

Improving Soil Moisture Assessment of Turfgrass Systems Utilizing Field Radiometry

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ACADEMIC ABSTRACT

The need for water conservation continues to increase as global freshwater resources dwindle. In response, many golf course superintendents are implementing new methods and tools to become more frugal with their water applications. For example, scheduling irrigation using time-domain reflectometer (TDR) soil moisture sensors can decrease water usage. Still, TDR measurements are time-consuming and only cover small scales, leading to many locations being unsampled. Remotely sensed data such as the normalized difference vegetation index (NDVI) offer the potential of estimating moisture stress across larger scales; however, NDVI measurements are influenced by numerous stressors beyond moisture availability, thus limiting its reliability for irrigation decisions. An alternative vegetation index, the water band index (WBI), is primarily influenced by water absorption within a narrow spectral range of near-infrared light. Previous research has established strong relationships between moisture stress of creeping bentgrass (CBG) grown on sand-based root zones, a typical scenario for golf course putting greens. However, this relationship characterizes only a small portion of total acreage across golf courses, which limits widespread adoption. In our research, '007' CBG and 'Latitude 36' hybrid bermudagrass (HBG) were grown on three soil textures, USGA 90:10 sand (S), sand loam (SL) and clay (C), arranged in a 2 x 3 factorial design, randomized within six individual dry-down cycles serving as replications. Canopy reflectance and volumetric water content (VWC) data were collected hourly between 0700 and 1900 hr using a hyperspectral radiometer and an embedded soil moisture sensor, until complete turf necrosis. The WBI had the strongest relationship to VWC ($r = 0.62$) and visual estimations of wilt ($r = -0.91$) compared to the green-to-red ratio index (GRI) or NDVI. Parameters associated with non-linear regression were analyzed to compare grasses, soils, indices, and their interactions. The WBI and GRI compared favorably with each other and indicated significant moisture stress approximately 28 hr earlier than NDVI ($P = 0.0010$). WBI and GRI respectively predicted moisture stress 12 to 9 hr before visual estimation of 50% wilt, whereas NDVI provided 2 hr of prediction time ($P = 0.0317$). When considering the time to significant moisture stress, the HBG lasted 28 hr longer than CBG, while S lasted 42 hr longer than either SL and C ($P \leq 0.0011$). Nonlinear regression analysis showed that WBI and GRI can be useful for predicting moisture stress of CBG and HBG grown on three diverse soils in a highly controlled environment. Our results provide substantial evidence and direction for future research investigating how WBI and GRI can expedite moisture stress assessment and prediction on a large-acreage basis.

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GENERAL AUDIENCE ABSTRACT

Managed turfgrasses provide several benefits including filtering pollutants, cooling their surroundings, generating oxygen, preventing erosion, serving as recreational surfaces, and increasing landscape aesthetics. Intensively managed turfgrass systems, such as on golf courses and sports fields, require more inputs to maintain acceptable conditions. Freshwater use is often excessive on intensively managed turfgrasses to maintain proper plant growth. Drought conditions often limit water availability, especially in regions with limited rainfall. Turf managers tend to over-apply water across large acreage when few localized areas begin to show symptoms of drought. Additionally, turf managers sometimes wrongly identify stressed areas from other factors as ones being moisture deprived. Advancements such as the use of soil moisture meters have simplified irrigation decisions as an aid to visual inspections for drought stress. While this method enhances detection accuracy, it still provides no solution to increase efficiency. Expanding our current knowledge of turfgrass canopy light reflectance for rapid moisture stress identification can potentially save both time and water resources. The objective of this research was to enhance our ability to identify and predict moisture stress of creeping bentgrass (CBG) and hybrid bermudagrass (HBG) canopies integrated into varying soil textures (USGA 90:10 sand (S), sand loam (SL) and Clay (C)) using light reflectance measurements. Dry-down cycles were conducted under greenhouses conditions collecting soil moisture and light reflectance data every hour from 7 am to 7 pm after saturating and withholding water from established plugs. Moisture stress was most accurately estimated over time using two vegetation indices, the water band index (WBI) and green-to-red ratio index (GRI), with approximately ninety percent accuracy to visible wilt stress. The WBI and GRI predicted moisture stress of CBG in all soil types and HBG in SL and C approximately 14 hours before the grasses reached 50% wilt. While light reflectance varies on exposed soils, our research shows that underlying soils do not interfere with measurements across typical turfgrass stands. This research provides a foundation for future research implementing rapid, aerial measurements of moisture-stressed turfgrasses on a broad application of CBG and HBG on constructed or native soils.

Dedication

I dedicate this thesis to my beautiful girlfriend, Jamie Hodnett for the support and encouragement she provided throughout my graduate career. She always provided nothing but absolute confidence in my abilities to accomplish my goals. She also spent countless hours accompanying me cleaning pots, transferring and planting plugs, gathering data, and compiling data. If it were not for you, this research project would never have reached its potential, and for that, I can never thank you enough. I would also like to dedicate this thesis to my amazing parents, Larry and Karen Roberson. Both parents instilled in me fundamental values that hard-work and courage are needed to overcome life obstacles. Their consistent support and guidance as I developed through challenges in life, and throughout my graduate school tenure, were invaluable. I cannot express enough gratitude for everything you all have done and taught me. If it were not for my parents, I would not be where I am today.

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List of Abbreviations

1. Creeping Bentgrass (CBG)
2. Evapotranspiration (ET)
3. Four-Parameter Logistic (4PL)
4. Green-to-Red-Ratio Index (GRI)
5. Hybrid Bermudagrass (HBG)
6. Hyper-Spectral Radiometer (HSR)
7. Inflection Point (IP)
8. Near Infrared (NIR)
9. Normalized Difference Vegetation Index (NDVI)
10. Photosynthetic Active Radiation (PAR)
11. Short-Wave Infrared (SWIR)
12. Unmanned Aerial Vehicles (UAV)
13. Time-Domain Reflectometer (TDR)
14. Turf Quality (TQ)
15. Vegetation Index (VI)
16. Volumetric Water Content (VWC)
17. Water Band Index (WBI)
18. Wilt Percent (WP)

Chapter 1. Literature Review

United States golf courses use approximately 2.93×10^{12} liters of water annually, which equates to 7.87×10^9 liters per day. Total water use amounts vary depending on regional variations influencing evapotranspiration amounts, the turfgrass species used, and underlying soil texture and properties. Several tools and methods are used to assist golf course superintendents in scheduling proper irrigation and minimizing water use such as underground soil moisture sensors, automated irrigation software based on estimated evapotranspiration rates, and handheld soil moisture time-domain reflectometers. The use of light reflectance's potential to identify and predict moisture stress is something that is being explored in numerous agronomic applications. However, turfgrass systems can change rapidly from hour to hour based on constructed soil structures found on golf courses. It is conceivable that turfgrass managers can minimize their total irrigation water applied on the golf course through the use of light reflectance. Our research shows the ability of light reflectance to indicate soil moisture stress in a highly controlled greenhouse setting with two grass species in a wide soil texture gradient. Drones equipped with sensors capable of collecting similar spectral data provide a promising future for rapid moisture stress identification across large acreage areas.

Water Usage

Total global water covers 71% of the earth and includes oceans, rivers, permafrost, lakes, etc. (Perlman, 2016). Total water on earth, including surface water and accounting for ground and atmospheric water, comprises a remarkable 1.41×10^{21} L (Gleick, 1993). Freshwater accounts for 3.4 % or 7.16×10^{19} L, but large amounts of this

freshwater is inaccessible for human use. In fact, of the total freshwater percentage, only 1.2% or 5.08×10^{17} L is, or could potentially be, used for human survival and signifies why useable water is such a vital global resource (Gleick, 1993). Lack of freshwater continues to be a concern when considering current and future populations.

In 2010, the world population was approximately 7 billion and the United Nations projects this to increase to 9.15 billion by 2050 (Alexandros et al., 2012). This drastic increase in human population creates concern because it generates a higher demand for agricultural food. More agricultural acreage will be required to meet these food demands causing an influx in irrigated acreage. Increasing agricultural acreage may not be achievable since total cropland in the United States has seen a gradual 17 percent decrease from 1949 to 2007 when first reported in 1949 that 165 million hectares were dedicated for food production (Nickerson and Borchers, 2012). Continual decreases in cropland require us to be more efficient on less land to meet growing populations all while water resources gradually deplete. The International Food and Policy Research Institute reported that in 1995 the total global freshwater withdrawal was quantified at 3.91×10^{15} L for human needs, such as food security and industrial growth (Rosegrant et al., 2002). The same report projects a 50% increase in water withdrawals by 2025 for the aforementioned reasons. Drastically increasing water withdrawals will limit the 250 million irrigated hectares worldwide (Rosegrant et al., 2002). Irrigation restrictions that limit agricultural production drive the accountability of humans to locate and diminish water consumption of other sectors that are not as critical as feeding a growing population.

Turfgrass maintained for recreational activities, such as golf, are non-essential to human survival, but still increase the strain on our water supply. In 2009, golf courses occupied 608,746 hectares of maintained turfgrass, in which, 484,978 hectares were irrigated (Throssell et al., 2009). Intensively managed greens, tees, and fairways were the main segments of golf that comprised irrigated land with some rough and practice areas as well. A study conducted by the Golf Course Superintendents Association of America reported that 2.93×10^{12} L of water were used across this total irrigated land in 2005, but was reduced by 21.8% in 2013 (Anonymous, 2009, 2014). Water savings between surveys are attributed to several factors such as the adoption of wetting agents, precise hand-watering, maintaining a drier soil profile, irrigation system improvements, etc. (Gelernter et al., 2015; Anonymous, 2014). Furthermore, golf course superintendents have begun to develop best management practice documents tailored to their specific golf course needs through the Golf Course Superintendents Association of America's best management practices planning guide. These documents help administer goals and plans on how to increase water use efficiency and conservation, among other management strategies (Carrow et al., 2007). Even with golf courses implementing these enhanced agronomic practices over the past decade, there is still significant room for improvements. Golf courses reducing their water usage allows for shifts to more pertinent issues, such as providing more water for increasing crop yields to meet the escalating human population. While the importance of water is evident, one must understand the relationship that water has with the soil and plant before they can properly employ conservation methods.

Water's Relationship to Soil and Plants

Water and Soil Relationships

Research has established existing relationships between soil texture, particle size, and organic matter as fundamental factors in determining soil properties such as water infiltration and water holding capacity (Ahuja et al., 1999; Ball, 2001; Gijssman et al., 2002; Gupta and Larson, 1979; Rawls et al., 1998). Water infiltration of soils is defined as a process when water on the soil surface enters through forces of gravity and capillary action (Mangala et al., 2016). Infiltration rates of soils typically increase as organic matter increases. Soil organic matter increases the macro and micro fauna populations that, in turn, generate organic waste products complementing the stability of soil aggregates. These soil aggregates lead to a higher percentage of pore spaces (Bot and Benites, 2005; Franzluebbers, 2002). Furthermore, soil texture is determined by the total percentage of particle sizes within a soil, as defined by the United States Department of Agriculture, and are as follows; “sand = 2.0 to 0.05 mm, silt = 0.05 to 0.002 mm, and clay = <0.002 mm” (Saxton et al., 1986). A study by Mazaheri and Mahmoodabadi (2012) showed that sand intensifies the influence on infiltration rates positively, whereas, silt and clay particles result in a negative relationship. In other words, as soil particle size decreases from sand to silt to clay, so does the soil pore size, decreasing infiltration capability (Nimmo, 2004). Along with particle size impeding water infiltration, their soil structure, or aggregate arrangement, also manipulates infiltration. The three primary particles form soil structures from single grain or granular with high sand percentages to platy or heavy soils with high clay amounts. Granular soils have high percolation rates, whereas, platy, dense soils severely limit the infiltration of water and lead to high erosion potential (Easton and Bock, 2016). Another factor related to the soil particles is bulk

density, which is a numerical ratio derived from the mass of dry soil within a known volume the soil occupies. Higher bulk densities are observed with sandier soils but have less probability of becoming compacted, and the inverse occurs with heavy clay soils. Increases in bulk density affect porosity that impacts the quantity and speed at which water will infiltrate through a soil (Easton and Bock, 2016). One grazing study investigated water infiltration of a sandy loam soil subjected to heavy grazing traffic and found a negative correlation of infiltration rate to bulk density (Franzluebbers et al., 2012). While soil texture alters several variables that influence water infiltration, it also impacts the amount withheld once it enters the soil.

