015) indicated that repeat applications of green pigments would cause photosynthetically active radiation to decline and quality to be reduced over time. However, the pigments tested by the authors were applied heavily as turf paints, unlike what was used in this study and were used on golf courses during active growth of creeping bentgrass. There is sparse evidence from research presented in this manuscript that phthalocyanine pigments applied at label rates impact normal light reflectance from photosynthetically active spectra. All treatments resulted in a decline in photosynthetic efficiency, as estimated using PRI, though no visible spectra were altered. Previously, reports indicated that repeat applications of four phthalocyanine-containing pigments reduced NDVI values and CO_2 exchange rate compared to nontreated turf (McCarty et al. 2014). A decrease in NDVI may be due to lower NIR reflectance because of the pigment effect described in this manuscript, rather than an actual decrease in turf density or other attributes that impact turf quality. The endogenous natural pigments, total chlorophyll and carotenoids, were not impacted by external applications of phthalocyanine pigments after seven days. Chlorophyll content and spectral indices were not generally impacted by phthalocyanine pigments. However, the photochemical reflectance index, which is commonly used as an indicator of photosynthetic efficiency was increased by each product tested. Overall, the canopy of creeping bentgrass treated with phthalocyanine pigments is more heavily influenced by plant reflectance than by the pigments on its surface. Data presented in this manuscript were collected after a single application, whereas many of these pigment-containing products are applied throughout the growing season. The impact of prolonged repeat applications of these pigment-containing products at normal application rates on spectral properties is unknown.

REFERENCES

Bartley, G. K. 2012. Applicability of Pigment Compounds for Reducing Light Stress in Bentgrass. Master of Science, Thesis. University of Tennessee. August, 2012.

Bell, G. and T. Danneberger. 1999. Temporal shade on creeping bentgrass turf. Crop Sci. 39(4): 1142-1146.

Blackburn, G. A. 2007. Hyperspectral remote sensing of plant pigments. J. Exp. Bot. 58(4): 855-867.

Bremer, D. J., H. Lee, K. Su, and S. J. Keeley. 2011. Relationships between normalized difference vegetation index and visual quality in cool-season turfgrass: I. Variation among species and cultivars. Crop Sci. 51(5): 2212-2218.

Carrow, R. N. 1996. Drought resistance aspects of turfgrasses in the southeast: Root-shoot responses. Crop Sci. 36(3): 687-694.

Carrow, R. N., J. M. Krum, I. Flitcroft, V. Cline. 2010. Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. Precision Agriculture 11(2): 115-134.

Dernoeden, P. H. 2012. Creeping bentgrass management, second editions. CRC Press. Boca Raton, FL, USA.

Ervin, E. H., X. Zhang, J. H. Fike. 2004. Ultraviolet-B radiation damage on Kentucky bluegrass. I. Antioxidant and colorant effects. HortScience 39(6): 1465-1470.

Gamon, J. A., J. Penuelas, C. B. Field. 1992. A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. Remote Sens. Environ. 41(1): 35-44.

Gamon, J. A., L Serrano, J. S. Surfus. 1997. The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. Oecologia 112(4): 492-501.

Garbulsky, M. F., J Penuelas, J. Gamon, Y. Inoue, I. Filella. 2011. The photochemical reflectance index (PRI) and the remote sensing of leaf, canopy and ecosystem radiation use efficiencies: a review and meta-analysis. Remote Sens. Environ. 115(2): 281-297.

Gitelson, A. A. and M. N. Merzlyak 1997. Remote estimation of chlorophyll content in higher plant leaves. Int. J. Remote Sens. 18(12): 2691-2697.

Gitelson, A. A. and M. N. Merzlyak. 2004. Non-destructive assessment of chlorophyll carotenoid and anthocyanin content in higher plant leaves: Principles and algorithms. Remote Sensing for Agriculture and the Environment. 2004: 78-94.

Hodgkinson, M. C., D. Johnson, and G. Smith. 2002. Causes of phytotoxicity induced by petroleum-derived spray oil. Spray oils beyond 2000. G. A. C. Beattie, D.M. Watson, M.L. Stevens, and a. R. N. S.-H. e. D.J. Rae, University of Western Sydney, Sydney, New South Wales, Australia**:** p. 170–178.

Hsiang, T., P. H. Goodwin, A. M. Cortes-Barco, B. T. Nash, and J. Tung. 2013. Activating disease resistance in turfgrasses against fungal pathogens: Civitas and Harmonizer. Plant and Microbe Adaptations to Cold in a Changing World, Springer Publishing, New York, NY, USA. p. 331-341.

Huang, B. 2003. Recent advances in drought and heat stress physiology of turfgrass-a review. I Int. Conf. Turf. Management and Sci. for Sports Fields 661.

Hüner, N. P. and W. G. Hopkins. 2008. Introduction to plant physiology, fourth edition. Wiley, New York, NY, USA.

Jackson, R. D. 1986. Remote sensing of biotic and abiotic plant stress. Annu. Rev. Phytopathol. 24(1): 265-287.

Kreuser, W. C. and F. S. Rossi. 2014. The Horticultural Spray Oil, Civitas™, Causes Chronic Phytotoxicity on Cool-season Golf Turf. HortScience 49(9): 1217-1224.

Latin, R. 2011. A practical guide to turfgrass fungicides, American Phytopathological Society, APS Press, Minneapolis, MN.

Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. (148C): 350-382.

Lucas, L. T. and L. C. Mudge. 1997. Fungicidal compositions for the enhancement of turf quality. U.S. Patent 5 643 852. Date issued: 3 April.

McCarty, L. B., J. R. Gann, C. E. Wells, T. F. Bruce, and P. D. Gerald. 2013. Physiological responses of creeping bentgrass to pigment-containing products. Agron. J. 105(6): 1797-1802.

McCarty, L. B., J. R. Gann, C. E. Wells, and P. D. Gerald. 2014. Creeping Bentgrass Field Response to Pigment-Containing Products. Agron. J. 106(4): 1533-1539.

Penuelas, J., I. Filella, C. Biel, L. Serrano, and R. Save. 1993. The reflectance at the 950–970 nm region as an indicator of plant water status. Int. J. Remote Sens. 14(10): 1887-1905.

Reynolds, W. C., G. L. Miller, and T. W. Rufty. 2012. Athletic field paint impacts light spectral quality and turfgrass photosynthesis. Crop Sci. 52(5): 2375-2384.

Reynolds, W. C., G. L. Miller, and T. W. Rufty. 2013. Athletic Field Paint Color Differentially Alters Light Spectral Quality and Bermudagrass Photosynthesis. Crop Sci. 53(5): 2209-2217.

Staff, U.S.G.A. 2004. Revising the USGA's recommendations for a method of putting green construction, United States Golf Association**:** 1-12.

Xu, Q. and B. Huang. 2000. Growth and physiological responses of creeping bentgrass to changes in air and soil temperatures. Crop Sci. 40(5): 1363-1368.

Xu, Q., B. Huang, and Z. Wang. 2002. Photosynthetic responses of creeping bentgrass to reduced root-zone temperatures at supraoptimal air temperature. J. Am. Soc. Hortic. Sci. 127(5): 754-758.

