ROOT CHARACTERISTICS OF WARM SEASON TURFGRASS SPECIES UNDER LIMITED SOIL WATER AND VARYING MOWING HEIGHTS

By

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Dedicated to my family and all the earthquake victims of Nepal

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TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CB	Common Bermudagrass
MRD	Maximum Root Depth
RDW	Root Dry Weight
RL	Root Length
RP	Root Proportion
RSA	Root Surface Area
SRL	Specific Root Length
SRSA	Specific Root Surface Area
STA	St. Augustinegrass
TRL	Total Root Length
TRSA	Total Root Surface Area
ZJ	*Zoysia japonica*
ZM	*Zoysia matrella*

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A greenhouse (GH) and a field drought (FD) study were conducted at the University of Florida in July 2012 and September 2013 (GH studies) and May 2013/14 (field studies) to examine the rooting characteristics of several turfgrass species under water limited (DD) conditions. Sixteen warm season turfgrass genotypes of bermudagrass (CB), St. Augustinegrass (ST), *Zoysia japonica* (ZJ) and *Zoysia matrella* (ZM) from commercial sources and the UF turfgrass breeding program were studied. The objective of these studies were to examine root architectural differences between species under DD. Partial dry down was imposed in the GH whereas irrigation was halted for 21 days in the FD. In the GH, the root surface area (TRSA), fine root length (FRL), and maximum root depth (MRD) were higher for ST and CB species and BA336 (ZM) and 5269-24 (ZJ) genotypes within respective species. In the FD, CB had more uniform root distribution at lower soil depths approximate (47-48%) in comparison to ST (19-29%), ZM (6-10%) and ZJ (7-11%) species. Overall in both study years at the 30-90 cm depth, CB had higher TRL, TRSA, FRL, and RP during DD in comparison to ST, ZM

and ZJ species. In another study (January-November, 2011 and January-December 2012/2013), the effect of mowing height on seasonal rooting variation of ‘Floratam’ and 2012/2013 for ’Tifway’ were evaluated. For Tifway, the 6.4 cm mowing height produced greater root growth compared to a 3.8 cm mowing height in 2012. In 2013, 1.3 and 3.8 cm had less root growth compared to 6.4 cm mowing height. For Floratam, the 10.0 cm mowing height produced greater root growth compared to the 5.0 cm mowing height. However, 7.6 cm and 10.0 cm as well as 7.6 m and 5 cm mowing height had similar root growth relative to time of the year. Information on differences between species under DD could be utilized as an approach for reduced water use in landscape turf. Similarly, the knowledge of seasonal variation in root growth as affected by different mowing heights could be utilized for fertilizer and irrigation management of Florida’s home lawns.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

U.S. Turfgrass Industry

The United States (US) turfgrass sod industry exceeds one billion dollars annually in turf sales alone (USDA census, 2002; 2007; 2012), but is often overlooked as an agricultural commodity. According to the the U.S. Department of Agriculture National Agricultural Statistics Service (NASS, 2012) reports, there are approximately 1,739 U.S. turfgrass sod farms in the US. Overall, the turfgrass and green industry (sod, landscape and golf courses) in the U.S. generates approximately $147 billion dollars in annual sales and provides employment to more than 1 million people (NASS, 2012). For Florida, the total turfgrass related revenues (sod, landscape mowing, management, golf courses, sports fields etc.) in 2007 were estimated to be approximately $6.26 billion; approximately a 6% increase in sod production and a 35% increase in overall sod sales over those amounts in 2002 (USDA census, 2002; 2007; Hodges and Stevens, 2010). In 2007, the state of Florida sold $175,945,432 worth of sod (USDA census, 2007), and in 2012 the sod sales were $170,944,240 (USDA census, 2012). Florida is responsible for roughly 12% of the total revenue from the U.S. sod industry.

The economic strength of the turfgrass industry justifies the importance of turfgrass research. But the high freshwater consumption of turf has always been an issue and is one of the major factors restricting expanded usage of turf in the U.S. An option to minimize the loss of fresh water consumption by turf in urban areas is the use of recycled water or grey water on golf courses and parks (Sammon, 2007). The National Gardening Association (NGA, 2013) estimates at least 25 million hectares of lawns are managed and irrigated with freshwater. In 2013, U.S. households spent $29.5

billion for home lawn management. Americans use as much as 320 gallons of water everyday of which 30% is directed towards landscape irrigation (EPA).

Erratic rainfall and long drought periods are other serious concerns that limit turfgrass water use and can have significant effects on turfgrass growth and development. Drought has serious impacts on growth and quality of turfgrasses (Beard, 1973) and has resulted in watering restrictions and bans on lawns, landscapes, and golf courses. Restriction of freshwater use in home lawns is enforced by water management districts in the state of Florida. Typically, home lawns can only be irrigated twice a week (SFWMD: South Florida Water Management Districts). In addition, in other states (Nevada and Arizona, for example) there are restrictions on planting turf altogether, including cash rebates from state governments for replacing or substituting turfgrass in home lawns with xeric plants (Sammon, 2007). Moreover, soil variability, climatic differences, and variation within turfgrass species with respect to drought resistance can affect the response of turf to water limited conditions. Inclusion of drought resistant turfgrasses would be a possible strategy to cope with water restriction mandates and drought conditions. Turfgrass water use could be reduced through inclusion of drought resistant turf in Florida home lawns. Research that emphasizes selection and evaluation of turfgrass species for root and canopy responses under drought conditions should be given high priority.

Warm-Season Turfgrass Species

Warm-season grasses are well adapted to Florida's warm and humid climate and common genera used as turfgrass include *Stenotaphrum, Cynodon, Zoysia, Paspalum* and *Eremochloa* (Busey, 1989). Warm season turfgrass species that are widely cultivated in Florida are primarily C_4 grasses (Trenholm et al., 2011). C_4 photosynthesis is thought to have evolved independently in grass and sedge families (Christin et al., 2007). The first carbon product formed during C_4 photosynthesis is a four carbon compound (oxaloacetate), thus they are called C_4 plants (Edward et. al., 2004).

Warm-season grass is preferred in Florida because of its green dense turf, quicker establishment, and ease of planting through sod, sprigs or plugs (Trenholm et al., 2011). Warm-season grass is also water-use efficient and performs well under water and fertilizer limited environments (Trenholm et al., 2011). There are a number of warm-season turgrass species utilized in the southern U.S. including St. Augustinegrass [*Stenotaphrum secundatum* (Waltz.) Kuntze], bahiagrass [*Paspalum notatum* Flugge], bermudagrass [*Cynodon dactylon* (L.) Pers], zoysiagrass [*Zoysia japonica* (Steud) and *Z. matrella* (L.) Merr], carpetgrass [*Axonopus fissifolius* (Raddi) Kuhlm], seashore paspalum [*Paspalum vaginatum* Swartz] and centipedegrass [*Ermochloa ophiruoides* (Munro)]. Among them, the species of the most significant commercial value in the southern U.S. are St. Augustinegrass, bermudagrass, bahiagrass and zoysiagrass. The primary use of these grasses differs with preference (home lawn, golf course, road side turf, pasture etc.) as they vary greatly in their establishment, growth rates, appearance and management. St. Augustinegrass is preferred in residential and commercial landscapes, bermudagrass in golf course putting greens, and zoysiagrass in both landscape and golf locations. The following are brief descriptions of the characteristics of the primary species utilized in Florida home lawns.

St. Augustinegrass

St. Augustinegrass is the primary home lawn species used for turf in the southeastern U.S. partially because it can acclimate to various soil and environmental conditions. The species is native to coastal regions of the Mediterranean and the Gulf of

Mexico (Trenholm et al., 2014). The first reported case of St. Augustinegrass used as a Florida home lawn was in 1880. Since that time, the cultivar was named in the 1920's and the first cultivar of known parentage was released in 1973 (Busey, 1995). St. Augustinegrass has five different ploidy levels: diploid, triploid, aneuploidy, tetraploid, and hexaploid with chromosome numbers ranging from 2n=2x=18 to 2n=6x=54 (Lewis et al., 2012). 'Floratam', a jointly released cultivar from the University of Florida and Texas A&M University in 1973, is the most widely used cultivar in Florida. 'Floratam' is coarse textured and resistant to drought but susceptible to cold. The cultivar is also susceptible to the polyploid damaging population (PDP) of southern chinch bug (Busey, 1993), and grey leaf spot (Trenholm et al., 2014).

Bermudagrass

Bermudagrass is a warm season perennial turfgrass species best adapted to tropical and subtropical climates and cultivated in many parts of the world for forage, but is also used in sports fields, golf courses, and home lawns (Taliaferro et al., 2004). Bermudagrass is native to Africa and Asia, has spread to different parts of the world (de Wet and Harlan, 1970; de Wet and Harlan, 1971) is now cultivated in more than 100 countries. There are two species of bermudagrass that are important in turf, common bermudagrass (*Cynodon dactylon* var *dactylon* (L.) Pers) and African bermudagrass (*Cynodon transvalensis* Burtt-Davy). Both species are self-incompatible. African bermudagrass is a diploid (2n=2x=18) with two floral branches per inflorescence; whereas common bermudagrass is a tetraploid (2n=4x=36) with 3-5 branches per inflorescence. The most commonly used improved bermudagrasses for turf are sterile, triploid hybrids (*C. dactylon* × *C. transvaalensis*) between common bermudagrass and African bermudagrass. Compared to other species, bermudagrass exhibits good drought tolerance (Qian and Fry, 1997), and therefore many breeding programs focus on selection of drought resistant bermudagrass cultivars for the southern U.S. (Huang, 2004).

Zoysiagrass

Another group of species often used in home lawn is Zoysiagrass (*Zoysia spp.*), a warm season grass native to Asia (Brosnan and Deputy, 2008). Zoysiagrasses are low maintenance grasses which require less fertilizer and have superior turf quality. Zoysiagrasses are tetraploid (2n=2x=40) and self-fertile. The two most important species of zoysiagrass used for turf are the coarse textured *Zoysia japonica* (Steud) and the fine textured *Zoysia matrella* (L.) Merr (Patton and Trappe, 2010, Qian and Fry, 1996). Zoysiagrass can be grown in latitudes ranging from 40° to 26° in the southern U.S. with *Z. japonica* being the most commonly grown (McCarty, 1995). Zoysiagrass is a dense, weed resistant turf, but has poor sod establishment and is susceptible to scalp damage. Current breeding programs in *Zoysia* are focused on selecting germplasm suited for golf courses and home lawns, with an emphasis on drought resistance.

Water Stress Physiology for Warm-Season Turfgrass

Water stress is a primary constraint for turfgrass growth and development (Carrow, 1996). Adequate soil water must be maintained in order to sustain growth, maintain proper shoot density and produce acceptable turf quality (Taliaferro, 2003; Taliaferro, 1995). Warm season turfgrass species are able to withstand drought effectively, but their ability to maintain growth and development under reduced or non-irrigated conditions is desirable (McCarty and Miller, 2002).

Water stress in turf could be triggered due to incidents such as uneven and/or inadequate precipitation, or rapid drainage on coarse soils (Zhou et al., 2014). Water deficits often result in reduced leaf area, slow leaf development, slow internode elongation and thus overall reduced growth (Taliaferro et al., 2004). Stress response in turfgrass varies as drought proceeds, resulting in mild or severe water stress conditions. Desired responses to drought include an ability to maintain photosynthetic activity with reduced leaf firing characteristics (Huang and Gao, 2000). Water stress primarily affects turf quality (TQ); TQ in general relates to texture, color, cover, uniformity and density (Huang et al., 1998; Jiang and Huang, 2000; Zhang, 2014). In general, drought response in turf initiates with wilting, then progresses towards leaf firing and chlorosis, and eventually leaf necrosis if drought symptoms worsen (Zhang, 2014; Poudel, 2010; Su et al., 2008; Thapa, 2010; Fuentealba, 2010). Moreover, the most common identifiable shoot characteristics in turf in response to drought includes less shoot biomass production, higher canopy temperatures, reduced photosynthetic activity, leaf wilting, leaf firing, and reduced TQ (Carrow, 1996; Su et al., 2013;Taliaferro et al., 2004).

Water Stress Resistance Mechanisms

Plants survive water stress through drought resistance. Drought tolerance and drought avoidance mechanisms are two major strategies involved in drought resistance of plants (Levitt, 1980). During water stress, turfgrass species might adopt one strategy over the other, or both drought avoidance and tolerance mechanisms could be utilized to sustain growth and physiological performance under drought (Huang, 2008). Drought resistance mechanisms include drought avoidance, drought tolerance and escape (Yue et al., 2006; Kim and Beard, 1988).

Drought avoidance mechanisms include improved water uptake via deeper and extensive root systems (Beard, 1989). Increased root numbers with deeper roots and higher root: shoot ratios in turf were associated with increased water uptake that resulted in improved drought avoidance characteristics (Karcher et al., 2008, Levitt, 1980). Turfgrasses capable of extraction of water via extensive root systems from deeper soil depths are also better capable of avoiding drought in comparison to shallow rooted turf (Boeker, 1974; Madison, 1971). Similarly, drought resistance through tolerance in turf is facilitated by accumulation of solutes during the process of osmotic adjustment (Qian and Fry, 1997). Osmotic adjustment (OA) is the accumulation of cellular solutes such as proline and salts in plant tissues. The process of OA helps maintain cellular turgor pressure that could help sustain cell elongation during water stress (Man et al., 2011). Moreover, drought escape via a short life cycle or developmental plasticity is another important strategy utilized by turf for drought resistance (Richardson et al., 2008). Drought escape in turf is primarily relevant in regions where rainfall is erratic and/or without irrigation. In this scenario escape becomes a viable alternative strategy for turf survival (Richardson et al., 2008). A drought escape strategy in turf facilitates turf growth and development when water is in available and initiates dormancy during a period of severe drought (Kramer, 1980). Thus, turfgrass drought resistance mechanisms might vary from one strategy over the other; i.e. production of deeper and more extensive root systems, osmotic adjustment, and/or special shoot characteristics that help reduce evapotranspiration losses (Qian and Fry, 1997; Huang et al., 1997b).

Water Stress and Turfgrass Performance Ranking

Understanding genetic variation between or within species for shoot and root morphological characteristics to rank species' performance under drought could be an important method in developing drought resistant turfgrass cultivars. Previous studies have documented canopy responses as prominent parameters to quantify drought resistance or susceptibility differences between turfgrass species (Huang et al., 1997b; Severmutlu et al., 2011). Extensive field research conducted by Huang et al. (1997b) documented turfgrass differences in the response of canopies of different species under 30 days of field drought. Four turfgrass species ((common bermudagrass, ‘TifBlair’ centipedegrass, seashore paspalum (four ecotypes), and ‘Emerald’ hybrid zoysiagrass)) were evaluated in the study to quantify the differences between species for their field drought performance. Species were ranked based on differences in canopy temperature and relative leaf water content in the order of Seashore paspalum (PI 509018) = TifBlair centipedegrass > common bermudagrass = Emerald zoysiagrass. This study suggests that, during drought, some species experience excessive canopy temperatures, indicating variability among turfgrass species in transpirational cooling (Huang et al., 1997b).

Similar results in turf were reported by Steinke et al. (2009), where four warm season turfgrass species (bermudagrass, St. Augustinegrass, and two zoysiagrass species) were evaluated for their field drought performance for 60 days. Steinke et al. (2009) reported that zoysiagrass canopy temperatures were significantly warmer than those of bermudagrass and St. Augustinegrass. On average, zoysiagrass exhibited 3 to 9 °C and 3 to 13 °C warmer canopy temperatures than St. Augustinegrass and bermudagrass, respectively.