Total soil water potential is a combination of several forces: gravitational, atmospheric, osmotic, and matric potentials. Matric, or suction, potential is the adsorption of water to soil particles and is governed by the type and size of soil particles in a given soil (Clark, 1990). After gravitational drainage, the suction potential of soil through capillary forces determines volumetric water content (VWC) amounts for plants. (Hillel, 1971; Kirkham, 2014). Definitions for soil VWC during unsaturated conditions are field capacity, permanent wilting point, and plant available water. Field capacity is the upper limit of a soil's water holding capacity after gravitational drainage approaches a negligible rate. Conversely, the permanent wilting point is the lower limit of a soil's water holding capacity. Soils approach a permanent wilting point once plants can no longer change their root osmotic potential to overcome the soil's suction potential and uptake of water from the soil. A region exists between field capacity and a wilting point when the plant completely senesces and is known as plant available water (Silva et al., 2014; Zotarelli et al., 2010). Regarding soil particle type and size, coarse particles have a lower

field capacity and permanent wilting point on a volume of water to volume of soil basis. However, as a soil's fine texture escalates, the water contents associated with field capacity and permanent wilting also increase. Furthermore, additions of organic matter increase any soil's water holding capacity beyond their normal thresholds (Anonymous, 2008b). There is a clear relationship to the amount of water that infiltrates and remains in the soil based on soil texture and organic matter, but once in the soil system, how does the plant uptake and utilize the water?

Water and Plant Relationships

All plants need water to conduct physiological functions necessary for survival. Some essential physiological functions that require water including creating hydrolysis and condensation reactions, maintaining cell turgor pressure and structure, regulating nutrient movement into cells through osmotic forces, transporting dissolved nutrients through the xylem and phloem, and producing food through photosynthesis (Sutcliffe, 1968). Plants must transport water from the soil through roots and distribute it throughout their tissues for the physiological functions listed above to occur. Accomplishing this requires a complex system of osmotic and tension (negative) pressure changes to fully move water from the soil throughout the entire plant (Steudle, 2001). Under normal conditions, plants will transpire through stomatal openings on the leaves, creating a negative pressure within the xylem and extending down to the roots. The xylem acts analogously to a straw by harboring this hydrostatic pressure gradient pulling water up the plant and allowing for passive transport through the root cells in a process known as mass flow (Steudle, 2000a; Steudle and Henzler, 1995; Steudle and Peterson, 1998; Tyree, 1997). The phenomenon is made possible because of water molecule properties.

Since the composition of water is hydrogen and oxygen atoms, their positive and negative charges generate attractive forces to each other (cohesion) and other surfaces (adhesion) (Perlman, 2018). Fiscus (1975) observed that as water uptake associated with transpiration increases, the xylem solute concentration becomes diluted. At high transpiration rates, the osmotic forces, or solute concentration differences, at the root level become insignificant. However, water flow movement from soils into the roots through osmotic gradient changes still play a crucial role, especially at low transpiration rates (Steudle, 2000a). Solutes, such as sodium (Na), potassium (K) and Calcium (Ca), are taken up by plant roots for osmotic water flow through two methods, apoplastically (in between cells) and transmembrane (across the cell membrane) movement (Ranathunge et al., 2017; Steudle, 2000b). When these solutes enter the root cells, there is typically a -0.5 to -2.4 MPa hydraulic gradient that creates water movement from the soil-water interface into the root (Kim et al., 2018; Lee et al., 2004). However, as the soil dries the matric potential will increase, causing the soil to approach a point known as 'residual water content.' At this point, the root cells cannot achieve a greater force than the soil's attraction to the remaining water molecules and reaches the permanent wilting point (Whalley et al., 2013). Once the water has entered the soil and translocated through the xylem by varying hydraulic pressures, it is essential to understand how soil water loss occurs.

Evapotranspiration: How Soil and Plants Lose Water

Soil water loss occurs through four main components: deep percolation, surface run-off, evaporation, and transpiration (Bice). Evaporation and transpiration rates are miniscule during a runoff event with their aggregate amounts occurring before or after

these runoff events. Evaporation is the change of water from a liquid or solid to a gaseous state by the transfer of heat energy. Transpiration, as discussed earlier, is the process of shifting pressures within the plant to pull water from the soil, up the plant, through plant pores (stomates) on the leaf surface and evaporated into the atmosphere. The combination of evaporation and transpiration is commonly known as evapotranspiration (ET) because the same environmental factors influence both components respectively (Bice). An adjusted definition for ET can then be thought of as the water lost from plant growth, consumptive use (water used to make plant tissues and sugars for energy), and evaporation from free surfaces (bare soil).

Evapotranspiration rates are regulated through combinations of several environmental factors including solar radiation, temperature, relative humidity, and wind speed (Anonymous, 2008a). Solar radiation drives heating and cooling on the earth's surface through atmospheric absorption and plays a primary role in ET of plants. Evapotranspiration rates influenced by solar radiation are not constant due to seasonality, sun position, and atmospheric turbidity. Higher air temperatures with sunny conditions accelerate water vaporization rates compared to a cool, cloudy day. Removal of vaporized water mainly occurs through air movement and relative humidity. When air is saturated (100% relative humidity), ET rates are meager because the relative humidity within leaves, 93 to 100%, matches that of the atmosphere, eliminating the vapor pressure gradient necessary for water to evaporate. Unsaturated air can store larger amounts of water thus creating accelerated rates of ET (Yarwood and Hazen, 1944). Lastly, as solar radiation heats the earth surface, it also generates energy for wind movement. In general, higher wind speeds expedite the removal of vaporized water. Even under low air

humidity conditions, if no air movement exists, the air above the evaporated surface, i.e., the boundary layer, gradually becomes saturated with moisture decreasing ET rates (Anonymous, 1990).

Botanical factors that influence ET include, genotype variations, canopy height, and ground cover percentage (Allen et al., 1998). The most influential factor at mitigating ET rates are genotype variations. Genotype properties that influence ET include increased leaf cuticle thickness, enhanced physiological responses to reduce leaf surface area, increased leaf hair density, and optimized stomatal opening (Chaves et al., 2002; DeLucia and Berlyn, 1984; Johnson et al., 1983; Jordan et al., 1984; Meinzer et al., 1995; Ristic and Jenks, 2002; Woolley, 1964). Stomatal closure governs photosynthetic efficiency, which impacts ET rates. Photorespiration increases as guard cells close stomata during drought conditions. (Katul et al., 2012). Photorespiration is the process of using oxygen (O_2), when the plant depletes total carbon dioxide (CO_2) concentrations, to produce carbon compounds used for carbohydrate production (Peterhansel et al., 2010). Even under moisture stress conditions, plants will satisfy their photosynthetic demands by opening their stomates for diffusal of atmospheric CO_2 . This physiological response is why ET can be considered a ‘penalty’ incurred by the plant (Cowan and Farquhar, 1977; Givnish and Vermeij, 1976; Hsiao, 1973). However, if opening stomates do provoke water loss but abbreviate ET and photorespiration rates by cooling the leaf surface, then this physiological function becomes beneficial (Igamberdiev et al., 2004; Katul et al., 2012). Here it is important to describe some primary pathways of photosynthesis and how some plants are more efficient than others at reducing photorespiration and minimizing water loss.

Most classifications of plants are either a C3 or C4 plant based on photosynthetic pathways. C3 plants use the Calvin Cycle (reactions that take place using light captured from chlorophyll as energy) within the chloroplast to fix CO₂ to ribulose 1,5 bisphosphate catalyzed by the rubisco enzyme. This reaction produces two-three carbon compounds called 3-phosphoglyceric acid that are used to make sugars for the plant (Lodish et al., 2000). For these reactions to occur, water must be present for adenosine triphosphate (ATP) hydrolysis and to provide hydrogen ions that reduce nicotinamide adenine dinucleotide phosphate (NADP⁺), both of which provide energy for the Calvin Cycle (Gowik and Westhoff, 2011). Plants limit water loss during heat stress by closing stomates and CO₂ concentrations within the plant become limited, resulting in O₂ to be used for sugar production. C3 plants expend energy to dispose of wasteful ammonia (NH₃) produced as a by-product before accumulating to harmful concentrations (Cornic and Briantais, 1991; Igamberdiev et al., 2004; Peterhansel et al., 2010).

The evolution of C4 plants developed specialized compartments to isolate chemical reactions that prevent photorespiration (Björkman, 1973; Ehleringer and Björkman, 1977; Hatch and Slack, 1966). The first step of CO₂ fixation by C4 plants is creating a four-carbon compound, malate, from oxaloacetic acid. Malate is produced within the mesophyll cells where O₂ is located and then diffused across the cell membrane into bundle sheath cells (Downton, 1970). Within the bundle sheath cells, malate goes through the Calvin Cycle to produce sugars. By isolating essential catalytic reactions, rubisco never encounters O₂, resulting in no photorespiration. Eliminating photorespiration alleviates the consequence associated with opening stomates for carbon dioxide that results in water loss during drought conditions (Waller and Lewis, 1979).

Understanding how plants and soils lose water improves how water loss is measured and prevented when intensively managing turfgrass areas.

Current Tools: How Water Loss is Measured and Prevented for Turfgrass

Turf managers have historically relied on their own previous experiences and scouting for drought symptoms to guide irrigation decisions, with the result being the overuse of water. Soil moisture measuring tools have yielded an opportunity to reduce water consumption through data-driven irrigation decisions. Researchers and turf practitioners, alike, are increasingly focusing efforts to improve these tools to use water more responsibly.

Several tools and methods are responsible within turf management for reducing water usage. Early methods for soil moisture assessment were physical core harvesting for laboratory calculations (gravimetric water) and probes that contained a water-air vacuum and a porous tip at the end inserted within the soil (i.e., tensiometers) measuring positive and negative tensions exerted by the soil (Johnson, 1962). These methods are time-consuming and not practical for large-scale adoption. Instead, a valuable tool for rapid soil moisture monitoring is the time-domain reflectometer (TDR). Time-domain reflectometers are portable moisture meters that emit high-frequency electromagnetic pulses through the soil and measure the travel time across metal probes (Kreuser, 2016; Topp et al., 1980). These portable meters provide an objective measurement of VWC for assisting turf managers to make water application decisions (Moeller, 2012). Mapping features incorporated with GPS technology on TDR's are being used to compare irrigation specifications such as nozzle size, the degree of rotation, and radial throw with soil moisture maps (Gatlin, 2011). However, assessing soil moisture using TDR meters is

similar to the aforementioned tools that were time-consuming, labor intensive and does not account for rapid VWC changes in the root-zones of golf courses when considering their implementation on large spatial scales. Some root-zones of golf courses can change drastically because of their high sand composition, and under the right conditions, turfgrass planted in these root-zones can lose 2.5 to 7.5 mm of water per day (Beard, 2002). Improvements came with the introduction of automated irrigation systems and the ability to schedule turfgrass watering needs based on historical weather data. Evapotranspiration-based irrigation allows turfgrass managers more significant control of water applications through established mathematical models that calculate reference crop ET (Davis and Dukes, 2010; Feldhake et al., 1983; Hubbard, 1992; Steiner et al., 1991). Research continues to evolve by enhancing models and devising new ones.

Employing best agronomic practices helps minimize how much water golf courses consume daily to maintain turfgrass health. While knowledge of agronomic practices is crucial, being proactive is just as vital. In 2008, the Golf Course Superintendents Association of America published data stating that nationally, only 15 percent of golf courses had done thorough irrigation audits (Throssell et al., 2009). Eighty percent of these golf courses had automated irrigation systems and irrigation components that are up to 16 years of age. Irrigation audits allow managers to detect defects before becoming too severe and make upgrades such as new sprinkler heads, nozzles, pumps, field controllers, main and lateral lines, etc. (Throssell et al., 2009). Other options beyond being proactive are available for managers. Some of the most popular options for limiting water consumption of golf courses involve the use of wetting agents, hand watering, keeping turf drier, utilizing irrigation scheduling techniques, establishing drought-tolerant

cultivars, and reducing overall managed acreage (James and Michael, 2008; Throssell et al., 2009).

An example of implementing some of these benefits exist in Nevada where water resources are already scarce. The Southern Nevada Water Authority in 2003 proposed that golf courses in the area be subjected to water budgets based on total managed acreage with penalties for not meeting these budget thresholds (Anonymous, 2012). Golf courses in the area converted more than 300 hectares of intensively-managed turfgrass to desert landscape areas which reduced water consumption from 1300 liters to 830 liters per day (Anonymous, 2012).