Zarco-Tejada, P. J., J. R. Miller, A. Morales, A. Berjon, and J. Aguera. 2004. Hyperspectral indices and model simulation for chlorophyll estimation in open-canopy tree crops. Remote Sens. Environ. 90(4): 463-476.

Chapter 4: Tables and Figures

Figure 1. Light reflectance of solidified water agar (solid line) amended with five Cu-based phthalocyanine (dashed line, averaged) and one Zn- and Ti-based phthalocyanine (broken line) turf pigments in ultraviolet (UV-A = 320 – 400 nm), visible (400 – 700 nm), and near infrared (700 – 1100 nm) regions of the electromagnetic spectrum.

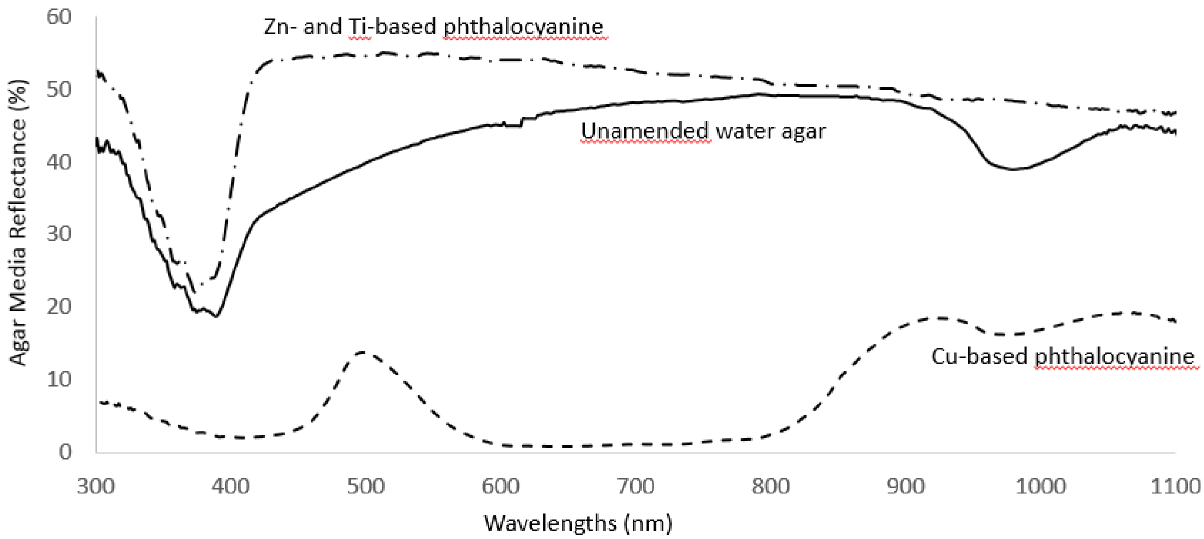

Figure 2. Percent canopy reflectance of six commercially available synthetic phthalocyanine pigment-containing products 24 hours after application to ‘L-93’ creeping bentgrass fairways at the Glade Road Research Facility, Blacksburg, VA.

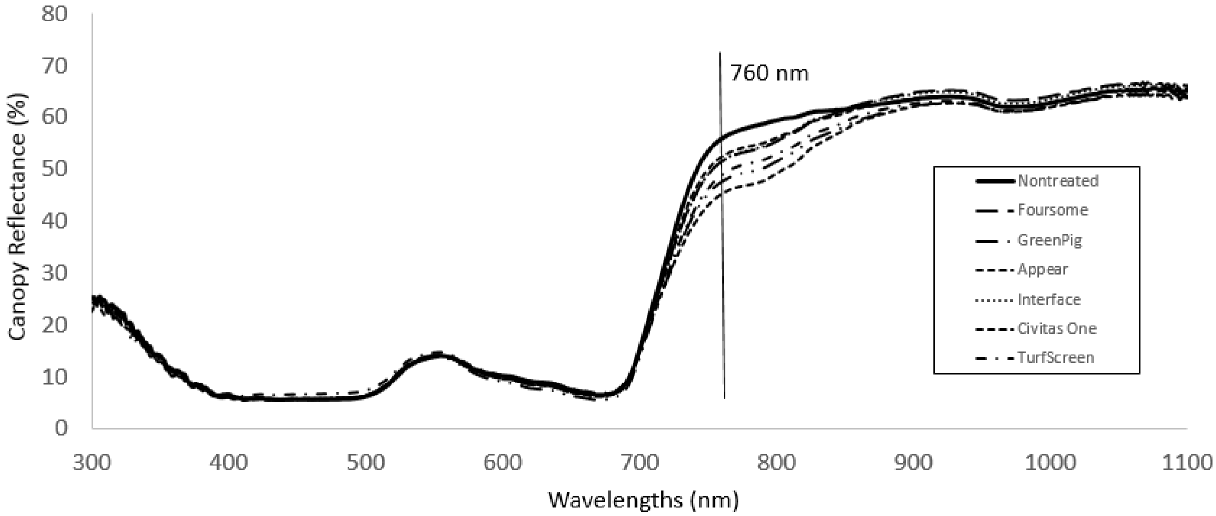

Table 1. Phthalocyanine synthetic pigment-containing products evaluated for their influence on spectral absorption characteristics of creeping bentgrass in Blacksburg, VA.

Product	**Use Rate**	**Manufacturer**	**Product Type**
Foursome	0.127 L ha^{-1}	Quali-Pro, Pasadena, TX	phthalocyanine spray pattern indicator
GreenPig	0.127 L ha^{-1}	Grigg Bros., Albion, ID	phthalocyanine spray pattern indicator
Interface	1.27 L ha^{-1}	Bayer Environmental Science, Research Triangle Park, NC	fungicide + phthalocyanine
Appear	1.27 L ha^{-1}	Syngenta Professional Products, Greensboro, NC	phosphite + phthalocyanine
Civitas Pre-M1xed	2.70 L ha^{-1}	Intelligro, Mississauga, Ontario, Canada	petroleum-derived spray oil + phthalocyanine
TurfScreen	0.79 L ha^{-1}	TurfMaxx, LLC., Erdenheim, PA	Zinc oxide, titanium dioxide, and phthalocyanine

Table 2. Analysis of variance probability values for reflectance data collected from near infrared, red edge, visible, and ultraviolet spectra obtained from creeping bentgrass grown as golf putting greens, golf fairways, and under greenhouse conditions at the Glade Road Research Facility in Blacksburg, VA.

Source	**760nm**	**Slope 760**	**Red Edge**	**Red**	**Green**	**Blue**	**UV-A**
Treatment	<.0001	<.0001	0.002	0.033	0.700	0.139	0.050
Trial	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Treatment x Trial	0.227	0.048	0.094	0.949	0.496	0.472	0.270

Table 3. Creeping bentgrass canopy reflectance at the red edge shoulder position (760 nm), average red edge (RE) reflectance (690 – 730 nm), average UV-A reflectance (320 – 400 nm), photochemical reflectance index (PRI = $(R_{531}-R_{570})/(R_{531}+R_{570})*1000$), and the slope of near infrared (760 – 900 nm), as influenced by synthetic phthalocyanine pigment-containing products on golf putting greens, golf fairways, and under greenhouse conditions at the Glade Road Research Facility in Blacksburg, VA.