Differences between genotypes for transpiration rate in response to soil drying was reported in Fuentealba (2010). In the study, genotype response to soil drying was reported with early or late decline of transpiration rate during the dry down. Genotypic differences for break point (BP: differences in the available soil water at which stomata begin to close) was also reported. Most of the zoysiagrass genotypes in the study maintained their transpiration rate longer into the drying cycle than bermudagrass and St. Augustinegrass. The normal transpiration rate for most bermudagrass genotypes was inherently higher, resulting in a more rapid depletion of soil moisture. ‘Floratam’ St. Augustinegrass exhibited properties that relate to an ability to tolerate a prolonged dry down. Floratam stomata begin to close at a higher available soil water, took longer to deplete the available soil water, and was able to extract more water from a confined pot a rooting zone than other genotypes under study.

In accordance with the above studies, Severmutlu et al. (2011) reported a strong positive relationship between drought and leaf firing in turf after 90 days of field dry down. In their study, field drought performance of seven turfgrass species (bermudagrass, zoysiagrass, buffalograss [(*Bouteloua dactyloides* (Nutt.)], bahiagrass, seashore paspalum, centipedegrass, and tall fescue [(*Schedonorus arundinaceus* (Schreb.)] were evaluated for the percentage of leaf firing, turfgrass quality, and percent green cover. Bermudagrass, bahiagrass and buffalograss were found to be superior with reduced leaf firing, and early shoot recovery in comparison to other species. Centipedegrass and zoysiagrass were found to be poor performers due to high rates of leaf firing and almost no recovery upon re-watering.

Drought responses of turfgrass species can be related to genetic variability and biotype (vegetatively propagated or seeded) (Croce et al., 2004). In a six year extensive study, biotypes of four turf species bermudagrass (5 vegetative and 11 seeded cultivars), zoysiagrass (5 vegetative and 4 seeded cultivars), paspalum (2 vegetative cultivars), and buffalograss (3 seeded cultivars) were evaluated for differences in canopy characteristics (Croce et al., 2004). It was concluded that vegetatively propagated cultivars performed better under water limited conditions in terms of TQ, shoot density and leaf blade width in comparison to seeded cultivars.

These studies suggest that quantifying canopy response to drought could play a very important role in understanding the drought resistant mechanisms associated with turfgrass species (Carrow, 1996). The most important shoot characteristics that seem to play a role in drought responses appear to be reduced chlorophyll content and increased canopy temperatures (Huang et al., 1997b). Therefore, it is likely that quantifying the performance ranking of species/cultivars based on canopy behavior under field drought conditions and elucidating their water uptake mechanisms would prove valuable for selecting drought resistant species/cultivars in a breeding program.

Turfgrass Root Architecture and Drought Responses

Root responses are important determinants of physiological response and survival under drought. Deeper root systems may be a plant defense mechanism to avoid drought, enabling plants to extract water from deeper soil layers (Chalmers et al., 2008). Superior drought resistance in turfgrass can be associated with extensive root growth, root water uptake from deeper soil depths, maintenance of viable roots in dry soil layers and effective root regeneration upon re-watering (Huang et al., 1997a). Species or cultivars with deep root systems could conceivably take advantage of a large volume of soil water (Boeker, 1974). In areas where rainfall is irregular, increased root growth to deep soil depths may be desirable for improving drought resistance.

Selecting species or cultivars for enhanced rooting characteristics or for high root/shoot ratios has been considered important in improving drought resistance (Bonos et al., 2004). Turfgrass species that have the ability to effectively access soil available nutrients or moisture would likely have favorable characteristics related to root length, root depth, and root distribution (architecture) (Bowman et al., 2002). Understanding root extension, root distribution, and its functionality could significantly improve our knowledge about root dynamics. Developing an understanding of root architecture supported by multi-year data on root growth and development in response to biological and environmental factors can provide a holistic approach in understanding root population dynamics and their functioning (Comas et al., 2013).

Understanding turfgrass genotypic differences for root architecture in water limited conditions is equally important to quantify drought resistance of turfgrass species (Hays et al., 1991). Selecting drought resistant turfgrass species through genetic manipulation can be assisted through assessment of inter-specific variation among turfgrass species for deep and extensive rooting systems (Huang, 1999; Huang and Gao, 2000).

For example, Hays et al. (1991) conducted a greenhouse study to understand the variation in root morphology between turf cultivars. In the study, the root morphology of ten bermudagrass cultivars was evaluated under drought conditions. The study found that turfgrass root mass at 30, 60, 90 and 150 cm depths were positively correlated with turf quality (Hays et al., 1991). Similarly, differences in drought resistance of turfgrass cultivars with varied root zone depths was reported in Steinke et al. (2010). In this experiment, seven St. Augustinegrass cultivars were evaluated for root architecture differences at 10 and 60 cm root zone depths. For both root zones, Floratam drought performance was reported to be superior over the other six cultivars (‘Amerishade’, ‘Common’, ‘Delmar’, ‘Palmetto’, ‘Raleigh’, and ‘Sapphire’). Floratam exhibited delayed leaf firing and elevated turfgrass quality in addition to a more extensive root architecture (Steinke et al., 2010).

Turfgrass species with deep root systems could take advantage of water that is available at soil depths not normally explored by most turfgrass species. Leksungnoen et al. (2012) evaluated two turfgrass species ‘Gazelle’ tall fescue (*Schedonorus arundinaceus* Schreb) and ‘Midnight’ Kentucky bluegrass (*Poa pratensis* L.) under field conditions and reported that Gazelle tall fescue had a deeper root system and was able to extract more water from deeper soil depths under reduced irrigation frequency in comparison to Midnight Kentucky bluegrass. Similarly, in a greenhouse study with 25 zoysiagrass genotypes (*Z. matrella, Z. japonica* and *Z. japonica Steud. x Z. tenuifolia* Willd. ex Trin), Marcum et al. (1995) reported that at 10 cm soil depth increments the average maximum root depth (AMRD) was positively correlated to total root weight and root number. Cultivars with higher AMRD, root number and root weight also ranked higher for their field drought performance.

Baldwin et al. (2006) documented variation in turfgrass cultivars for root growth and development in a field dry down study. Baldwin et al. (2006) assessed six bermudagrass cultivars (‘SWi-1021’, ‘Arizona Common’, ‘Tif no.3’, ‘TifSport’, ‘Aussie Green’, and ‘Celebration’) under three irrigation intervals of 5, 10 and 15 days. In data pooled across all irrigation treatments, Celebration was reported to produce 114% and 97% greater root weight than TifSport and Aussie Green. Similarly, Huang et al (1997a) reported differences in root re-growth and development of turf cultivars after periods of drought. Huang et al. (1997a) evaluated four turfgrass species [common bermudagrass, centipedegrass, seashore paspalum and hybrid zoysiagrass] for their root dry weight recovery upon re-watering and found that among the four turfgrass species, root dry weight recovery of centipedegrass and seashore paspalum were found to be equal to that of well-watered control plants.

Not only soil water but also the growing season and soil available nutrients can have a significant impact on turfgrass root growth and development. Rowland et al. (2014) evaluated three warm season turfgrass species, 'Tifdwarf' and 'Tifeagle' bermudagrass, 'SeaDwarf' seashore paspalum and 'ToccoaGreen' zoysiagrass at varied irrigation and potassium (K) levels in the fall/spring of 2009 and spring of 2010. In the study during spring, ToccoaGreen zoysiagrass showed the lowest thatch and root weight at the 10-20 cm soil depths whereas SeaDwarf paspalum showed the highest root weight at the 10-20 cm soil depths in the fall.

Cultivars selected for a high root/shoot ratio under greenhouse conditions could potentially demonstrate superior drought performance when assessed under field drought conditions (Karcher et al., 2008; Brady et al., 1995). Poudel (2010) documented the genetic potential of bermudagrass for improved drought performance through extensive rooting and high root/shoot ratios. Based on the overall results, differences for field drought performance in vegetative bermudagrass were identified. Bermudagrass genotypic variation for TQ in response to deficient irrigation levels (0% and 33% of ET) were reported. The study concluded that future work should focus on potential genetic variation that confers physiological attributes that contribute to differences in drought resistance.

These studies suggest that understanding species variation in root morphology is equally important as utilizing canopy characteristics when evaluating drought resistance. Genetic manipulation for drought resistance may depend upon the extent of available variation in the ability of turfgrass species to develop and maintain an extensive root system (Duncan, 1994). Differences within or between species or genotypes are key sources for identification of current drought resistance characteristics in turf and will help in the development of drought resistance turfgrass cultivars in the future. Understanding drought resistance rooting characteristics of turfgrass species or genotypes through their performance ranking under drought conditions could help in identifying breeding techniques that might be crucial in the future of drought tolerance turfgrass breeding.

Approaches to Study Turfgrass Root Architecture

Different approaches are utilized to study turf root architecture, with the most common root sampling methods utilizing transparent PVC tubes to grow samples of turfgrass and ultimately harvesting the intact root system in greenhouse studies (Su et al., 2008), or soil core sampling in field studies (Hendrick and Pregitzer, 1996). Recently, a non-destructive way of root visualization utilizing minirhizotrons is gaining popularity for the study of turfgrass root architecture in field conditions (Bonin et al., 2013).

Studies that utilize transparent polyvinyl chloride (PVC) tubes in greenhouse studies typically aim to understand the rate of root growth and development (RRD) on a daily, weekly or monthly basis (Su et al., 2008; Poudel, 2010; Thapa, 2010; Fuentealba, 2010). PVC tubes are filled with suitable growth medium, sand or fritted clay, and RRD is noted for the deepest root growth along the tube. Although PVC tubes are easy to handle in most cases, a significant amount of root mass from these tubes is lost during washing of soil sections. This is the same case for field root sampling methods that utilize the manual extraction of root cores (Fitter, 1991). Destructive soil core sampling also has significant loss of root tissue during the handling and washing of soil cores (Hendrick and Pregitzer, 1996). Even the up-graded soil core sampling technique ‘shovelomics’ (a field protocol where roots are excavated within a 20 cm radius around the plant hypocotyl and 20 cm below the soil surface) can have significant drawbacks in assessing root properties related to the labor cost while collecting root samples, especially when the root sample size is large (Trachsel et al., 2011).

In contrast to traditional soil coring methods or transparent greenhouse PVC tubes, the minirhizotron camera system is a nondestructive way to study below ground plant root responses over time. The minirhizotron camera system has been widely used to study root morphological characteristics in field studies of other plant species such as bahiagrass, cotton, peanut, and castor (Rowland et al., 2015, Loison et al., 2012,) and in limited amounts in turfgrass (Murphy et al., 1994; Aryal et al., 2015). The technology has been used to study root parameters of cool season grasses (Murphy et al., 1994; McMichael and Taylor, 1987) and a few perennial warm season grasses (Bonin et al., 2013; Aryal et al., 2015; Han and Young, 2014). Minirhizotrons have also been used to understand rooting behavior of turfgrass species for management aspects such as mowing heights and its effect on root morphology (Beyrouty et al., 1990). The technology consists of a high-resolution camera that is inserted into a transparent plastic tube (minirhizotrons) that are typically inserted in the root zone at a 45° angle to the soil surface (tube angle can vary depending on the type of system that is used and could range from 30° to 60°). Images are acquired using a computer system and software that allows for still images of the root profile at different depths over time. In addition, the tubes typically have a locking mechanism that allows for the repeated imaging of the same section of soil repeatedly through a growing season. Since the introduction of the technology, it has been used on a limited basis primarily because the technologies are costly. Root analysis techniques such as minirhizotrons could be alternative nondestructive methods to study root phenotyping of crops in field conditions (Bonin et al., 2013).

Goals and Objectives

Drought and root morphological response is known for many agronomic row crops but such data are limited for the most common warm season turfgrasses species (McCarty and Miller, 2002). A sod based field study and a greenhouse tube study can help to identify the most drought resistant species in several U.S. turfgrass breeding programs, including the breeding program at the University of Florida. Findings from research should lead to understanding of root architectural differences as well as turfgrass response during water limited environments, thus obtained information could thus be utilized for turfgrass water and nutrient management in the state of Florida. The proposed research was conducted to establish rooting characteristics in greenhouse and field experiments for turfgrass species and genotypes grown under limiting soil moisture imposed with a dry-down treatment and in response to three mowing heights. The research approach comprises traditional root screening methods in the greenhouse and a modern nondestructive fashion of root screening utilizing minirhizotrons in the field to quantify rooting behavior of warm season turfgrass species.

This research will provide important information that should be useful in breeding programs aimed at developing drought resistant cultivars, with the following objectives: 1) understand root architecture of warm season turfgrasses in greenhouse tubes under limited soil water condition; 2) understand root architecture of warm season turfgrasses in the field under limited soil water condition; and 3) compare the effects of mowing height on root architecture and root distribution of St. Augustinegrass and bermudagrass.

Hypotheses A: Warm season turfgrass species from different genera will have variability in root architecture that will respond differentially to a controlled greenhouse drydown and in a field drydown. In turn, these characteristics will give insight into the root characteristics that are most highly related to limited soil water environment. Hypothesis B: Different mowing heights will have effect in turfgrass root architecture.

CHAPTER 2

ROOTING CHARACTERISTICS OF WARM SEASON TURFGRASS SPECIES UNDER LIMITED SOIL WATER DURING GREENHOUSE DRY DOWN

Introduction

Drought limits turfgrass growth and development by decreasing quality, shoot growth and root biomass (Huang et al., 1997a; Huang et al., 1997b). Achieving stress resistance in cultivars through genetic manipulation requires an understanding of the inter- and intraspecific variation in root extension, soil depth exploration (Duncan, 1994; Liu et al., 2005) and root/shoot ratios (Bonos et al., 2004). Water stress in turf could be triggered due to events such as uneven and/or inadequate precipitation, or rapid drainage on coarse soils (Zhou et al., 2014). Water deficits often result in reduced leaf area, slow leaf development, and slow internode elongation, thus leading to overall reduced growth (Taliaferro et al., 2004). Turfgrasses, similar to other plant species, resist drought either through escape, avoidance or tolerance (Levitt, 1980). Drought tolerance and avoidance mechanisms can include improved and prolonged water uptake via deep and extensive root systems (Beard, 1989) or through osmotic adjustment (Yue et al., 2006), while drought escape can be accomplished via a short life cycle or developmental plasticity, often involving changes in root architecture. Therefore, extensive root systems are very important plant defense mechanisms to avoid drought stress (Hurd, 1975; Boeker, 1974). Selection and development of turfgrass cultivars that can develop and maintain deep and extensive root systems can provide excellent drought resistance potential (Cross et al., 2013; Carrow, 1996).

There are several examples of studies that have screened turf genotypes for rooting characteristics related to drought tolerance. For example, Marcum et al. (1995) reported that deep roots with extensive root branching and volumes at deep soil depths were desirable traits of a drought resistant cultivar. Fuentealba (2010) also reported differences between zoysiagrass genotypes for their rooting characteristics during drought stress. In their study, an experimental genotype, ‘BA182’, and a commercial cultivar, ‘JaMur’, were reported superior in root length density (RLD) and root volume (RV). Despite the importance of root traits for drought tolerance, assessments of drought performance in turfgrass are typically evaluated by examining aboveground characteristics. For example, Severmutlu et al. (2011) assessed drought resistance characteristics of seven turfgrass species, bermudagrass [(*Cynodon dactylon* (L.) Pers)], zoysiagrass [(*Zoysia japonica* (Steud.)], buffalograss [(*Buchloe dactyloides* (Nutt.) Engelm], bahiagrass [(*Paspalum notatum* (Flugge.)], seashore paspalum [(*Paspalum vaginatum* (Swartz.)], centipedegrass [(*Eremochloa ophiuroides* (Munro.)], and tall fescue [(*Schedonorus arundinaceus* (Schreb.)] for percentage leaf firing, turfgrass quality, and percentage green cover. In 90 days of field drought, bermudagrass, bahiagrass and buffalograss demonstrated superior drought resistance with reduced leaf firing, and better shoot recovery in comparison to other species, while centipedegrass and zoysiagrass had high leaf firing and almost no recovery from drought upon re-watering However, in some studies, the causal link between below and aboveground characteristics is clear. A good example is the greenhouse and field study by Qian et al. (1997) using ‘Mildlawn’ bermudagrass, ‘Meyer’ zoysiagrass, ‘Mustang’ tall fescue’ and ‘Prairie’ buffalograss showed the extensive rooting of bermudagrass and buffalograss and deep extensive rooting of tall fescue as important features related to leaf wilt resistance.