Some of the newest adaptations turf managers are applying are deficit irrigation programs and underground soil moisture sensors. Deficit irrigation is the principle of returning water amounts less than actual ET amounts without compromising turf quality (TQ). Fu (2004) proved that for three grass species, ‘Meyer’ zoysiagrass, ‘Midlawn’ bermudagrass, and ‘Falcon II’ tall fescue, 60 to 80 percent deficit irrigation resulted in the maintenance of acceptable TQ and only 100 percent for ‘Brilliant’ kentucky bluegrass. In California, where water costs regularly increase, Friendly Hills Country Club favored an invasive turf species, kikuyugrass, that requires less irrigation. In 2010, the golf course irrigated 65% of reference ET for the kikuyugrass while maintaining TQ. This decision reduced summer water use and consequently saved approximately 55 million liters (Anonymous, 2012). Evapotranspiration-based irrigation is generally considered the most appropriate method, but limitations still exist with microclimates that influence site-specific ET needs for optimal turfgrass growth (Snyder et al., 2015). Improvements for assessing site-specific water needs were introduced with the advent of

wireless soil moisture sensors (Bremer and Ham, 2003; Ritsema et al., 2009). Grabow et al. (2012) reported that using one or more underground soil-moisture sensors with smart irrigation systems reduced water consumption by 39% compared to timer and reference ET based treatments over three years. Researchers and golf course superintendents recognize the opportunity for water conservation improvements through continual research to revise current methods and unfold novel methods. The present struggle is the urgency for a technique that accurately assesses soil moisture stress rapidly and non-destructively, but the potential of light reflectance may alleviate this need.

Light Reflectance of Plants, Turfgrass, and Water Availability

Solar radiation provides the necessary energy for plants to synthesize compounds for energy. This provided energy is in the form of light, or electromagnetic energy, that plants absorb for photosynthetic use. This fundamental relationship of plants with light is of great importance with botanist, ecologist, dendrologist, and agronomist researchers (Gates et al., 1965). Plant foliage absorbs a percentage of light over a spectral range of 400 to 3,000 nanometers (nm) with the remaining light transmitted through the leaves or reflected towards the sun. There are three main categories within this entire region of light: visible, commonly known as the photosynthetic active radiation (PAR) spectrum (400–700nm), near-infrared (NIR) (700-1,100nm), and shortwave-infrared light (SWIR) (1,100-3,000nm). Absorption within the total light range is greatest with PAR, excluding a peak at green light or 550 nm. Furthermore, it increases to approximately 50 to 60 percent reflectance across the NIR region and gradually decreases within the SWIR region (Knipling, 1970). Plants strongly absorb light within the PAR due to various pigments, mainly chlorophyll, but also carotenoids, xanthophylls, and anthocyanins. The

PAR region, in order by highest energy state, includes: violet (400-420nm), blue (420-490nm), green (490-570nm), yellow (570-585nm), orange (585-620nm), and red (620-700nm) light. The reflectance peak around 550 nm within the green light region and strong absorption occurring in the blue and red light regions represent a spectral pattern of healthy plant growth (Rabideau et al., 1946). Furthermore, the robust absorption features at key regions from 1300nm and beyond are developed from water within the leaf. Studies that isolated the thickness of water within fully hydrated corn and cotton leaves show these absorption trends when compared to SWIR reflectance (Allen et al., 1969; Gausman et al., 1970). Light reflectance increases within the NIR light region, caused by the cellular arrangement within the leaf (Mestre, 1935). Photosynthetic active radiation and infrared light can penetrate through the cuticle and epidermis of leaves. Once the light penetrates through these layers, further scattering occurs to mesophyll cells where hydrated portions of the cells and air chambers reside. Since the plant uses PAR light for energy production and the longer SWIR wavelengths are absorbed by moisture, the remaining NIR light is scattered through moisture influences. Quantitatively, 40 to 60% of NIR light is scattered upward (reflected) and the remaining downward (transmitted) (Allen and Richardson, 1968; Knipling, 1970). Using these proven trends, researchers have developed mathematical formulas to quantitatively describe many relationships of plants to causal variables through light reflectance studies.

One of the earliest studies involving turfgrass and spectral research was using a spectroradiometer and examining the potential of objectively, rather than subjectively, measuring turfgrass quality (Birth and McVey, 1968). The authors found it possible to use NIR and red light reflectance in a simple mathematical ratio (R_{745}/R_{675}). Simple ratios

and linear combinations of reflective light percentages within these regions are how earlier vegetation indices (VI) were developed (Huete and Jackson, 1987). Vegetation indices are quantitative values generated from some mathematical formula that is indicative of key plant features such as health, biomass, or pigment concentrations (Asrar et al., 1984; Bannari et al., 1995; Campbell and Wynne, 2011; Sims and Gamon, 2002). Early vegetation indices, like the simple ratio used by Birth and McVey (1968), are susceptible to variables that produce background noise not directly related to plant vegetation.

Interference from variables such as soil can be minimized through normalized vegetation indices (Rouse Jr et al., 1974). The normalized difference vegetation index (NDVI) uses the contrasting high red light absorption and scattering of NIR light to normalize variability and is calculated as follows: $[NDVI = ((NIR-VR)/(NIR+VR))]$, where VR equals visible red light (Carlson and Ripley, 1997; Myneni et al., 1997). The NDVI is the most ubiquitous vegetation index for light reflectance assessment of turfgrass along with other agronomic applications (Carrow, 2010). Early research of NDVI involved establishing the relationship to leaf area index ($LAI = m^2 \text{ leaf area}/m^2 \text{ ground area}$) of native grassland dryness for fire assessment (Paltridge and Barber, 1988). However, limitations occur as NDVI becomes saturated when observations of $2 m^2/m^2$ or higher LAI values occur because as the total amount of vegetation and biomass can increase but the total amount of light reflectance remains constant. The NDVI has been used for agronomic assessment of parameters such as, but not limited to, N fertilization of sweet pepper (*Capsicum annuum*), leaf chlorophyll and pigments of sunflower (*Helianthus annuus*) and tobacco (*Nicotiana tabacum*), and yield of wheat (*Triticum*

aestivum) and corn (*Zea mays*) (Gamon et al., 1990; Serrano et al., 2000a; Shanahan et al., 2001; Thomas and Oerther, 1972).

Within turfgrass systems, established correlations of NDVI exist with N fertilization of creeping bentgrass (*Agrostis stolonifera*), plant biomass, structure, canopy density, canopy uniformity, color and other factors associated with TQ (Beard, 1972; Bell et al., 2002; Bremer et al., 2011a; Fitz-Rodríguez and Choi, 2002; Gallo et al., 1985; Jiang and Carrow, 2005; Lee and Bremer, 2008; Sellers, 1987; Stiegler et al., 2005; Trenholm et al., 1999). The NDVI has not only been correlated with TQ components but also with stressors such as brown patch in tall fescue (*Festuca arundinacea*) and bermudagrass (*Cynodon dactylon*) soil compaction (Green et al., 1998; Guertal and Shaw, 2004). Since TQ assessments are visual subjective ratings, variations of light reflectance and absorption between red and green light within the PAR region influence factors of TQ (Bremer et al., 2011b; Morris and Shearman, 1998). Red light reflectance becomes more prominent while the absorption of green light increases, causing TQ and associated components to subside (Baldwin et al., 2009). However, the NIR components can cause fluctuations of NDVI values that humans may not detect upon visual inspection. There can be several possibilities at the cellular level (cell wall, protoplast, cytoplasm, stomatal changes) within leaves that cause variations of light scattering (Brosnan et al., 2005; Fu and Huang, 2003; Gausman, 1973). Abiotic and biotic stresses such as low temperature, UV-B radiation, wounding, nutrient deficiencies, compaction, bacterial and fungal infections, and other pests can cause reductions of absorbed PAR light by plants, deviations from normal NIR light scattering, and accessory pigment accumulation (Chalker-Scott, 1999).

The two most abundant endogenous pigments, chlorophylls *a* and *b*, are directly linked to photosynthetic production (Curran et al., 1990). Chlorophyll *a* absorbs mostly light energy from the red region (600-700nm) and blue region (400-500nm); compared to chlorophyll *a*, *b* has greater absorption in the blue region and is limited in the red region. Both pigments allow plants to maximize their range of light absorption for energy use (Gitelson et al., 2003). When plants are stressed, foliage accumulates other pigments such as anthocyanins, carotenoids, and xanthophylls. These pigments help absorb other wavelengths of PAR light during stressful periods to avoid photoinhibition caused from free radicals associated with ultraviolet or other radiation (Demmig-Adams and Adams, 1996; Gitelson et al., 2001). Knowing how pigments interact with light radiation and the low leaf reflectance within the PAR region, researchers hypothesized a strong relationship would exist between light reflectance amounts and pigment concentrations (Horler et al., 1983; Stiegler et al., 2005; Zarco-Tejada et al., 2004). Maas and Dunlap (1989) found a significant relationship to reflectance at 550 nm and 670 nm when compared to carotenoid and chlorophyll concentrations of a normal corn plant, one grown in the dark, and an albino one induced with fluridone ($r > 0.91$). Several factors that were previously mentioned can cause a decrease or increase in pigment concentrations that influence NDVI values. While it is difficult to isolate components manipulating light absorption or reflectance amounts in the PAR region, it may be possible when moisture stress is the limiting factor.

Moisture content within the leaves of plants is the most prominent factor influencing NIR and SWIR regions. Chlorophyll concentrations correlate with NDVI but during drought conditions tend to degrade at the same time, or in some cases, after

significant stress occurs (DaCosta et al., 2004). A hormonal response in plants when moisture stress is present helps to explain the resulting simultaneous or delay in chlorophyll degradation. Previous studies show how warm- and cool-season turfgrasses can preserve their TQ during moisture stress conditions (Fu and Huang, 2001; Huang, 1999; Huang et al., 1997; Huang and Fu, 2000). Turfgrasses preserve quality through metabolic and physiological responses that occur under drought conditions causing stomatal closure and reduced leaf surface area (Kramer, 1988; Shinozaki and Yamaguchi-Shinozaki, 1997). A chemical hormone known as abscisic acid is created within the roots in response to mild moisture stress and then transported to shoots for chemical signaling of plant responses to preserve moisture levels at acceptable levels (Davies et al., 2002). Once severe moisture stress occurs, the normal functions of related pigments diminish to varying degrees (Hopkins, 1999). Since associations of NDVI, TQ and pigment concentrations are present, the capability of turfgrasses to preserve TQ make it difficult to distinguish severe moisture stress conditions before they occur. Water stress is one of the most prominent restrictions reducing TQ and correlating light reflectance with plant water stress is, and has been, challenging, but gaining tremendous interest (Howell et al., 1984; Olson Jr, 1977).

Before the solid establishment of light reflectance measurements, measuring plant water status through considerations of many factors such as relative water content (RWC = difference between fresh and dry weights), water potential, transpiration or photosynthetic rates, stomatal conductance, and canopy temperature were the most reliable means (DaCosta and Huang, 2006; Pearcy et al., 1989). These methods can be labor- and time-intensive for individuals to manually determine plant water needs.

Researchers have used light reflectance and water absorption features within the near- and far-infrared light regions with centers located at 970, 1,200, 1,400, and 1,940 nm, to devise water indices (WI) (Curran, 1989; Roberts et al., 1997) Water indices such as the shortwave infrared water stress index [SIWSI = (1230-1250 nm)], three-band ratio indices [$RATIO_{975} = 2((R_{960} - 990) / ((R_{920} - 940) + (R_{1090} - 1110))))$] and [$RATIO_{1200} = 2(R_{960} - 990) / ((R_{920} - 940) + (R_{1090} - 1110))$], equivalent water thickness (EWT = foliar water volume per leaf area m^3m^{-2}) and the normalized difference water index [$NDWI = (R_{860} - R_{1240}) / (R_{860} + R_{1240})$] are derived from these water absorption features (Gao, 1995; Gao et al., 1993; Serrano et al., 2000b; Sims and Gamon, 2003; Yilmaz et al., 2008). Water indices have been applied for agronomic applications such as water stress assessments for rice, forest canopies, and Sudan grass (Dennison et al., 2005; Hanna and Girmay-Gwahid, 1999; Panigrahi and Das, 2018). However, the optimal absorption features occur at 970 and 1200 nm. These bandwidths are optimal because light within the NIR region has higher energy potentials, lower absorption coefficients and ability to penetrate plant canopies, whereas SWIR wavelengths have less energy and higher absorption coefficients due to atmospheric interference (Bull, 1991; Claudio et al., 2006; Palmer and Williams, 1974).

