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Water-Use Characteristics of Warm-Season Putting Green Cultivars and Management Practices Associated with New Putting Green Genetics

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Water-use characteristics of warm-season putting green cultivars and management practices associated with new putting green genetics

By

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Bermudagrass (*Cynodon* spp.) is the most common turfgrass used on golf course putting greens in the southeastern United States (Lyman et al., 2007). In 2013, the National Turfgrass Evaluation Program (NTEP) started a 5-year trial of warm-season putting green cultivars. One of the bermudagrass cultivars in the study is MSB-285 (experimental cultivar). MSB-285 is a sister plant of MSB-264 (Philley and Munshaw, 2011) and is a distinct cultivar of *C. dactylon* × *C. transvaalensis*. MSB-285 has a more extensive root system and upright growth habit than traditional bermudagrass putting green cultivars (Philley and Munshaw, 2011). Due to MSB-285's unique genetic make-up and growth habit, the objectives of this research were to determine if best management practices used to maintain ultradwarf bermudagrasses would be suitable for MSB-285 and to determine the water-use characteristics of MSB-285 compared to industry standard cultivars.

DEDICATION

To my kids; Dylan, Meredith, and Loralei, thank you for the patience and consideration as I worked long days and nights the past two years. To my mom; Janice Wait, thank you for always being there for me when I fell. Your kindness, work ethic, and strong mental attitude are attributes I strive to achieve in my daily life. To my wife; Michelle Aucoin Wait, you have been the best thing to ever happen to me. You are my world and my heart; I love you more and more every day. Your passion, love, and drive, are more than I deserve. I will spend the rest of my life appreciating the fact that I get to navigate this world with you, as a team, forever united.

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Maroon, White, Fight, Fight, Fight. Hail State!!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

LITERATURE REVIEW

Golf Putting Greens

History and Genetics

Turfgrass scientists generally agree that the origin of bermudagrass (*Cynodon* spp.) is Africa, but some researchers have stated the origin could be Australia, Eurasia, India or Indo-Malaysian (Mitich, 1989). Kopec (2003) hypothesized that common bermudagrass (*C. dactylon*) seed initially arrived in America from hay that was used as bedding on transport ships carrying slaves from Africa. Although the origin of bermudagrass has not been undeniably pinpointed, many of the improved varieties used today are from African stock (Mitich, 1989).

The ability to sustain growth and density under humidity and heat has made hybrid bermudagrass the most utilized warm-season turfgrass on golf courses in the southeastern United States (Hartwiger and O'Brien, 2006). In 2007, an estimated 80% of golf course putting green surfaces in the Southeast were bermudagrass (Lyman et al., 2007). McCullough et al. (2004a) classified turf-type bermudagrasses into four categories: common bermudagrasses (*C. dactylon*) that are tetraploid and have a total of 36 chromosomes, African bermudagrasses (*C. transvaalensis*) that are diploid and have 18 chromosomes, hybrid Magennis bermudagrasses (*C. magennisii*) that are naturally triploid and have 27 chromosomes, and Bradley bermudagrasses (*C. bradleyi*) which are aneuploids with 18 chromosomes. Two turf-type cultivars that do not fit into the four categories listed above are ‘Tifton-10’ (*C. dactylon*) and ‘Tifgreen’ [*C. dactylon* (L.) Pers. × *C. transvalensis* (Burtt-Davy)] (experimental designation 328). Tifton-10 is registered as a hexaploid with 54 chromosomes (Hanna et al., 1990), and Tifgreen is an interspecific hybrid bermudagrass commonly used on golf courses in the Southeast (Beard, 2002).

Golf course putting greens in the Southeast have undergone major transformations in the past 60 years. The high temperatures and humidity in the Southeast forced golf courses to use common bermudagrass as a putting green surface while their northern and western counterparts were able to grow creeping bentgrass (*Agrostis stolonifera* L.). Creeping bentgrass proved itself to be a more desirable putting surface than common bermudagrass because of its ability to be mown at lower heights while still maintaining density and desired green color. In 1956, the dwarf-type bermudagrass Tifgreen became available for use as a putting surface. Dwarf-type bermudagrasses, such as Tifgreen, have a lower growth habit and produce a higher shoot density than other hybrid bermudagrasses (Brosnan and Deputy, 2008). Soon after the release of Tifgreen, vegetative mutations (off-types) were discovered in both Georgia and South Carolina. The putting green characteristics of these off-types was superior to Tifgreen, therefore they were subsequently propagated and distributed as ‘TifDwarf’ (Burton, 1966). With the introduction of these new dwarf-type putting green surfaces, many golf courses in the Southeast converted from common bermudagrass to dwarf cultivars. While the dwarf-type bermudagrass cultivars were a vast improvement to common bermudagrass, they were still considered inferior to creeping bentgrass putting surfaces. In the mid-1990’s, more heat resistent varieties of creeping bentgrass were developed and introduced on many golf courses in the Southeast (Bigelow, 2007). Creeping bentgrass improved the playability of putting greens in the Southeast, but the more heat and drought resistent varieties of creeping bentgrass proved to be very expensive and labor intensive to manage. Humid summers in the Southeast impede movement of water through the plant, so many courses installed large fans around greens complexes and hand-watered greens throughout the day. There was a constant balancing act of ensuring adequate air movement and water was applied to the green to prevent wilt, while producing a firm playing surface. Due to the constant presence of water, fungicide applications became routine, which further increased costs associated with maintaining these superior putting surfaces. At the turn of the century, mutations of Tifgreen and Tifdwarf were released under the names 'MiniVerde' (Kaerwer, 2001), 'Champion' (Brown et al., 1997), and 'TifEagle' (Hanna and Elsner, 1999). These new cultivars were labeled as ultradwarfs because they exhibited even more dwarf characteristics than their predecessors (Figure 1.1). Ultradwarfs gave superintendents the ability to mow greens lower and increase ball roll distance while still maintaining firmness and desired green color (Brown et al., 1997; Hanna, 1999; Kaerwer, 2001). The majority of commercially grown ultradwarf cultivars are genetically related to Tifgreen or Tifdwarf, and have been found to be genetically unstable (Reasor et al., 2016).

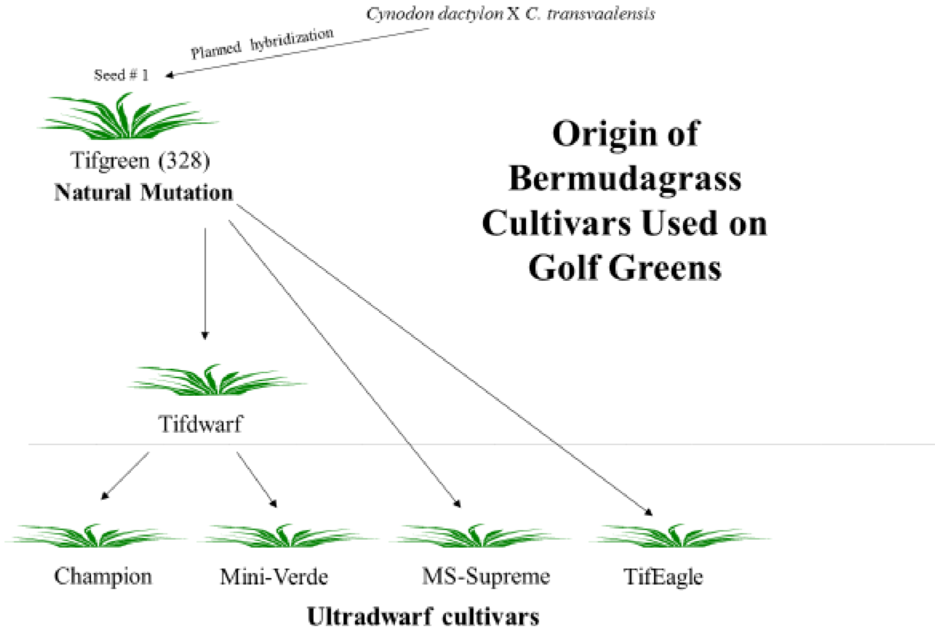

Figure 1.1 Origin of bermudagrass cultivars used on golf greens

Growth habit representation and genetic lineage of (Champion, MiniVerde, and TifEagle).

Bermudagrass genetic instability has led to the presence of off-types (phenotypically different) on many putting greens in the Southeast. Off-type grasses in a putting green system can cause the surface to become non-uniform in growth habit and color. Zhang (1999) concluded that vegetative distribution of ultradwarf bermudagrass was a possible source of contamination that contributed to the production of off-types. Reasor et al. (2016) concluded that management practices should be addressed to prevent off-type grasses from developing. Turfgrass breeders continue to develop experimental warm-season putting green cultivars, as seen in the 2013 National Turfgrass Evaluation Program (NTEP) warm-season putting green trial. One of the bermudagrass cultivars in the 2013 NTEP trial is MSB-285 (experimental cultivar). MSB-285 is a sterile triploid interspecific hybrid between selected genotypes of *C. dactylon* and *transvaalensis*. When amplified with SSR marker ES295668, MSB-285 and its sister plant, MSB-264, generated a unique allele compared to Tifgreen derived-cultivars, Tifdwarf, TifEagle, Champion, and MiniVerde (Personal communication, Harris-Shultz, 2013). MSB-285, much like MSB-264, has a more upright leaf orientation and a finer leaf texture than the standard ultradwarf bermudagrasses and retains color longer into the fall and winter (Figure A.2) (Figure A.4) (Liu, 2008; Philley and Munshaw, 2011)

Two of the non-bermudagrass putting green cultivars in the 2013 NTEP trial are 'Diamond' zoysiagrass [*Zoysia marrella* (L.) Merr]) and 'SeaDwarf' seashore paspalum (*Paspalum vaginatum* O. Swartz). Diamond has 40 chromosomes and is distinguished from other zoysiagrasses by its characteristics of shade and salinity tolerance, as well as its visual turfgrass quality (TQ) (Qian and Engelke, 1999). Environmental Turf, who holds the distribution rights to SeaDwarf, states that SeaDwarf is a dwarf cultivar of seashore paspalum and can tolerate mowing heights from 2.5 to 100 mm (www.environmentalturf.com).