	Creeping Bentgrass Canopy Reflectance (%)				
Treatment	**760 nm**	**RE**	**UV-A**	**PRI**	**Slope 760**
Non-treated	52.0 a†	24.2 a	11.4 a	-1.07 c	0.031 e
Foursome	46.2 cd	21.9 bc	10.8 bc	8.45 ab	0.079 cd
GreenPig	43.8 de	21.2 cd	10.6 bc	15.26 a	0.092 b
Appear	49.2 b	22.9 b	11.0 ab	8.55 ab	0.068 d
Interface	48.4 bc	21.9 bc	10.9 ab	6.67 b	0.082 c
Civitas Pre-M1xed	42.3 e	20.4 d	10.2 c	9.48 ab	0.111 a
TurfScreen	47.6 bc	21.7 bc	10.9 bc	10.37 ab	0.079 c
			Analysis of variance		
Treatment	***	**	*	**	***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

†Means within columns followed by the same letter are not significantly different according to Fisher's protected LSD (*P* = 0.05).

Table 4. Analysis of variance probability values for chlorophyll content collected seven days after treatment (DAT) with synthetic pigment-containing products and reflectance-derived chlorophyll indices obtained one DAT from creeping bentgrass grown under field and greenhouse conditions at the Glade Road Research Facility in Blacksburg, VA.

Source	**Chl† *a***	**Chl *b***	**TCC**	**Cars**	**Chl VI‡1**	**Chl VI2**	**PRI#**
Treatment	0.43	0.87	0.75	0.53	0.009	0.87	0.008
Trial	<.001	<.001	<.001	<.001	<.001	<.001	<0.001
Treatment x Trial	0.22	0.56	0.40	0.38	0.54	0.18	0.54

†Pigment concentrations for chlorophylls *a* and *b* (Chl), total chlorophyll (TCC), a total carotenoids (Cars).
‡Previously documented chlorophyll vegetation indices (Chl VI) (VI1 = R_{790}/R_{715}) and (VI2 = $(R_{675}*R_{690})/R_{683}^2$).
#Photochemical reflectance index (PRI = $(R_{531}-R_{570})/(R_{531}+R_{570})$).

CHAPTER 5. Quantifying the impact of air movement from turf fans on creeping bentgrass putting greens:[3]

I. Defining the relationship between wind velocity and microenvironment growing conditions.

II. Rapid estimation of relative plant health variables using spatial mapping of spectral reflectance.

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ABSTRACT

Heat stress of creeping bentgrass (*Agrostis stolonifera* L.) is one of the most limiting factors of successful putting green management in the Southeastern and Mid-Atlantic regions of the US. The installation of turf fans along the perimeter of golf putting greens has become a common practice to alleviate heat stress and poor air circulation. There is limited scientific literature available to support claims of improved creeping bentgrass performance with the use of high-output turf fans during heat stress. Individualized sampling across putting surfaces is time consuming and does not accurately depict benefits across an entire putting green. Researchers

[3] Submitted for publication MM-DD-YYYY

have adopted spectral reflectance indices as viable options for objective turfgrass quality measurements, though these data are seldom linked to direct measureable turfgrass attributes. Geo-referenced reflectance data has been utilized to generate turf quality maps across a variety of turf surfaces. The objectives of this research were to 1) quantify wind output across putting greens with turf fans, 2) define the relationship between wind velocity and root zone temperature, canopy temperature, volumetric soil water content, and root depth across putting greens with fans, 3) explore the relationship between spectral reflectance and physical measurements collected from putting greens with turf fans, and 4) estimate root depth and root zone temperature across sampled and un-sampled locations of a putting green using spectral reflectance. Trials were established at the Turfgrass Research Center (TRC) in Blacksburg, VA and Willow Oaks Country Club (WOCC) in Richmond, VA to explore the impact of turf fans on creeping bentgrass from 2013-2015. Data collection included root zone temperature, volumetric soil water content, canopy temperature, and wind velocity. Root depth and spectral reflectance were collected at WOCC. Industrial, direct-drive fans ran for four days at TRC in August 2014 and 2015 prior to data collection every meter along a linear transect from 1 m to 6 m. Data were collected at WOCC from in-play creeping bentgrass putting greens equipped with permanent 5hp oscillating turf fans (TurfBreeze TB-50 Premium) and compared against comparable putting greens without fans. Fans at WOCC ran continuously from May through data collection in August of 2013 and 2015. Each fan treatment was replicated on three greens and for each green, three subsamples were collected every 3m between 6m and 21m. Fan had a significant effect on all response variables for each trial, except soil water content for one trial at WOCC. Average wind speed across trials by location were 1.8 m s^{-1} at TRC and 2.9 m s^{-1} at WOCC. Wind velocity nearest the fan was 3.6 m s^{-1} and 5.3 m s^{-1} at TRC and WOCC, respectively. Wind velocity was most closely related to root zone temperature (r = 0.75 – 0.91) and root depth (r = 0.69, WOCC only). Canopy reflectance was also most closely related to root zone temperature and root depth at WOCC. The simple ratio vegetation index of near infrared to red edge (RVI2 = R_{760}/R_{730}) was negatively correlated to root zone temperature (r = -0.84) and positively correlated to root depth (r = 0.67). RVI2, root zone temperature, and root depth were assigned classifications used for building surface maps to estimate each variable across sampled and un-sampled locations.

INTRODUCTION

Creeping bentgrass (*Agrostis stolonifera* L.) is widely considered desirable for golf putting green surfaces, but the range of use is often pushed beyond its zone of adaptation into areas of supraoptimal temperatures (Dernoeden, 2012). Roots of creeping bentgrass and other cool-season grasses grow optimally between 10 to 18°C, with maximum shoot growth between 15 and 24°C (Beard, 1973). The physiological effects of heat stress to creeping bentgrass are well documented (Huang, 2003). Manipulation of the microenvironment to lessen environmental stress improves the ability to manage creeping bentgrass in suboptimal growing conditions (Stier, 2006).

Air and soil temperatures in excess of 35°C are common during summer months throughout the Transition Zone and Southeastern United States. Previous research has shown that high soil temperatures are more detrimental to root growth than high air temperatures (Beard, 1966, Xu and Huang, 2000). Root growth and root/shoot ratio of 'L-93' and 'Penncross' creeping bentgrass were reduced after 7 d under high soil/air temperature stress (35/35°C) compared to control (20/20°C) (Xu and Huang, 2001). Reducing soil temperatures while maintaining air temperatures (24/35°C) improved root growth and root/shoot ratio of each cultivar to levels comparable to control (20/20°C). 'L-93' root growth increased as soil temperatures were lowered to 29°C and 32°C, while air temperature remained the same. Growth of 'Penncross' roots increased when soil temperatures cooled to 29°C but not 32°C. Root fresh weight of each cultivar was not impacted by reducing soil temperature to 32°C and 29°C, but increased at 24°C. Xu and Huang (2001) concluded that reducing soil temperature by as little as 3°C can improve potential for creeping bentgrass survival during prolonged periods of high air temperature stress.