Clearly, quantifying species variation for root architecture is important in screening for drought resistant turfgrass cultivars. Genetic variation for drought resistance could be associated with extensive root systems and may help explain the differences among turfgrass species in their ability to withstand drought conditions (Duncan, 1994). These species were evaluated for their drought performance under both dry down and well-watered conditions. Although total root length was assessed, additional root architectural parameters were also evaluated to determine their utility in evaluating drought responses. The specific objectives of the study were to: 1) compare the root system architecture of the species under limited soil water and well-watered conditions; 2) identify rooting differences of genotypes within species under limited soil water; 3) identify root traits of interest that are associated with species, and genotype within species, under limited soil water; and 4) determine the root traits that are the most closely associated with canopy drought responses under limited soil water condition.

Materials and Methods

To quantify the differences in root architecture among warm season turfgrass, a greenhouse study was initiated to evaluate root distribution patterns of commercial and experimental genotypes from the University of Florida turfgrass breeding program. The study was conducted at the University of Florida, Gainesville, FL in a greenhouse during 2012 and 2013. The study was arranged as a randomized complete block design (RCBD) with four replications. Two separate experiments were conducted, one withholding water to allow for the development of dry conditions over time (DD), and one under well-watered (WW) conditions (arranged in RCBD with four replications) where soil moisture was kept at field capacity (approx. 322 ml of water in each tube).

Sixteen warm season turfgrass genotypes were utilized in both experiments and were comprised of both commercial cultivars and experimental genotypes currently utilized in the UF turfgrass breeding program; the genotypes included were: ‘Celebration’, ‘UFCD 347’, ‘UFCD 12’, and ‘PI 289922’ bermudagrass (CB); ‘JaMur’, ‘4360’, ‘5269-24’, and ‘BA182’ *Zoysia japonica* (ZJ); ‘ToccoaGreen’, ‘Zeon’, ‘BA336’, and ‘BA374’ *Zoysia matrella* (ZM); and ‘Floratam’, ‘Captiva’, ‘Palmetto’, and ‘Sapphire’ St. Augustinegrasses (ST).

All entries were clonally propagated in a greenhouse and a single 3.8 × 3.8 cm plug was transplanted into a 90 × 3.5 cm clear plastic tube filled with Turface calcined clay (Turface® MVP® fritted clay, Profile Products LLC, Buffalo Grove, IL) that was saturated prior to planting. Plugs used for planting were collected from field grown sod on October, 2011 and acclimated in the greenhouse before planting. Planting was performed in December, 2011 for first experiment and in February, 2013 for second experiment. Plugs, 3.8 cm diameter, were washed free of soil and roots were trimmed prior to planting. The fritted clay had a dry bulk density of 0.67 kg L^{-1}, a particle density of 2.50 kg L^{-1}, a total porosity of 0.73, and a saturated hydraulic conductivity of 9.5×10^{-4} m sec^{-1} (van Bavel et al., 1978). Each clear tube was then inserted into an opaque, white PVC tube and held at a 30° angle to vertical by a metal wire grid rack system during the entire experiment. Plants were irrigated at a rate of 73.33±6.67 ml of water per minute with a drip irrigation system, with one drip emitter on each tube. Natural light was supplemented with overhead lamps set to maintain a photoperiod of 16 hours daylight and an average photosynthetically active radiation (PAR) of 1000 μmol m^{-2} sec^{-1}. Grasses were trimmed once a week at 5 cm height throughout the study period (during partial dry down). Fertilizer was applied once a week at 73 kg ha^{-1} N, 11 kg ha^{-1} P and 41 kg ha^{-1} K using Miracle Gro® (24-8-16) fertilizer (Scotts Miracle Grow®, Marysville, OH). Fritted clay contains only trace amounts of N (Dovel et al., 1993), and water drains easily so higher N fertilizer was applied to maintain grass growth and development (Su et al., 2008; Poudel, 2010; Thapa, 2010).

A gradual DD experiment was initiated for 15 days during both 2012 and 2013. The first experiment started 17 July, 2012 and ended to 1 August, 2012; the second experiment started 26 September, 2013 and ended 10 October, 2013.Tubes from the WW and DD treatments were weighed at 8:00 am (EST) every day and tube weight was recorded. For the WW experiment, water lost through evapotranspiration was fully replenished every day to keep the soil at or near field capacity during the study period. For the gradual DD experiment, plant available water (calculated for each cultivar individually) was allowed to deplete by 10% each day. Plant available water for a given cultivar was calculated based on the root depth for that cultivar and using the value of 322 ml of plant available water for the entire tube determined in a preliminary study (data not reported). For example, if the root depth of the ZM cultivar was 30 cm (one third of the maximum root length of 90 cm), the plant available water was computed to be 107 ml (one third of 322 ml). For this same example, tubes of ZM were not allowed to lose more than 10% (~11 ml) of water each day in the DD experiment. To monitor partial DD progression, time domain reflectometry (TDR) probes (Spectrum Technologies Inc., Plainfield, IL) were used to record percent soil volumetric moisture depletion at a 5 day interval at 15 and 60 cm tube depths. TDR probes of 5 cm in length were inserted into the tubes at 15 or 60 cm depths along pre-drilled holes present at these depths.

At harvest, tubes were cut into three 30 cm sections and roots within each section were washed, scanned and analyzed using WinRHIZO software (Regent Technology, Canada) to determine total root length (TRL, in cm), average root diameter (ARD, in mm), total root surface area (TRSA, in cm^2), maximum rooting depth (MRD, in cm), and fine root length at 0-1.5 mm diameter class (FRL, in cm). Fine root class selected in this study were based on previous studies where root ≤ 1.0 mm were categorized as fine roots that were associated with increased root surface area in two tallgrass prairie *Bromus inermis* (L.) and *Poa pratensis* (L.) (Reinhardt and Miller, 1990). Similarly, Meier and Leuschner (2008) had categorized roots at 1.15-1.58 mm diameter class as fine roots in European beech (*Fagus sylvatica* L.) tree species. In addition, canopy response of leaf firing (LF) and turf quality were recorded during dry down. Leaf firing was based on visual basis by examining the severity of the burn present at the shoot tips. Turf quality and leaf firing ratings were based on a 1-9 scale, where a value of 1 represents poor TQ or severe LF and a 9 is excellent TQ or no LF. A turf canopy rating of 6 represents acceptable TQ or LF response. TQ and LF data were obtained from a concurrent study Zhang (2014). In addition, roots were dried for 48 h at 65 °C, weighed and total root dry weight (RDW, in g) was determined. Root proportion was calculated for 0-30 cm depth as TRL at 0-30 cm/ TRL at 0-90 cm depths.

Statistical Analysis

Analyses of variance (ANOVA) were used to determine differences in TRL, TRSA, ARD, RDW, RP, MRD and FRL across species and genotypes and genotypes within species to determine any interaction of factors in the DD experiment and across species separately in the WW experiment. The GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC) was used, incorporating a mixed model across species, and genotypes within species. Year, species or genotype and depth were fixed factors and replication nested within years was a random factor. Root parameters were analyzed using depth as a factor in the model with two levels: 0-30 and 30-90 cm sections. For factors with no depth interactions, analysis was performed at the level of the entire tube (0-90 cm). Data analysis was limited to two way interaction such that the tabulation and interpretation of the results would be meaningful, statistical analysis at each level species, and genotypes within species were thus limited to two way interactions as specified through SAS codes. Whenever there was not a significant interaction by species, or genotypes within species, data for the two years were pooled. For year interactions, data were presented by each year. For year and depth interactions, data were presented by each experiment year and depth. When factors were determined as significant at the p=0.05 significance level, means were separated using Tukey's honestly significant difference test (HSD).

In addition to ANOVA, a multivariate analysis of variance using canonical discriminate analysis (CDA) was performed to determine the root traits of interest that would help quantify species, and genotype within species variability. CDA analysis was selected for multivariate analysis in our study for its capability to separate classes for correlated data (Zhao and Maclean, 2000). "In CDA, the matrix algebraic equation which provides the canonical discriminate functions are constrained such that the successive discriminate functions define the maximum possible differences between experimental treatments" (Matthew et al., 2010). In order to compute the desirable root traits that could be associated with less canopy leaf firing response, a stepwise regression analysis was performed. The relevant root traits that could be associated with less leaf firing for greenhouse DD were computed incorporating the following model into the stepwise regression analysis: Ln_LF11 = Ln_TRL Ln_TRSA Ln_FRL Ln_MRD Ln_RDW Ln_ARD Ln_RP…….equation (1). Root traits will be assessed in the model one at a time at 0.05 significance level. Trait significant at 0.05 level will be included in the model. Species canopy response for LF at day 11, and the root traits thus quantified by the model would help explain the association of important root traits with canopy LF response. Similar, statistical analysis was performed with TQ rating at day11 (TQ11), using the following model into the stepwise regression analysis: Ln_TQ11 = Ln_TRL Ln_TRSA Ln_FRL Ln_MRD Ln_RDW Ln_ARD Ln_RP (equation 2).

Results

Evidence of Dry Down Progression

The direct measurement values from the TDR instrument, TDR readings (reflective of volumetric soil moisture) were analyzed at 1, 5, 10 and 15 days after dry down (DADD) at two soil depths, 15 and 60 cm (Table 2-1). For reflective volumetric soil moisture, there was no species by year interaction for 1, 5, 10 and 15 days after DADD (Table 2-1), thus the data at days 1, 5, 10, and 15 DADD were pooled across years. By day 5, significant soil moisture depletion had occurred in all species at both soil depths (Table 2-2). Moisture loss continued through day 10 and 15 for both soil depths. At 15 cm at the end of the DD cycle, ST had the highest TDR readings, exceeding ZM and ZJ; while at 60 cm, ST soil moisture was lowest in comparison to the other three species (Table 2-2).

In addition, the TQ and LF visual rating collected at the beginning (TQ1 and LF1) and at day 11 of DD (TQ11 and LF11) were analyzed (Table 2-3). There was species by year interaction for TQ1, LF1, TQ11 and LF11 (Table 2-3), thus data were reported by year. In 2012, the initial TQ1 differences evident in ZM and ZJ (higher TQ) compared to ST and CB was not evident at the end of DD (Table 2-4). This signifies that ZJ and ZM relatively lost TQ during progressive DD compared to ST and CB. In contrast, the initial differences in LF1 at the beginning of the DD between ST (less LF) and ZM, ZJ or for CB and ZM, ZJ was still evident at the end of DD, where ST and CB had less LF compared to ZJ and ZM (Table 2-4). In 2013, the only variation for TQ1 was observed for ZJ with higher TQ value compared to CB (Table 2-4). However, by day 11, TQ was higher for ST and ZM compared to CB and ZJ. Similarly, ST also had less LF compared to CB and ZJ at day 11. In both study years, ST consistently showed less LF compared to ZJ (Table 2-4).

For TQ11 and LF11 comparison for genotypes within species, both traits TQ11 and LF11 were significant only for ZJ genotypes (LF11=13.47, TQ11=15.81 p<.0001) and showed a year interaction. For CB genotypes, only TQ11 was significant (TQ11=6.25 p<.01, LF11=0.92 NS). For ST, both traits LF11 and TQ11 were insignificant (ST=1.84, 0.24 NS), similar was the result for ZM genotypes (LF11=2.78, TQ11=1.61 NS). Within ZJ, in 2012 genotypes did not differed for LF ratings at day 11 (5269-24=JaMur=BA182=4360). However in 2013, 4360 and 5269-24 had less LF at day 11 compared to BA182 and JaMur (4360=5269-24>BA182=JaMur). Similar was the trend for TQ at day 11 for ZJ genotypes. No differences was observed between ZJ genotypes in 2012. However in 2013, 4360 and 5269-24 maintained higher TQ compared to JaMur (4360=5269-24>JaMur) however TQ was similar between 5269-24, and BA182. Similar was the result for BA182 and JaMur. Within CB at day 11, 289922 and Celebration maintained higher TQ compare to UFCD12 (289922=Celebration>UFCD12) however TQ was similar between UFCD347 and UFCD12 (data not shown).

Comparisons Among Genera During DD and WW at Two Depths (0-30, 30-90 cm): the Traits of TRL, TRSA, ARD and FRL

In the DD experiment, TRL, TRSA, ARD and FRL showed no year by species interactions, but did have species by depth interactions (Table 2-5); therefore, data were pooled across both years but shown separately by the two depth classes (0-30 and 30-90 cm). In the upper soil depth (0-30 cm), ST had higher TRL, TRSA and FRL than the other three species, while CB, ZJ and ZM were all similar for these traits (Table 2-6). Bermudagrass produced the largest diameter roots (ARD) compared to ZM, while ST and ZJ were intermediate. Deeper in the soil, 30-90 cm, for DD, the separation between species for TRL and TRSA was similar to that found at the shallower depth, with ST having higher TRL and TRSA compared to the other species. However, TRL and TRSA values were not different between CB and ZM, or between ZM and ZJ. For ARD, ST, CB and ZM were not different; however, ST and CB had greater diameter roots than ZJ. At the other end of the root diameter spectrum, ST had greater FRL deeper in the soil than the other three species, with CB having greater FRL than ZJ and ZM (Table 2-6). Under DD, ST had an approximately 50:50 split between the upper and lower soil depths, while both ZJ and ZM proliferated at least 80 percent of their roots in the 0-30 cm soil depth (Table 2-6).

In the WW experiment, there were year by species and species by depth interactions (Table 2-5) so data are reported by species, year, and depth. In this WW condition in 2012, species separation for TRL, TRSA and FRL within the upper soil depth (0-30 cm) was the same as observed for the DD experiment at the same depth; ST had the highest values and the other species were not different from each other (Table 2-7). However in 2013, the separation of species was not as straight forward; trends were similar to 2012 in that ST was always ranked within the highest statistical group for TRL, TRSA, and FRL. Bermudagrass was not different from ST for TRSA and had greater TRL and TRSA than ZJ (Table 2-7). At the deeper soil depth (30-90 cm), ST was always in the top statistical group for TRL, TRSA, ARD and FRL in both years (Table 2-7). The other three warm season species were not different for TRL, TRSA, or FRL in either year. Bermudagrass and ZM were both statistically similar to ST for ARD in both years; while the coarser textured ZJ had the lowest ARD in both years (Table 2-7). As observed in DD tubes, ST had an approximately 50:50 split in RP under WW conditions in both years (Table 2-7). Bermudagrass had more than 70 percent of its roots in the upper soil profile in both years. Both ZM and ZJ had significantly more roots in their upper profile for both years.

Comparing Genotypes within Species During DD

In the DD experiment only, genotypes within CB, ST, ZJ and ZM were compared to determine if variability in these root traits existed at the species level (Table 2-8). Whenever there were not significant genotype by depth interactions, data for these traits were analyzed for the entire tube (0-90 cm) versus individually within the two depth classes; when interactions existed, average trait values were presented for each depth.

For genotypes within CB, no differences were found for ARD, RDW and MRD but TRL, TRSA, FRL and RP were different among individual genotypes (Table 2-8). The RP trait did not have a year by genotype interaction, so data were pooled for analysis. For TRL, TRSA and FRL, no genotype by depth interaction existed, so data presented were analyzed across the entire tube length (0-90 cm). Table 2-9 provides the analysis of the entire tube for TRL, TRSA, and FRL, showing that TRL and FRL were not different among genotypes within CB, but differences were observed for TRSA. Examining the TRSA data by year, CB genotypes differed only due to a low value for UFCD12 in 2012; there were no differences in 2013 (Table 2-10). The CB genotype, 289922 had the lowest root proportion, but was not different from Celebration or UFCD347. These genotypes produce more roots lower in the soil profile than UFCD 12 (Table 2-10).