Management Practices

Golf course putting green cultivars represent the smallest amount of acreage on a golf course. However, they are often the most intensely managed and receive the highest frequency of inputs. During an average round of golf, 75% of golf strokes involve the putting green, either through incoming golf shots, or putts (Beard, 2002). Golf course superintendents balance their putting green management strategy to meet the needs of the golfers, while still maintaining healthy turfgrass. Best management practices for bermudagrass putting greens are always evolving and vary depending on environmental conditions, location, and the species/cultivar used. During the growing season, ultradwarf greens are often rolled and mowed, or double-mowed, daily (McCarty and Miller, 2002). Ultradwarfs are often verticutt, top-dressed, and groomed weekly, and aerified once or multiple times a year using both hollow and solid-tines (Bevard, 2005; Cisar, 1999; McCarty and Miller, 2002; Unruh and Elliott, 1999; Craft, 2016).

Mowing

Of all the management practices used on ultradwarfs, mowing is performed the most frequently. Mowing golf course putting greens not only adds to the aesthetics of the golf course but also improves the greens playability. Golf course putting green speed, measured by ball roll distance (BRD), is a measurable objective of playability that golf course superintendents use to adjust management practices. Friction caused by shoot growth can reduce BRD, slowing the speed of the green (McCullough et al., 2006). To counteract friction, ultradwarf putting greens are mown at heights of 4.0 mm or lower while harvesting clippings (Sorochan, 2014). Lower mowing heights on putting greens can produce longer BRD because of the reduction in friction, but mowing greens lower is not always the solution to increasing BRD. Mowing greens too low can cause scalping, which lowers TQ and could create a surface that is non-uniform. Reduced clipping yields are directly correlated to longer BRD in ultradwarf putting greens; however, turfgrass uniformity also plays a factor in BRD. A more uniform turfgrass height of cut produces a smoother, more unimpeded ball roll. Ball roll distance is not only affected by friction from shoot growth, but it is also affected by shoot growth rate uniformity (McCullough et al., 2006).

Plant growth regulators

To counteract shoot growth, increase uniformity, and increase BRD, putting greens are often treated with plant growth regulators (PGR) (McCullough et al., 2004b; McCarty et al., 2011; Rademacher, 2000). The PGR's used on turfgrass can be classified into two types. Type I PGR's are cell division (mitosis) inhibitors, and Type II PGR's are gibberellin biosynthesis inhibitors (Murphy et al., 1998). Type II PGR's can be further separated into classes A and B. Class A is a gibberellin biosynthesis inhibitor absorbed through the foliage and inhibits biosynthesis of gibberellin late in the biosynthesis pathway (Murphy et al., 1998). Class B is a gibberellin inhibitor that inhibits biosynthesis in the early stages of the biosynthesis pathway (Murphy et al., 1998). Class A inhibitors are the most commonly used PGR's on bermudagrass putting greens, and studies show that Class A inhibitors cause less injury and physiological disruption to turfgrass species (Murphy et al., 1998). Trinexapac-ethyl (TE) is a Class A PGR used by turfgrass professionals, homeowners, and landscapers. Trinexapac-ethyl blocks 3ß-hydroxylation, inhibiting the formation of highly active gibberellic acid (GA) from inactive precursors (Rademacher, 2000). McCullough et al. (2005) conducted two greenhouse studies to evaluate the effects of TE on six dwarf-type bermudagrasses. The TQ rating of turfgrass treated every 10 d with 0.0125 kg TE ha^{-1} during all observations from 20 to 60 days after initial treatment (DAIT) were higher, relative to the untreated controls. Also, an average increase of 12% TQ was observed across all cultivars when compared to the control. Clipping yield was reduced by 46-69% among all cultivars treated with TE. The clipping yield was highest among TifEagle and Champion with respect to all un-treated cultivars, while TE-treated TifEagle was the only TE treated cultivar with <60% reduction in clipping yield. The researchers concluded that TE is a viable method to reduce clipping yields in dwarf-type cultivars. In another study using TifEagle, Bunnell et al. (2005) concluded frequent TE applications increased total shoot chlorophyll and total non-structural carbohydrates (TNC), relative to the untreated control.

Researchers at Clemson University evaluated the effects of TE on turfgrass with varying amounts of nitrogen (N) (McCullough et al., 2006). The experiment was conducted on TifEagle mowed 6 d wk^{-1} at 3.2 mm. Plots were fertilized with either 6, 12, 18, or 24 kg N ha^{-1} wk^{-1}. Half of the plots were treated every 21 d with 0.05 kg TE ha^{-1} while the other half received no TE. The BRD of plots treated with TE was longer than the untreated plots, regardless of fertilizer application regimen. The BRD of TE-treated plots was longer during evening measurements than the evening measurements of untreated plots, suggesting that TE treatments increased overall BRD throughout the day. The study estimated that there was a 50% reduction in clipping yield when TifEagle was treated every 21 d with 0.05 kg TE ha^{-1} compared to untreated TifEagle. Other results indicated the initial TE treatments reduced TQ compared to non-treated turfgrass, but in both years of study, the TQ recovered to within 10%-25% of the untreated turfgrass by 2 weeks after initiation (WAI). An increase in N fertility linearly increased TQ for TE and untreated plots, but BRD linearly decreased as fertility rates increased. This research suggests increased fertility rates decreased BRD because of greater friction on the ball due to an increased and less uniform shoot growth (McCullough et al., 2006).

For a turfgrass to perform to maximum potential, it must have a well-formed root structure. Roots are the site of initial water and nutrient uptake in the plant. When using PGR’s to increase BRD, turfgrass managers ensure that root growth is not negatively impacted. McCarty et al. (2011) noted that TifEagle increased root length density (RLD) 33% when treated every 14 d with 0.0175 kg TE ha^{-1} relative to the untreated control. In other studies, TE applications did not significantly effect rooting parameters, relative to the controls (McCullough et al., 2005).

Water-use

Municipal water restrictions have become more common in much of the Southern United States (Wherley et al., 2014). Lack of knowledge in turfgrass water-use has caused many municipalities and communities to remove turfgrass with the goal of reducing water usage (Kopp and Jiang, 2013; Vickers, 2001). A 2013 study estimated that golf courses in the Southeast used ~ 490,000 kL of water per year (GCSAA, 2015). In 2010, the Alliance for Water Efficiency (AWE) published a report entitled Golf Course Water Efficiency Introduction. One of their primary goals of the AWE is to promote the efficient and sustainable use of water. The AWE stated that a more collaborative approach to water management on the part of not only the course owners, who pay the water bill, but also the golf course superintendent, who maintains the course's turfgrass, is the best solution for conserving water. Through a collection of water audits, the AWE determined that golf courses, on average, over water their turfgrass by 20-50%. Data collected from the water audits suggests that the leading causes of over watering are: irrigation timing, irrigation uniformity, and the lack of weather-based irrigation controllers (AWE, 2010). In the report, the AWE did not address the issue of turfgrass selection with respect to water conservation. However, other studies have focused on the selection of turfgrass species/cultivars that use water more efficiently (Zhou et al., 2013; Rowland et al., 2014).

Breeding and Selection

Turfgrass often requires supplemental irrigation to maintain a quality playing surface that is aesthetically pleasing. Turfgrass breeders have long selected and bred turfgrass for improved characteristics such as uniformity, shoot density, and desired green color, but as water conservation efforts continue, many turfgrass breeders may focus on selecting and breeding turf-type grasses that can maintain playability and desired color with less water.

Researchers at the University of Queensland, in Australia, conducted two experiments using 460 genotypes, as well as 3 commercial cultivars of bermudagrass (Zhou et al., 2013). The objective of the experiment was to determine if bermudagrass genotypes collected in 4 different climatic regions in Australia expressed drought stress characteristics based on the region they were collected. In study I, there were 120 rain free days. In study II, plants were grown under a rainout shelter, and water was withheld for 290 days. Drought treatment was not significant among genotypes and cultivars until late stages of water deficiency. The authors also noted commercial cultivars were not the most drought resistant. Regarding the location of collection, genotypes from dry regions were not the most drought resistant. The authors concluded that genotypes collected in the Mediterranean region (dry, hot, summers and wet winters) were most drought resistant, while genotypes from the subtropical region showed the least amount of drought resistance. The researchers speculated that genotypes from the dry region had never recuperated from drought stress in, unlike the genotypes from the Mediterranean region. The environment in which the plant was adapted to, led to a weaker plant in respect to drought stress for the genotypes from the dry region, and a more drought resistant turfgrass in the Mediterranean region. Certain characteristics of a cultivar are used by breeders when selecting plants that are more drought resistant. Root biomass is one of those characteristics. Roots that have more surface area and are longer, have the ability to reach more water in the soil than roots that are smaller and shorter (Duncan and Carrow, 1999).