The use of high-output turf fans has become a common practice on golf putting greens in the Transition Zone and Southeastern US to improve growing conditions through improved air circulation and increased transpiration (Beard, 1998, Stier, 2006). The USGA has taken a stance on promoting the benefits of turf fans for creeping bentgrass putting greens for over two decades, with a combination of anecdotal evidence and limited scientific literature (Moeller, 2011, O'Brien, 1996, O'Brien, 2009, Zontek, 1992). The earliest documentation of turf benefits with increased air movement (1.8 m s^{-1}) at the canopy level reported a maximum surface cooling of 7.2°C and a 6.1°C decrease in soil temperature (Duff and Beard, 1966). However, these results were inconsistent with more recent studies (Koh et al., 2003, Taylor et al., 1994). Taylor et al. (1994) reported no significant reduction in air temperature 3 cm above the canopy, small decreases in soil and canopy temperatures, and decreased soil moisture when wind velocities were increased from 1.4 to 2.6 m s^{-1}.

In a subsequent study, the combined impact of turf fans and syringing was evaluated (Guertal et al., 2005). As this study was not designed to evaluate variable wind speeds, wind velocities were reported as means across plots (1.7 m s^{-1}) and were comparable to previous reports (Duff and Beard, 1966, Taylor et al., 1994). The authors reported a small decrease in mean soil temperatures with air movement alone (<1°C) in only one of two years. The mean soil cooling effect of air movement with light syringing was greater (1 to 2.6°C). Plots receiving air movement alone had greater root-length density on 5 of 17 sampling dates compared with no-fan, no-syringe control.

Turf fans used by Guertal et al. (2005) were non-oscillating, and produced an average wind speed of 1.7 m s^{-1} over 21-m^2 plots for 7 hr in the afternoon. Turf fans are typically run continuously with oscillation during stressful conditions, often on putting greens in excess of 500

m^2. Turf fan output and efficiency have improved substantially in comparison to fans previously reported. The most commonly used turf fans on golf courses in 2015 were 127 cm in diameter, powered by a 3.7-kW engine capable of producing wind velocities of 22.2 m^3 s^{-1} (personal communication from SubAir Systems, LLC). Based on data collected for this manuscript, average wind speeds of 1.7 m s^{-1} in previous research is equivalent to the expected output at 21 m from the fan base (data not shown). The impact of air movement at greater velocities has not been documented.

One obstacle with quantifying impacts of air movement over large surfaces is the inability to rapidly and accurately assess changing microclimate conditions. The literature is replete with use of spectral reflectance to estimate plant responses to biotic and abiotic stresses (Jackson, 1986, Krum et al., 2010, Murphy et al., 2014, Raikes and Burpee, 1998, Sullivan and Holbrook, 2007). The most common approach to utilize such data for turfgrass evaluations is through the use of vegetation indices (Bremer et al., 2011, Stiegler et al., 2005, Trenholm et al., 2000). Bremer et al. (2011) reported that the normalized difference vegetation index of near infrared (NIR) and visible red (VR) (NDVI = (NIR-VR)/(NIR+VR)) can be used as an acceptable alternative to subjective visual turf quality ratings. NDVI has been associated with chlorophyll concentrations and soil moisture content of creeping bentgrass (Johnsen et al., 2009, Stiegler et al., 2005). The simple vegetation ratio (RVI = NIR/VR) may correlate more closely with turf quality when the turf canopy is full (McCurdy, 2014). Alternative indices have been proposed that utilize the region of sharp change in reflectance between red and NIR, or the red edge (RE) (Clevers and Gitelson, 2013, Gitelson et al., 1996). The relationship of NDVI, RVI, and related vegetation indices to relative soil temperature, canopy temperature, and root depth has not been established. Geo-referenced reflectance measurements have been utilized to create reflectance maps for precision turf management decisions (Krum et al., 2010). This is based on principles of precision agriculture, which are designed to account for spatial variability and changing microclimate conditions (Carrow et al., 2010).

Research is unavailable on the influence of air movement at higher outputs, across larger surface areas, and using modern turf fans. For this reason, the objectives of the this research are to a) define the relationship of wind velocity with root zone temperature, canopy temperature, and soil moisture content at incremental increases in wind output in a controlled field research environment b) determine whether these associations apply to in-play golf putting greens equipped with high-output turf fans, c) establish relationships of vegetation reflectance to root zone temperature and root depth as impacted by air movement, and d) explore reflectance mapping as a rapid non-destructive estimation of relative root zone temperature and root depth across entire putting surfaces.

MATERIALS & METHODS

Blacksburg site

Field studies were conducted on 'Penncross' and '007' creeping bentgrass putting greens in August 2014 and 2015, respectively, at the Virginia Tech Turfgrass Research Center (TRC) in Blacksburg, VA (Trials 1 & 2). The 25 year old 'Penncross' research putting green was originally built to USGA specifications (Staff, 2004), with high organic matter accumulation creating a loamy-sand root zone. The year old '007' research putting green was built to USGA specifications, using a 90:10 sand/peat root zone mixture. The putting greens were mowed three times per week at 4 mm throughout the study duration. Nitrogen was applied as a foliar spray application bi-weekly throughout the summer at a rate of 7.32 kg N ha^{-1} (Bulldog Bentgrass

Special, 28-8-18, N-P-K plus micronutrients). A complete fungicide program was applied throughout the summer to prevent diseases common in the region. No plant growth regulators or herbicides were applied prior to or during the studies. The entire putting surfaces were irrigated to field capacity as needed to prevent drought, including on the day of trial initiation.

Two treatments of i) fan and ii) no fan were arranged as a randomized complete block design over three replications. Treatments were blocked over replication to address potential shade effects that may have been present. Individual plots measured 3.7 m wide by 6.5 m long, with one non-oscillating 76 cm direct-drive fan (0.33 kW, 4.3 m^3 s^{-1}; Q Standard Industrial, Model#10380, Burnsville, MN) centered on the ground at the edge of each plot along the width to blow over the plot length. Fans ran continuously for 4 d in August with maximum daily air temperatures ranging from 27 to 33°C and no rainfall. In both years, data were collected between 1300 and 1600 hr along a linear transect of each plot every 1 m through 6 m. The maximum wind speed was determined at ground level for each sampling location using a Kestrel 3000 anemometer (Weather Republic, LLC., Downington PA). Soil temperature at 3.8 cm was measured using a 6300 Digital Soil Thermometer (Spectrum Technologies, Aurora, IL). Surface temperature measurements were collected using a Craftsman 50455 IR Thermometer (Sears Holdings Corp., Hoffman Estates IL). A FieldScout TDR 300 (Spectrum Technologies, Aurora, IL) equipped with 3.8 cm probes was used to measure volumetric water content.

Richmond site

Studies were also conducted on in-play golf putting greens at Willow Oaks Country Club (WOCC) in Richmond, VA (Trials 3 and 4). Greens were seeded in 2007 with a blend of Penn A1, Penn A4, and Penn G2 creeping bentgrass. Soil type was designed to USGA specifications, with 4% organic matter added to coarse sand. Putting greens were cut daily at 3.2 mm and rolled with a light-weight roller three times per week. Chemical inputs were identical on all putting greens, and included a routine fungicide program, plus bi-monthly flurprimidol (420 g ha^{-1}) and foliar-applied nutrition (2.9 kg N ha^{-1}). Cultivation consisted of core aeration in the spring and fall (1.6 cm diameter hollow core tines), plus vertical mowing (2 mm diameter) in the spring, followed by sand topdressing. Supplemental solid tine aeration (0.64 cm tine diameter) and light topdressing was performed monthly between events when environmental conditions allowed. Overhead irrigation was applied only when deemed necessary by the golf course superintendent, with supplemental irrigation applied by hand-watering when volumetric water content (VWC) dropped below 13% at 7.6 cm depth in localized areas. VWC was monitored two or three times daily at approximately 25-30 locations per green using a FieldScout TDR 300 Soil Moisture Meter (Spectrum Technologies, Aurora, IL).