For the genotypes of ST, there were no differences in TRL, TRSA, ARD, FRL, RDW or MRD (Table 2-8). The only trait with genotype differences was RP, which also had a significant year by genotype interaction. In 2012, Sapphire and Floratam each had 0.46 and 0.44 RP, respectively; while Palmetto and Captiva had 0.55 and 0.60 percent respectively (data not shown). In 2013, there were no differences between ST genotypes, and all had a higher percentage of their roots in the lower soil profile 30-90 cm (data not shown).

Within ZJ genotypes, there were differences for all traits except ARD (Table 2-8). Significant year by genotype and genotype by depth interactions occurred for TRL, and TRSA (Table 2-8); therefore, data were reported by year and depth for these traits. In 2012, at the upper soil depth (0-30 cm), ZJ genotypes did not differ in TRL and TRSA.

In contrast in 2013, genotype 4360 produced higher TRL and TRSA compared to JaMur and BA182 and higher TRSA than 5269-24 (Table 2-11). At the deep soil depth (30-90 cm), 5269-24 had greater TRL and TRSA compared to the other three genotypes in 2012; whereas, in 2013, 4360 was not different from 5269-24. The remaining traits (RDW, MRD, RP and FRL) for ZJ were analyzed using the entire tube, but each trait had a significant year by genotype interaction (Tables 2-8 and 2-9); therefore, years were examined separately. Root biomass production (RDW) was similar for ZJ genotypes in 2012, however 4360 produced greater RDW among ZJ genotypes in 2013 (Table 2-12). In both experimental years, 5269-24 had the greatest MRD among ZJ genotypes, and greatest FRL in comparison to JaMur. The RP trait for ZJ indicates all the genotypes concentrated the majority of roots in the upper 0-30 cm (Table 2-12); however there were some exceptions. Notably 5269-24 consistently had more roots below 30 cm than other genotypes, while JaMur and BA182 consistently had fewer roots below 30 cm.

Within ZM, genotypes were different for all traits except ARD (Tables 2-8 and 2-9). There were no genotype by depth interactions so analyses were performed for the entire tube. Significant year by genotype interactions were evident for TRL, TRSA, RDW, MRD, FRL and RP (Table 2-8 and 2-9); therefore results are provided by year. Among ZM genotypes in 2012, BA336 had TRL, TRSA and FRL compared to the other three genotypes (Table 2-13). For MRD, BA336 and Zeon were not different, but BA336 did have deeper roots than BA374 and ToccoaGreen. In 2013, BA336 and BA374 were similar and had higher values for TRL, TRSA, RDW, MRD and FRL than Zeon and ToccoaGreen. The single exception was that ToccoaGreen was not different from

BA336 for its rooting depth in 2013 (Table 2-13). There were minimal differences for RP among ZM genotypes (Table 2-13). In 2013, Zeon concentrated more roots in the upper profile (0-30 cm) than did the other genotypes.

Discriminate Analysis: Testing Combinations of Root Traits

A multivariate analysis of variance was performed using canonical discriminate analysis (CDA) to test the species differences on a linear combination of seven root and shoot variables: TRL, TRSA, ARD, MRD, RDW, FRL, RP, TQ15 and LF15. The across species comparison was significant (Wilk's λ =14.59, canonical correlation CAN1=0.89 and CAN2=0.66 respectively, p<.0001) and the two functions generated (CAN1 and CAN2) accounted for 98% of the across species variance (Tables 2-14 and 2-15). Standardized variable weight within factors indicates that CAN1 had a high positive correlation with FRL, TRSA and RDW; whereas, CAN2 was positively correlated with TRL, ARD, RDW, LF day 15 and MRD (Table 2-15). The FRL, TRSA and RDW values associated with CAN1 helped to explain 82% of the across species variability (Table 2-15). Within class mean (Table 2-16) values were plotted to illustrate the grouping of species (Figure 2-1). St. Augustinegrass had higher FRL and TRSA values and was separated from ZJ, ZM and CB. Bermudagrass (CB) was negative for CAN1 and positive for CAN2, while ZJ and ZM were negative for both CAN1 and CAN2. These results were very consistent with those obtained from ANOVA where ST showed higher FRL and TRSA compared to ZM, ZJ and CB species.

A second discriminate analysis was performed to determine the combination of traits that were important for the differentiation of genotypes within individual species. The multivariate analysis for genotypes within species was only significant for ZJ and ZM (Table 2-14). Thus, results are only reported for ZJ and ZM. The multivariate analysis for genotypes within ZJ species showed only CAN1 (CAN1=0.87, p<.01) to be significant; CAN1 explained 72% of the variability within ZJ genotypes and showed a high positive correlations with FRL, MRD, TRSA and LF day 15 (Table 2-17). When class means (Table 2-18) for ZJ genotypes were plotted in the quadratic space (Figure 2-2), 5269-24 for FRL, MRD, TRSA distanced itself from the other three genotypes in the multivariate space. In contrast, JaMur and BA182 grouped in the lower end in the negative side of the quadrant, suggesting they had relatively poor FRL, MRD and TRSA compared to 5269-24 during DD.

The multivariate analysis within ZM genotypes had both canonical vectors significant (CAN1=0.88, p<.0001 and CAN2=0.72, p<.05) (Tables 2-19). These two factors were able to explain 90% of the variability within ZM genotypes (Table 2-19). Factor CAN1, was dominated with FRL, TRSA, RDW and RP; while CAN2 with FRL, TRSA and LF day 15. When plotting the class means for ZM genotypes in the quadratic space (Table 2-20 and Figure 2-3), genotypes BA336 and BA374 for FRL, TRSA, RDW and RP and were separated from Zeon and ToccoaGreen in the multivariate space. Additionally, ToccoaGreen was in the negative quadrant while Zeon was intermediate.

Stepwise Regression Analysis

In the regression model, TRL, FRL, TRSA, ARD, MRD, RDW, and RP were regressed against leaf firing (LF) rated at day 11. In our study, the results from stepwise regression analysis identified MRD was associated with improved canopy response (less LF rating) of species or genotypes during DD (Table 2-21).

Similarly, a regression model with combination of traits, TRL, FRL, TRSA, ARD, MRD, RDW, and RP were regressed against turf quality (TQ) rated at day 11. In our study, the results form stepwise regression analysis identified TRSA was associated with canopy response (TQ11) of species or genotypes during DD (Table 2-22).

Discussion

Studying drought tolerance in turfgrass has important impacts not only to aid in homeowner and recreational user requirements, but in providing improved environmental stewardship since turf is a primary source of landscape water use [(National Gardening Association (NGA), 2013)]. This study was aimed at determining if some turgrass species and individual genotypes within species may have varied root response to surface soil drying under water limited environment. The level of evaluation was focused on the root system because many below ground traits have the capacity to impart important drought tolerance traits. The results clearly show that across species and genotypes within species, the primary root growth was distributed in the upper 0-30 cm of the soil column. For example, ZM and ZJ species had 72 to 94% of their TRL distributed within the top 30 cm of soil, whether in well-watered or water scarce conditions. These results are in agreement with other turfgrass rooting studies. Huang and Fry (1998), Qian et al., (1997), Poudel (2010) and Thapa (2010) also reported high relative percentages of roots distributed in the upper 30 cm of soil. However, even this pattern showed some variability among species, such that ST typically exhibited very uniform rooting across the 0-90 cm soil column. The impact of a root architecture that concentrates most root growth and development in top soil layers may lead to susceptibility to plant water stresses under conditions of limited soil water. Thus, this study suggests that the ZM and ZJ species may be more desirable for regions that receive adequate and frequent rainfall or in areas with shallow soils.

The relatively deep root systems of the ST species might allow this species to take advantage of deeper soil moisture while maintaining growth and development under water scarce conditions. This hypothesis was partially supported by the actual TDR readings that were reflective of the soil volumetric moisture in this study. In the upper 15 cm soil depth, the actual TDR values between species after 15 days of DD showed similar water use trends suggesting that all the species accessed soil water equally at this shallow depth. However at the 60 cm soil depth, TDR values for ST (9.4) and CB (10.6) were lower than those for ZM (11.1) or ZJ (11.8) after 15 days of DD, indicating that ST and CB species accessed deep soil water to a greater degree than the zoysiagrass species. This may be due to the relatively shallow root growth and development for zoysiagrass in comparison to ST species. The significance of extensive and deep roots as evident in ST in 2012 and 2013 and in CB in 2012 could be associated with relatively less leaf firing at the end of DD in comparison to ZJ species as reported in concurrent study Zhang (2014). These results indicate an advantage of deep and extensive root systems to sustain growth in water limited environments.

Most drought associated research has focused on and advocated the importance of deep roots for drought tolerance, while there has been relatively little investigation of the role that other root traits may play a role (Doussan et al., 2006; Comas et al., 2013). These could include fine root distribution, average root diameter, or surface area, or rooting volume when determining optimal root architectures for drought resistance. The FRL is a particularly important trait for water and nutrient uptake because diameter influences the absorption area for both nutrients and water (Lynch, 2007; Rewald et al., 2011). In fact, FRL have been shown to be responsible for 35-50% of total water uptake for plants (Segal et al., 2008), including turfgrass (Green et al., 1991; Huang and Gao, 2014). Increasing the FRL also translates into increased total root surface area (Kim, 1987; Green et al., 1991). This is because surface area relates to the potential for continued water uptake in drying soils (Sun et al., 2013; Huang et al., 1997a). In the current study, these traits also varied among species; in particular ST had greater fine root length followed by CB, ZM and ZJ species. In the 30-90 cm depth under DD, the fine roots contribution towards TRL (FRL /TRL) for ST was approximately 52%, indicating that the fine root length likely contributed to the greater water uptake of ST at this depth than for the other species. This high FRL for ST was likely also linked to its high TRSA values, with increased surface area also likely contributing to ST's greater water uptake than for zoysiagrass (ZJ) at deep soil depths. Similar variation for fine root production and its relationship to TRL between species was reported in Green et al. (1991) who found the fine root contribution towards TRL for species to be as low as 1% for 'Emerald' zoysiagrass, and 85 to 95% 'FB119' and 'Texturf 10' bermudagrass.

This study has demonstrated that screening for water limited environments should take into consideration root traits beyond just total root length as mentioned in other turfgrass studies. However, combining traits in a meaningful holistic manner can be challenging. Multivariate analyses such as discriminate analysis is a useful and quantitative way to determine which trait drive differences among genotypes to the largest extent and can then differentiate among genotypes that might be selected in a breeding program (Johnson et al., 2003; Matthew et al., 2010; Zhao and Maclean, 2000; Cordukes and Williams, 1981). From the discriminate analysis in the current study, the two canonical functions (CAN1 and CAN2) explained 98% of the root trait differences across species, 72% of the genotypes within ZJ and 90% of the genotypes within ZM species variability. Overall, the most dominant root traits that were relevant to help separate species and genotypes within species were FRL, TRSA, and MRD. These traits showed a separation of ST from the other three species, as well as two primary separations of particular genotypes within ZJ or ZM. The importance of fine roots, root surface area and root depth in the soil profile for CAN1 and CAN2 follows the results discussed above reiterating that both traits are likely crucial during DD for enhanced water uptake (Huang, 1999).

Finally, in an effort to relate these belowground results to performance of the turfgrass genotypes above ground, the results from Zhang (2014) were utilized, a concurrent study addressing the aboveground responses in the same experiment. Traits measured in Zhang (2014) simultaneously with the rooting traits reported here included LF and TQ. We focused on the relationship between root traits and leaf firing (LF) or turf quality (TQ), primarily because these traits would be used to evaluate genotypes for their drought tolerance when examining aboveground traits only. By regressing TRL, TRSA, ARD, RDW, MRD, FRL and RP with LF, the trait associated with LF was MRD under limited soil water environment. Similarly, by regressing TRL, TRSA, ARD, RDW, MRD, FRL and RP with TQ, the trait associated with TQ was TRSA under limited soil water environment. Finding a link with above- and belowground traits is not too surprising; a multivariate principal component analysis was used by Sun et al., (2013) to identify important shoot physiological responses and associated root phenotypes of seven tall fescue genotypes. Root length, root dry weight and its association with canopy relative water content were compared to determine variability among genotypes. In the study, a combination of traits such as total root length, root dry weight, root elongation and specific root length were identified as important traits that might contribute towards better TQ in tall fescue during drought.

Summary

Overall, results from this study have shown that not only root length, but FRL, TRSA, and MRD could also be relevant for separating genotypes for drought tolerance. Mechanistically, it has been reported that these traits may provide improved water uptake during water scarce conditions. For turfgrass in particular, high or prolonged water uptake during dry conditions could maintain acceptable turfgrass quality in drought prone environments.

This study further suggests that understanding species and genotype variation in root morphology is very important for screening for drought resistance. However, this study also shows that the historical and current concentration of efforts on determining differences in TRL alone is not adequate for effective genotype selection. In overall the TRL value could be similar between species, but partitioning of root between depths could be different. As, this study also showed that an important difference among genotypes was in the overall arrangement of roots between the two depth zones studied; with some utilizing a fairly uniform distribution across the 90 cm examined, while others preferentially concentrated roots in the shallow soil profile. This root architecture alone has important implications about the ability to access adequate soil water under drought conditions.

Differences between species or genotypes for these root parameters mentioned above has provided important information that can be utilized in selection of improved drought tolerant genotypes. Based on this study, it is clear that the inclusion of species such as ST (in 2012 and 2013) and CB (in 2012) and specific genotypes identified in other species such as BA4360 and 5269-24 (ZJ) that have deep and extensive root systems are important contributors for root architectural improvements related to drought avoidance. Because of the utility of the traits examined in this study, future work should be focused on variation that considers root physiological attributes, including root water uptake in field conditions, along with root morphological characteristics among species and genotypes to improve breeding efforts for drought resistance in turfgrass.

Table 2-1: Analysis of Variance F-statistics for time domain reflectometer (TDR) of four warm season turfgrass species at two soil depths (15, 60 cm) for the dry down (DD) treatment tubes.

Effect	DF	Day 1	Day 5	Day 10	Day 15
Year	1	16.06**	25.33**	8.48*	29.84**
Species	3	5.22**	8.04***	10.01***	4.68**
Year*Species	3	2.53	0.67	0.30	0.77
Depth	1	27.46***	132.02***	187.77***	392.07***
Year*Depth	1	80.75**	59.58***	13.44***	18.34***
Species*Depth	3	15.63***	23.03***	19.86***	13.41***

*, **, *** significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table 2-2: Average values for time domain reflectometry (TDR) readings (reflective of volumetric soil moisture), of four warm season turfgrass species at 15 and 60 cm depths for dry down (DD) tubes.

	15 cm soil depth [π]				60 cm soil depth [π]			
Species	TDR (Day 1) VSM% [π]	TDR (Day 5) VSM%	TDR (Day 10) VSM%	TDR (Day 15) VSM%	TDR (Day 1) VSM%	TDR (Day 5) VSM%	TDR (Day 10) VSM%	TDR (Day 15) VSM%
ST[¥]	21.0 a[Ŧ]	10.3 a	8.9 a	7.6 a	19.6 b	9.8 c	9.5 c	9.4 c
CB	17.8 b	8.5 b	8.1 b	7.3 ab	20.3 a	11.4 b	11.0 b	10.6 b
ZM	18.9 b	8.7 b	8.2 b	7.0 b	20.5 a	13.8 a	12.7 a	11.1 ab
ZJ	18.7 b	8.6 b	8.1 b	7.0 b	20.6 a	14.4 a	13.6 a	11.8 a

[Ŧ] Means followed by same letters within each column were not significantly different, P ≤ 0.05. Means tabulated were sorted separately for each day.

[π] Time domain reflectometry values for reflective of volumetric soil moisture (VSM%) recorded at 15 and 60 cm depths within the tubes respectively.

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table 2-3: Analysis of Variance F-statistics of species for turf quality (TQ) and leaf firing (LF) at beginning (TQ1, LF1) and end of dry down (TQ11 and LF11).