Rowland et al. (2014), at the University of Florida, conducted three experiments to investigate the impact of the N to potassium (K) ratio on drought resistance of warm-season putting green cultivars. Putting green cultivars included TifDwarf and TifEagle bermudagrasses, SeaDwarf and 'PristineFlora' zoysiagrass [*Z. japonica* Stued. by *Z. tenuifolia* (L.) Merr.]. Rates of N to K, ranging from 1:1, 1:2, 1: 3, and 1:4, were applied to separate plots as well as multiple irrigation intervals. Irrigation intervals were calculated using the Blaney-Criddle method of estimating evapotranspiration (ET) (Ponce, 1989). Plots were further sub-divided into sub-plots that were watered at either 25% ET, 50% ET, or 100% ET. Results indicate that K, at a ratio of 1N:1K, is more K than the plant needs, even under drought stress. SeaDwarf and PristineFlora consistently had a higher drought resistance than TifEagle and TifDwarf, with respect to normalized difference vegetation index (NDVI), relative chlorophyll (RCI), and visually observed leaf wilting. Volumetric soil water content (VWC) recorded before watering, and rainfall events revealed that under all ET percentages, SeaDwarf VWC was consistently higher, leading the researchers to believe that root growth and thatch development may have more of an impact on drought resistance than the plant's morphological leaf structure. This conclusion contrasts with previous research where morphological adaptation, such as horizontal leaf growth, slow vertical growth, and high shoot density, contributed to a more drought resistant plant (Brian et al., 1981: Kim and Beard, 1988).

Physiology

Plants must maintain a balance between carbon dioxide absorption and water loss. Although, there are multiple mechanisms and processes by which a turfgrass uses water. The turfgrass itself spends minimal energy moving water from the soil to leaf tissue and then outward into the environment (Kopp and Jiang, 2013). While physical forces move water from the soil through the plant into the atmosphere; the plant uses energy to develop and maintain structures that allow the process of water movement to occur. The physical forces that move water from the soil upward can be expressed and calculated using water potential. The water potential in the soil and surrounding areas dictates the ability of an environment to move water from the soil upward. Plant structures develop in a manner that makes water potential gradients benefit the overall growth of the plant (Sterling, 2004).

Evapotranspiration is the amount of water lost by a plant in a given area, in a given span of time. Since physical forces are the driving factor behind water movement throughout a plant, this process can be referred to, and calculated as, potential evapotranspiration (PET). Energy balance calculations must be used to determine PET, and the calculations must take into account the soil type, plant type, and other factors that affect the energy balance between the soil, turfgrass, and atmosphere (Stier et al., 2013).

To better understand the relationship between water quality, TE, and drought stress, a ten-week greenhouse study was conducted by Hejl et al. (2015). Tifway bermudagrass was watered twice weekly to either fully watered (1.0 x ET) or to a deficit

(0.3 x ET), using reverse osmosis (RO), sodic potable, or saline water. ET rates were significantly higher among plants that received sodic potable water vs. plants that received RO water. The lowest TQ recorded was turfgrass watered with RO; researchers attributed the lower TQ rating in the RO treated turfgrass to a lower initial reference ET for RO. Two key findings include TQ and canopy temperatures. Across all treatments, TQ was lower, and canopy temperatures were higher in the turfgrass watered at a deficit (Hejl, 2015). Drought stress induces many physiological responses in the plant. In the turfgrass literature, the most common physiological parameters include: osmotic adjustment (OA), proline accumulation, leaf relative water content (RWC), canopy temperatures, and TNC levels.

Osmotic adjustment is defined as the accumulation of solutes in plant tissue as a response to dehydration (Turner and Jones, 1980). Osmotic adjustment helps a plant maintain turgor pressure and allows for cell elongation during times of dehydration. Jiang and Huang (2000) reported prior exposure to drought stress, known as preconditioning, increased the subsequent heat tolerance of Kentucky bluegrass (*Poa pratensis* L.). A greenhouse study conducted by Jiang and Huang (2001) investigated whether the subsequent heat tolerance of drought preconditioned Kentucky bluegrass was associated with OA and root growth. After 14 and 21 d of heat stress, preconditioned Kentucky bluegrass OA was 17 and 48% higher and TQ was 13 and 21% higher, respective to d of acclimation, than the non-preconditioned plants.

The amino acid proline, accumulates within a plant in response to drought stress and plays an important role in OA and plant cell protection during drought stress (Shaoyun et al., 2009; Zhang et al., 2009). In previous research conducted by Da Man et al. (2011), an increase or accumulation of proline was detected in tall fescue subjected to drought stress, and proline levels were higher for more drought resistant cultivars.

Leaf relative water content is an estimate of current water content in a leaf's tissue relative to the maximum water content it can hold at full turgidity (Barrs and Weatherly, 1962). Values vary for each variety of plant tested, but normal values in plants range from 85-95%. Leaf wilting has been observed in turfgrass at 70-80% relative water content (Cockerham and Leinauer, 2011). Levels of 40-50% have been associated with leaf desiccation or even plant death. Da Man et al. (2011) reported a 20% reduction in tall fescue RWC after 10 d of drought treatment.

Mechanisms of drought stress are probably associated with the closure of stomata as indicated by higher canopy temperatures (Kopp and Jiang, 2013). Turfgrasses that are subjected to drought stress have canopy temperatures that range from 5-13 ˚C higher than when watered adequately (Leksungnoen et al., 2012).

Conclusion

Golf course superintendents are often judged by the quality of the putting greens they manage. The vast majority of ultradwarf putting greens available are derived from Tifgreen and have been found to be genetically unstable (Reasor et al., 2016). This genetic instability has caused off-types within the putting green system, which can reduce the quality of a putting surface. When replacing these contaminated putting surfaces, golf course superintendents must weigh a multitude of factors. With the ongoing water conservation efforts in the United States, water-use efficiency may be one of the factors superintendents consider. The 2013 NTEP warm-season putting green trial is a source of quantitative and qualitative data that can assist golf course superintendents as they decide which cultivar to replace their putting surface with. However, data collection does not include water-use characteristics and does not include best management practices associated with experimental cultivars, such as MSB-285. Research was conducted in order to investigate effects of typical best management practices upon MSB-285 as well as to compare its water-use characteristics to commercially standard warm-season putting green cultivars.

CHAPTER II

MANAGEMENT PRACTICES ASSOCIATED WITH NEW PUTTING GREEN GENETICS

Introduction

Golf course superintendents in the southeastern United States balance multiple factors when implementing their putting green management practices. Factors that affect management practices include: location and environment, climate and micro-climates, local weather, stakeholder opinion, labor and equipment resources, and availability of inputs. Creating and implementing a putting green management strategy must be balanced with the cultivar used as the putting greens surface. Standard management practices, such as mowing frequency, height of cut, and plant growth regulator (PGR) application frequency and rate, have been investigated (McCarty and Canegallo, 2005; McCullough et al., 2004b; McCullough et al., 2007). Ultradwarf putting greens are typically mown to heights of 4.0 mm or lower (McCarty and Canegallo, 2005; McCullough et al., 2007; Sorochan, 2014). Shoot growth causes friction to occur between the golf ball and the putting surface. Sorochan (2014) stated that decreasing mowing height from 4.0 mm to 3.2 mm on ultradwarf bermudagrass increased ball roll distance (BRD). However, decreasing mowing heights from 4.0 mm to 3.2 mm can negatively affect turfgrass quality (TQ), and if scalping occurs, can affect uniformity of the turfgrass surface. To increase shoot growth uniformity, reduce vertical growth, and increase BRD, PGR's are applied on ultradwarf putting greens. Trinexapac-ethyl (TE), a Type II, class A PGR, is commonly applied when managing hybrid bermudagrass (Rademacher, 2000). McCullough et al. (2005) concluded that ultradwarf bermudagrass treated with 0.0125 kg TE ha^{-1} 10 d^{-1}, clipping yield was reduced 46-69% and TQ increased 12%, relative to the untreated control. McCullough et al. (2006) concluded that ultradwarf bermudagrass, mown at 3.2 mm 6 d wk^{-1} and treated with 0.05 kg TE ha^{-1} 3 wk^{-1}, clipping yield was 50% less and BRD increased, compared to the untreated control.

Frequent TE applications and mowing heights of 4.0 mm or lower are standard management practices used on ultradwarf bermudagrass putting greens in the Southeast (McCullough et al., 2007; Sorochan, 2014). The use of TE and reduced mowing heights create a smooth putting surface with adequate BRD and desired green color. Management practices associated with industry standard, 'Tifgreen' [*C. dactylon* (L.) Pers. × *C. transvalensis* (Burtt-Davy)] derived cultivars, 'TifEagle' (Hanna and Elsner, 1999), 'MiniVerde' (Kaerwer, 2001), and 'Champion' (Brown et al., 1997), are unique to the cultivars themselves and may not be suitable for new experimental cultivars.

'MSB-285' (experimental cultivar) is a new interspecific hybrid bermudagrass that was bred and selected by the Mississippi State University (MSU) turfgrass breeding program. Much like its sister plant 'MSB-264' (Philley and Munshaw, 2011), MSB-285 retains color longer into the winter and exhibits a more upright leaf orientation than the standard ultradwarf cultivars (Figure A.2) (Philley and Munshaw, 2011; Liu, 2008). MSB-285 can be maintained at putting green heights (4.0 mm or lower) while still producing quality growth and density. The leaf orientation, growth habit, and unique genetic allele (Personal communication Harris-Schultz, 2013), make MSB-285 a unique plant from the standard ultradwarf cultivars (Personal communication Philley, 2015). Due to these factors, MSB-285 may require different management strategies to produce a commercially acceptable putting green surface. Therefore, the objective of this research was to quantify the response of new putting green genetics to industry standard ultradwarf cultivars in response to various mowing heights and PGR regimes.