One of the more studied water indices is the water band index [$WBI=(R_{970}/R_{900})$] (Penuelas et al., 1993). Researchers initially measured light reflectance of gerbera daisies (*Gerbera jamesonii*) to indicate their plant water status, but similar research of pepper and bean plants and wheat validated their results. Fewer reflectance changes occurred when RWC was above 90% than at or below 85% (Peñuelas et al., 1993; Penuelas et al., 1996). Furthermore, Sims and Gamon (2003) found that WBI correlated with thinner

tissue structures (> 5mm in thickness) ($r^2=0.59$) and direct EWT measurements ($r^2=0.66$). The WBI's capability to assess canopy water content of thinner tissues and its weak absorption coefficient makes it an optimal water index for assessment of turfgrass systems. Creeping bentgrass planted within a sand-based rootzone had the strongest correlation to soil VWC compared to six other VI's across two trials ($r \geq 0.80$) (McCall et al., 2017). The green to red ratio index [(GRI = R_{550}/R_{670})] was also significantly related to soil VWC ($r \geq 0.50$), but NDVI or other investigated indices were not (McCall et al., 2017). However, other research has found significant correlations between NDVI and soil VWC ($r = 0.22-0.30$; Jiang et al., 2009) and ($r = 0.28-0.64$; Johnsen et al., 2009).

The potential of using turfgrass canopies as a medium for observing soil moisture stress through narrowband light reflectance exists because of the known existing properties within the NIR region. NIR light being located beyond the PAR region make it an area of interest for early detection of moisture stress conditions. Most research making comparisons of soil VWC to remotely sensed data of turfgrass systems make this connection under deficit irrigation or when moisture stress is already present (Caturegli et al., 2015; Jiang and Carrow, 2007; Sönmez et al., 2008). Dettman-Kruse et al. (2008) used partial least square analysis of the overall PAR and NIR regions to predict moisture stress one day before observing visual symptoms for creeping bentgrass, and perennial ryegrass. Similar to McCall et al. (2017), Dettman-Krus et al. established a relationship between limited soil VWC and light reflectance with one species of grass in one soil type. No apparent research has investigated the feasibility of using the nondestructive, rapid potential of remotely sensed data, specifically in the NIR region, to estimate or predict soil moisture stress across different grass species integrated into various soil types.

Soil Moisture Assessment through Unmanned Aerial Vehicles

Unmanned aerial vehicles (UAV) are gradually becoming a viable tool to enhance how turfgrass is managed (Valente et al., 2011). Their popularity comes from the lower cost of use, flexible flight planning, ability to operate in various weather patterns, and quicker access to big data collected when compared to high-resolution satellite imagery (Xiang and Tian, 2011). Small unmanned aerial vehicles create more opportunities for precision agriculture, or precision turfgrass management (PTM), practices to be employed by all levels of turfgrass managers (Bremer, 2016). Precision turfgrass management is the practice of using an integration of global guiding technologies, remote sensing to identify field variability, and geographic information software for spatial data processing to determine and apply the minimum inputs such as pesticides, water, or nutrients, in specific areas. Another component of PTM is the prevention of limiting variables from occurring by monitoring things such as pests, nutrient amounts, irrigation defects, and underlying soil problems (Carrow et al., 2010). Available literature shows handheld remote sensors distinguishing plant aspects by using them as an objective measurement and comparing them to ground-based observations or manually extracted data (Caturegli et al., 2016). Small unmanned aerial vehicles equipped with sensors that have a similar proficiency as handheld sensors can provide the needed platform to rapidly estimate biophysical and vegetation stressors such as soil moisture stress (Watts et al., 2012). Research using UAV's for moisture stress assessment is in the early stages, but Hong et al. (2018) research is an example of using UAV to remotely sense within the near-infrared range of turfgrass under deficit irrigation and comparing to soil VWC. Hong's research is a small piece of an extensive puzzle displaying UAV's potential of

rapidly assessing soil moisture on large scale areas. However, research using this same technology studying contrasting turfgrass species integrated into different soil types is at the theoretical stage. Adopting UAV for this purpose could be integrated with irrigation systems to drastically increase water savings and be redirected for more pertinent uses. Nonetheless, UAV's full potential cannot be unlocked, until a solid foundation is established at the smallest scale to avoid data misinterpretation and, in turn, wasting vast amounts of water.

Research Objectives

The objectives of this research were to 1) determine whether vegetation indices (NDVI, GRI, and WBI) can objectively estimate moisture stress by soil texture (sand, sand loam, clay), grass species (creeping bentgrass and bermudagrass), or their interaction and 2) investigate vegetation indices as early predictors of moisture stress compared to visual symptom development of bent and bermudagrass in varying soil textures

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Chapter 2. Improving Soil Moisture Assessment of Turfgrass Systems Utilizing Field Radiometry

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Abstract: The need for water conservation continues to increase as global freshwater resources dwindle. Golf course superintendents are adapting to these concerns by implementing new tools to reduce water consumption. For example, scheduling irrigation using time-domain reflectometer (TDR) soil moisture sensors can decrease water usage, but unsampled locations become a risk. Soil moisture stress has successfully been estimated using the normalized difference vegetation index (NDVI) derived data through remote sensing. However, numerous stressors other than moisture constraints impact NDVI values. An alternative index, the water band index (WBI), uses near-infrared light reflectance to estimate moisture limitations within the plant canopy. Previous research has established relationships between soil moisture stress of creeping bentgrass (CBG) grown on sand-based root zones. '007' CBG and 'Latitude 36' hybrid bermudagrass (HBG) grown on three soil textures (USGA 90:10 sand (S), sand loam (SL) and clay (C)) were arranged in a 2 x 3 factorial design randomized across six dry-down cycles. Reflectance and volumetric water content (VWC) data were collected between 0700 and 1900 hr using a hyper-spectral radiometer and imbedded soil moisture sensors, respectively, until complete turf necrosis. The WBI had the highest relationship to VWC ($r = 0.62$) compared to the green-to-red ratio index (GRI) or NDVI. WBI and GRI were statistically the same and identified significant moisture stress approximately 28 hr earlier than NDVI ($P = 0.0010$). Both WBI and GRI predicted moisture stress 12 to 9 hr, respectively, before fifty percent visual estimation of wilt, whereas, NDVI provided 2 hr of prediction time ($P = 0.0317$). Nonlinear regression analysis showed that WBI and GRI can be useful for predicting moisture stress of CBG and HBG grown on three diverse soils in a highly controlled environment.

Keywords: CBG, creeping bentgrass; 4PL, four-parameter logistic non-linear regression; ET, evapotranspiration; GRI, green-to-red ratio index; HBG, hybrid bermudagrass; HSR, hyper-spectral radiometer; IP, inflection point; NDVI, normalized difference vegetation index; UAV unmanned aerial vehicles; TDR, time-domain reflectometer; TQ, turf quality; VI, vegetation index; VWC, volumetric water content; WBI, water band index

1. Introduction

Water conservation is an increasing concern for the agricultural sector. As the global population increases, commitments to increase crop production will be required to meet an exponentially increasing demand for food [1]. Reducing golf course water consumption provides potential to redirect these savings to accomplish this goal. Currently, golf courses within the United States occupy 608,746 hectares of maintained turfgrass, of which 484,978 hectares are irrigated [2]. The Golf Course Superintendents Association of America reported in 2005 that golf courses used 2.934×10^{12} L of water, whereas in 2013 they reported a 21.8% reduction [3]. These water savings can be attributed to golf course superintendents adapting new management methods such as wetting agents, hand-watering, enhanced-efficiency irrigation systems, keeping turf soils drier, and more frequent use of irrigation audits and upgrades [4,5]. The most significant water conservation advancements within the golf industry are the use of evapotranspiration (ET) based irrigation and handheld moisture meters. Scheduling irrigation based on ET allows turfgrass managers to deliver water applications over large acreage from previously recorded weather data [6,7]. Furthermore, the evolution of moisture meters, such as time-domain reflectometers (TDR), offers turfgrass managers the capability to objectively quantify when water availability is limited. However, microclimates on golf courses cause variations in ET replacement needs for optimal turfgrass growth [8]. These technologies become inadequate when considering large acreage areas because they are too labor intensive, time-consuming or associated with possibilities of misidentifying areas of localized moisture stress. The development of underground soil moisture sensors integrates the benefits of handheld TDRs and automated irrigation. In fact, in one study water usage was reduced by 39% compared to using ET-based irrigation alone [9]. The progression of water conservation on golf courses has been ascertainable, but the difficulty is making soil moisture assessments over large acreage rapidly, non-destructively, and accurately.

The interest of using light reflectance over turfgrass canopies for soil moisture assessment is escalating. Most light reflectance research involves transforming reflectance data into quantitative values known as vegetation indices (VI). The most widely used VI for plant performance or stress assessment of crops and maintained turfgrass is the normalized difference vegetation index ($NDVI = (R_{760} - R_{670}) / (R_{760} + R_{670})$) [10,11]. NDVI is strongly associated with turfgrass quality (TQ) and its associated components (shoot density, color, and canopy uniformity) [12-16]. Furthermore, visible (400-700nm) light absorption is driven by overall plant pigment concentrations ($r = 0.97$) [17-20]. Research shows that restricting the total available visible light for proper turfgrass photosynthesis to occur manipulates underlying TQ parameters and, thus, reduces overall turf quality [15,21,22]. Therefore, abiotic or biotic stressors that cause plants to senesce (nutrient issues, diseases, insects, excess UV radiation, etc.) result in higher visible light reflectance [23-25]. Soil volumetric water content (VWC) is often used by researchers and turf practitioners as an objective measurement of moisture stress. Previous research has shown a significant relationship between VWC and NDVI ($r = 0.22 - 0.68$) [26,27]. The association NDVI has with visible and NIR light constitutes the variability seen when multiple stressors are present. Therefore, spectral reflectance can indicate the onset of plant stress only through controlled studies or field validation [10]. Furthermore, as chlorophyll concentrations degrade, often simultaneously with drought stress, it becomes difficult to discern moisture stress from other stressors with using VI's with relationships to visible light [28,29]. Thus, isolating moisture

stress detection utilizing narrowband reflectance beyond wavelengths associated with pigment concentration is conceivable.

Water within the plant influences NIR and short-wave infrared (SWIR = 1,100-3000 nm) differently based on total tissue water content. Once NIR light waves penetrate the tissue, they are scattered or transmitted by changes within the turgor pressure of spongy mesophyll cells [30]. Portions of the SWIR region are strongly absorbed by leaf water content once these lower energy wavelengths diffuse inside the leaf [31]. Both regions have key water absorption troughs with centers at 970, 1,200, 1,450, 1,950, and 2,250 nm [32]. These water absorption bands are used to estimate the relative water content (RWC) and tissue water content determined by dry mass [33,34]. Water absorption features occurring at 1,200 nm and beyond possess higher absorption coefficients and penetrate less into the tissue before being absorbed, whereas the 970 nm has the lowest absorption coefficient [35]. Penuelas et al. [36] found a negative correlation ($r \geq -0.79$) between the water band index ($WBI = R_{900}/R_{970}$) and RWC with gerbera (*Gerbera jasmonii*) plants and saw significant changes with the WBI when RWC was $\geq 85\%$. Sims and Gamon [32] demonstrated how both equivalent water thickness (kg m^{-2} ; $r^2 = 0.66$) and WBI ($r^2 = 0.59$) had the highest correlations with water content of thin plant tissue (< 5 mm). Furthermore, the authors reported a weaker relationship ($r^2 \leq 0.35$) when considering total canopy water content. WBI has also been used for water stress assessment for several plant systems such as rice, forest, chaparral and Sudangrass canopies [37-40]. Within turfgrass systems, McCall et al. [41] found that WBI had the strongest relationship to soil VWC ($r = 0.80 - 0.81$) compared to the green-to-red ratio index ($GRI = R_{550}/R_{670}$; $r = 0.50 - 0.73$) and NDVI ($r = \text{NS} - 0.43$, where NS = nonsignificant) for creeping bentgrass (CBG) grown in a sand medium. Another study identified the capability of predicting CBG water stress symptoms one day prior to the onset of drought symptoms using the spectral region where the WBI is located (750 – 1,100 nm) [42]. Neither of these studies investigated these relationships with multiple grass species in soils other than a sand based root-zone.

The potential accrues daily for integrating unmanned aerial vehicles (UAV) with sensors capable of gathering spectral data beyond the visible light range. Current research has found that UAV's can be used to discern differences between ET-based irrigation treatments using near-infrared spectral reflectance [43]. As these sensors become cheaper the demand or collecting water stress detection data non-destructively and rapidly will inflate. More than one grass species is typically grown on a golf course with often highly variable underlying soil characteristics. A controlled experiment is required for correct UAV data interpretation to avoid wasteful water applications. Therefore, the objectives of this research were to (i) determine whether previously investigated vegetation indices can objectively estimate moisture stress of two common grass species (bent and bermudagrass) grown in different soil textures under controlled environments, and (ii) investigate vegetation indices as early predictors of moisture stress compared to visual symptom development of bent and bermudagrass in varying soil textures.