This study was arranged as a modified randomized complete block design over three replications for two treatments of fan or no fan. Each putting green served as an experimental unit, with data collected every 3 m in three linear subsets between 6 m and 21 m, for a total of 18 sampling locations per green. Electric TB-50-Premium (3.7 kW, 22.2 m^3 s^{-1}; SubAir Systems, LLC, Graniteville, SC) fans were installed approximately 3 m from the putting surface in locations with the least impact on playability. Fans were mounted 1 m from the ground with 140° oscillation. Fans were activated in the spring when average daily air temperature consistently exceeded 21°C (10 May 2013 and 03 May 2015) and ran continuously throughout the summer. Because fan placement was dependent on putting greens with the greatest need, the no-fan control within each replication was placed at a nearby location with similar growing conditions. The experiment was repeated in summer 2013 and 2015.

Ground truth measurements included maximum wind speed, soil and canopy temperatures, root zone moisture content, and rooting depth. Data collection occurred between 1000 and 1700 h during the hottest part of the day. All measurements for each of 18 sampling locations per green were collected within the same timeframe. Data were collected consecutively from all three greens within a replication to minimize temporal changes throughout the day. Data were collected using previously described methods (above). Maximum wind speed was determined by placing an anemometer at ground level for three fan oscillations per sampling location. Rooting depth was measured using a 9- by 2-cm AMS Turf Profiler (AMS, Inc., American Falls ID) to provide a larger surface area for root length estimation compared to previous studies (Guertal et al., 2005) and because of limited sampling space within each measurement unit. Average root depth across each profile was measured, with deeper bentgrass roots in aeration holes excluded.

Reflectance Mapping

Putting green boundaries and individual subsample coordinates were geo-referenced with differential GPS (Phoenix 300, Raven Industries, Sioux Falls, SD) using OmniStar HP subscription for sub-decimeter resolution. Spectral reflectance in the visible red (VIR; 670-22 nm), red edge (RE; 730 nm), and near infrared (NIR; 760 nm LWP) was captured using a Crop Circle ACS 470 (Holland Scientific, Lincoln, NE), with geo-referenced coordinates assigned to each data point using a GeoSCOUT GLS-400 (Holland Scientific, Lincoln, NE). The sensor was mounted 70 cm from the turf canopy to provide a 46 cm field of view. Continuous reflectance data (10 measurements s^{-1}) were collected by walking the mounted unit across the putting surface, using daily walk-mow patterns (54 cm width) for guidance. Raw geo-referenced reflectance data were uploaded to an online GIS data processing service (TurfScout, LLC, Greensboro NC) for creation of reflectance maps of entire putting greens and 1-m diameter representation of each subsampling location. Raw data were processed via TurfScout, LLC and transformed into a simple ratio vegetation index (RVI = NIR / Red) (Birth and McVey, 1968), with erroneous outliers removed. Co-located ground truth data along with reflectance outputs were joined into a single table. For reflectance outputs, data falling within a 0.5-m radius of the sample location were averaged. A random subset of 15% of sample locations from three greens was retained to test for accuracy.

Statistical Analysis

Data were subjected to analysis of variance using JMP Pro 11.2.0 (SAS Institute, Inc., Cary NC). General linear regression analysis was performed to quantify the relationship between distance (m) from the fan or theoretical fan base and each variable of interest. Variables of interest included wind velocity, root zone temperature, canopy temperature, volumetric soil water content, root depth, and spectral reflectance. Analysis of variance and t-test were used to evaluate differences in slope and intercept of each variable ($\alpha = 0.05$), to quantify where air movement from fans was no longer influential. Pearson correlation coefficients ($\alpha = 0.05$) were calculated to develop a relationship between wind velocity and each variable of interest.

Additionally, Pearson correlation coefficients ($\alpha = 0.05$) were calculated between reflectance measurements and root zone temperature or root length as a method to evaluate the application of spectral reflectance for estimation of root zone temperatures and root depth. The relationship between each ground truth variable and canopy reflectance was established using simple linear regression and then applied to the entire map. Estimates of each variable at known locations were compared to observed values and used to calculate the root mean square errors (RMSE). Observed data for canopy reflectance, root zone temperature, and root depth were divided into five quantiles to define the relationship of canopy reflectance to root zone temperature or root depth across non-sampled map locations.

RESULTS & DISCUSSION

ANOVA revealed significant treatment, location, and trial effects for wind speed, root zone temperature, soil moisture content, canopy temperature, and root depth across all distances (Table 1). However, the distance of effect between fan and no fan was always significant within a trial ($P < 0.05$) for each variable of interest, except soil water content in Trial 3 at WOCC. Fan by location and fan by trial interactions were primarily due to changes in ambient conditions at the time of data collection. For instance, mean canopy temperatures of putting greens without fans ranged from 30.9 to 35.5 °C across trials ($P < 0.0001$, data not shown). Root depths were only measured at WOCC because data were collected after a full season of fan influence, whereas data collected at TRC occurred after only four days which would have had limited impact on root development. The significance of root depth was more dependent on trial than on fan treatment (Table 1).

Surface mean wind velocities and their intercept and slope across six distances on putting surfaces with fans is presented in Table 2. The mean wind velocity on putting surfaces without fans is also listed, though convergence criteria of the linear relationship to distance were not met and therefore slope and intercept are not listed. Data from two trials at TRC and two trials at WOCC were pooled by location because there was no trial by fan interaction within location ($P \geq 0.27$). Mean wind velocities across all distances of data collection on putting surfaces with fans were 1.76 and 2.91 m s^{-1} for TRC and WOCC, respectively ($P < 0.0001$). The mean wind velocity at TRC was comparable to those reported in previous research (Duff and Beard, 1966, Guertal et al., 2005, Taylor et al., 1994). However, the intercept at distance 0 was 3.55 m s^{-1} with a negative slope of -0.511. Measurements at TRC were collected each meter between 1 and 6 m. This decrease in wind speed by approximately 1 m s^{-1} for every 2 m distance from fans indicates that wind velocity will fluctuate dramatically across a putting surface. Data were collected from six distances across full, in-play putting greens at WOCC every 3 m, from 6 m to 21 m from the fan. The average wind speed across these surfaces was 2.91 m s^{-1}, with a slope of -0.681 that intercepts the fan base at a velocity of 5.29 m s^{-1}. Velocity ranged from a maximum of 6.9 m s^{-1} at 6 m to a minimum of 0.8 m s^{-1} at 21 m across all WOCC data points (data not shown). The velocities nearest fan bases were over three times greater than those reported in previous research. Additionally, large turf fans installed adjacent to putting greens oscillate and are capable of covering large portions of the surface, albeit at variable velocities.