Materials and Methods

Research was conducted at the R. R. Foil Plant Science Research Center, Starkville, MS, from 15 June to 1 September 2015 and 2016 (Figure A.1). The experiment was conducted as a split-plot design with whole plot units (13.7m x 13.7m) in a randomized complete block design and 2 subplot units (4.6m x 4.6m) in a split-plot in strips design. There were 3 blocks with a main plot factor of cultivar. Trinexapac-ethyl application rate and mowing height were factors applied in perpendicular strips across the cultivars. Plots were not re-randomized in 2016. Soil profile was an 85:15 sand: peat mixture constructed per USGA specifications (United States Golf Association Green Section Staff, 1993). Bermudagrass cultivars included MiniVerde, TifEagle and MSB-285.

Cultivars were established by sprigs on 20 August 2014 at a sprigging rate of 100 $m^3\ ha^{-1}$. In 2015, fertilization was applied weekly using an Anderson's (The Andersons, Montgomery, AL) 16N-2P_2O_5-18K_2O granular fertilizer to deliver 12.2 kg N ha^{-1}. In 2016, plots were fertilized weekly using a combination of 10N-1.3P-4.2K and 5N-0P-5.8K liquid fertilizers (50:50 quantity of N) (Progressive Turf, LLC, Ball Ground, GA) at a rate of 3.1 kg N ha^{-1}. Liquid fertilizer was applied weekly using a CO_2 pressurized backpack sprayer. Plots were sand top-dressed at a depth of 12.3 mm. Topdressing was conducted 1, 6 and 11 weeks after initiation (WAI); ~ 24 hours after each clipping yield collection. Trimec Bentgrass (PBI Gordon; Kansas City, MO) at 0.298 kg a.i. ha^{-1} MCPP, 0.185 kg a.i. ha^{-1}2, 4-D, 0.075 kg a.i. ha^{-1} Dicamba, was applied on 26 June 2015 and 27 June 2016 to control prostrate spurge [*Chamaesyce maculata* (L.) Small], while iprodione + trifloxystrobin (Interface StressGuard; Bayer; Research Triangle Park, NC) was applied at 2.18 kg a.i. ha^{-1} and 0.15 kg a.i. ha^{-1} respectively, on 26 June 2015 and 29 July 2016 to control leaf spot (*Bipolaris cynodontis*). 0.12 kg a.i. ha^{-1} Bifenthrin (Quali-Pro Golf & Nursery 7.9F, Pasadena, TX) was applied on 26 June 2015 and 27 June 2016 to control armyworms [*Spodoptera frugipeda* (J. E. Smith)]. Irrigation was applied uniformly over all plots as needed to prevent wilt.

Plots were mown using a Toro Greensmaster Flex 2100 (The Toro Company, Bloomington, MN) 5 d wk^{-1} at either 3.2 mm or 4.0 mm. One mower was used for both cutting heights and height of cut was adjusted before each mowing height was applied. Trinexapac-ethyl (Primo Maxx, Syngenta, Basel, Switzerland) was applied weekly using a CO_2 pressurized backpack sprayer at either 0, 0.02 or 0.05 kg a.i. ha^{-1}. The sprayer was calibrated to deliver 161 L ha^{-1} through TeeJet TP 8004VS spray tips (TeeJet Technologies, Springfield, IL).

Data collection included TQ, surface firmness, BRD, clipping yield, shoot total non-structural carbohydrates (TNC), relative chlorophyll index (RCI), normalized difference vegetation index (NDVI), and root biomass.

Turfgrass quality was recorded weekly based on color, texture, density, and uniformity of the putting surface. Quality was visually evaluated from 1-9, 1=poorest quality brown turfgrass, 6=minimally acceptable turfgrass, 9=ideal uniform, dense, fine textured, dark green turfgrass (Morris, 2017).

Surface firmness was measured weekly using a USGA TruFirm, Turf Firmness Meter (USGA, Far Hills, NJ). TrueFirm measures the maximum penetration of an impact hammer with a hemisphere-shaped end that resembles a golf ball. The impact hammer was dropped on the putting surface and values from three single drops, in randomly selected locations per subplot, were averaged. The average of the three drops was recorded, and results represent the depth of penetration (cm), where higher depth values indicate a softer surface.

Ball roll distance was measured 3, 7, and 11 WAI. To determine BRD, a modified USGA stimpmeter was used and lengths were recorded (cm). Detailed methodology of the modified USGA stimpmeter is described by Gaussoin et at. (1995). Three standard golf balls were rolled in opposite directions on each plot; the six recorded distances were averaged and multiplied by two, to represent the BRD of each plot.

Clipping yield (g m^{-2}) was collected 1, 6, and 11 WAI. Mowing was postponed for 3 d before each clipping yield collection. The Toro Greensmaster Flex 2100 mower was used to collect clipping yield samples from each plot. Clippings from each plot were placed in a zip-lock bag and stored in an ice chest during collection. From each zip-lock bag, 200 mg of clippings were transferred to a heavy-duty aluminum foil packet and stored at -80^{o}C for future analysis. The remaining clippings were placed in individual paper bags and dried in a forced air oven (Precision Science Company, Chicago, IL) at 65^{o}C for a minimum of 48 h. Dried clippings and their respective paper bags were weighed and recorded. Clippings were removed from the bags, and the weight of the bag was recorded. The total of the dried bag and clippings minus the dried bag alone, was recorded as the clipping yield for each plot.

Clippings were used to determine shoot TNC (mg g^{-1}). Shoot TNC was analyzed using Nelson's assay and determined the amount of glucose and fructose in the plant tissue (Nelson, 1944; Somogyi, 1952). Detailed methodology is described in Waltz and Whitwell (2005).

Relative chlorophyll index, using a FieldScout CM1000 (Spectrum Technologies Inc., Aurora, IL), was recorded weekly. CM1000 Chlorophyll meter (Model 2950), wave bands are 700 nm (red) and 840 nm (NIR) and receptors include 4 photodiodes, 2 for ambient light and 2 for reflected light from the sample (turf, leaf). With an output scale of 0 to 999 for RCI, the equation is as follows: Index=[(S840/A840)/(S700/A700)]*1000 with S = sensor and A = ambient light (Personal communication, Sayre, 2017). Six readings were obtained by holding the meter ~ 1.5 m from the turfgrass at systematically chosen locations on a central transect down the center of the plot. The 6 readings were averaged to represent the RCI for each respective plot.

Normalized difference vegetation index was measured with a Field Scout Turf Color Meter (TCM) 500 NDVI (Spectrum Technologies, Inc., Aurora, IL) at turf canopy level. The TCM500, wave bands are 660 nm (± 5 nm) and 850 nm (± 5 nm) and the output is in NDVI based on the following: NDVI = (NIR-RED) / (NIR+RED) (Personal communication, Sayre, 2017). Six subsamples within the center of each plot were averaged and recorded as the NDVI value for each plot.

Root biomass was measured on 1 September in 2015 and 2016, by removing one plug with roots from each plot. Plugs were removed by hammering in a 5.1 cm schedule-

40 polyvinyl chloride pipe (Silver-Line, Asheville, NC) that was cut at a 35-degree angle, and measured 30.5 cm from one side to its shortest angled end. Roots were air-dried at 65°C (Precision Science Company, Chicago, IL) for 48 h and weighed for dry-matter determination. To account for possible soil contamination, all data were converted to an ash-free dry weight (AFDW) basis by ashing samples in a muffle furnace (Blue M Electric Company, Blue Island, IL) at 550 °C for 3 h. The ash weight was subtracted from the original dry weight to determine the total root biomass (g m^{-2}).

Statistical Design and Analysis

Factorial effects were evaluated using analysis of variance with the MIXED procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC). Means separation was accomplished using a pdmix800.sas macro (Saxton, 2000) and Fisher's protected LSD at $P \leq 0.05$. When a cultivar by year interaction did not occur, data collected over the 2-yr study were pooled.

Results and Discussion

Turfgrass Quality

In year I, there was a significant cultivar by mowing height interaction with respect to TQ (Table 2.1). However, mowing height did not have a significant effect on TifEagle or MiniVerde's TQ. The 3.2 mm mowing height reduced MSB-285 TQ 15% compared to the 4.0 mm mowing height. MSB-285 TQ averaged 5.6 and 6.6 respective to 3.2 and 4.0 mm mowing heights. Scalping was observed on MSB-285 plots at the 3.2 mm mowing height, which is similar to previous research (Liu, 2008).

Surface Firmness

In year I, there was significant main effect mean for cultivar with respect to surface firmness (Table 2.1). Higher surface firmness readings equate to a softer surface. In year I, surface firmness was 15% higher for MSB-285 compared to TifEagle and MiniVerde, while in year II, all cultivars were similar.

Ball Roll Distance

In year I, BRD differed due to cultivar by mowing height and cultivar by TE interactions (Table 2.2). MiniVerde BRD did not differ due to mowing height. However, Ball roll distance for MiniVerde mown at 4.0 mm was greater than MSB-285 and TifEagle, regardless of the mowing height or TE rate applied. When the highest rate of TE was applied to MSB-285, only non-TE-treated TifEagle BRD was similar to MSB-285. MiniVerde BRD increased at both rates of TE, while TifEagle's BRD increased from the control to the 0.02 kg ha^{-1} wk^{-1} rate, but did not significantly increase when comparing the 0.02 and 0.05 kg ha^{-1} wk^{-1} rate.