2. Materials and Methods

2.1 Study Area and Organization

A dry-down study was conducted under greenhouse conditions at the Glade Road Research Facility in Blacksburg, VA from June to September 2018. Treatments of two grass species, '007' CBG and 'Latitude-36' hybrid bermudagrass (HBG), planted into three soil types, USGA 90 USGA sand:10 peat moss (Clay: 1.4%, Silt: 0.8%, Sand: 97.8%), sand loam (Clay: 18%, Silt: 46.7%, Sand: 35.1%) and clay (Clay: 41.4%, Silt: 19.8%, Sand: 38.9%) were arranged in a 2 x 3

factorial design randomized within six replications. Creeping bentgrass plugs were harvested in March and June 2018 using a R&R (Tuscan, AZ) 10.2 cm cup cutter from a putting green built to USGA specification. Roots were washed and pruned to 0.64 cm to remove pre-incorporated soil and planted into the three soil types. Bermudagrass plugs were established from sprigs starting in February 2018 into metal autoclave trays filled with 0.014 cm³ of USGA 90:10 sand, top-dressed with sand and grown in for two months. In April 2018, HBG plugs were extracted from established trays using the same methods described for the CBG transfer. Bermudagrass plugs were grown under lights supplying an average of 600 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ during a 14 hr photoperiod [44]. All HBG experimental units were supplemented with growth lights because of their high light requirements compared to CBG, in which case natural sunlight was sufficient to meet lighting requirements [45,46] All CBG and HBG treatments were transplanted into Kord 155mm STD horticultural pots (Toronto, Canada) with dimensions 15.3 cm x 14.6 cm and an 1,835 cm³ capacity; a minimum of two months of root growth was ensured before using experimental units within dry-down cycles. To ensure both CBG and HBG treatments with the same soil were treated similarly, soils were sieved with a #4 U.S standard sieve (4.75 mm openings), oven-dried at 100 °C for 48 hours and filled to a specified mark on three pots to determine average bulk densities for each soil type (USGA 90:10 sand – 1.03 g/cm³, Loam – 0.97 g/cm³, and Clay – 0.80 g/cm³).

During the grow-in period, plugs were treated with SQM's 28-8-18 Bulldog fertilizer (Atlanta, GA) at 2.5 kg N ha⁻¹ for CBG and 12 kg N ha⁻¹ for HBG on a weekly basis. To prevent disease, fluxopyroxad + pyraclostrobin (Lexicon, BASF) was applied at 0.24 and 0.49 kg ai ha⁻¹ on a 28-day interval, and fluazinam (Secure, Syngenta) at 0.78 kg ai ha⁻¹ on a 14-day interval. All inputs were applied using a CO₂ pressurized spraying system calibrated to deliver 813 L ha⁻¹ through TeeJet TTI11004 flat spray nozzles (Glendale Heights, IL). Treatments were maintained at a height of 1.27 cm with Black & Decker garden shears (Towson, MD) and trimmed twice a week. Prior to initiating a dry-down replication, plugs were irrigated with two ten-minute applications of overhead irrigation spaced two hours apart. Plugs were placed in the middle of a 0.91 x 3.35 m table with a custom made irrigation system that was one foot above the plugs and equipped with nozzles 0.84 m apart and an output of 0.76 liters per minute at 137.9 kPa. Treatments were allowed 12 hours of gravitational drainage before inserting Meter Environment (Pullman, WA) 5TM soil moisture sensors with a 1639 cm³ volume of influence . The turf canopy was carefully separated through pilot holes before inserting soil sensors to the top of the black molding with orientation considered because the zone of influence is emitted in one direction from the two dielectric permittivity prongs labeled accordingly on the sensor. All treatments were blocked within replication because of sensor quantity limitations, and each dry-down cycle consisted of one replication. All treatments within each replication were randomized around a Lasko wind tunnel fan (West Chester, PA), ensuring that no one treatment was in the same location more than twice. The fan was used to mimic low humidity and high ET associated with rapid dry-down under real-world conditions and blew an average of 9.7 to 11.3 kmh across treatment location. Plots ran perpendicular to the fan with locations 1 - 3 0.75 m from the fan at and locations 4 - 6 directly behind those at a distance of 0.90 m. The fan ran continuously from 0700 and 1900 hr in conjunction with all data collection until all pots reached complete necrosis. The data collection interval was determined by the time of approximate sunrise to the average time our greenhouse fan turned off, which was set not to run when below 27°C. While the risk of nighttime stomatal conductance is possible, especially with contrasting warm- and cool-season turfgrasses, the lack of an automated system inhibited the ability for nighttime data collection [47]. To help exclude possible confounding variables, several supplemental studies were executed. Such studies included: soil moisture release curves, light reflectance of dry and

hydrated soils independent of turfgrass, soil VWC sensor calibrations with specific soils, and root length and biomass.

2.2 Data Collection and Analysis

2.2.1 Soil Moisture Release Curves

This study was conducted in the Soil Science Laboratories at Smyth Hall in Blacksburg, VA in January 2017. USGA 90:10 sand, sand loam, and clay soils were sieved, oven-dried, and loosely packed into soil sleeves with dimensions 2.5 cm x 1.2 cm. Sleeves loosely packed with soils were placed onto porous plates within trays filled with water for 24 hours ensuring each soil was saturated from upward water movement and air did not become trapped. Three replications of each soil were placed into soil moisture pressure plate extractors (Soilmoisture, Inc., Santa Barbara, CA) at 33, 100, 300, 500, and 1,500 kPa. Each pressure chamber had a drainage tube; when water ceased exiting this tube, soil sleeves were considered to have reached equilibrium with the applied chamber pressure. Once at equilibrium, soil sleeves were removed, weighed with a Mettler Toledo analytical scale (Columbus, OH), oven dried at 105°C for 48 hours, then reweighed. Mass differences were used to calculate gravimetric water content [$\theta_g = (M_{wet} - M_{dry})/M_{dry}$] where M_{wet} = the mass of wet soil and M_{dry} = mass of oven dry soil. Gravimetric values were converted to VWC [$\theta_v = \theta_g * \beta$], where θ_v = volumetric water content and β = the mass of dry solids divided by total sleeve volume and the three replications of each soil were averaged together. Water retention parameters for the van Genuchten model [48] were fit by minimizing the residuals between modeled and average measured volumetric water content for each pressure head.

2.2.2 Specific Soil Sensor Calibration

VWC from the 5TM soil sensors were calibrated for each soil based on manufacturer recommendations [49]. Four liters of each soil was sieved and oven-dried at 105 °C for 48 hours in preparation for sensor calibration. The soil was added to a mixing container, and for each data point 100 to 200 mL of water was added. Once mixed, the soil was added to the same Kord horticultural pot used in the dry-down study and packed to the same specific soil bulk density of the plugs when were transplanted. Raw soil sensor data was collected, and 103 cm³ of soil was excavated into soil sleeves, weighed, oven dried for 48 hours at 105 °C, and reweighed to determine gravimetric water content. The bulk densities of soil sleeves were used to convert gravimetric water content to volumetric water content through the same calculations used for soil retention curves. Soil sensor raw data was plotted against calculated volumetric water content, and the regression analysis that best fit the data was used to adjust the output data from the 5TM sensors. The USGA 90:10 sand was best explained with linear regression ($r^2 = 0.99$), while the heavier textured sand loam and clay soils had the highest relationships with logarithmic regression analysis ($r^2 \geq 0.98$). These equations allowed for data corrections to establish continuously adjusted VWC.

2.2.3 Root Length and Biomass

Roots from supplemental plugs of each treatment listed in Section 2.1 were washed, measured, and harvested through four replications. Roots were washed of soil by placing plugs onto a #4 U.S. standard soil sieve and water slowly running down the side to minimize root destruction. Root length (cm) was measured using a ring stand to allow untangled roots to

suspend through the ring. Root length was measured from the bottom of the ring to the longest three roots. After measuring length, roots were harvested by using the ring as a guide to trim all suspended roots. Harvested roots were used for root biomass calculations to ensure no root differences between soil types for each grass species were present when dry-down cycles were initiated. Harvested roots were weighted and then dried at 70 °C for three days for dry root weight plus any soil still attached to the roots. A 240 V Thermo Scientific Muffle Furnace (Waltham, MA) was used to ash root samples at 500 °C for 12 hours. Root weight differences were calculated by first taking the difference of dried roots and ashed roots to achieve soil weight and then subtracted from dried root weight.

2.2.4 Collection

Spectral reflectance data were always collected first using the Spectral Evolution HSR equipped with a contact probe measuring a spot size of 2.5 cm to take direct measurements from the canopy or soil surface. The HSR measures narrowband reflectance from 512 different wavelengths within a range of 320 to 1100 nm at a 1.4 nm sampling bandwidth. Any time light reflectance data was collected, a BaSO₄ panel was used for white reference calibration by placing the contact probe flush with the pad immediately before data collection. For the dry-down study, calibration readings were taken every hour to 1) ensure no irregularities came about with background noise and 2) help separate readings when sorting data during the extraction process. Other data collected after light readings were as follows: visual turf quality (TQ), visual estimation of wilt percent (WP), and air temperature. Turf quality was assessed using a 1 to 9 scale (6 = minimum acceptable quality and 9 = highest acceptable quality) according to guidelines established by the National Turfgrass Evaluation Program [50]. Wilt ratings were based on previously established literature and guidelines that were modified from a 1 to 9 scale (1 = 100 percent wilt and 9 = no detectable wilt stress) to 0 to 100% wilt coverage of treatment area. Guidelines for rating WP are described as when leaf firing, dormancy, or no plant recovery occurs [50,51]. Lastly, soil VWC was collected continuously using a Campbell Scientific CR300 datalogger (Linden, NJ) equipped with 5TM soil sensors. Campbell Scientific PC200W data logger software was used to extract raw data to be transformed to calibrated VWC from established equations mentioned in section 2.4.

2.2.5 Analysis

Light reflectance data was extracted using Spectral Evolution DARWin SP v.1.2.5093 software and converted to an excel file format using SED to CSV v.1.2.0.0 conversion software. Raw reflectance values were transformed into three vegetation indices (VI) used within in this study. The normalized difference vegetation index [$NDVI = (R_{760} - R_{670}) / (R_{760} + R_{670})$], green-to-red ratio index [$GRI = (R_{550} / R_{670})$], and water band index [$WBI = (R_{900} / R_{970})$] were all calculated from previously established equations [10,36,52]. Converted data were organized by replication, date, and time that each data collection was taken. All data, including supplemental studies, were analyzed using JMP Pro 13 (Cary, NC). Vegetation index data was modeled using a non-linear regression four parameter logistic (4PL) each treatment by replication (Figure 1) where y = index type, a = growth rate, b = the inflection point, c = lower asymptote, d = upper asymptote, and HAI = hours after initiation of the dry-down cycle.

$$y = c + \frac{(d - c)}{\left(1 + \text{Exp}((-a)(HAI - b))\right)} \quad (1)$$

This equation was chosen based on the goodness of fit model comparison and biological significance. Each indices' inflection point (IP) was determined from the 4PL equation stated above and used to analyze differences in index types, grass species, soil type, and their interactions of detecting significant soil moisture stress. An analysis of variance was used to explain source and source interaction significance for (IP) and wilt prediction to 50% wilt (WP⁵⁰). Means were separated using the students' t-test ($\alpha = 0.05$) where appropriate. Furthermore, each indices' values that occurred before three or more readings consistently occurred below the upper asymptote was considered the point a plant physiological response occurred due to moisture stress. To reduce variability, data distribution were analyzed, and one standard deviation was subtracted from the average to provide a conservative value at the time this plant physiological response occurred. The time at which 50% wilt occurred and the time where the physiological response occurred were determined using a custom inverse prediction model within the JMP software based on our 4PL regression models. The time to 50% wilt was subtracted from the time this moisture stress response was evaluated to provide the hours of predicted wilt for each index; their efficiency by grass species, soil type, and their interactions were examined.

2 Results and Discussion

Table 1. Pearson correlation coefficients (r) of vegetation indices to turf quality (TQ), wilt percent (WP), soil volumetric water content (VWC) and said vegetation indices derived from light reflectance data. Light reflectance data and other parameters collected from '007' creeping bentgrass and 'L-36' hybrid bermudagrass integrated into three soil types and subjected to drought stress under greenhouse conditions in Blacksburg, VA.