Effect	DF	TQ 1	LF1	TQ11	LF11
Year	1	0.43	1.43	22.87**	0.54
Species	3	11.74***	14.71***	4.26**	8.05***
Year*Species	3	6.40***	6.86***	3.57*	5.98***

*, **, *** significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table 2-4: Species averages for turf quality (TQ1, TQ11) and leaf firing (LF1, LF11) at the beginning and at the end of greenhouse DD.

	2012				2013			
Species	TQ1	TQ11	LF1	LF11	TQ1	TQ11	LF1	LF11
ST[¥]	6.3 b[‡]	4.4 a	9.0 a	5.3 a	7.0 ab	5.5 a	7.8 b	5.4 a
CB	6.7 b	4.4 a	8.8 a	5.5 a	6.8 b	4.4 b	8.8 a	4.7 bc
ZJ	7.3 a	4.1 a	8.1 b	4.1 b	7.2 a	4.7 b	8.0 b	4.5 c
ZM	7.5 a	4.3 a	7.6 b	4.3 b	7.1 ab	5.4 a	7.9 b	5.2 ab

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05.

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table 2-5**:** Analysis of variance F-statistics for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL) and root proportion (RP) across species and root depths for DD and WW tubes in 2012 and 2013.

Effect	DF	TRL	TRSA	ARD	FRL	RP[£] (0-30 cm)
DD						
Year	1	2.53	10.49*	2.92	1.72	7.12***
Species	3	159.89***	130.16***	5.54***	159.17***	57.20***
Year*Species	3	2.06	0.71	0.37	2.13	0.78
Depth	1	98.75***	153.23***	40.01***	90.54***	NA
Year* Depth	1	3.19	0.00	1.04	4.00*	NA
Species*Depth	3	27.69***	15.96***	2.84*	29.07***	NA
WW						
Year	1	32.58***	4.67	1.03	36.72***	9.53*
Species	15	163.60***	155.26***	24.23***	163.55***	73.76***
Year*Species	15	6.13***	5.74***	3.42*	6.29**	3.09*
Depth	1	205.65***	322.61***	102.80***	192.15**	NA
Year* Depth	1	0.22	2.65	14.29***	0.03	NA
Species*Depth	15	25.3***	13.89***	12.30***	21.71***	NA

NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

[£] Root proportion (RP) parameter is a ratio of total root at 0-30/30-90 cm soil depth classes; therefore, depth is not a parameter in the analysis of this characteristic.

Table 2-6: Average values across 2012 and 2013 for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL) and root proportion (RP) at 0-30 and 30-90 cm depths for the DD tubes.

	TRL (cm)		TRSA (cm^2)		ARD (mm)		FRL (cm)		RP (cm/cm)
Species	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm
ST[¥]	5476 a[‡]	6128 a	744 a	726 a	0.44 ab	0.38 a	5241 a	5977 a	0.48 c[£]
CB	3358 b	2031 b	502 b	244 b	0.46 a	0.39 a	3202 b	1969 b	0.64 b
ZJ	3193 b	1034 c	428 b	117 c	0.43 ab	0.27 b	3072 b	1010 c	0.83 a
ZM	3751 b	1238 bc	480 b	155 bc	0.42 b	0.36 ab	3610 b	1197 c	0.80 a

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05.

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

[£] Root proportion (RP) parameter is a ratio of total root at 0-30/30-90 cm soil depth classes; therefore, depth is not a parameter in the analysis of this characteristic.

Table 2-7: Average values in 2012 and 2013 for total root length (TRL), total root surface are (TRSA), average root diameter (ARD), fine root length (FRL) and root proportion (RP) at 0-30 and 30-90 cm depth classes for species in WW tubes.

	TRL (cm)		TRSA (cm^2)		ARD (mm)		FRL (cm)		RP (cm/cm)
Species	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm
					2012				
ST[¥]	5908 a[‡]	5792 a	807 a	659 a	0.43 a	0.38 a	5701 a	5702 a	0.50 c[£]
CB	3447 b	1569 b	465 b	186 b	0.42 a	0.36 a	3330 b	1488 b	0.71 b
ZJ	3213 b	746 b	402 b	86 b	0.40 b	0.24 b	3130 b	726 b	0.85 a
ZM	3593 b	1538 b	440 b	172 b	0.38 b	0.34 ab	3520 b	1509 b	0.72 b
					2013				
ST	4224 a	4491 a	624 a	563 a	0.46 b	0.39 a	4015 a	4381 a	0.49 c
CB	3467 b	1249 b	567 ab	161 b	0.52 a	0.41 a	3236 b	1210 b	0.75 b
ZJ	2762 c	365 b	396 c	42 b	0.45 b	0.09 b	2642 b	358 b	0.94 a
ZM	3246 bc	642 b	471 bc	81 b	0.45 b	0.28 a	3100 b	624 b	0.88 a

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05.

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

[£] Root proportion (RP) parameter is a ratio of total root at 0-30/30-90 cm soil depth classes; therefore, depth is not a parameter in the analysis of this characteristic.

Table 2-8: 2012 and 2013 combined analysis of variance F-statistics for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), root proportion (RP), fine root length (FRL), root dry weight (RDW) and maximum root depth (MRD) for genotypes within species for DD tubes.

Species	DF	TRL	TRSA	ARD	FRL	RP$^{\pounds}$	RDW (0-90 cm)	MRD (0-90 cm)
CB								
Year	1	4.99	9.08*	2.18	4.72	8.87*	13.53*	20.05***
Genotype	3	4.21*	10.04***	1.31	3.44*	4.70*	0.97	2.44
Year*Genotype	3	2.97*	7.63***	2.27	2.77	0.79	1.61	1.59
Depth	1	78.30***	155.92***	14.77***	68.87***	NA	NA	NA
Year*depth	1	2.15	0.07	1.99	2.47	NA	NA	NA
Genotype*depth	3	2.21	0.68	1.19	2.35	NA	NA	NA
ST								
Year	1	0.71	0.84	1.66	0.77	10.98*	6.98*	-∞
Genotype	3	0.47	0.69	0.49	0.38	3.47*	1.84	-
Year*Genotype	3	5.61***	4.38***	0.33	5.32***	10.31***	1.66	-
Depth	1	5.68*	0.23	45.42***	7.56***	NA	NA	NA
Year*depth	1	12.44**	7.36***	1.37	13.17***	NA	NA	NA
Genotype*depth	3	0.92	0.85	0.37	0.90	NA	NA	NA
Z. japonica								
Year	1	4.14	14.60***	1.83	3.49	3.07	31.12***	10.91*
Genotype	3	21.32***	11.30***	0.82	22.12***	26.71***	12.26***	21.55***
Year*Genotype	3	11.20***	15.56***	4.96***	10.70***	6.06***	12.33***	4.26*
Depth	1	160.01***	187.83***	18.42***	154.01***	NA	NA	NA
Year*depth	1	0.12	3.22	0.18	0.03	NA	NA	NA
Genotype*depth	3	4.15*	4.34***	2.63	3.97	NA	NA	NA

Table 2-8: Continued.

Species	DF	TRL	TRSA	ARD	FRL	RP[£]	RDW (0-90 cm)	MRD (0-90 cm)
Z. matrella								
Year	1	2.83	7.83*	3.00	2.07	0.01	14.25***	6.48*
Genotype	3	38.78***	35.57***	1.49	38.07***	6.94***	5.30***	9.91***
Year*Genotype	3	8.82***	7.15***	1.08	8.84***	4.46*	4.72*	7.17***
Depth	1	267.14***	245.75***	3.26	263.19***	NA	NA	NA
Year*depth	1	1.15	6.22***	0.02	0.76	NA	NA	NA
Genotype*depth	3	0.29	1.19	0.52	0.32	NA	NA	NA

NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

[£] Root proportion (RP) parameter is a ratio of total root at 0-30/30-90 cm soil depth classes; therefore, depth is not a parameter in the analysis of this characteristic.

∞ All ST genotypes had similar MRD of 90 cm.

Table 2-9: Whole tube (0-90 cm) analysis of variance F-statistics for total root length (TRL), total root surface area (TRSA), and fine root length (FRL) for genotypes within species for DD tubes.

Species	DF	TRL	TRSA	FRL
CB				
Year	1	4.99	9.08*	4.72
Genotype	3	2.65	8.74***	2.11
Year*Genotype	3	1.87	6.65***	1.70
ZJ				
Year	1	NT	NT	3.49
Genotype	3	NT	NT	12.96***
Year*Genotype	3	NT	NT	6.27***
ZM				
Year	1	2.83	7.83*	2.07
Genotype	3	24.85***	21.91***	24.70***
Year*Genotype	3	5.66***	4.41*	5.74***

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

NT, Not tested indicates there was a significant depth interaction. These traits were analyzed by depth in Tables 1-6.

Table 2-10: Whole tube (0-90cm) average for total root surface area (TRSA) and root proportion (RP) at 0-30 cm depth of CB genotypes for DD tubes

Genotypes	2012 TRSA (cm^2)	2013 TRSA (cm^2)	RP (0-30 cm)
289922	817 a[‡]	949 a	0.57 b
Celebration	689 a	785 a	0.65 ab
UFCD347	595 a	755 a	0.61 ab
UFCD12	330 b	873 a	0.71 a

[‡] Means followed by same letters within each column were not significantly different, $P \leq 0.05$; these traits are reflective of characteristics across the entire root system.

Table 2-11: *Zoysia japonica* genotype averages for total root length (TRL), total root surface area (TRSA), at 0-30 cm and 30-90 cm depths and root proportion (RP) at 0-30 cm depth for DD tubes.

	2012				2013			
	TRL (cm)		TRSA $(cm)^2$		TRL (cm)		TRSA $(cm)^2$	
Genotypes	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm
5269-24	3748 a[‡]	2456 a	399 a	229 a	3640 ab	2642 a	479 b	312 a
4360	2554 a	217 b	247 a	17 b	4749 a	2194 a	749 a	262 a
BA182	3010 a	196 b	403 a	31 b	2812 b	233 b	394 b	38 b
JaMur	2591 a	335 b	356 a	49 b	2739 b	0 b	393 b	0 b

[‡] Means followed by same letters within each column were not significantly different, $P \leq 0.05$.

Table 2-12: Whole tube (0-90cm) average root dry weight (RDW), maximum root depth (MRD) and fine root length (FRL) of ZJ genotypes for DD tubes.

	2012				2013			
Genotypes	RDW[†] (gm)	MRD (cm)	FRL (cm)	RP (0-30 cm)	RDW[†] (gm)	MRD (cm)	FRL (cm)	RP (0-30 cm)
5269-24	0.68 a[‡]	71 a	6094 a	0.64 b	1.25 b	90 a	6095 a	0.58 b
4360	0.71 a	33 b	2422 b	0.95 a	2.20 a	74 a	6639 a	0.70 b
JaMur	0.77 a	34 b	2808 b	0.89 a	0.78 b	29 b	2603 b	1.00 a
BA182	0.66 a	35 b	3084 ab	0.94 a	0.74 b	42 b	2905 b	0.91 a

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05; these traits are reflective of characteristics across the entire root system.

Table 2-13: Whole tube (0-90cm) average for total root length (TRL), total root surface are (TRSA), root dry weight (RDW), maximum root depth (MRD), fine root length (FRL) in 2012 and 2013 and root proportion (RP) at 0-30 cm depth of ZM genotypes for DD tubes.

	2012						2013					
Genotypes	TRL (cm)	TRSA (cm^2)	RDW[†] (gm)	MRD (cm)	FRL (cm)	RP (0-30 m)	TRL (cm)	TRSA (cm^2)	RDW[†] (gm)	MRD (cm)	FRL (cm)	RP (0-30 cm)
BA336	7934 a[‡]	1005 a	0.76 a	78 a	7702 a	0.66 a	6961 a	1003 a	1.85 a	80 ab	6622 a	0.70 b
Zeon	3519 b	405 b	0.60 a	52 ab	3442 b	0.78 a	3116 b	424 b	0.68 b	41 c	3001 b	0.95 a
BA374	3991 b	464 b	0.81 a	42 b	3891 b	0.84 a	7123 a	967 a	1.59 a	90 a	6822 a	0.69 b
ToccoaGreen	3145 b	362 b	1.04 a	40 b	3070 b	0.89 a	4127 b	624 b	1.00 b	60 bc	3902 b	0.82 b

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05; these traits are reflective of characteristics across the entire root system.

[†] Dry weights were determined after drying at 65° C for 48 hours.

Table 2-14: Multivariate analysis of variance F-statistics across species, across genotypes, genotypes within CB, genotypes within ST, genotypes within ZJ, and genotypes within ZM for DD experiment.

Variables	Wilks' Lambda
Across Species	14.59***
Genotypes within CB[ǂ]	1.33^{NS}
Genotypes within ST	1.14^{NS}
Genotypes within ZJ	2.81***
Genotypes within ZM	3.26***

NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

[ǂ] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table 2-15: Within class standardized canonical coefficient for root and shoot parameters across species for DD experiment.

Variables	CAN1	CAN2
TRL	-7.4882880	8.65385
TRSA	0.9045773	-2.37160
ARD	-0.0604810	0.59025
MRD	-1.0714270	0.29537
RDW	0.3002290	0.45657
FRL	7.8633476	-7.84610
TQ day15	-0.2618900	-0.57960
LF day 15	0.1896373	0.38251
RP	-0.5233590	-1.26430
[ǂ]Can R^2	0.89***	0.66***
[‡]PR	0.82	0.16
[ǂ]Cu PR	0.82	0.98

[ǂ]Canonical Correlation, [‡]Proportion, [ǂ]Cumulative proportion

*** Significant at $P \leq 0.0001$.

Table 2-16: Class mean for canonical variables across species for DD experiment.

Genotype	CAN1	CAN2
CB[ǂ]	-0.9756670	1.43563
ST	3.3802875	-0.06930
ZJ	-1.2483860	-0.59700
ZM	-1.1562350	-0.76940

[ǂ]ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table 2-17: Within class standardized canonical coefficient for root and shoot parameters for genotypes within ZJ species for DD experiment

Variables	CAN1	CAN2
TRL[ǂ]	-19.9680	-6.25930
TRSA	0.43020	3.51823
ARD	-0.06790	-1.99750
MRD	0.98749	-0.50550
RDW	-0.31520	1.37160
FRL	18.9005	2.89055
TQ day15	-0.82930	-0.01950
LF day 15	0.70618	-0.40810
RP	-0.62980	-0.37310
[ǂ]Can R^2	0.87**	0.70^{NS}
[‡]PR	0.72	0.21
[ǂ]Cu PR	0.72	0.93

[ǂ]Canonical Correlation, [‡]Proportion, [ǂ]Cumulative proportion
NS, *, **, *** Non-significant or significant at $P \leq 0.05$, 0.01 or at 0.0001 respectively.

Table 2-18: Class mean for canonical variables within ZJ genotypes for DD experiment.

Genotype	CAN1	CAN2
5269-24	2.93538	-0.2252
BA182	-0.92240	-0.8118
4360	-0.54960	1.5644
JaMur	-1.46340	-0.5275

Table 2-19: Within class standardized canonical coefficient for root and shoot parameters for genotypes within ZM species for DD experiment.

Variables	CAN1	CAN2
TRL[ǂ]	-4.28900	-37.10800
TRSA	0.72496	8.39966
ARD	0.00229	-2.15270
MRD	-1.23530	-0.28400
RDW	0.48271	-0.46820
FRL	5.79040	30.24960
TQ day15	-0.63910	-0.28410
LF day 15	-0.22920	1.21946
RP	0.10009	-0.04710
[ǂ]Can R^2	0.88***	0.72*
[‡]PR	0.69	0.21
[ǂ]Cu PR	0.69	0.90

[ǂ]Canonical Correlation, [‡]Proportion, [ǂ]Cumulative proportion
NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table 2-20: Class mean for canonical variables within ZM genotypes for DD experiment.