In year II, there was a significant main effect mean for cultivar, mowing height and TE (Table 2.2). MiniVerde BRD was 19 and 13% greater than that of MSB-285 and TifEagle, respectively, while TifEagle BRD was 7% greater than MSB-285. The 3.2 mm mowing height BRD was 9% greater than the 4.0 mm mowing height, which is consistent with results reported by Sorochan (2014). Across all cultivars and mowing heights, TE applied at 0.02 and 0.05 kg ha^{-1} wk^{-1}, BRD was 6 and 10% greater respectively, compared to the control. Results, with respect to BRD for MSB-285, are consistent with the findings of Liu (2008) and the 2013 NTEP trial.

Clipping Yield

In year I, clipping yield differed due to TE by cultivar and cultivar by mowing height interactions (Table 2.3). Compared to the control, TifEagle clipping yield was reduced by 5 times when treated with 0.02 kg a.i. ha^{-1} wk^{-1} TE. This result is consistent with McCullough et al. (2005) which showed that TE applied at < 0.02 kg. ha^{-1} significantly reduced ultradwarf bermudagrass cultivar clipping yield. MSB-285, having the highest clipping yield at all rates, displayed a similar significant reduction in response to TE at both 0.02 and 0.05 kg a.i. ha^{-1} wk^{-1}, when compared to the control. MiniVerde's clipping yield was not significantly reduced by TE in year I.

In year II, there was a significant main effect mean for cultivar, mowing height, and TE (Table 2.3). MSB-285 clipping yield was 7 times greater than TifEagle and MiniVerde. At the 3.2 mm mowing height, 1.5 times more clippings were harvested than the 4.0 mm height, across all cultivars and TE rates. Trinexapac-ethyl applied at 0.02 and 0.05 kg. ha^{-1} wk^{-1} reduced clipping yield by 64% compared to the control.

Shoot Total Non-Structural Carbohydrates

In year I and II, there was a significant main effect mean for mowing height (Table 2.4). Shoot TNC (mg g^{-1}) content for all cultivars mown at 4.0 mm was 2.5 times greater than cultivars mown at 3.2 mm. A reduction in green leaf tissue reduces turfgrass potential to conduct photosynthesis. Therefore, frequent low mowing heights directly correlate to a decrease in carbohydrate reserves (Hull, 1992; McCullough et al., 2007).

Relative Chlorophyll Index

In year I and II, there was a significant main effect mean for mowing height (Table 2.4). Relative chlorophyll index (0-999) for all cultivars mown at 4.0 mm was 7% higher than cultivars mown at 3.2 mm.

Normalized Difference Vegetation Index

In year I and II, there was a significant main effect mean for mowing height (Table 2.4). The NDVI (0-1) rating for all cultivars mown at 4.0 mm was 4% higher than cultivars mown at 3.2 mm. Results from year I and II for NDVI and RCI were similar, indicating that the canopy of the cultivars mown at 4.0 mm contained more chlorophyll per unit leaf area, than cultivars mown at 3.2 mm.

Root Biomass

In year I and II, root biomass differed due to mowing height by TE and cultivar by TE interactions (Table 2.5). MSB-285 root biomass was 2.5 times greater than MiniVerde and TifEagle. As TE rate increased to 0.05 kg ha^{-1} wk^{-1}, MSB-285 root biomass decreased 38% compared to the control. However, MiniVerde and TifEagle root biomass was similar at all TE application rates. MSB-285 treated with 0.05 kg TE ha^{-1} wk^{-1}, root biomass was 2 times greater than MiniVerde and TifEagle. Root biomass of cultivars not treated with TE and mown at 4.0 mm was significantly greater than the 3.2 mm mowing height (no TE) (Table 2.5). With respect to MiniVerde and TifEagle, results are consistent with McCullough et al. (2005) where TE did not have detrimental effects on root growth compared to untreated turfgrass. Higher mowing heights create more leaf surface area which enhances the turfgrass ability to capture more sunlight, which can increase root depth (Hull, 1992).

Conclusion

MSB-285 clipping yield was 7 times greater than MiniVerde and TifEagle. Throughout the study, MSB-285 BRD was shorter than TifEagle and MiniVerde, which corresponds to greater shoot growth than that of other varieties tested. Although TE reduced the clipping yield of MSB-285, clipping yield was similar to non-TE-treated TifEagle and was significantly greater than non-TE-treated MiniVerde. Further research into higher TE application rates and/or the use of different PGR active ingredients and modes of action is warranted to manage MSB-285 clipping yield and to increase BRD. However, data from the 2013 warm-season putting green trial suggests MSB-285 BRD could be comparable to other non-bermudagrass cultivars that are currently used as putting green surfaces today. Further research should be conducted to compare the recuperative capability of MSB-285 in comparison to other bermudagrass cultivars. Further research also should include investigations into the playability of MSB-285 as a golf green.

Table 2.1 Turfgrass quality (1-9) and surface firmness (cm) influenced by three bermudagrass cultivars, two mowing heights, and trinexapac-ethyl at Mississippi State University, Starkville, MS from 1 June to 31 August 2015 and 2016.

Turfgrass Quality (1-9)†						Surface Firmness (cm)‡			
Year 1			Year 2			Year 1		Year 2	
Cultivar	Mowing Height (mm)	(1-9)	Cultivar	Mowing Height (mm)	(1-9)	Cultivar	(cm)	Cultivar	(cm)
MSB-285	3.2	5.6b§	MSB-285	3.2	6.5a	MSB-285	1.19a	MSB-285	1.30a
TifEagle		6.4ab	TifEagle		7.5a	TifEagle	1.04b	TifEagle	1.25a
MiniVerde		6.4ab	MiniVerde		6.5a	MiniVerde	0.99b	MiniVerde	1.17a
MSB-285	4.0	6.6a	MSB-285	4.0	6.7a				
TifEagle		6.7ab	TifEagle		7.3a				
MiniVerde		6.5b	MiniVerde		6.7a				

†Turfgrass quality recorded weekly for 13 weeks and then averaged. 1-9, 1=poorest quality brown turfgrass, 6=minimally acceptable turfgrass, 9=ideal uniform, dense, fine textured, dark green turfgrass.
‡Surface firmness [depth of penetration (cm)] recorded weekly for 13 weeks and then averaged.
§Means within each column followed by the same letter are not significantly different according to Fischer's Protected LSD (P>0.05).

Table 2.2 Ball roll distance (cm) influenced by three bermudagrass cultivars, two mowing heights and trinexapac-ethyl rates at Mississippi State University, Starkville, MS from 1 June to 31 August 2015 and 2016.

Ball Roll Distance (cm)†							
Year 1						Year 2	
Cultivar	Mowing Height (mm)	(cm)	Cultivar	Trinexapac-ethyl (kg ha^{-1})	(cm)	Cultivar	(cm)
MSB-285	3.2	215d‡	MSB-285	0	204e	MSB-285	212c
TifEagle		257b	TifEagle		229de	TifEagle	228b
MiniVerde		280ab	MiniVerde		248cd	MiniVerde	263a
						Mowing height (mm)	
MSB-285	4.0	207e	MSB-285	0.02	212e		
TifEagle		248c	TifEagle		260bc	3.2	245a
MiniVerde		286a	MiniVerde		287b	4.0	224b
						Trinexapac-ethyl (kg. ha^{-1})	
			MSB-285	0.05	218e		
			TifEagle		268bc	0	222c
			MiniVerde		315a	0.02	235b
						0.05	246a

†Average ball roll distance taken at 3, 7 and 11 WAI: Weeks after initiation, using a USGA modified stimpmeter.
‡Means within each column followed by the same letter are not significantly different according to Fischer's Protected LSD (P>0.05).

Table 2.3 Clipping yield (g m^{-2}) influenced by three bermudagrass cultivars, two mowing heights and trinexapac-ethyl rates at Mississippi State University, Starkville, MS from 1 June to 31 August 2015 and 2016

Clipping Yield (g m^{-2}) †							
Year 1						Year 2	
Cultivar	Mowing height (mm)	(g m^{-2})	Cultivar	Trinexapac-ethyl (kg a.i. ha^{-1})	(g m^{-2})	Cultivar	(g m^{-2})
MSB-285	3.2	9.21a‡	MSB-285	0	12.04a	MSB-285	7.29a
TifEagle		2.24c	TifEagle		3.87b	TifEagle	1.78b
MiniVerde		1.57cd	MiniVerde		1.60c	MiniVerde	0.98b
MSB-285	4	5.36b	MSB-285	0.02	5.02b	Mowing height (mm)	
TifEagle		1.33de	TifEagle		0.81c	3.2	4.34a
MiniVerde		0.39e	MiniVerde		0.98c	4	2.36b
			MSB-285	0.05	4.79b	Trinexapac-ethyl (kg a.i. ha^{-1})	
			TifEagle		0.67c	0	5.83a
			MiniVerde		0.36c	0.02	2.27b
						0.05	1.94b

†Average of clipping yield taken from 3, 6 and 11 WAI: Weeks after initiation, using a Toro Greensmaster Flex 2100
‡Means within each column followed by the same letter are not significantly different according to Fischer's Protected LSD ($P>0.05$).

Table 2.4 Shoot total non-structural carbohydrates, relative chlorophyll index, and normalized difference vegetation index impacted by two mowing height regimes when pooled across three bermudagrass putting green cultivars and trinexapac-ethyl rates at Mississippi State University, Starkville, MS from 1 June to 31 August 2015 and 2016.

	Year I and II†	Year I and II	Year I and II
Mowing height (mm)	TNC ($mg\ g^{-1}$)‡	RCI (0-999)§	NDVI (0-1)¶
3.2	0.024b	230.39b	0.67b
4.0	0.066a	248.76a	0.70a

†Average weekly readings from 13 weeks in Year I and Year II

‡TNC, Shoot total non-structural carbohydrates.