Index	Turf Quality [‡]	WP [‡]	Volumetric Water Content ^ψ	WBI [°]	NDVI ^α	GRI ^γ
WBI	0.66 [‡]	-0.92	0.62	-	0.83	0.89
NDVI	0.60	-0.80	0.47	0.83	-	0.92
GRI	0.65	-0.89	0.56	0.89	0.92	-

[‡] All (r) values significant ($P \leq 0.0001$)

[‡] Based on a 1 to 9 scale, where 1 = dead turf, 6 = minimally acceptable quality, and 9 = dense turf

[‡] Based on a percentage scale where 0% = no visible wilt and 100% = leaf firing of the experimental unit

^ψ Soil water content calculated from adjusted raw data values using a 5TM soil moisture sensor

[°] the water band index (WBI), calculated as follows: R_{900}/R_{970}

^α the normalized difference vegetation index (NDVI), calculated as follows: $(R_{760}-R_{670})/(R_{760}+R_{670})$

^γ the green-to-red ratio (GRI), calculated as follows: R_{550}/R_{670}

3.1 Pearson Correlations

Pearson correlation coefficients were analyzed to compare the relationships between WBI, NDVI, GRI, TQ, WP, and VWC (Table 1). Correlation values are based on an average of 108 collection events per dry-down replication across six treatments, providing approximately 3,900 data points for each parameter. All reported coefficients ranged from -0.92 to 0.92, with all relationships being highly significant ($P \leq 0.0001$). All indices were negatively correlated to WP, with WBI having the strongest relationship ($r = -0.92$). The WBI also had the strongest relationship to VWC ($r = 0.62$) compared to NDVI ($r = 0.47$) and GRI ($r = 0.56$), which follows the same trend observed by McCall et al. [41]. Furthermore, VWC had a negative relationship with WP ($r = -0.66$), validating that wilt observed was in response to soil moisture stress. The relationship between WBI and WP were plotted over time to show the inverse relationship (Figure 1). Values of the WBI were consistently high while CBG was not expressing drought symptoms. However, WBI values began to decline rapidly in conjunction with CBG visual wilt. As the entire CBG canopy became necrotic from drought stress, WBI values again held consistently low. The strong relationship between WBI and WP, along with the strong inverse relationship over time, provide sound evidence that WBI is a suitable index for estimating soil moisture stress.

All three indices had a moderate relationship to TQ ($r = 0.60 - 0.66$) with WBI having the strongest correlation. This is congruent with previous studies that documented these indices are useful to estimate TQ under the conditions tested [14,16,41]. However, because of the inverse relationship observed between TQ and WP ($r = -0.68$; data not shown), TQ will not be discussed throughout this manuscript. All indices tested compared favorably to one another. The NDVI and GRI had the strongest affiliation ($r = 0.92$), whereas NDVI and WBI had the lowest correlation ($r = 0.83$). The WBI compared favorably to GRI with a correlation of $r = 0.89$. The relationship of these indices to one another suggests that GRI may be more impacted by other stressors than limited moisture conditions alone. However, GRI still has a significant relationship with WBI and is therefore potentially useful for moisture stress identification.

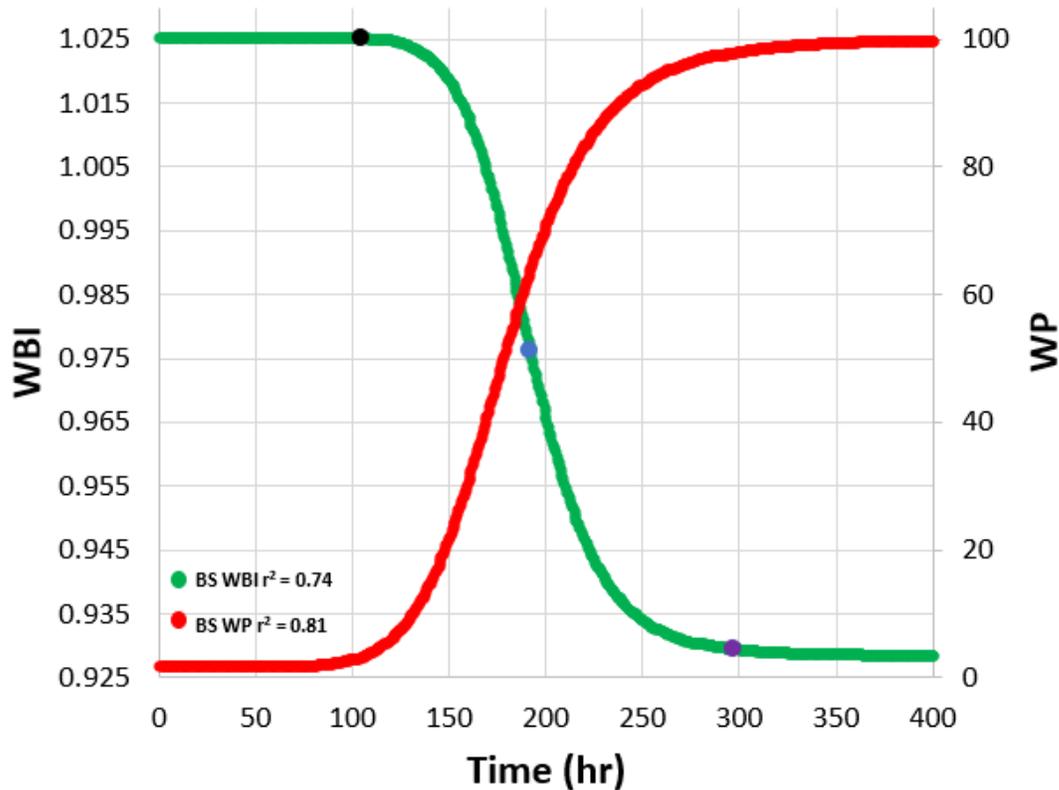


Figure 1. Four-parameter nonlinear logistic regression analysis of spectral reflectance gathered from creeping bentgrass (B) established in USGA 90:10 sand (S) subjected to greenhouse conditions in Blacksburg, VA. The water band index ($WBI = R_{900}/R_{970}$) was pooled over six dry-down cycles to observe the response to soil moisture stress over time (hr) from initiation of each cycle. The wilt percent (WP) is plotted against the WBI relationship to show their inverse relationship over time from dry-down cycle initiation. The inflection point indicated by the blue dot is a critical point of significant moisture stress and is the transition from the upper, no stress, or lower, permanent wilting, asymptotes indicated by the black and purple dots, respectively.

3.2 Four-Parameter Logistic Model

Because WBI had the highest relationship with WP and VWC, this variable will be used to explain how key values were derived from our four-parameter logistic (4PL) regression analysis. Figure 1 shows how the 4PL regression analysis is used can be used to explain the relationship of WBI to soil moisture stress of CBG planted into USGA 90:10 sand across six dry-down replications ($r^2 = 0.74$). Parameter estimates of upper and lower asymptotes along with calculated IP were derived from the 4PL equation. The inflection point (IP) represented by the blue dot is the halfway point between no moisture stress, the upper asymptote represented by the black dot, and complete necrosis of turfgrass, the lower asymptote represented by the purple dot. The WBI intersects with WP in close proximity to fifty percent 50% wilt, which we deemed as significant wilting. The comprehension of the WBI compared to visual 50 percent wilt and their intersection point occurring in close proximity when considering the hours when WBI's IP occurs is essential for understanding the results discussed throughout this manuscript.

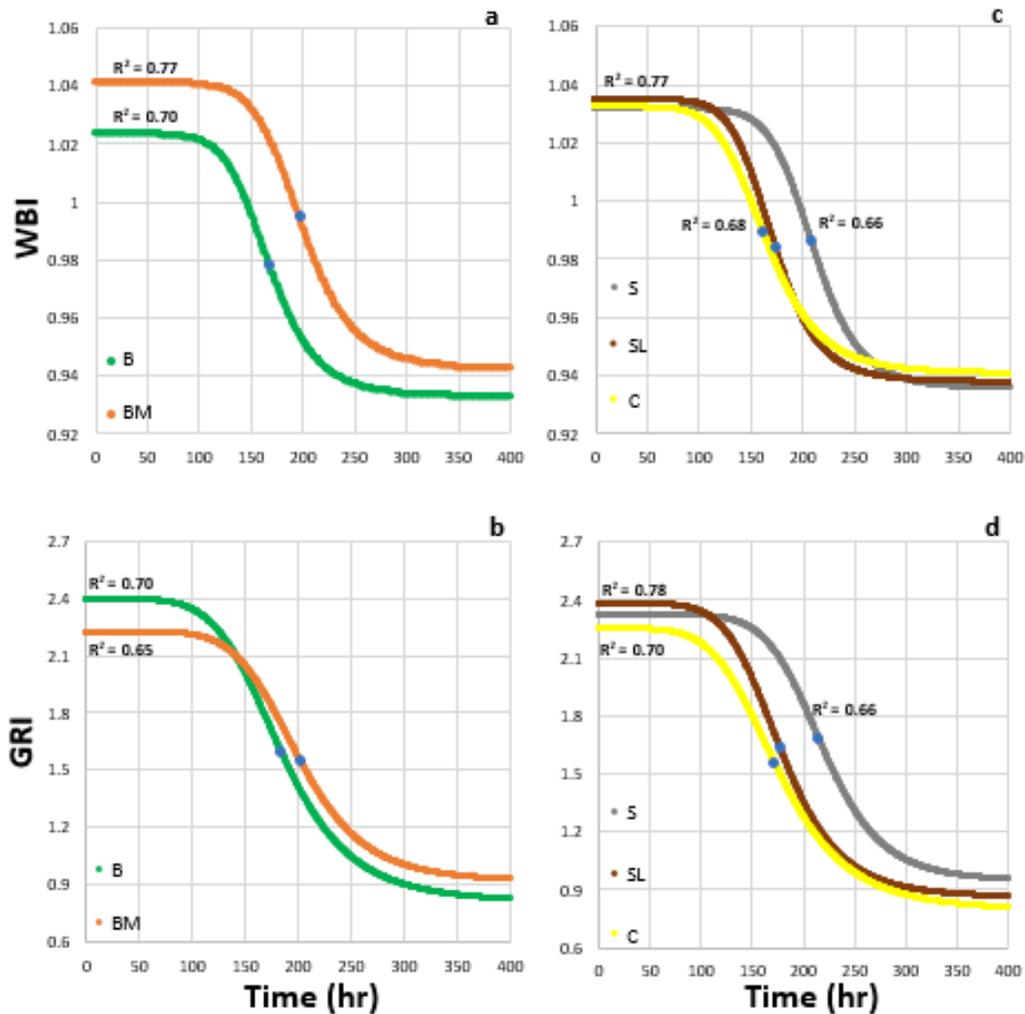


Figure 2. Four-parameter nonlinear logistic regression analysis of spectral reflectance gathered from two grass species (bentgrass (B) and bermudagrass (BM)) established into three soil types (USGA 90:10 sand (S), sand loam (SL), and clay (C)) subjected to greenhouse conditions in Blacksburg, VA. The water band index ($WBI = R_{900}/R_{970}$) and green-to-red ratio ($GRI = R_{550}/R_{670}$) were pooled over six dry-down cycles and separated by grass species, a) and b), and soil type, c) and d), to observe each factors response to soil moisture stress over time (hr) from when dry-down cycles were initiated. The inflection point as indicated by the blue dot is a critical point marked as the transition from the upper to the lower WBI or GRI values.

Grass species and soil type were separated by WBI and GRI for 4PL regression analyses based on analysis of variance effects test of IP (Table 2, Figure 2 a-d). Regarding grass species, the lowest relationship was observed with bermudagrass using GRI ($r^2 = 0.65$), whereas, the highest occurred with bermudagrass utilizing WBI ($r^2 = 0.77$). Interestingly, the same relationship was observed with bentgrass between both WBI and GRI ($r^2 = 0.70$). This is presumed to be caused by the physiological differences both species of turfgrasses display when coping with drought conditions. Bermudagrass uses water more efficiently during drought conditions resulting in delaying of wilt conditions by closing their stomates and eliminating the possibility of photorespiration, while CBG rapidly progresses to wilting when soil water content becomes the limiting factor [53]. We hypothesize that the observed differences between GRI and WBI occurred

because of bermudagrass' ability to delay drought symptoms and preserve TQ longer. Furthermore, WBI has the highest relationship because it is influenced by cellular arrangements within the tissue determined by internal moisture content for both bent and bermudagrass [36,54-55]. However, CBG's same relationship between GRI and WBI seems to occur because pigment concentrations may degrade at the same time, causing both to be influenced simultaneously [56]. Furthermore, when using these same indices to compare soil types, the lowest and same relationship is observed with the USGA 90:10 sand ($r^2 = 0.66$), while the sand loam and clay treatments had the highest coefficients of determinations with GRI ($r^2 = 0.68$ for sand loam and $r^2 = 0.78$ for clay).