The linear relationship between air movement and other variables of interest were significant for putting green surfaces with fans (Table 3). Data are presented as pooled within location for TRC and WOCC ($P < 0.01$), but also as individual trials within location because of trial by fan treatment interactions. Slopes between trials within location were consistent, though the magnitude or intercept changed considerably by trial. Wind velocity was negatively correlated with root zone temperature, soil water content, and canopy temperature at TRC (r = -0.48 - -0.59, $P < 0.01$). The relationship of wind velocity to root zone temperature and soil water content was significant at WOCC (r = -0.55 - -0.59, $P < 0.05$), while the relationship with canopy temperature was insignificant ($P > 0.05$). Root depth was highly, positively correlated to wind speed at WOCC ($r = 0.69$, $P < 0.0001$), the only location where these data were collected. While the correlation of air movement to other variables of interest does not conclude prove, it is very likely that wind velocity drives these factors.

Root zone temperature was most closely and consistently related to wind speed, regardless of location or trial ($r = 0.48 – 0.91$, $P \leq 0.0031$). Root zone temperature decreased by approximately 1 °C and 0.75 °C for every 1 m s^{-1} increase in wind velocity at TRC and WOCC, respectively (Figure 1). As previously stated, the total distance of data collection was 6 m at TRC and 21 m at WOCC. Root zone temperature at WOCC increased from 30.1 °C nearest the fan to 33.1 °C 21 m from the fan (data not shown). Corresponding wind speeds at these distances were 4.8 and 1.1 m s^{-1}, respectively. Volumetric water content of soil was significantly related to wind velocity across trials at TRC and WOCC ($r = 0.28 - 0.31$, $P < 0.001$). However, there was no significant relationship between soil water content and wind velocity for Trial 3 at WOCC. Similarly, there was a significant relationship between canopy temperature and wind speed at TRC but not WOCC when pooled over trials. ANOVA revealed a highly significant trial by fan interaction for soil water content and canopy temperature (Table 1). Further investigation showed no significant fan influence on soil water content in Trial 3 (data not shown). Fans impacted canopy temperature in all trials, but the relationship was independent of wind velocity based on these data. Rooting depth was not measured at TRC, as previously noted, but was strongly related to wind speed within and across trials at WOCC ($r = 0.53 – 0.81$, $P < 0.05$). Using the linear relationship, root depth was increased by 10 mm for every 1 m s^{-1} increase in wind speed (Figure 2). Across trials, root depth and wind speed increased from 63 mm and 1.1 m s^{-1} at 21 m from the fan base to 92 mm and 4.8 m s^{-1} at 6 m from the fan, respectively.

Of the variables related to wind velocity, root zone temperature and root depth should have the greatest impact on overall plant health. Xu and Huang (2001) concluded that soil temperatures have a greater impact on the health of creeping bentgrass than air temperature. Soil water content does not have as strong of an impact on root health as soil temperature when water availability is not limiting. The relationships built from these data were from different climates in Virginia and from two sources of air movement with drastically different output. Data from each location suggests that air movement has a significant influence on root zone temperature. Data from six unique, in-play putting greens collected over two years also suggest that air movement can strongly influence the root depth of creeping bentgrass throughout stressful summer growing conditions. The overall impact of artificial air movement at higher outputs will likely depend on a variety of extraneous factors. Ambient air temperatures and prevailing wind will most certainly influence how fans impact the growth of creeping bentgrass. These data were collected on sand-based root zones built to USGA specification. Water holding capacity and thermal fluctuations will likely vary by soil structure and organic matter accumulation.

Remote sensing with spectral reflectance can be used to rapidly estimate characteristics about plant canopies when clear relationships have been established (Jackson, 1986). However, due to variability in atmospheric conditions within and across sampling times, accurate estimates of soil or plant attributes necessitate proper ground-truth validation. Reflectance data from an equipment-mounted sensor were compared against measurements of root zone temperature, soil water content, canopy temperature, and root depth collected at WOCC (Table 4). Comparisons were made against reflectance of red (670 nm), near infrared (760 nm), red edge (730 nm), and three simple ratio vegetation indices derived from these spectra. There were no significant relationships between soil water content or canopy temperature and any spectral reflectance parameter tested ($P > 0.05$). No individual spectra were significantly related to root zone temperature, though all calculated vegetation indices were negatively correlated ($r \geq -0.60$, $P < 0.01$). R_{760} and RVI2 were positively related to root depth ($r \geq 0.54$, $P < 0.05$) while all other reflectance data were unrelated. RVI2 had the strongest relationship of all reflectance data to root zone temperature (r = -0.84, $P < 0.0001$) and root depth (r = 0.67, P = 0.0026). RVI2 is derived using reflectance from two important regions outside of the visible light spectrum. Near infrared light reflectance has been closely associated with leaf canopy structural integrity, while light reflected from the red edge is most closely related to endogenous chlorophyll concentrations (Gitelson, et al., 2005, Knipling, 1970). Green plant tissue strongly absorbs red light, though dark green tissue does not always equate to highest turf quality. These data suggest that a ratio of near infrared to red edge light reflectance may be a stronger indicator of a healthier stand of creeping bentgrass than traditional near infrared to red light, with a denser root system.

The relationships between RVI2 and root zone temperature and root depth are linear and can therefore be useful for estimating these characteristics at non-sampled locations using reflectance mapping. Using quantiles of these data estimates, RVI2 values can be equated to estimated root zone temperatures and root depth of sampled and non-sampled locations (Table 5). Estimates of root zone temperature and rooting depth at unsampled locations were derived from reflectance maps using the relationship between spectra and ground truth and sampled locations. It is important to note that any relationship to vegetation reflectance applies only to a particular collection period. For example, these data showed a linear relationship between RVI2 and root zone temperature across all trials ($r > 0.75$, $P < 0.0001$, Fig. 1). Using this estimated model, an RVI2 value of 2.22 may equate to a root zone temperature of 30°C and an RVI2 value of 2.03 has an estimated root zone temperature of 33.7°C. In another study, these values cannot be used to estimate temperatures without validation. However, the relationship established in this manuscript may be useful to make generic estimations that higher RVI2 values may indicate lower root zone temperatures.

CONCLUSIONS

The output of turf fans has increased substantially in recent history. Fans used on in-play golf putting greens in this study produced a range of wind velocities from a maximum of 6.9 m s^{-1} at 6 m from the fan base to a minimum of 0.8 m s^{-1} at 21 m across the putting surface. Root zone temperatures at 3.8 cm depth were reduced by 2.1 to 3.9°C across putting surfaces. Linear relationships of wind velocities were established against root zone temperature, root depth, canopy temperature, and soil moisture content using in-play putting surfaces and controlled research putting greens with lower air output. These relationships set a baseline for the anticipated benefits of turf fans over a variety of wind velocities. Additionally, reflectance using near infrared (760 nm) and red edge (730 nm) light spectra were linearly related to soil temperature and root depth of creeping bentgrass built on sand-based root zones. This relationship provides a method for rapidly estimating these factors across a putting surface with minimal sampling requirements.

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LITERATURE CITED

Beard, J. 1998. Heat stress causes and prevention. TUR FAX 6: 1-6.

Beard, J.B. 1973. Turfgrass: Science and culture. Prentice-Hall, Englewood Cliffs, NJ.