§Relative chlorophyll index: recorded weekly for 13 weeks and then averaged (CM 1000; Spectrum Technologies, Aurora, IL).

¶Normalized difference vegetation index: recorded for 13 weeks and then averaged (FieldScout TCM 500 NDVI Turf Color Meter, Spectrum Technologies, Aurora, IL).

Table 2.5 Root biomass (g m^{-2}) influenced by three bermudagrass cultivars, two mowing heights and trinexapac-ethyl rates at Mississippi State University, Starkville, MS from 1 June to 31 August 2015 and 2016.

Root biomass (g m^{-2}) 13 WAI†							
Cultivar	(g m^{-2})	Mowing height (mm)	Trinexapac-ethyl (kg a.i. ha^{-1})	(g m^{-2})	Cultivar	Trinexapac-ethyl (kg a.i ha^{-1})	(g m^{-2})
MSB-285	5.7a‡	3.2	0	3.5b	MSB-285	0	7.4a
TifEagle	2.3b		0.02	3.1b	TifEagle		2.2c
MiniVerde	2.2b		0.05	3.2b	MiniVerde		2.1c
Mowing height (mm)		4	0	4.3a	MSB-285	0.02	5.1b
3.2	3.2a		0.02	3.3ab	TifEagle		2.3c
4	3.5a		0.05	2.9b	MiniVerde		2.2c
Trinexapac-ethyl (kg a.i. ha^{-1})					MSB-285	0.05	4.6b
0	3.8				TifEagle		2.4c
0.02	2.9				MiniVerde		2.2c
0.05	2.1						

†Weeks after initiation: Root biomass pooled from 13 WAI in 2015 and 2016.
‡Means within each column followed by the same letter are not significantly different according to Fischer's Protected LSD (P>0.05).

CHAPTER III

WATER-USE CHARACTERISTICS OF WARM-SEASON PUTTING GREEN CULTIVARS

Introduction

Water is the universal solvent that sustains life in both humans and turfgrass. Due to the widespread need for water, much debate occurs over water resource allocation. In 2013, it was estimated that golf courses in the Southeast used ~ 490,000 kL of water per year (GCSAA, 2015). In 2010, the Alliance for Water Efficiency (AWE) stated that 20-50% of water used on golf courses was unnecessary (AWE, 2010). The AWE concluded that irrigation timing, irrigation uniformity, and the lack of weather-based irrigation controllers are the leading causes of over watering golf course turfgrass (AWE, 2010). Many factors are involved when determining how much water is required on a golf course. Each definable area on a golf course requires its own specific amount of water. Of all the definable areas on a golf course, golf course putting greens consist of the least acreage, but receive the most attention from golfers and golf course superintendents alike. The water-use characteristics of warm-season turfgrass species has been well documented (Qian and Fry, 1997; Duncan and Carrow, 1999; Huang et al., 1997); however, few studies have reported on the water-use characteristics of warm-season putting green cultivars (Rowland et al., 2014)

Zhou et al. (2013) collected bermudagrass (*Cynodon* spp.) genotypes from 4 different climatic regions of Australia. Both commercial cultivars and experimental selections were tested to examine drought resistance. The researchers stated that the commercial cultivars were not the most drought resistant. Regarding the drought resistance of experimental selections from different climatic regions, selections from dry arid regions were not as drought resistant as cultivars collected from the Mediterranean region (dry summers, moist winters). The researchers concluded that cultivars from dry regions never recuperated from drought in their native settings and therefore were not adapted to do so.

Morphological adaptation such as: horizontal leaf growth, slow vertical growth, and high shoot density, have been reported to contribute to a more drought resistant turfgrass (Brian et al., 1981: Kim and Beard, 1988). However, Rowland et al. (2014) reported that morphological adaptation was not the only factor to drought resistance in turfgrass cultivars. Three experiments conducted by Rowland et al. (2014) found that 'SeaDwarf' seashore paspalum (*Paspalum vaginatum* Swartz), and 'PristineFlora' zoysiagrass [*Z. japonica* Stued. by *Z. tenuifolia* (L.) Merr.], were consistently more drought resistant than 'TifEagle' (Hanna and Elsner, 1999), and 'TifDwarf' [*C. dactylon* (L.) Pers. × *C. transvalensis* (Burtt-Davy)] with respect to leaf wilting. Results suggest that root growth and thatch development have more of an impact on drought resistance than the plant's morphological leaf structure (Rowland et al., 2014).

The 2013 National turfgrass evaluation program (NTEP) warm-season putting green trial contains 11 experimental bermudagrass cultivars, 8 experimental zoysiagrass cultivars and 1 experimental seashore paspalum cultivar (www.ntep.org). Previous water-use studies have only investigated industry standard putting green cultivars (Rowland et al., 2014), but further research is needed to explore the water-use characteristics of experimental cultivars. MSB-285, an experimental cultivar developed at Mississippi State University (MSU), is a new interspecific hybrid bermudagrass that was bred and selected by the MSU turfgrass breeding program (Personal communication, Philley, 2015). MSB-285 is phenotypically and genotypically different from the standard ultradwarfs used on putting greens in the Southeast (Personal communication, Harris-Schultz, 2013). Therefore, the objective of this research is to investigate the response of ‘Diamond’ zoysiagrass (*Zoysia marrella* L.), SeaDwarf, TifEagle and MSB-285 bermudagrass when grown under various water regimens.

Materials and Methods

A greenhouse study was conducted at the R. R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS. Study I was conducted from 16 May to 29 June 2016 and study II was conducted from 11 July to 24 Aug. 2016. The greenhouse was cooled using an evaporative cooler and fans (Quietaire, Houston, TX) that were regulated with a QCOM central control system (Temecula, CA). Day/night temperature set points were 30^{o}C/20^{o}C, and the relative humidity in all greenhouses using evaporative coolers was ~ 100%. Although the day/night temperature set points were 30^{o}C/20^{o}C, the evaporative cooler and fans were not able to lower the temperature to those degrees at all times. The average ambient temperature in the greenhouse during times when readings were taken was 34^{o}C for study I and 36.6^{o}C for study II.

Cultivars included: zoysiagrass; Diamond, paspalum; SeaDwarf, and bermudagrasses; TifEagle, and MSB-285.

Two weeks prior to initiation of water regimens, plugs were collected from MSU's turfgrass research plots using a standard 11 cm diameter golf course cup cutter. The roots of each plug were removed below the thatch layer and plugs were thoroughly washed to remove any thatch, debris, or soil (Figure B.4). Plugs were transferred into lysimeters and allowed to establish (Figure B.1).

Three water regimens were created using a combination of drip irrigation and calcined clay (CC) aggregate sizes. An automated drip irrigation system, controlled by a RainBird SST-600i controller (Rainbird, Azusa, CA) and faucet timers (Orbit, Bountiful, UT), were used in conjunction with 0.5 GPH drip stake assemblies (Hummert Int., Earth City, MO) and 1.27 cm Rainbird green-stripe tubing (Rainbird, Azusa, CA). Soil moisture content was measured using a Decagon EM50G wireless cellular data logger (m^3 m^{-3}) and 3 5TM Soil moisture sensors (Decagon, Pullman, WA). Soil moisture sensors were placed in one lysimeter in each water regimen with data logged every 4 h. The three water regimens consisted of a 0.31, 0.15, and 0.05 m^3 m^{-3} water volumes (Figure B.3). All plants were grown in PVC lysimeters (35 cm tall / 10 cm in diameter) that were packed with CC aggregates. The 0.31 m^3 m^{-3} regimen growth medium consisted of Axis Ceramic 30/60 CC (0.595 / 0.250 mm) (EP Minerals, Middleton, TN). The 0.15 m^3 m^{-3} regimen consisted of Red Diamond RBI 10/20 CC (2.00 / 0.841 mm) [Southern Athletic Fields (SAF), Columbia, TN]. The 0.05 m^3 m^{-3} regimen consisted of 516 Mule Mix 6/20 CC (3.36 / 0.841 mm) (SAF). The 0.31 m^3 m^{-3} regimen reached equilibrium 5 days after initiation (DAI) of water regimens, while the 0.15 and 0.05 m^3 m^{-3} regimens reached equilibrium at 4 DAI (Figure B.3).

Response variables measured included leaf firing (LF), normalized difference vegetation index (NDVI), relative chlorophyll index (RCI), canopy temperature, osmotic adjustment (OA), root biomass, root length density (RLD), and specific root length (SRL).

Weekly LF was visually rated using a 0-100% scale, where 0% represents 0% desiccation, and 100% represents complete desiccation.

Normalized difference vegetation index was measured with a Field Scout Turf Color Meter (TCM) 500 "NDVI" (Spectrum Technologies, Inc., Aurora, IL) at turf canopy level. The TCM500, wave bands are 660 nm (± 5 nm) and 850 nm (± 5 nm) and the output is in NDVI based on the following: NDVI = (NIR-RED) / (NIR+RED) (Personal communication, Sayre, 2017). One NDVI value was recorded (per lysimeter) weekly.

Relative chlorophyll index, using a FieldScout CM1000 (Spectrum Technologies Inc., Aurora, IL), was recorded weekly. CM1000 Chlorophyll meter (Model 2950), wave bands are 700 nm (red) and 840 nm (NIR) and receptors include 4 photodiodes, 2 for ambient light and 2 for reflected light from the sample (turf, leaf). With an output scale of 0 to 999 for relative chlorophyll content index (RCI) the equation is as follows: Index=[(S840/A840)/(S700/A700)]*1000 with S = sensor and A = ambient light. (Personal communication, Sayre, 2017). Two readings were recorded weekly by holding the CM1000 ~ 30 cm above the turf canopy facing east and west. The two readings were averaged to represent the RCI for each lysimeter.