Genotype	CAN1	CAN2
BA336	2.45297	0.86401
BA374	0.48346	-0.44270
ToccoaGreen	-0.43090	-1.42720
Zeon	-2.50560	1.00589

Table 2-21: ΨStepwise regression analysis for root traits that were significant at 0.05 levels that could be associated with better canopy response during greenhouse DD.

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	LN_MRD	1	0.2402	0.2402	3.0807	39.84	<.0001

[ϕ] Significant at P ≤ 0.05 as identified by stepwise regression model.

ΨStepwise regression model: Ln_LF11 = Ln_TRL Ln_TRSA Ln_ARD Ln_FRL LN_RDW Ln_MRD Ln_RP.

Table 2-22: ΨStepwise regression analysis for root traits that were significant at 0.05 levels that could be associated turf quality (TQ11) during greenhouse DD.

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	LN_TRSA	1	0.2184	0.2184	5.2655	35.2	<0.0001

[ϕ] Significant at P ≤ 0.05 as identified by stepwise regression model.

ΨStepwise regression model: Ln_TQ11 = Ln_TRL Ln_TRSA Ln_ARD Ln_FRL LN_RDW Ln_MRD Ln_RP.

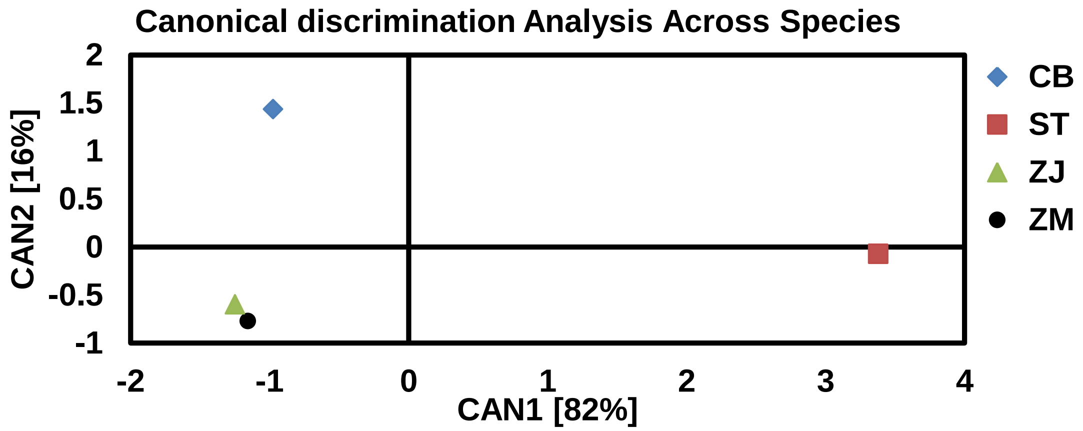

Figure 2-1: Canonical discriminate analysis class means plotted across four warm season turf species.

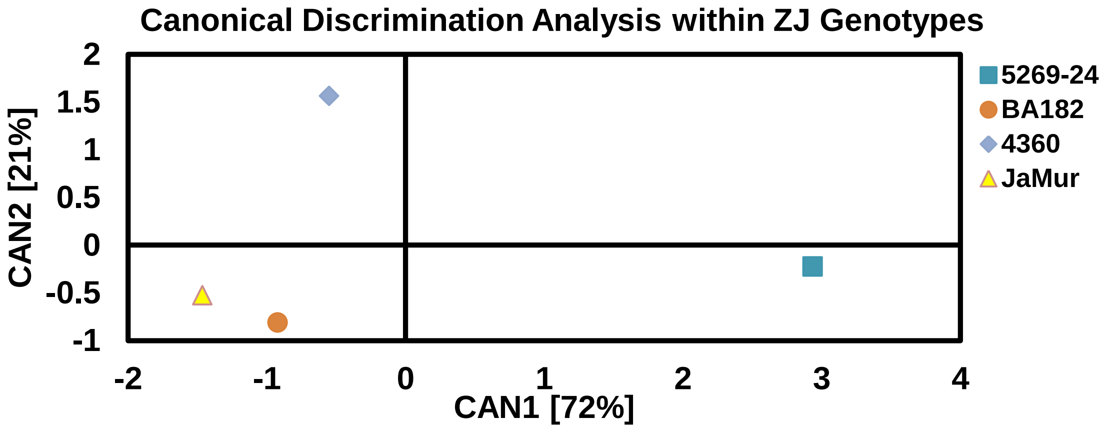

Figure 2-2: Canonical discriminate analysis class means plotted for genotypes within ZJ species.

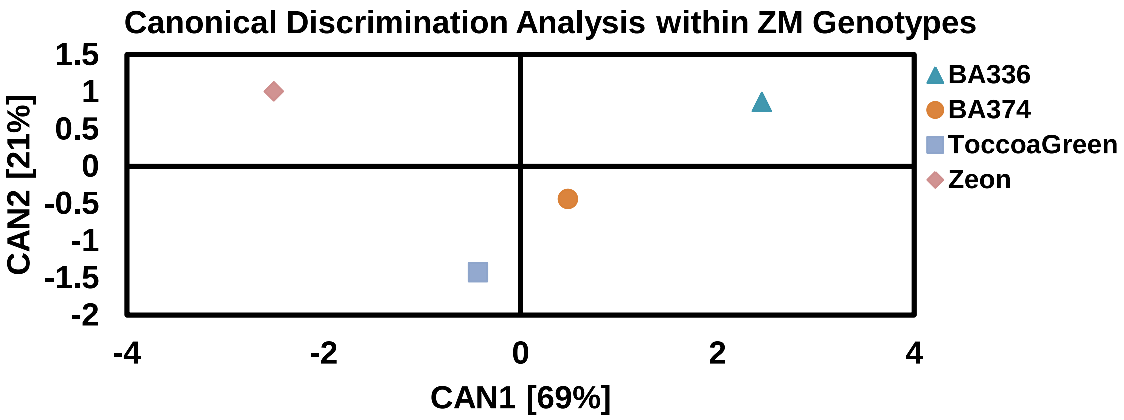

Figure 2-3: Canonical discriminate analysis class means plotted for genotypes within ZM species.

CHAPTER 3

ROOTING CHARACTERISTICS OF WARM SEASON TURFGRASS SPECIES UNDER LIMITED SOIL MOISTURE DURING FIELD DRY DOWN

Introduction

Different approaches have been used to study turf root architecture. The most common greenhouse method has been the use of clear tubes constructed of transparent polyvinyl chloride (PVC) that allow for visual observation of (Su et al. 2008). This method is useful to study root growth and development in short duration studies (Thapa, 2010; Fuentealba, 2010; Poudel, 2010). Although the handling of these PVC tubes and harvest of intact root systems is convenient and logistically simplified there is a significant loss of root mass during washing of samples to separate the roots from the soil. Similarly, root core samples are the most common method to evaluate root growth under field conditions (Hendrick and Pregitzer, 1996), but also result in a loss of roots during sampling and washing. Both of these common screening techniques are destructive sampling methods which do not allow for the evaluation of the same root segments over time.

To address this limitation, minirhizotrons have been utilized to understand *in situ* turf root architecture differences over time under field conditions (Murphy et al., 1994, Aryal et al., 2015). This technology uses still images acquired using a camera and computer software that quantifies root parameters such as total root length, root surface area, root volume and different root diameter classes. This non-destructive method has been widely used to study root morphological characteristics in field studies of other plant species such as cotton, peanut, and bahiagrass (Rowland et al. 2015; Loison et al. 2012) and in a limited number of studies for turfgrass (Murphy et al. 1994; Aryal et al., 2015). In particular, minirhizotrons have been utilized to study root parameters of cool season grasses (Bonin et al. 2013, Murphy et al., 1994) and a few perennial warm season grasses (Bonin et al. 2013; Aryal et al., 2015). The technology allows quantification of rooting depth, diameter, volume and surface area (Guo et al., 2007) which may be associated with a plant's relative ability to extract nutrients and water from the soil (Ingram and Leers, 2001). In addition, because the minirhizotron technology allows for repeated imaging of the same locations within the root system, this technique is a powerful means of evaluating changes in root architecture over time in response to treatment or season.

The most common warm season turfgrass species grown in Florida are St. Augustinegrass [*Stenotaphrum secundatum* (Waltz.) Kuntze], bermudagrass [*Cynodon dactylon* (L.) Pers], and two zoysiagrass species [*Zoysia japonica* (Steud) and *Zoysia matrella* (L.) Merr] (Trenholm et al., 2011, McCarty, 1995). St. Augustinegrass is the primary home lawn species used for turf in the southeastern U.S. The grass is native to coastal regions of the Mediterranean and the Gulf of Mexico (Trenholm et al., 2014), and known to grow well in a variety of soil types. Bermudagrass is a warm season perennial turfgrass species best adapted to tropical and sub-tropical climates and is cultivated in many parts of the world mainly for forage; however, it is also utilized for sports fields, golf courses, and home lawns (Taliaferro et al., 2004). Bermudagrass is native to Africa and Asia, but has naturalized in many regions of the world (de Wet and Harlan, 1970; de Wet and Harlan, 1971) and is now cultivated in more than 100 countries. Another species gaining popularity in Florida is zoysiagrass, a warm season grass native to Asia. Zoysiagrasses are low maintenance species that require less fertilizer and have superior turf quality (McCarty, 1995).

Turfgrass water use is of major concern in Florida home lawns (Haley et al., 2007). Every year, erratic rainfall and weather conditions bring periods of drought in most regions of Florida. Growing urbanization in Florida has also increased the turf and landscape acreage across the state, bringing with it, an increase in the amount of water used for maintaining turf (Trenholm et al., 2000; Trenholm et al., 2011). Drought conditions combined with state laws for water conservation can affect turf management. Currently, water management district regulations for turfgrass in home lawns only allow irrigation twice a week (St. John River Water Management District, SJRWMD) in Florida. These restrictions on lawn irrigation often result in drought stress in most Florida home lawns (Ozan and Alsharif, 2013).

Recent studies have been focused more on understanding turfgrass water use and selection and development of turfgrass cultivars that have drought resistant characteristics (Zhou et al. 2014). Different studies have been undertaken to understand performance ranking or to quantify drought resistance characteristics within turfgrass species or genotypes (Zhou et al. 2014; Sun et al. 2013; McCready and Dukes, 2011; Thapa; 2010; Poudel, 2010; Fuentealba, 2010; McCann and Huang, 2008; Baldwin et al. 2006; DaCosta and Huang, 2006; Huang and Gao, 2000; Huang, 1999). For example, Severmutlu et al. (2011) assessed drought resistance characteristics of seven turfgrass species (bermudagrass, zoysiagrass, buffalograss, bahiagrass, seashore paspalum, centipedegrass, and tall fescue) using measurements of percent leaf firing, turfgrass quality, and percent green cover. They found that bermudagrass, bahiagrass and buffalograss demonstrated superior drought resistance with less leaf firing and better shoot recovery in comparison to other species while centipedegrass and zoysiagrass exhibited significant leaf firing and almost no recovery from drought upon re-watering (Severmutlu et al. 2011).

Baldwin et al. (2006) evaluated six bermudagrass cultivars (‘SWI-1021’, ‘Arizona Common’, ’Tif No.3’, ‘TifSport’, ’Aussie Green’, and ‘Celebration’) under three irrigation intervals of 5, 10 and 15 days and found, when pooled across all irrigation treatments, that ‘Celebration’ produced 114% and 97% greater root weight than ‘TifSport and ‘Aussie Green’. Steinke et al. (2009) evaluated four warm season turfgrass species, bermudgrass, St. Augustinegrass, and two zoysiagrass species, over 60 days of drought and reported that zoysiagrass exhibited higher canopy temperatures than bermudagrass or St. Augustinegrass. On average, zoysiagrass exhibited 4-9 and 4-13 °C warmer temperatures than St. Augustinegrass or bermudagrass, respectively. Similarly, Steinke et al. (2010) evaluated seven St. Augustinegrass cultivars for drought response, and reported that St. Augustinegrass cultivars differed in their drought response measured using digital image analysis for different environmental stressors such as heat, soil drying or drought. In the same experiment, among seven St. Augustinegrass genotypes (Amerishade, Common, Delmar, Floratam, Palmetto, Raleigh, Sapphire, Mean), ‘Floratam’ had delayed leaf firing and elevated quality in comparison to other genotypes under water stress.

Examining only turfgrass canopy responses to drought is not enough to fully quantify drought resistant characteristics. It is equally important to understand below ground rooting responses by undertaking root morphological studies to understand above ground canopy response in a non-destructive fashion. In previous turfgrass studies, minirhizotron technology was used to evaluate creeping bentgrass (*Agrostis* *stolonifera* L.) rooting behavior in response to deficit irrigation in a non-destructive manner over two year time. In this study conducted by Fu and Dernoeden, (2009), drought led to increases in total root number. Utilizing the ability of minirhizotrons to assess roots at the exact same location of the root system repeatedly over time, Murphy et al. (1994) studied the rooting behavior of two cool season species, 'Penncross' creeping bentgrass (*Agrotis plustris* Huds.) and annual bluegrass [*Poa aannua* L. *var. reptans* (Hausskn.)] and found that total root number was greatest during June and early July with a gradual decline during late August and early September. This would suggest that plants may alter root morphology to compensate for temperature stress during the season.

The objectives of this field study was to compare the rooting response of four warm season turfgrass species (four genotypes of each species) as they progress through a field dry down imposed by elimination of water over a four week period. Genotypes were selected based on a previous greenhouse study (Fuentealba, 2010) that assessed these genotypes (and others) for high or low transpiration rates and late or early break points (BP: early or late stomatal closure in response to available fraction of transpirable soil water). We hypothesized that under field dry down, genotypic variation in root architecture would be evident among the selected genotypes of the four turfgrass species. The specific objectives were: 1) compare the changes in root system architecture of these species during a 21 day dry down; 2) identify rooting differences between genotypes within each species during dry down; 3) determine which root parameters are the most effective to screen relative to dry down responses; and 4) determine if the traits deemed most useful during drought are also observed during non-drought stressed periods.

Materials and Methods

A field study was conducted at the University of Florida’s Plant Science Research and Extension Unit (PSREU) in Citra, FL to assess the root responses of warm-season turfgrass species to a limited soil moisture conditions or sequential surface soil drying (DD). The study was arranged as a randomized complete block design (RCBD), with three replications of sixteen warm season turfgrass genotypes comprised of commercial cultivars and experimental genotypes available from the UF turfgrass breeding program. Genotypes within species included: ‘Celebration’, UFCD347, UFCD12, and 289922 bermudagrasses (CB); ‘JaMur’, 4360, 5269-24, and BA182 for *Zoysia japonica* (ZJ); ‘ToccoaGreen’, ‘Zeon’, BA336, and BA37 for *Zoysia matrella* (ZM); and ‘Floratam’, ‘Captiva’, ‘Palmetto’, and ‘Sapphire’ St. Augustinegrasses (ST). All entries were clonally propagated in a greenhouse and ten 3.8 × 3.8 cm plugs were transplanted into 1.83 × 2.14 m^2 field plots in June, 2011. Plots were fertilized with 49 kg^{-1} ha^{-1} $month^{-1}$ of N for the months of April, June, July and September using applications of 15-1-15 fertilizer (Signature brand fertilizer, Greeley, CO). For weed control, preemergence herbicides (Oxadiazon and prodiamine, 3.36 kg a. i. ha^{-1}) were applied during the months of February, May and October in 2012; March and September in 2013; and February in 2014. The herbicide 2,4-D, 2-ethylhexyl ester was applied in spring and summer to control broadleaf weeds (0.96 kg a. i. ha^{-1}). Bifenthrin and diafenthiuron (insecticides) were applied to control mole crickets (*Scapteriscus Borelli* Giglio-Tos) and caterpillars (*Crambus spp.*) in April, September, and November of 2013 (0.44 kg a. i. ha^{-1}). Grasses were mowed once a week with a rotary mower and maintained at 6.4 cm mowing height throughout the study period.