Beard, J.B. and W.H. Daniel. 1966. Relationship of creeping bentgrass (*Agrostis palustris* Huds.) root growth to environmental factors in the field. Agron. J. 58: 337-339.

Birth, G.S. and G.R. McVey. 1968. Measuring the color of growing turf with a reflectance spectrophotometer. Agron. J. 60: 640-643.

Bremer, D.J., H. Lee, K. Su and S.J. Keeley. 2011. Relationships between normalized difference vegetation index and visual quality in cool-season turfgrass: II. Factors affecting NDVI and its component reflectances. Crop Sci. 51: 2219-2227.

Carrow, R.N., J.M. Krum, I. Flitcroft and V. Cline. 2010. Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. Precision Agriculture 11: 115-134.

Clevers, J.G. and A.A. Gitelson. 2013. Remote estimation of crop and grass chlorophyll and nitrogen content using red-edge bands on Sentinel-2 and-3. Int. J. Appl. Earth Observation and Geoinformation 23: 344-351.

Dernoeden, P.H. 2012. Creeping bentgrass management, Second Edition. CRC Press. Boca Raton, FL, USA.

Duff, D.T. and J.B. Beard. 1966. Effects of air movement and syringing on the microclimate of bentgrass turf. Agron. J. 58: 495-497.

Gitelson, A.A., M.N. Merzlyak and H.K. Lichtenthaler. 1996. Detection of red edge position and chlorophyll content by reflectance measurements near 700 nm. J. Plant Physiol. 148: 501-508.

Gitelson, A.A., A. Vina, V. Ciganda, D.C. Rundquist and T.J. Arkebauer. 2005. Remote estimation of canopy chlorophyll content in crops. Geophysical Research Letters 32.

Guertal, E., E. van Santen and D. Han. 2005. Fan and syringe application for cooling bentgrass greens. Crop Sci. 45: 245-250.

Huang, B. 2003. Recent advances in drought and heat stress physiology of turfgrass-a review. Int. Conf. Turf. Management and Science for Sports Fields 661.

Jackson, R.D. 1986. Remote sensing of biotic and abiotic plant stress. Annu. Rev. Phytopathol. 24: 265-287.

Johnsen, A.R., B.P. Horgan, B.S. Hulke and V. Cline. 2009. Evaluation of remote sensing to measure plant stress in creeping bentgrass (L.) fairways. Crop Sci. 49: 2261-2274.

Knipling, E.B. 1970. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. Remote Sens. Environ. 1: 155-159.

Koh, K., G. Bell, D. Martin and N. Walker. 2003. Shade and airflow restriction effects on creeping bentgrass golf greens. Crop Sci. 43: 2182-2188.

Krum, J., R. Carrow and K. Karnok. 2010. Spatial mapping of complex turfgrass sites: Site-specific management units and protocols. Crop Sci. 50: 301-315.

McCurdy, J. D., C. M. Baldwin, H. W. Philley, B. R. Stewart, and J. Ruffin. 2014. Comparison of visual rating techniques and spectral reflectance characteristics during turf variety trial evaluations. ASA, CSSA, & SSSA International Annual Meeting, Long Beach, CA. November 5, 2014. Crop Science Society of America.

Moeller, A., and Chapin, B. 2011. Using turf fans in the Northeast. Limited air movement isn't just a southeast or transition-zone problem. USGA Green Section Record 49: 1-4.

Murphy, J.T., C.E. Owensby, J.M. Ham and P.I. Coyne. 2014. Estimation of vegetative characteristics by remote sensing. Academic Research Journal of Agricultual Science and Research 2: 47-56.

O'Brien, P. 1996. Optimizing the turfgrass canopy environment with fans. United States Golf Association. USGA Green Section Record. 34(4) 9-12.

O'Brien, P. 2009. The costs of blowing wind. A guide to the selection, installation, and operation of fans for better summer performance of bentgrass putting greens. USGA Green Section Record 47: 7-9.

Raikes, C. and L. Burpee. 1998. Use of multispectral radiometry for assessment of Rhizoctonia blight in creeping bentgrass. Phytopathology 88: 446-449.

Staff, U.S.G.A. 2004. Revising the USGA's recommendations for a method of putting green construction, United States Golf Association**:** 1-12.

Stiegler, J., G. Bell, N. Maness and M. Smith. 2005. Spectral detection of pigment concentrations in creeping bentgrass golf greens. Int. Turf. Soc. Res. J 10: 818-825.

Stier, J. C. 2006. Understanding and managing environmental stresses of turfgrass. XXVII International Horticultural Congress-IHC2006: International Symposium on Horticultural Plants in Urban and Peri-Urban 762.

Sullivan, D.G. and C.C. Holbrook. 2007. Using ground-based reflectance measurements as selection criteria for drought- and aflatoxin-resistant peanut genotypes. Crop Sci. 47: 1040-1050. doi:10.2135/cropsci2006.08.0511.

Taylor, G., C. Peacock, J. DiPaola, L. Lucas and U. Blum. 1994. Influence of wind velocity on temperature, leaf water potential and soil moisture percentage of creeping bentgrass golf greens. 1995 Agronomy abstracts. ASA, Madison, WI: 187.

Trenholm, L., M. Schlossberg, G. Lee, W. Parks and S. Geer. 2000. An evaluation of multi-spectral responses on selected turfgrass species. Int. J. Remote Sens. 21: 709-721.

Xu, Q. and B. Huang. 2000. Growth and physiological responses of creeping bentgrass to changes in air and soil temperatures. Crop Sci. 40: 1363-1368.

Xu, Q. and B. Huang. 2001. Lowering soil temperatures improves creeping bentgrass growth under heat stress. Crop Sci. 41: 1878-1883.

Zontek, S.J. 1992. Measuring air movement for better grass. USGA Green Section Record 30: 38-39.

CHAPTER 5: TABLES AND FIGURES

Table 1. Analysis of variance on the impact of turf fans for surface wind speed, root zone temperature, soil water content, canopy temperature, and root depth to creeping bentgrass putting greens from two locations in Virginia.

	Wind (m s^{-1})	Root zone temperature (°C)	Soil water content (%)	Canopy temperature (°C)	Root depth (mm)
Fan	***	***	**	***	*
Location	***	***	**	***	---
Trial	***	***	***	***	***

***, **, * significant at the 0.001, 0.01, and 0.05 level, respectively.

Table 2. Average wind velocity across bentgrass green units with and without fans for two locations in Virginia. Coefficient of determination (R^2) and root mean square error (RMSE) calculated on greens with fans using linear regression across six distances from fan base.

	Wind (m s^{-1})					
	TRC[†]			WOCC		
	Mean	Intercept	Slope	Mean	Intercept	Slope
Fan	1.76***	3.55***	-0.511***	2.91***	5.29***	-0.846***
No Fan	0.28	---	---	0.88	---	---
SE	0.12	0.18	0.045	0.19	0.38	0.104
		R^2	0.79		R^2	0.59
		RMSE	0.46		RMSE	1.00

*** Significant at the 0.001 level. --- Convergence criteria not met for linear regression.

[†] Trial by fan interaction was not significant within location ($P \geq 0.27$), therefore data were pooled over trials within TRC and WOCC locations.