Canopy temperatures were taken weekly using an infrared thermometer (Everest InterSci. Inc., Chino Hills, CA) held ~3 cm above the turfgrass surface. Once all readings were taken, an ambient temperature was obtained. The ambient temperature was obtained by holding the thermometer ~ 50 cm above the lysimeters while the thermometer was in ambient mode. Before analyzing canopy temperature data, the ambient temperature from each lysimeter was subtracted from the canopy temperature resulting in a number that represented the difference in canopy temperature and ambient. When the resulting number was negative, it represented a canopy temperature that is lower than the ambient temperature.

Osmotic adjustment was collected 4 weeks after initiation (WAI) by measuring the osmotic potential of leaf sap at full turgor. Leaf tissue (200 mg) was submerged in deionized water and placed in a refrigerator at 4^{o}C for 12 h to hydrate the plant tissue. Samples were shaken/blotted dry by using a reusable k-cup (Keurig, Reading, MA). Leaf tissue was transferred to a 2-mL centrifuge tube and flash frozen in liquid nitrogen. Leaf tissue was then ground in a micro-pestle to express plant sap. Plant sap (10 µL) was inserted into a vapor pressure osmometer (Wescor, ELITech, Princeton, NJ) to determine the osmolality (mmol kg^{-1}). Osmotic potential was calculated using the formula: osmotic potential = – ([osmolality][0.001][2058]) (Blum, 1989; Burgess and Huang, 2014).

For rooting parameters, plants were excavated from their respective lysimeters and washed free of all debris and CC on a 1-mm sieve. Roots were clipped from the shoot base and then carefully washed again (Figure B.2). Root response variables (RLD and SRL) were measured by positioning roots on a 30 cm by 20 cm Plexiglas® [poly (methyl methacrylate)] tray with 5 mm of water. The roots were scanned with a flatbed scanner (EU-88, EPSON, Nagano, Japan) (resolution 800 by 800 dpi) and saved in grayscale as a .tiff file. Root images were then analyzed using WinRHIZO (Pro Version 2005; Regent

Instruments Inc., Quebec, QC). WinRHIZO is a system that includes digital image analysis software and the capability of integrating a scanner to measure RLD, total root length (mm) per volume of soil (cm^3), and is described at (http://regent.qc.ca/assets/winrhizo_software.html). To calculate SRL, a ratio of root length to root dry weight was calculated to determine the amount of root length per grams of dry weight (cm g^{-1}). Roots were air-dried at 65°C (Precision Science Company, Chicago, IL) for 48 h and weighed for dry-matter determination. To account for possible soil contamination, all data were converted to an ash-free dry weight basis by ashing samples in a muffle furnace (Blue M Electric Company, Blue Island, IL) at 550 °C for 3 h. The ash weight was subtracted from the original dry weight to determine the total root biomass (g m^{-2}).

Statistical Design and Analysis

The orientation of the greenhouse benches created 3 lines of lysimeters from the back (closest to the evaporative cooler) to front (closest to fans and door). Each of the three water regimens in both studies involved a separate randomized complete block design, with three blocks of four cultivars in each regimen (Figure B.1). The General Linear Model procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC) was used to compare cultivars within each water regimen. Means were separated using Fisher's protected LSD at $P \leq 0.05$, and when a cultivar by study interaction was not observed ($P > 0.05$), data was pooled across studies.

Results and Discussion

0.05 m^3 m^{-3} Regimen

At 2 WAI, TifEagle LF was 32% which was 5 and 3 times higher than MSB-285 and SeaDwarf, respectively (Table 3.1). At 4 WAI, Diamond and TifEagle LF was 2 times higher than SeaDwarf and MSB-285. At 6 WAI, SeaDwarf and TifEagle LF was 2 times higher than Diamond and MSB-285. At 6 WAI, Diamond, MSB-285, SeaDwarf and TifEagle LF was 36%, 24%, 68%, and 64%, respectively. Results from 2 WAI are consistent with Rowland et al. (2014) who observed less leaf wilting (rolled and fired leaf tissue) at 2 WAI of a 50% ET water regimen from SeaDwarf compared to TifEagle.

At 2 and 4 WAI, MSB-285 NDVI was 25% and 28% higher than Diamond and TifEagle, respectively, while SeaDwarf NDVI was 23% higher than TifEagle (Table 3.1). The NDVI rating at 2 and 4 WAI was consistent with LF at 2 and 4 WAI with respect to MSB-285 and SeaDwarf compared to TifEagle. Results, with respect to SeaDwarf and TifEagle, NDVI is consistent with Rowland et al. (2014) where the NDVI of SeaDwarf in 2010 was higher than TifEagle.

At 2 WAI, SeaDwarf RCI was 31% higher than that of TifEagle, which is consistent with the 31% higher LF observed in TifEagle compared to SeaDwarf at 2 WAI (Table 3.1). At 4 WAI, MSB-285 RCI was 26% higher than Diamond and TifEagle. This result is consistent with LF taken at 4 WAI, where MSB-285 LF was 65% less than TifEagle. At 6 WAI, MSB-285 RCI was 26% higher than Diamond and TifEagle, while SeaDwarf was similar to all cultivars.

Canopy temperatures recorded weekly did not differ between cultivars (data not shown). However, the average canopy temperature of all cultivars growing in the 0.05 m^3

m^{-3} regimen was 14C° above the ambient air temperature at 6 WAI (data not shown). The higher canopy temperatures measured are consistent with Heijl (2015), who observed higher canopy temperatures in Tifway bermudagrass that was irrigated at a deficient level (0.3 x ET). The high canopy temperatures measured in all plants suggests the 0.05 m^3 m^{-3} volumetric water content was effective at creating drought stress (Leksungnoen et al., 2012). Physical forces move water from the soil, through the plant, and into the atmosphere. Water that moves through the plant is not only used for photosynthesis but also acts as a coolant. The lack of water movement through the plant removes the cooling effect of water and can cause increased canopy temperatures (Leksungnoen et al., 2012; Kopp and Jiang, 2013).

At 4 WAI, Diamond OA was lower than all other cultivars (Table 3.1). Osmotic adjustment aids in maintaining turgor pressure and the ability to retain water in the leaf (Burgess and Huang, 2014). Qian and Fry (1997) noted a correlation between turfgrass recovery from drought stress with higher OA levels. Jiang and Huang (2001) stated Kentucky bluegrass preconditioned by means of drought stress OA levels were higher than the non-preconditioned control.

MSB-285 root biomass was 2.5 and 3.4 times greater than TifEagle and Diamond, respectively (Table 3.1). In contrast, SeaDwarf and MSB-285's root biomass was similar. Rowland et al. (2014) found similar results in rooting parameters of SeaDwarf in comparison to TifEagle and PristineFlora. MSB-285 and SeaDwarf RLD was 1.5 times higher than Diamond and TifEagle. An extensive root zone with a high RLD provides buffering capacity to the plant during stress adaptation. Therefore, turfgrasses with a high RLD have better survivability when intensely managed (Duncan and Carrow, 1999).

Baldwin et al. (2009) found similar results where RLD measurements for Sea Isle 2000 (*Paspalum vaginatum* Swartz) were significantly higher than Diamond when grown in a greenhouse. Diamond and TifEagle SRL was 1.5 times higher than MSB-285 and SeaDwarf. Plants with a higher SRL have more root length per given dry biomass and are generally seen as having higher rates of water and nutrient uptake (Perez-Harguindeguy et al., 2013). However, Perez-Harguindeguy et al. (2013) stated that a high SRL can be the result of a low diameter in the roots and a low tissue density. MSB-285 and SeaDwarf root diameter was 25% greater than Diamond and TifEagle. The smaller root diameter produced by Diamond and TifEagle could be the reason for the lower SRL (Harguindeguy et al., 2013).

0.15 m^3 m^{-3} Regimen

At 2 WAI, TifEagle NDVI was 7% higher than Diamond, while MSB-285 and SeaDwarf NDVI was similar to Diamond and TifEagle (Table 3.2). At 2 and 4 WAI, all cultivars were similar with respect to NDVI.

At 6 WAI, Diamond RCI was 17 and 21% greater than MSB-285 and TifEagle. Meanwhile, SeaDwarf's RCI was similar to all cultivars (Table 3.2). Rowland et al. (2014) concluded that TifEagle and SeaDwarf RCI was higher than PristineFlora and TifDwarf when watered at 100% ET.

0.31 m^3 m^{-3} Regimen

At 2 and 4 WAI, SeaDwarf's RCI was 12 % higher than Diamond. Similarly, at 2, 4 and 6 WAI, SeaDwarf RCI was higher than TifEagle (Table 3.3). The higher RCI for SeaDwarf suggests it is more tolerant than Diamond and TifEagle to saturated conditions.

Zong et al. (2015) found that the shoot and root biomass of SeaDwarf was reduced after 30 d of waterlogged (over saturated) conditions.

Conclusion

Although Diamond was unable to produce a RLD or biomass similar to MSB-285 or SeaDwarf, it was able to grow under the 0.05 m^3 m^{-3} water regimen while producing significantly less LF at 6 WAI than SeaDwarf and TifEagle. Response variables measured suggest similarities between SeaDwarf and MSB-285, with the most prevalent being rooting parameters. Rowland et al. (2014) concluded that the superior drought resistance of SeaDwarf was due in part to its dense thatch layer and deep rooting. Further study should be conducted to conclude that MSB-285's deep rooting could make it a superior turfgrass while under drought stress. Results indicate that MSB-285 is able to withstand growing in a 0.05 m^3 m^{-3} water regimen more efficiently than the ultradwarf TifEagle. Future research is needed to quantify the exact amount of water needed to sustain a high quality warm-season putting green surface.