3.3 Analysis of Inflection Points

Table 2. Analysis of variance of the inflection points and hours to reach 50% wilt (WP⁵⁰) estimation for three vegetation indices based on light reflectance data collected from '007' creeping bentgrass and 'L-36' hybrid bermudagrass integrated into three soil types and subjected to greenhouse conditions in Blacksburg, VA across six dry-down cycles.

Source	IP [±]	WP ⁵⁰ ^ψ
Index Type (IT)	<0.0001	0.0093
Grass Species (GS)	<0.0001	<0.0001
IT x GS	0.3383	0.3781
Soil Type (ST)	<0.0001	<0.0001
IT x ST	0.9525	0.7611
GS x ST	0.2520	0.0191
IT x GS x ST	0.9999	0.4652

[±] Inflection points (IP) derived from four-parameter logistic regression analysis of the water band (R_{900}/R_{970}), normalized difference vegetation ($(R_{760}-R_{670})/R_{760}+R_{670}$), and the green-to-red ratio (R_{550}/R_{670}) indices.

^ψ Calculated from the difference when 50% wilt was observed to the upper data cloud before wilt occurrence. The hours where a physiological change occurred was determined by subtracting one standard deviation from the mean of the upper data cloud of the water band (R_{900}/R_{970}), normalized difference vegetation ($(R_{760}-R_{670})/R_{760}+R_{670}$), and the green-to-red ratio (R_{550}/R_{670}) indices using a four parameter logistic regression analysis.

Analysis of variance for IP revealed highly significant differences for index type, grass species, and soil type ($P < 0.0001$) (Table 2). While all these factors had strong significance alone, no interaction in any combination had significance ($P \geq 0.25$). Therefore, index data were pooled over grass species and soil type, grass species were pooled over index type, and soil type was pooled over index type and grass species.

Table 3. Mean hours of inflection points for three vegetation indices based on light reflectance data collected from creeping bentgrass and hybrid bermudagrass integrated into three soil types and subjected to greenhouse conditions in Blacksburg, VA across six dry-down cycles.

Index Type	IP (hours) [±]
WBI	181.35 a
GRI	183.08 a
NDVI	210.99 b

Means followed by the same letter in a column are not significantly different

Means were separated using the students' t-test ($\alpha = 0.05$)

[±]IP = Inflection point derived from four-parameter logistic regression analysis of the water band (WBI = R_{900}/R_{970}), the green-to-red ratio (GRI = R_{550}/R_{670}) and the normalized difference vegetation ((NDVI = $R_{760} - R_{670}) / (R_{760} + R_{670})$) indices and pooled by grass species and soil type.

Because of the strong relationship between indices and wilt stress, the IP of each index were used to determine the time from dry-down initiation to when significant wilt stress was detected. To corroborate the validity of using the IP as the critical point for detecting significant moisture stress, the IP occurred 9 to 23 hours after the point where visual 50% occurred based on 4PL for all treatments (data not shown). The visual representation of the IP location can be seen in Figures 1 and 2 (a-d).

The WBI and GRI performed similarly with regards to hours to IP, whereas the IP associated with moisture stress for NDVI was approximately 27 hours later than the other two indices (Table 3). This result is congruent with variable soil moisture stress detection observed with the use of NDVI from the literature [26,41] and NIR light reflectance associated with WBI's ability to detect leaf water content independent of degrading pigment concentrations [10,32,36,57,58]. The strong GRI relationship to both WBI and visible wilt is an important consideration for future research as the ability to detect moisture stress using visible light is more practical and cost-effective than using narrowband NIR light reflectance. However, typical reflectance measurements of plant canopies within the visible range are limited because of high light absorption within the photosynthetically active region. Subtle changes in ambient light conditions during remote data collection can significantly alter GRI and other visible-light indices. The high overall absorption within the visible light region creates sensitivity to other stressors such as brown patch (*Rhizoctonia solani*) in tall fescue (*Festuca arundinacea*) and compaction in bermudagrass that causes shifts from normal spectral reflectance [60,61].

Inflection points of WBI were used to explain the significance observed between grass species and soil type sources (Table 2) because of the strong significance observed between WBI and VWC for our research and congruent literature [41]. The GRI and NDVI data were removed because of the strong evidence that supports WBI being more closely associated with VWC and WP (Table 1). The WBI IP were pooled by soil type and separated by grass species (Figure 3a) and pooled by grass species and separated by soil type (Figure 3b) because of the strong treatment effects with no interaction. Moisture stress across all soils, as defined by the IP, occurred 35 hr earlier in CBG than HBG (Figure 3a). Bermudagrass is considered a warm-season plant due to its isolation of the Calvin cycle around high concentrations of carbon dioxide (CO₂). In comparison, CBG CO₂ fixation reactions are not isolated and stomates must stay open for longer periods of time to maintain adequate concentrations. During drought stress, open stomata of CBG result in

higher water loss required to maintain efficient photosynthesis and cool the plant [62]. Our findings of slower development of wilt symptoms is likely attributed to differing photosynthetic pathways of the grasses tested. Furthermore, root length and biomass were collected to supplement our research findings. Root length and biomass were comparable among soil types but differed by grass species (data not shown). Root length and biomass were taken from experimental units not subjected to drought stress but grown in same greenhouse conditions as ones used for dry-down cycles. Results showed that before any moisture stress, HBG had 19.5 cm roots and CBG had 13 cm root length, while HBG had 1.79 g and CBG had 0.25 g of root biomass weight and were statistically different ($P < 0.0001$). Before any irrigation was restricted from treatments, HBG had a significantly greater root system compared to CBG and likely aided in accessing more water deeper within the soil profile to delay moisture stress symptoms. Index IP varied by soil type when grass species were pooled (Figure 3b). The USGA 90:10 sand treatments displayed significant moisture stress approximately 42 hr later than the finer textured SL and C soils. The 6 hr difference between SL and C soils was insignificant. The USGA 90:10 sand likely allowed the CBG and HBG treatments to have more accessibility to plant-available water at lower soil VWC. The organic matter content within the USGA 90:10 sand (1.2%) and the custom blend of sand particles (0.15 – 0.50 mm) compared to the heavier soil textures is one reason for this balance between water retention and enhanced plant accessibility. Furthermore, comparing soils with similar upper and lower VWC field capacity limits (sand: 5 – 15%, loam: 10 – 25%, clay: 25 – 40%), we know that the permanent wilting point occurs at higher VWC as soil texture decreases in size [62,63].

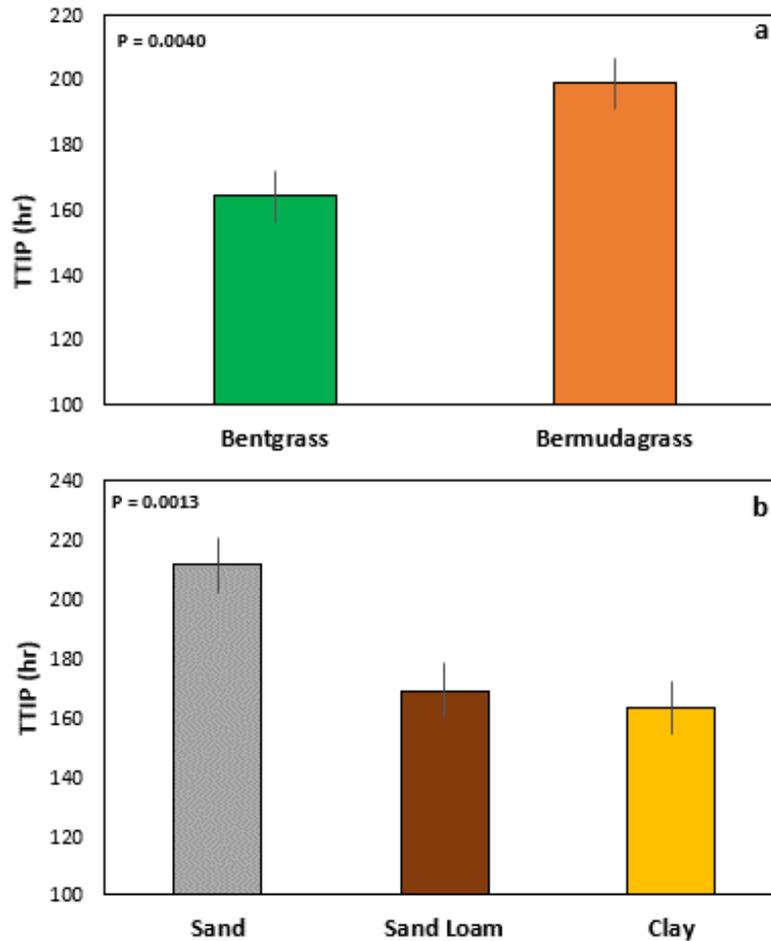


Figure 3. Mean hours to the inflection point of creeping bentgrass and hybrid bermudagrass in three soil types subjected to drought stress and modeled using the non-linear four-parameter logistic regression after initiating six dry-down cycles conducted under greenhouse conditions in Blacksburg, VA. The inflection points of the water band ($WBI = R_{900}/R_{970}$) and the green-to-red ratio $GRI = R_{550}/R_{670}$) were used to observe significant moisture stress over hours from dry-down cycle initiation and represented as time to inflection point (TTIP). Inflection points were derived from spectral reflectance data and pooled across **a)** WBI, GRI, and soil types **b)** WBI, GRI and grass species.

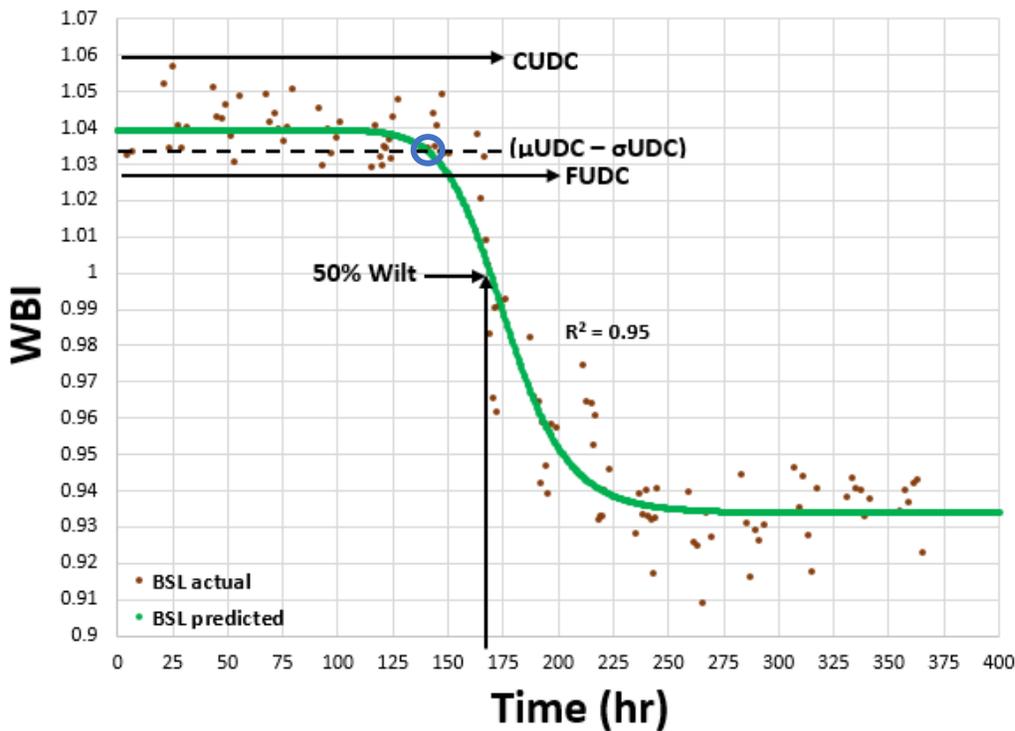


Figure 4. Four-parameter nonlinear logistic regression analysis of spectral reflectance data gathered from ‘007’ creeping bentgrass (B) established in sand loam (SL) and of one dry-down cycle subjected to greenhouse conditions in Blacksburg, VA. The water band index ($WBI = R_{900}/R_{970}$) is plotted against the time (hr) from initiating the dry-down cycle to illustrate the relationship to soil moisture stress over time. To eliminate variability for wilt prediction, mean values (μ) and standard deviation (σ) within the floor and ceiling of upper data cloud (FUDC and CUDC, respectively) were calculated. The blue circle represents the estimated point of a plant physiological response to soil moisture stress derived from the difference of the μ UDC and one σ UDC. The hours where 50% wilt occurred is subtracted from hours of the adjusted value to provide the total wilt prediction time observed.