To study rooting characteristics, transparent 5.10 cm (inner diameter) by 1.83 m minirhizotron tubes were inserted into each field plot at a 45° angle to the soil surface in July 2011 and roots along the tube walls were periodically imaged using a digital camera system (Bartz Technology Corp., Carpinteria, CA). The experiment was well-watered until the imposition of a dry-down treatment. In both study years, May 7 was the initiation of the DD in both 2013 and 2014 and represented a well-watered (WW) condition (day 1); while May 28 in 2013 and May 29 in 2014 were the ending points of the DD and represented a severe drought condition (SD, day 21). During the three consecutive weeks (May 7-28 in 2013 and May 7-29 in 2014) irrigation water was withheld and rainfall excluded using a 15.3 × 30.5 m tarp (Agri Supply, Valdosta, GA) in 2014 to cover the field plots during periods of rain. Digital images of the root system were collected on May 7, 14, 20 and 28 in 2013 and on May 7, 14, 22 and 29 in 2014 and were analyzed for root parameters including: total root length (TRL, in mm), total root surface area (TRSA, in mm^2), average root diameter of roots present (ARD, in mm), maximum root depth (MRD, cm), root proportion (RP) and fine root length (FRL, in mm). Fine root class selected in this study were based on previous studies where root ≤ 1.0 mm were categorized as fine roots that were related with increased root surface area in two tallgrass prairie *Bromus inermis* (L.) and *Poa pratensis* (L.) (Reinhardt and Miller, 1990). Similarly, Meier and Leuschner (2008) had categorized roots at 1.15-1.58 mm diameter class as fine roots in European beech (*Fagus sylvatica* L.) tree species. Root proportion (RP) was calculated as the TRL at 0-30 cm/ TRL for the entire tube (0-90 cm depths). Visual canopy responses for leaf firing at beginning (LF1) and at the end of DD (LF21) and turf quality (TQ1 and TQ21) were recorded. Turf quality and leaf firing were rated using a 1-9 scale, where a value of 1 represented severe loss of TQ or high LF (brown plot) and a value of 9 represented excellent TQ or no LF; visual ratings of 6 represented acceptable TQ/LF response. TQ and LF ratings were obtained from concurrent study Zhang (2014).

Statistical Analysis

Mixed model analysis of variance (ANOVA) was performed using Proc GLIMMIX (SAS Institute Inc., Cary, NC) to determine differences in TRL, TRSA, ARD, FRL, MRD and RP across species and genotypes within each species. In each analysis, year, day, depth, species and/or genotype (as relevant) were treated as fixed factors and replication with nested year as a random factor. Data for TRL, TRSA, ARD and FRL was square root transformed to meet to the assumptions of normality (Bartlett, 1947). Root parameters during DD were analyzed across the entire tube (0-90 cm) and using depth as a factor in the model with two levels: 0-30 and 30-90 cm sections. Individual tubes also represented the genotypes or species under study, and were recognized as repeated measures. Thus, in the analysis, depths nested within tubes was also utilized as a repeated measure (root images were collected within each tube at two depths). Data analysis was limited to two way interaction such that the tabulation and interpretation of the results would be meaningful, statistical analysis at each level: species, and genotype within species were thus limited to two way interactions. Whenever an interaction with year was not significant, data for the two study years were pooled. For separation of means, a Tukey’s honestly significant differences (HSD) test was performed.

A multivariate analysis of variance using canonical discriminate analysis (CDA) was performed to determine the root traits that were most effective at separating species, genotype within species variability at the end of DD (day 21). CDA analysis was selected for multivariate analysis in our study for its capability to separate classes for correlated data (Zhao and Maclean, 2000). "In CDA, the matrix algebraic equation which provides the canonical discriminate functions are constrained such that the successive discriminate functions define the maximum possible differences between experimental treatments" (Matthew et al., 2010).To determine the relationship between root traits measured and level of leaf firing and turf quality, a stepwise regression analysis was performed at day 21 (DD) and 1 (WW). At day 21, species had varied response for LF, where some species maintained acceptable LF rating of 6 compared to others (i. e. differences between CB and ST, ZJ or ZM species). Traits thus quantified by the model would help explain the association of important root trait with canopy LF response. Similar, statistical analysis was performed with TQ rating at the end of DD (TQ21). Root traits will be assessed in the model one at a time at 0.05 significance level. Trait significant at 0.05 level will be included in the model. Species response for LF/TQ at day 21, and the root traits thus quantified by the model would help explain the association of important root traits with canopy response. To determine if the traits deemed most useful during drought are also observed during non-drought stressed periods a similar stepwise regression analysis was also performed for TQ and LF rating at day1, using the following models into the stepwise regression analysis: Day 21: Ln_LF21 = Ln_TRL Ln_TRSA Ln_FRL Ln_ARD Ln_MRD Ln_RP (Equation-1). Day21: Ln_TQ21 = Ln_TRL Ln_TRSA Ln_FRL Ln_ARD Ln_MRD Ln_RP (Equation-2). Day 1:

Ln_LF1 = Ln_TRL Ln_TRSA Ln_FRL Ln_ARD Ln_MRD Ln_RP (Equation-3). Day1: Ln_TQ1 = Ln_TRL Ln_TRSA Ln_FRL Ln_ARD Ln_MRD Ln_RP (Equation-4).

Results

Evidence of Dry Down

The turf canopy response of TQ and LF during dry down were analyzed at the beginning of the dry down (TQ1 and LF1) and at the end of dry down (TQ21 and LF21). Species differences were only evident for TQ21 and LF21 (Table 3-1). Species by year interaction did not occurred thus data were pooled for two years (Table 3-1). By the end of DD (day 21), CB had higher TQ with less LF followed by ST, ZJ and ZM species (Table 3-2). For genotype within species comparisons, LF21 and TQ21 was significant only for ST genotypes (LF21=3.40, TQ21=3.46 $p<.05$), these traits were insignificant for CB (LF21= 2.06, TQ21=1.75 NS), ZJ (LF21=2.79, TQ21=2.83 NS), and ZM (LF21= 2.68, TQ21=2.83 NS). In ST, Sapphire had higher TQ than Captiva at day 21 (Sapphire> Captiva) however TQ was similar for Sapphire, Floratam, and Palmetto. Similar was the result for Floratam, Palmetto, and Captiva. For LF, Floratam had less leaf firing compared to Captiva at day 21 (Floratam>Captiva) however LF was similar for Floratam, Sapphire, and Palmetto. Similar was the result for Sapphire, Palmetto, and Captiva (data not shown).

Across Species During Periods of DD (day 1, 7, 14, 21): at 0-30 and 30-90 cm

The effect of surface soil drying (limited soil moisture) caused an overall increase in TRL, TRSA, and FRL over the 21 day dry down when comparing across species and depths (Table 3-3). However, species were not different for ARD (Table 3-3). For TRL, the increase was seen in a relatively short time period, developing during the first seven days (Table 3-4). For TRSA and FRL, differences developed more slowly in a 14 day period. Examining the details of the root responses showed that TRL, TRSA, and FRL differences between upper (0-30 cm) and lower (30-90 cm) soil depths showed year by species as well as species by depth interactions (Table 3-3); thus data are presented for year by species and depth. Although day was significant, none of these traits produced a significant species by day interaction. The RP had a significant year by species interaction (Table 3-3); therefore, data were reported for both years.

In 2013, average TRL and TRSA in the 0-30 cm depth were similar between species; while at the 30-90 cm depth, TRL and TRSA were greater for CB compared to ST, ZJ, and ZM species (Table 3-5). Fine root length was also greater for CB at this depth compared to the other three species. In 2014, TRL and FRL in the 0-30 cm depth was similar across all species; while at the 30-90 cm depth, CB and ST overall had greater TRL, TRSA and FRL compared to ZJ and ZM species that were similar across these traits (Table 3-5). When looking at the relative proportion of root distribution in the 0-30 and 30-90 cm depths, RP varied dramatically between species across years. In 2013 and 2014, ZJ and ZM species allocated 90% or more of their TRL in the upper soil depth, followed by ST that allocated 71-81% of its roots in the shallow depth (Table 3-5). Alternatively, CB had a relatively balanced root distribution between upper and lower depth categories with an RP of 53 and 52 in 2013 and 2014, respectively.

Genotypes within Species

There were no significant differences in root traits among genotypes within a given species by depth (CB =2.92, 3.21, 5.35, 1.63, 1.28 ; ST= 2.17, 2.01, 0.40, 3.18, 2.91; ZJ = 1.81, 1.90, 0.01, 1.51, 1.08; ZM = 0.72, 0.72, 0.49, 0.70, 0.94; P-values for TRL, TRSA, ARD, FRL and RP respectively for genotype within each species); thus all results shown are for traits across the entire tube length (0-90 cm) (Table 3-6).

The CB genotypes did differ for TRL, TRSA and ARD but showed no year by genotype or day by genotype interaction (Table 3-6); therefore, data from both years were pooled to produce genotype means (Table 3-7) and daily means across all CB genotypes (Table 3-8). For FRL, MRD and RP, CB genotypes were not different (Table 3-6). Among genotypes, UFCD347 and Celebration had higher TRL compared to UFCD12; while root length was similar for UFCD347, Celebration, and 289922, and for 289922 and UFCD12 (Table 3-7). The TRSA was lowest for UFCD12 compared to UFCD347, 289922 and Celebration; while for ARD, the only differences were seen for 289922 that had larger ARD than UFCD12 (Table 3-7). As was seen in the species comparisons, TRL, TRSA and FRL increased during the dry down period for CB genotypes (Table 3-8).

Within ST and ZM species, differences between genotypes were only identified for MRD (Tables 3-6 and 3-9). No interactions occurred for MRD allowing genotype means for each of these species to be pooled for the two experimental years. For ST, Sapphire and Captiva produced the deepest roots (75 and 68 cm respectively), while Floratam had the shallowest roots (51 cm). Palmetto (59 cm) was not different from Captiva or Floratam (Table 3-9). Within ZM, BA374 had the deepest roots (60 cm) and Zeon had the shortest roots (42 cm) (Table 3-9). Within ZJ, genotypes were indifferent for all the traits under study (Table 3-6).

Discriminate Analysis

Across Species at the End of DD (day 21)

The multivariate analysis of variance examined eight linear combinations of root or shoot variables: TRL, TRSA, ARD, MRD, FRL, RP, TQ21 and LF21. The multivariate analysis across species was significant (Wilk's λ = 10.05, Can1=0.90, p<.0001 and

CAN2=0.45, p<.05) (Tables 3-10 and 3-11). Of the two canonical functions generated, the canonical functions CAN1 explained 93% and CAN2 explained 5% of the species variability; thus, two canonical functions explained 98% of the root trait variation across species. The function CAN1 showed higher positive correlation with TQ21, MRD and TRL (Table 3-11); whereas, CAN2 was driven by TRSA, ARD, RP, MRD and FRL. When class means on canonical variables for each species (Table 3-12) were plotted (Figure 3-1), CB separated distantly in the multivariate space from the other three species. The patterns of separation in Figure 3-1 support that at day 21 of DD, the separation of CB from ST, ZJ and ZM species was highly influenced by MRD, and TRL. The discriminate analysis also revealed the relationship of the fours species to surface soil drying with respect to MRD, TRL (CAN1) and TRSA, ARD, FRL and RP (CAN2).

Genotypes within Species (day 21)

Discriminate analysis was performed to determine the root traits of interest that would differentiate genotypes within species. The multivariate analysis for genotype within species was significant for all species (Table 3-10). Multivariate analysis results for genotypes within respective species are reported in Tables 3-13, 3-15, 3-17, and 3-19.

Discriminate analysis for genotypes within CB species were analyzed using combinations of eight traits: TRL, TRSA, ARD, MRD, FRL, RP, TQ21, and LF21. Of the two factors, only CAN1 was significant [(CAN1=0.86, p<.01, CAN2=0.67 (NS)] and explained 70% of the within genotypes variability (Table 3-13). TRSA and FRL were positively correlated and dominant in CAN1 (Table 3-13). When class means (Table 3-14) for each genotype were plotted in the quadratic space (Figure 3-2), UFCD347, Celebration and 289922 were different in compared to UFCD12. When differentiating genotypes of CB under drought conditions it may be more useful to consider TRSA and FRL compared to other root traits.

Multivariate analysis comparison for genotypes within ST was significant (Wilk's λ=3.83, p<.0001) (Table 3-10). For ST, CAN1 was significant and explained 83% of the variability within ST genotypes whereas CAN2 was not significant [(CAN1=0.95, p<.0001, CAN2=0.78 (NS)] (Table 3-15). Three variables, MRD, TRL, and RP contributed the most in CAN1 and were positively correlated. When class means for each genotype (Table 3-16) were plotted (Figure 3-3), Sapphire and Captiva were separated from Floratam and Palmetto. Root traits that were most useful for separating ST genotypes during DD were MRD, TRL and RP.

Within ZJ only canonical factor 1 was significant (CAN1=0.94, p<.0001, and CAN2=0.85 NS) (Table 3-17). Four variables, LF21, FRL, TRSA and MRD were positively correlated with CAN1 and explained 76% of the within genotype variability. Class means for each genotype in the quadrant (Table 3-18), illustrate that JaMur was separated from the other genotypes and was positive for CAN1; whereas, 4360 was negative for CAN1 (Figure 3-4). The variables FRL, TRSA and MRD were the most effective traits for differentiating ZJ genotypes during DD.

Only CAN1 was significant (CAN1=0.88, p<.01) foe ZM and explained 64% of the variability within genotypes (Table 3-19). Positively correlated variables with CAN1 were TRSA, RP, FRL, and MRD (Table 3-19). When class means for canonical factors (Table 3-20) were plotted (Figure 3-5), BA336, BA374 and ToccoaGreen separated from Zeon and were positively correlated with CAN1. Root traits that most contributed for genotype grouping within ZM species were TRSA, RP, FRL, and MRD.

Stepwise Regression Analysis to Quantify Relevant Root Traits at the End of DD (day 21)

Stepwise regression analysis was performed to quantify the root traits that could be associated with a LF rating of 6 and above at the end of DD (day 21). Similar stepwise regression analysis was performed to quantify the root traits that could be associated with TQ at the end of DD (day 21). The most important root traits identified could thus be utilized to quantify the performance of species or genotypes under water limited condition. These root parameters could also be utilized in screening of species or genotypes in the future of turf breeding programs. Root variables included in the stepwise regression model were TRL, FRL, TRSA, ARD, RP and MRD. Drought response canopy variable was LF and TQ rating from day 21 (CB had LF rating of at least 6 or within the acceptable range even at the end of DD). Log transformation of all the variables was performed to standardize the traits for comparison during stepwise regression analysis. In the stepwise regression model, LF was regressed for all the root traits studied, and similar statistical analysis was performed for TQ (equations-1 and 2: materials and methods). Associated root traits with LF and TQ that are significant at 0.05 level in a stepwise fashion were selected by the model (fitness of root variable in the model was computed by taking one root variable at a time at 0.05 significance level). From the stepwise regression analysis RP, FRL and MRD were root traits that were associated with shoot response of LF and TQ rating during field DD (Tables 3-21 and 3-22).

Stepwise Regression Analysis to Determine if the Root Traits Deemed Most Useful at the End of DD (day 21) Can be Obtained During Day 1 (WW)

Stepwise regression analysis was also performed to quantify if root traits relevant at the end of DD (day 21) could also be identified during non-drought stressed period, day 1 (WW). These root traits could be utilized to understand if root parameters deemed important at the end of DD are also relevant during WW. Root traits included in the model were TRL, FRL, TRSA, ARD, RP and MRD. Visual LF and TQ ratings from day 1 (during WW) were included in the model as a canopy response. At day 1, all turf genotypes had LF or TQ ratings of 6 and above (LF or TQ ratings ranged from 6-9 for all genotypes). As day 1 was a non-drought stress period, the stepwise regression model was computed such that a LF rating was interpreted as a turf canopy response. Similar analysis was performed for TQ ratings from day 1. Log transformation was performed on all the variables to standardize the traits for comparison during stepwise regression analysis. Taking into account the censoring variables, LF or TQ ratings, the stepwise regression model (equations- 3 and 4: materials and methods) would then censor the corresponding associated root traits in a stepwise fashion at 0.05 significance level (fitness of root variable in the model was computed by taking one root variable at a time). From stepwise regression analysis MRD was identified as the root trait that would represent ideal turf LF response during non-drought stress periods, or under WW conditions (Table 3-23). However, ARD and RP were associated with TQ response at day 1 (Table 3-24).