Table 3.1 Leaf firing, relative chlorophyll index, normalized difference vegetation index (NDVI), osmotic adjustment, root biomass, root length density, and specific root length of warm-season putting green cultivars grown under a 0.05 m^3 m^{-3} water regimen at the R.R. Foil Plant Science Research Center greenhouse in Starkville, MS from May 16, 2016 to June 29, 2016 and July 11, 2016 to August 24, 2016.

	Leaf Firing (%) [†]			Relative Chlorophyll Index (0-999)[‡]			
Cultivar[§]	2 WAI[¶]	4 WAI	6 WAI	Cultivar	2 WAI	4 WAI	6 WAI
Diamond	25.8ab[#]	34.2a	35.8b	Diamond	147ab	125b	117b
MSB-285	6.7b	13.3c	24.2b	MSB-285	175ab	170a	159a
SeaDwarf	10.0b	17.5bc	67.5a	SeaDwarf	192a	149ab	136ab
TifEagle	32.0a	40.8a	64.2a	TifEagle	133b	128b	119b
LSD	20.51	19.55	26.95	LSD	26.6	20.7	33.7

	NDVI (0-1 scale) [††]			Osmotic Adjustment (MPa)
	2 WAI	4 WAI	6 WAI	4 WAI
Diamond	0.51bc	0.51bc	0.47	0.53b
MSB-285	0.68a	0.65a	0.58	0.83a
SeaDwarf	0.64ab	0.62ab	0.53	0.77a
TifEagle	0.49c	0.46c	0.49	0.90a
LSD	0.137	0.127	NS[‡‡]	0.232

Table 3.1 (Continued)

	Root Biomass ($g\ m^{-2}$)	Root Length Density ($mm\ cm^{-3}$)	Specific Root Length ($cm\ g^{-1}$)	Root Diameter (mm)
Cultivar	6 WAI	6 WAI	6 WAI	6 WAI
Diamond	2.2b	14.38b	2312a	0.23b
MSB-285	7.4a	25.96a	1224b	0.33a
SeaDwarf	6.5a	24.44a	1312b	0.31a
TifEagle	3.0b	16.63b	1945a	0.25b
LSD	2.44	5.969	577.3	0.039

†Leaf firing: Percentage of visual desiccation to the entire plant.
‡Chlorophyll rated using a CM 1000 chlorophyll meter based on a scale of 0-999
§Bermudagrass cultivars: MSB-285, TifEagle. Zoysiagrass cultivar: Diamond. Paspalum cultivar: SeaDwarf Seashore Paspalum
¶WAI, weeks after initiation
#Means within each column followed by the same letter are not significantly different according to Fischer's Protected LSD (P>0.05).
††NDVI, normalized difference vegetation index, 0 to 1 scale
‡‡NS: Not Significant

Table 3.2 Normalized difference vegetation index, and relative chlorophyll index of warm-season putting green cultivars grown under a 0.15 m^3 m^{-3} water regimen at the R.R. Foil Plant Science Research Center greenhouse in Starkville, MS from May 16, 2016 to June 29, 2016 and July 11, 2016 to August 24, 2016.

	NDVI [†]				Relative Chlorophyll Index (0-999)[‡]		
Cultivar[§]	2 WAI[¶]	4 WAI	6 WAI	Cultivar	2 WAI	4 WAI	6 WAI
Diamond	0.68b[#]	0.67	0.65	Diamond	267	203	195a
MSB-285	0.71ab	0.66	0.58	MSB-285	252	187	162b
SeaDwarf	0.71ab	0.66	0.61	SeaDwarf	278	194	164ab
TifEagle	0.73a	0.65	0.57	TifEagle	275	180	155b
LSD	0.044	NS[††]	NS	LSD	NS	NS	31.6

[†]NDVI, normalized difference vegetation index, 0 to 1 scale
[‡]Chlorophyll rated using a CM 1000 chlorophyll meter based on a scale of 0-999
[§]Bermudagrass cultivars: MSB-285, TifEagle. Zoysiagrass cultivar: Diamond. Paspalum cultivar: SeaDwarf Seashore Paspalum
[¶]WAI, weeks after initiation
[#]Means within each column followed by the same letter are not significantly different according to Fischer's Protected LSD ($P>0.05$).
[††]NS: Not Significant

Table 3.3 Relative chlorophyll index of warm-season putting green cultivars grown under a 0.31 m^3 m^{-3} water at the R.R. Foil Plant Science Research Center greenhouse in Starkville, MS from May 16, 2016 to June 29, 2016 and July 11, 2016 to August 24, 2016.

	Relative Chlorophyll Index (0-999) †		
Cultivar‡	2 WAI§	4 WAI	6 WAI
Diamond	165b¶	156b	160ab
MSB-285	198ab	186ab	153ab
SeaDwarf	226a	213a	182a
TifEagle	170b	146b	141b
LSD	43.7	48.3	40.2

†Chlorophyll rated using a CM 1000 chlorophyll meter based on a scale of 0-999

‡Bermudagrass cultivars: MSB-285, TifEagle. Zoysiagrass cultivar: Diamond. Paspalum cultivar: SeaDwarf Seashore Paspalum

§WAI, weeks after initiation

¶Means within each column followed by the same letter are not significantly different according to Fischer's Protected LSD (P>0.05).

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APPENDIX A

R.R. FOIL PLANT SCIENCE RESEARCH CENTER FIELD PLOTS IN STARKVILLE, MS FROM 1 JUNE TO 31 AUGUST 2015 AND 2016.

Figure A.1 Field Plots: 16 Sept. 2015

9 randomized plots; MSB-285, TifEagle, MiniVerde; 3 TE application rates.

Figure A.2 Field plots 20 Nov. 2015

Extended fall color of MSB-285; Front, Middle Right, Back Left

Figure A.3 MSB-285, upright growth habit.

The growth habit of MSB-285 compared to the ultradwarf bermudagrass, Champion.

Figure A.4 MSB-285 fall/winter color

MSB-285 (front), in comparison to MiniVerde (middle). Picture taken: 9 Dec. 2014, at the R. R. Foil Plant Science Research Center, Starkville, MS.

Figure A.5 MSB-285, Spring Color

MSB-285 (middle / right), spring color. Picture taken: 11 Apr. 2016, at the R. R. Foil Plant Science Research Center, Starkville, MS.

APPENDIX B

R.R. FOIL PLANT SCIENCE RESEARCH CENTER GREENHOUSE, STARKVILLE, MS FROM MAY 16, 2016 TO JUNE 29, 2016 AND JULY 11, 2016 TO AUGUST 24, 2016.

Figure B.1 Left: 0.31 m^3 m^{-3} Regimen. Middle: 0.15 m^3 m^{-3} Regimen. Right: 0.05 m^3 m^{-3} Regimen.

Three blocks of four cultivars in each regimen.

Figure B.2 Picture taken: 6 WAI from 0.05 m^3 m^{-3} regimen.

From left, to right: TifEagle, SeaDwarf, MSB-285, Diamond

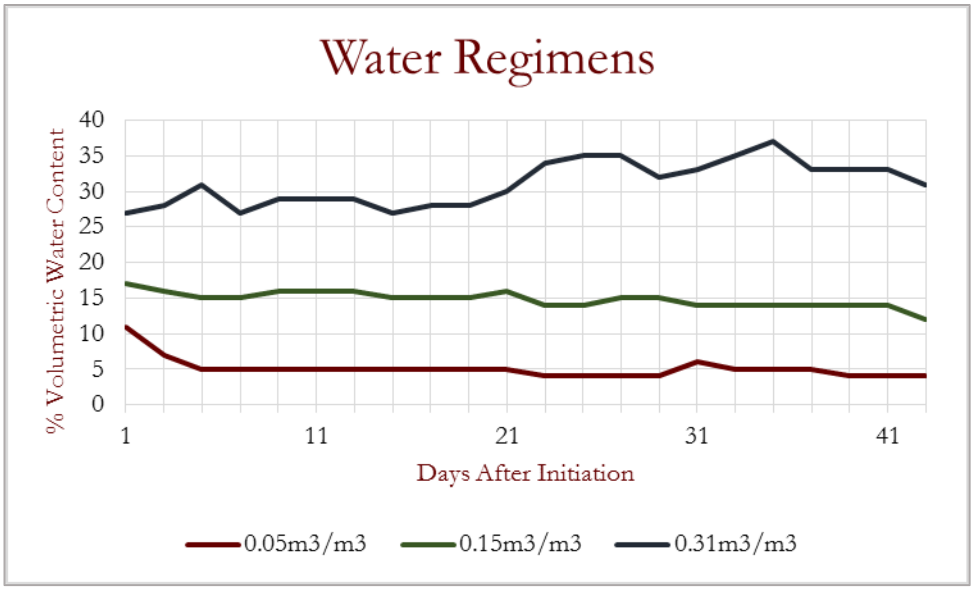

Figure B.3 Three water regimens (m^3 m^{-3})

Graphed results from Decagon EM50G wireless cellular data logger (m^3 m^{-3}) and 3 5TM Soil moisture sensors (Decagon, Pullman, WA).

Figure B.4 Cleaning station at R.R. Plant Science Research Center, Starkville, MS.

Greenhouse, mesh-sieve washing station and sun shade.

Figure B.5 MSB-285 at 6 WAI of 0.31 m^3 m^{-3} Regimen

Blue-green algae (cyanobacteria) build up within turfgrass canopy.