Table 3. Linear relationship between wind speed (m s^{-1}) and root zone temperature, soil water content, canopy temperature, and root depth from creeping bentgrass putting greens in Blacksburg, VA (TRC) and Richmond, VA (WOCC). Convergence criteria were not met on greens without fans.

	Root zone temperature (°C)			**Soil water content (%)**			**Canopy temperature ((°C)**			**Root depth (mm)**		
	R^2	**Intercept**	**Slope**	**R^2**	**Intercept**	**Slope**	**R^2**	**Intercept**	**Slope**	**R^2**	**Intercept**	**Slope**
TRC	0.23†**	27.7 (0.7)	-1.08 (0.3)	0.28	28.4 (1.3)	-2.34 (0.65)	0.35	29.8 (0.6)	-0.85 (0.22)	---‡	---	---
Trial 1	0.69	26.0 (0.4)	-1.08 (0.18)	0.3	30.9 (1.9)	-2.44 (0.93)*	0.36	29.3 (1.0)	-1.43 (0.47)**	---	---	---
Trial 2	0.56	29.5 (0.5)	-1.12 (0.25)	0.5	26.0 (1.1)	-2.22 (0.55)**	0.4	30.3 (0.8)	-1.27 (0.39)**	---	---	---
WOCC	0.35	33.3 (0.4)	-0.51 (0.12)	0.31	29.8 (0.7)	-0.85 (0.22)	NS	32.1 (0.9)	NS	0.48	45.6 (6.0)	10.3 (1.8)
Trial 3	0.75	35.0 (0.4)	-0.75 (0.11)	NS	27.5 (0.7)	NS	NS	33.7 (1.4)	NS	0.65	67.7 (5.2)	7.3 (1.4)
Trial 4	0.82	33.0 (0.1)	-0.74 (0.09)	0.57	32.3 (1.1)	-1.88 (0.41)	0.73	33.0 (0.5)	-1.25 (0.19)	0.28*	43.5 (6.4)	6.2 (2.5)*

†Values significant at 0.001 unless otherwise noted as ** or * for *P* = 0.01 and 0.05, respectively. NS = not significant at *P* = 0.05.
‡Root depth not collected at TRC.

Table 4. Pearson's correlation coefficients relating spectral reflectance to root zone temperatures, soil water content, canopy temperature, and root depth of creeping bentgrass from golf greens in Richmond, VA.

Reflectance	Root zone temperature (°C)	Soil water content (%)	Canopy temperature (°C)	Root depth (mm)
R_{670} †	NS	NS	NS	NS
R_{760}	NS	NS	NS	0.54*
R_{730}	NS	NS	NS	NS
RVI1‡	-0.75***	NS	NS	NS
RVI2	-0.84***	NS	NS	0.67**
RVI3	-0.60**	NS	NS	NS

***, **, and * significant at 0.001, 0.01, and 0.05, respectively. NS = not significant at P = 0.05.

†R_x indicates spectral reflectance at specified wavelength (nm).

‡RVI, the simple vegeation ratio index, calculated as RVI1 = R_{760}/R_{670}; RVI2 = R_{760}/R_{730}; and RVI3 = R_{730}/R_{670}.

Table 5. Root zone temperature and root depth estimation using the simple ratio index (RVI2 = R_{760}/R_{730}), based on data collected from creeping bentgrass putting greens in Richmond, VA.

Quantiles	RVI2	Root zone temperature (°C)	Root depth (mm)
> 90%	> 2.20	< 30.1	> 119
75 – 90%	2.17 -2.20	30.1 -31.2	107 - 118
25 – 74%	2.03 -2.169	31.19 - 33.5	82 - 106
10 – 24%	1.95 - 2.029	33.49 - 34.3	76 - 81
< 10%	< 1.95	> 34.3	< 76

Figure 1. Linear relationship between wind velocity (m s^{-1}) and root zone temperature (°C) of creeping bentgrass putting greens for two trials for two location (left column TRC = Blacksburg, VA; right column WOCC = Richmond, VA).

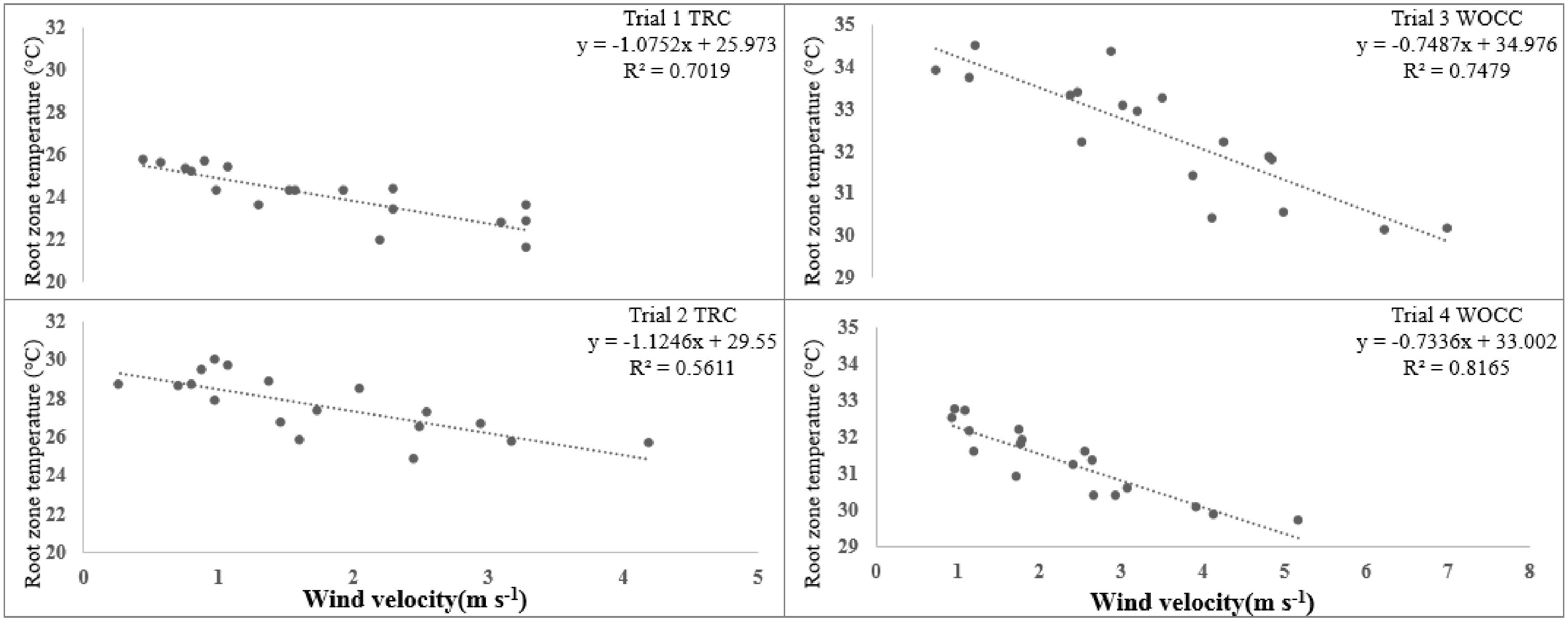

Figure 2. Relationship between wind velocity (m s^{-1}) and root depth (mm) of creeping bentgrass grown on golf putting greens with turf fans in Richmond, VA between 2013 and 2015.