3.4 Inverse Prediction of Drought Stress

The ability to use this data for wilt prediction would be a considerable step towards utilizing light reflectance as a tool for rapid moisture stress prediction across larger turf surfaces, such as golf course fairways. A representation of the non-linear 4PL regression model of the WBI for CBG integrated into sand loam for one dry-down cycle is used to show how the upper data cloud (UDC) value is calculated (Figure 4) and represented by the blue circle. Once three or more data WBI points occurred below the upper data cloud, the floor (FUDC) and ceiling (CUDC) of the upper data cloud were established. The distribution was analyzed, and one standard deviation (σ) was subtracted from the mean (μ) of all data within the UDC to represent the point of initial physiological change in response to drought stress, based on the 4PL model’s inverse relationship to WP. The hour of wilt prediction (WP^{50}) represents the difference between visually estimated 50% wilt and μ UDC – σ UDC. There was no interaction between index type with any other main effect ($P \geq 0.3781$; Table 2). All main effects were significant ($P \leq 0.0093$), as was the interaction between grass species and soil type ($P \leq 0.0191$). Therefore, indices are presented with

all data pooled over grass species and soil type, and individual treatments of grass and soil type are presented separately.

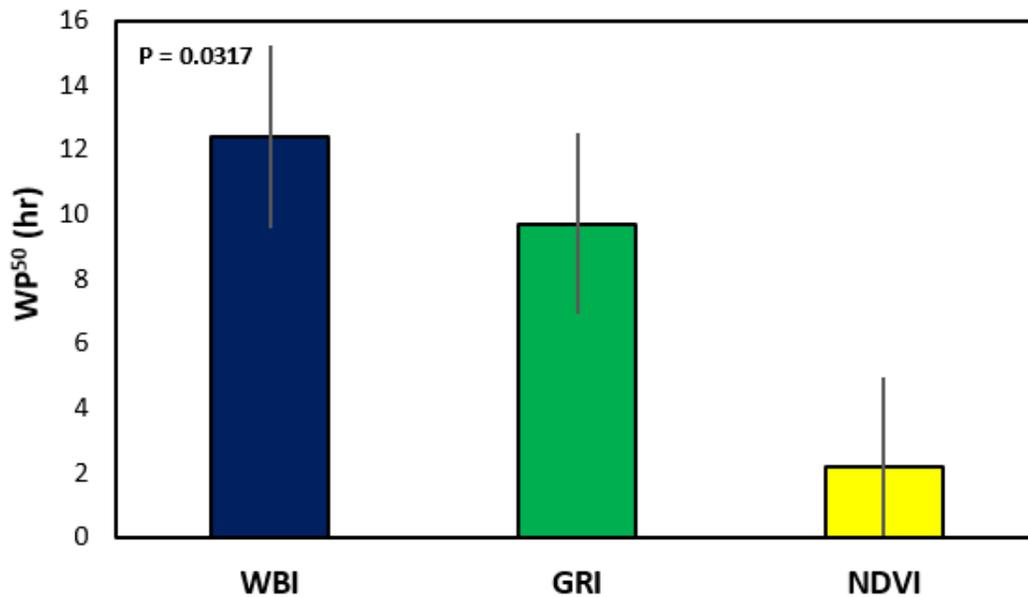


Figure 5. Hours between drought stress prediction with vegetation indices and 50% visible wilt (WP⁵⁰) of turfgrass canopies. Data were pooled across six replications of two grass species: creeping bentgrass and hybrid bermudagrass, and three soil types: sand, sandy loam, and clay. The WP⁵⁰ of values represent mean responses from six drydown cycles of creeping bentgrass and hybrid bermudagrass in three soil types conducted under greenhouse conditions in Blacksburg, VA. The WP⁵⁰ was calculated from the difference of hours at observed 50% wilt to the upper data cloud before wilt occurrence. The hours where a physiological change occurred was determined by subtracting one standard deviation from the mean of the upper data cloud. The wilt prediction values of the water band (WBI = R_{900}/R_{970}), the green-to-red ratio (GRI = R_{550}/R_{670}), and the normalized difference vegetation ((NDVI = $(R_{760}-R_{670})/(R_{760}+R_{670})$)) indices derived from spectral reflectance data analyzed using a non-linear four-parameter logistic regression were pooled across grass species and soil type.

Wilt prediction of WBI and GRI were similar, with 12 and 9 hr WP⁵⁰, respectively (Figure 5). The NDVI predicted significant moisture stress by 2 hr but was not as effective as either WBI or GRI ($P = 0.0317$). This trend is congruent with other data presented in this manuscript that suggest NDVI is not as closely associated with drought stress as WBI or GRI. Previous research has also established a correlation of NDVI to soil moisture content, but with variable efficiency ($0.28 \leq r \leq 0.64$) [27,41]. The documented relationships of NDVI to other stressors further discourage its use as a drought stress predictor. Furthermore, as with the IP results, subsequent data for WP⁵⁰ is explained with WBI. While GRI was statistically the same compared to WBI in regard to wilt prediction capabilities, GRI has the potential to be influenced from other stressors because of its relationship with visible light. For these reasons, the interaction between grass species and soil type is reported using WBI and excluding GRI and NDVI.

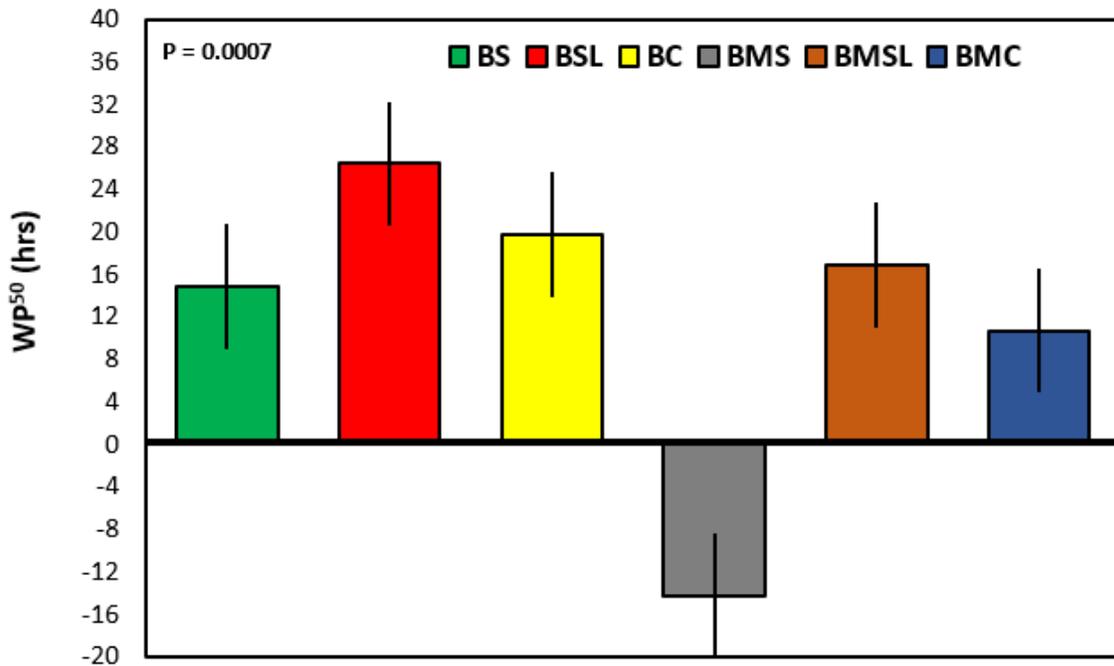


Figure 6. Mean hours of 50% wilt prediction (WP⁵⁰) after initiating six drydown cycles of six treatments, bent sand (BS), bent sand loam (BSL), bent clay (BC), bermuda sand (BMS), bermuda sand loam (BMSL) and bermuda clay (BMC), under greenhouse conditions in Blacksburg, VA. The WP⁵⁰ was calculated from the difference of hours at observed 50% wilt to the upper data cloud before wilt occurrence. The hours where a physiological change occurred was determined by subtracting one standard deviation from the mean of the upper data cloud. The wilt prediction values of the water band (R_{900}/R_{970}) and the green-to-red ratio (R_{550}/R_{670}) indices derived from spectral reflectance data analyzed using a non-linear four-parameter logistic regression were pooled across treatments.

The WP⁵⁰ of individual treatments of grass species grown on different soils were highly significant ($P = 0.0007$) (Figure 6). Bermudagrass grown on sand (BMS) was the only treatment where we were unable to predict wilt, with WP⁵⁰ occurring 10 hr after BMS reached 50% visible symptom expression. The inverse prediction of drought stress using 4PL parameters were positive for all other treatments. Wilt prediction of all treatments, excluding BMS, compared favorably with each other and had WP⁵⁰ of approximately 11 to 26 hr. The difference between BMS and all other treatments are best explained through the combination of bermudagrasses having enhanced physiological capabilities to void drought stress and of USGA 90:10 sand having organic matter and a custom blend of sand particle size compared to other tested soils. These factors allowed BMS to avoid drought stress and access most of the VWC until reaching the permanent wilting point which VWC varies by soil type at this point (sand: 5%, sand loam: 10%, and clay: 20%) [62,63]. Once this point was reached for the BMS, the accelerated wilting was so rapid that light reflectance lagged behind moisture stress.

3 Conclusions

Global concerns of freshwater availability for an increasing human population necessitate efficient consumption, especially for non-vital uses such as golf course management. Best management practices are implemented on golf courses to minimize water requirements using data-driven irrigation strategies. However, most of the strategies depend on local weather data and do not necessarily reflect irrigation needs for microclimates within a larger sward of turfgrass. Light reflectance measurement of turfgrass canopies is a promising tool to identify moisture stress with other variables eliminated, using data transformations through vegetation indices. We know that the WBI occurs outside of electromagnetic regions manipulated by endogenous pigment concentrations that allow the index to effectively discern moisture stress from other stressors. Furthermore, WBI was reported to detect moisture stress of CBG within a sand-based root zone that is typical of golf course putting greens. Our research contributes to the overall understanding of estimating moisture stress across turfgrass systems using spectral reflectance. We demonstrate how three vegetation indices can be used to estimate and predict moisture stress across a variety of turfgrass canopies prior to visible wilt symptom expression. Two indices, WBI and GRI, were more strongly correlated to visual estimations of wilt and to VWC than the more commonly-researched NDVI. Regression analysis of WBI and GRI data shows temporal index changes in response to turfgrasses approaching PWP. Overall, the WBI is most consistently associated with limited water availability but the GRI was almost as effective in all metrics compared, with both indices providing an approximate 12 hr lead time prior to extensive drought stress. The NDVI, which is used most commonly in agricultural settings, was the least reliable at estimating drought stress in our study.

Data within this manuscript were collected on CBG and HBG grown on varying soil textures under greenhouse conditions. These two grasses represent the most common species grown on golf course fairways worldwide. Additionally, the soil types examined represent a wide range of underlying root zones that these grasses may be grown on. The grasses and soils evaluated in this study provide a wide array of real-world turfgrass canopy scenarios. Our results should serve as a fundamental approach for expanding rapid drought stress detection across golf courses using aerial imagery. The strong relationship between WBI and GRI provide flexibility for choosing sensors for future drought studies collected across data collection platforms. A sensor capable of collecting reflectance values within the near-infrared range cost significantly more compared to a visible-light camera needed to estimate GRI. The readily available data acquisition of GRI from visible light implies a more direct practical use. However, high absorbance of photosynthetically active light may limit GRI effectiveness under variable solar conditions. Previous reports of WBI detecting moisture stress independent of other stressors using narrowband reflectance of more stable near-infrared light is encouraging when paired with our results, though current technology and associated costs may limit immediate application.

Exploring the practicality of these indices under real-world application is an important step in being able to rapidly, and non-destructively, identify and predict soil moisture stress. Results from these data may be used to help develop index thresholds that imply early onset wilt occurrence for future research. Applying index thresholds to remotely sensed data might allow for computer automation of wilt detection and linkage to automated irrigation systems. Precise, accurately, and timely irrigation across large turfgrass systems will improve efficiency by applying water only when and where it is needed.

Data Set: <https://doi.org/10.7294/W4-MGPR-CZ59>

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