Discussion

This *in-situ* root study utilizing a minirhizotron camera system was successful in quantifying turf rooting dynamics, including architectural changes over time during a field experiment achieved through a sequential dry-down over a three week period. Unlike destructive greenhouse and field studies that utilize transparent PVC tubes, soil core sampling methods (Qian et al. 1997; Hendrick and Pregitzer, 1996), this system acquired quantifiable digital images of the root system and was able to pinpoint soil dynamic root production or turnover by depth over three weeks of dry down (Bonin et al. 2013). Similar to other studies (Han and Young, 2014; Aryal et al., 2015) this study showed the advantages of the minirhizotoron system for addressing questions of root growth in response to surface soil drying on turfgrass genotypes.

The overall results from the field DD suggests root partitioning between species varied by year and by depths upper (0-30 cm) and deeper (30-90 cm) soil depths. Although data were generated once every week during the dry down period, interestingly, day (1, 7, 14 or 21) was not significant at all levels of comparisons (across species, or genotype within species). This might be due to the short period of dry down (21 days) that occurred during the study. The common response appeared to be an overall increased total root production (compared to day 1 with day 21), across all species and genotypes within species while accessed under limited soil moisture or surface soil drying of three weeks period.

The results of the study suggest that most of the turf root growth was concentrated in the upper 30 cm of soil; a pattern that was very evident for ZJ and ZM that had 6-11% of their respective root systems below 30 cm. The ST genotypes were moderately shallow in their architectures with 19-29% of their roots below 30 cm. The exception was that CB had a more uniform root distribution with a range of 47-48% of its roots below 30 cm.

These results were somewhat consistent in reference to the results from our greenhouse study (see Chapter II), where ZM and ZJ species had higher RP (80-90%) in the upper 0-30 cm of the soil depth and were expected to exhibit poor drought avoidance characteristics in the field DD. In contrast, ST and CB species had relatively high percentages of roots (30-50%) at the deep soil depth (30-90 cm) and were expected to have higher genetic potential for drought avoidance if screened under field DD. These assumptions based on the greenhouse experiment were supported by the results from the field DD experiment, as evidenced by the superior performance in CB and ST compared to ZJ and ZM (Zhang, 2014) under surface soil drying. In addition, CB and ST showed better TQ and less LF during the 21 days of field dry down compared to ZJ and ZM species. The relatively deep and extensive root systems of ST and CB in the field would allow them to take advantage of deeper soil moisture and maintain better root growth and development under water scarce conditions to resist LF during the field DD and maintain TQ.

In addition to TRL, the related traits of TRSA, ARD, and FRL were also enhanced at the deep 30-90 cm depth for CB and ST compared to ZJ and ZM species. This could also help explain the low LF rating for CB and ST in that improved root length, surface area, and active fine roots were enhanced at deeper depths. In a similar study, Carrow (1996) ranked species for deep root production at 20-60 cm depth in the order, CB (‘Tifway’) > tall fescue (‘Rebel II’) > ST (‘Raleigh’) > common centipedegrass > ZJ (’Meyer’). Qian et al. (1997) also reported in another field DD study that ZJ at a 60-90 cm depth had the lowest root growth and development in comparison to CB (‘Midlawn’). Shallow rooting of ZJ (Meyer) compared to CB (Midlawn) was also associated with higher leaf wilting or drought susceptibility (Qian et al., 1997). Similarly, the importance of deep and extensive roots for drought avoidance was reported by Steinke et al. (2010) for ST genotypes.

For genotypes within species, variability in root architecture under limited soil water environment or surface soil drying was evident. The CB genotype, UFCD12 had an overall poor root performance, with less TRL and TRSA, compared to other three CB genotypes when assessed across all soil depths (0-90 cm). These field DD results were consistent with the results from the greenhouse study where UFCD12 was noted with low root development compared to the other CB genotypes.

In ST, Floratam and Palmetto had poor root growth and development in 2013, likely due to poor initial stand establishment. For Floratam, this poor root system establishment was likely linked to its low percent green cover (PGC) in comparison to other ST genotypes in this field experiment (Zhang, 2014). Among ST genotypes, Sapphire had increased MRD than the other two genotypes, which translated at least for Sapphire into greater canopy response with improved TQ (Zhang, 2014).

For individual ZM and ZJ genotypes, there was little variability in root traits across the length of the tube; with the exception of the ZM genotype BA374 having a deeper root system compared to Zeon. Interestingly, when examined across sixteen genotype comparison Zeon had delayed leaf firing despite its shallow roots (Zhang, 2014); indicating the action of other non-root associated drought mechanisms (i. e. leaf osmotic adjustment) in this genotype. This situation underscores the need for understanding other drought resistance mechanisms along with root system effects in turfgrass breeding programs.

The association of deep root systems and improved response to limited soil water, as was noted for CB in the current study, was also found by Huang et al. (1997a) who reported improved drought resistance in warm season turf (centipede grass or

Paspalum compared to zoysiagrass) that had extensive root growth and enhanced root water uptake at deeper soil depths, with maintenance of root viability in the drying soil surface. Steinke et al. (2011) reported improved drought resistance in Celebration bermudagrass with a deep and extensive rooting system (Steinke et al., 2009) that had 50% green ground cover, delayed leaf firing and turf quality even after 60 days of dry down. Bermudagrass also had greater mean days50 value (or took 50-60 days to lose 50% of ground green cover) compared to St. Augustine in a similar study (Steinke et al., 2010). In another study, Fu et al. (2004) reported bermudagrass with acceptable turf quality (TQ rating of 6) even at a significantly reduced irrigation level (40% of ET) compared to zoysiagrass, tall fescue, and Kentucky bluegrass (*Poa pratensis* L.).

A discriminate analysis (CDA) was performed to quantify the important root traits associated with limited soil moisture environment that could be utilized to help explain the species/genotype root variability during DD. The purpose of a CDA analysis is to reduce the set of inter-correlated variables of large dimension data into smaller unrelated dimensions (Matthew et al., 2010), thus improving the efficiency of determination of differences between species or genotypes and their association to root or shoot traits of interest in relation to DD. Discriminate analysis has been utilized in different studies to quantify various traits of interest, including: the importance of lateral roots (fine roots) in relation to improved yield in pea (*Panicum sativum* L.; Androsiuk, 2012); the association of the number of lateral roots with herbage yield in alfalfa (*Medicago sativa* L.; Lamb et al., 2000); and the association of physiological and emergence characteristics under different soil moisture regimes in cool-season grass species (Gazanchian et al., 2006). Similar to these studies, the canonical functions

(CAN1 or CAN2) were able to explain 98% of the across species, 89% of the genotypes within CB, 97% of the genotypes within ST, 99% of the genotypes within ZJ, and 86% of the genotypes within ZM species variability in root traits under study. Overall the most dominant root traits that were relevant to help explain species (CB versus ST, ZJ and ZM) and genotypes [UFCD347, Celebration (CB); Sapphire and Captiva (ST); and BA336, BA374, Zeon (ZM)] grouping were FRL, TRSA, MRD and RP.

The results from the stepwise regression analysis further supported the results of the CDA analysis. During surface soil drying, FRL, MRD and RP were identified as the most significant root parameters that were associated with the LF and TQ during DD. Similar to this result, in the CDA analysis, FRL and MRD were also the most important root parameters that played important roles in separating species and genotypes within species. A similar stepwise regression analysis was utilized by Leksungnoen et al. (2012) to understand drought tolerance or susceptibility of tall fescue (*Festuca arundinacea*) and Kentucky bluegrass (*Poa pratensis* L.) in relation to soil water depletion pattern at different soil depths (0-20, 20-40, 40-60, 60-80 and 80-100 cm). In their analysis, different soil depths were treated as independent variables and stomatal conductance as a dependent variable. Tall fescue was found to have extracted more water from deep soil layers (80-100 cm) and maintained turf quality compared to Kentucky bluegrass that was more sensitive to soil water stress in the mid soil layer (40-60 cm). This deep rooting pattern for tall fescue was also found by Su et al. (2008), that reported tall fescue to have 3-12 times greater root mass at deeper soil depths compared to Kentucky bluegrass. The relationship between deep rooting and drought tolerance was supported by the current study that found bermudagrass had more deep roots at 30-90 cm soil depth and was able to sustain 21 days of drought with no loss of leaf firing or turf quality; in contrast to the shallow rooted zoysiagrasses that lost turf quality and were found susceptible to drought. These results indicate that turf species with deep and extensive root systems could sustain performance during dry down longer in water-limited environments.

However, it is always necessary to quantify turf root and quality traits under well-watered conditions as well, either to assess the utility of species or genotypes under adequate irrigation or during periods of abundant rainfall. In the current study, a stepwise regression revealed that MRD was the only trait linked to LF whereas ARD, and RP were associated with TQ when water was adequate; however, this same trait (MRD and RP) was linked to LF and TQ under DD, indicating perhaps a universal importance of MRD and RP to turf quality across all water environments.

While TRL has been the focus in many root studies assessing drought tolerance, the current results indicate the importance of other root traits for tolerance to water deficit conditions as well. The root parameters of FRL, MRD and RP were important root traits during DD. The significance of fine root production during drought has been reported in other studies. Fine roots are reported to be active sites of water uptake and increase the surface area for uptake (Rewald et al., 2011). The importance of fine roots has been reported in herbaceous (Hernandez et al., 2010) and woody plants (Henry et al., 2012), where plants in dry conditions typically have small diameter roots with large specific root length. Maximum root depth has also been found to be related to turf canopy responses in other studies (Su et al., 2008; Riaz et al., 2010; Rimi et al., 2012). In Su et al. (2008), only tall fescue had measurable root surface area at 90-120 cm soil depth. The results from these and the current study suggest that FRL, MRD and RP could be important traits of interest in addition to TRL for screening species under limited soil water conditions as these traits were associated with improved canopy response during field DD. All four traits could be associated with species, genotype or genotype within species separation during DD. These traits could be utilized in screening drought avoidance characteristics of species or genotypes in turf breeding programs, since they show significant associations with turf canopy response during water scarce conditions. Thus, species or genotypes that have deeper and more extensive rooting systems as evident in Sapphire (ST), and BA374 (ZM) could have the ability to maintain water and nutrient uptake during drought conditions.

Summary

This study quantified the root traits of importance that could be utilized to separate species and genotypes during a dry down period. Aside from TRL, a trait commonly chosen in drought studies examining impacts to the root system, this current study found that FRL, MRD and RP were also important root traits for separating species under surface soil drying or water limited environment. Screening species just on the basis of TRL alone, therefore, might not always adequately represent true performance of species under limited soil water; thus a screening program should include other parameters including FRL, MRD and RP. Moreover, while assessing turf during limited soil water, greenhouse and field studies must be employed. In our study, turf species showed different responses to limited soil water in the field experiment compared to the greenhouse experiment. Bermudagrass showed excellent performance with less leaf firing and sustained turf quality (LF of 6.4 and TQ of 5.6) even after 21 days of field dry down; in contrast, ST did not show adequate performance under limited soil water in the field in comparison to its relatively acceptable performance (LF ratings of 4.8 and 4.4 in 2012 and 2013 respectively) in the greenhouse experiment.

Importantly, root traits at the relatively shallow depth of 0-30 cm showed little variability among species or genotypes. Therefore, it is important to assess deep root traits as in this study at 30-90 cm. In addition, separation among species or genotypes may be more apparent during longer dry down periods. In this study, not every genotype with a shallow root system (or the majority of TRL confined to the 0-30 cm) showed poor response to soil drying. Exceptions included Floratam (ST) with LF rating of 4.8 and TQ rating of 3.9 at the end of DD that had shallow root systems but were better drought performers by exhibiting delayed leaf firing and a relatively green canopy during the dry down. Therefore, it is equally important to explore root and shoot responses simultaneously to truly understand turfgrass performance under drought.

Table 3-1: Analysis of Variance F-statistics of species for turf quality (TQ) and leaf firing (LF) at the beginning (TQ1, LF1) and at the end of dry down (TQ21 and LF21) in the field DD.

Effect	DF	TQ1	LF1	TQ21	LF21
Year	1	13.03*	0.00	6.81*	5.79*
Species	3	0.34	2.24	37.85***	35.86***
Year*Species	3	0.88	0.74	0.08	0.10

*, **, *** significant at $P \leq 0.05$, 0.01 or at 0.0001 respectively.

Table 3-2: Species averages for turf quality (TQ21) and leaf firing (LF21) at the end of field DD.

Species	TQ21	LF21
ST[¥]	3.4 b[‡]	3.6 b
CB	5.6 a	6.4 a
ZJ	1.9 c	1.9 c
ZM	1.8 c	1.8 c

[‡] Means followed by same letters within each column were not significantly different, $P \leq 0.05$

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table 3-3: Analysis of variance at (0-30, 30-90 cm) depths for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL) and root proportion (RP) across species during periods of DD (day 1, 7,14, 21).

Effect	DF	TRL	TRSA	ARD	FRL	RP (0-30 cm)
During DD (Day 1,7,14, 21)						
Year	1	25.53***	1.83	10.30	28.82***	0.54
Day	3	5.46***	3.62*	1.27	4.63***	0.01
Year*Day	3	0.82	0.84	0.51	2.45	0.03
Species	3	10.45***	10.58***	2.35	9.78***	52.67***
Year* Species	3	28.06***	22.18***	1.19	29.41***	24.04***
Species*Day	9	0.23	0.14	0.19	0.36	0.03
Depth	1	99.87***	97.21***	5.80*	104.5***	NA
Year*Depth	1	0.44	1.16	4.89*	15.37***	NA
Day*Depth	3	0.61	0.24	1.49	0.44	NA
Species*Depth	3	10.99***	8.9***	1.93	8.38***	NA

NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

[£] Root proportion (RP) parameter is a ratio, the parameter was analyzed at (0-30) cm depths.

Table 3-4: Average TRL, TRSA and FRL across species and depth during drought periods (day 1,7,14, 21).

Day	TRL (mm)	TRSA (mm^2)	FRL (MM)
1	722 b[‡]	538 b	231 c
7	761 a	566 ab	243 bc
14	778 a	574 a	254 ab
21	779 a	567 ab	265 a

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05; these traits are reflective of characteristics across the entire root system.

Table 3-5: Species average for total root length (TRL), total root surface are (TRSA), fine root length (FRL) and root proportion (RP) at 0-30 and 30-90cm depths during periods of drought (day 1,7,14,21) in 2013 and 2014.

	TRL (cm)		TRSA (cm^2)		FRL (cm)		RP (cm/cm)
Species	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm
2013							
ST[¥]	1224 a[‡]	282 b	1138 a	274 b	132 b	18 b	0.81 a
CB	1157 a	1055 a	897 a	830 a	385 a	184 a	0.53 b
ZJ	1127 a	95 b	929 a	86 b	276 ab	11 b	0.89 a
ZM	1478 a	135 b	1188 a	129 b	403 a	29 b	0.90 a
2014							
ST	1763 a	695 a	1253 a	515 a	716 a	243 b	0.71 b
CB	1338 a	1228 a	906 ab	703 a	679 a	620 a	0.52 c
ZJ	1183 a	56 b	714 b	32 b	620 a	27 c	0.93 a
ZM	1482 a	80 b	889 ab	53 b	783 a	35 c	0.94 a

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05.

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table 3-6: Whole tube (0-90 cm) analysis of variance for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL) and maximum root depth (MRD) for genotypes within species during period of DD (day 1, 7, 14, 21).

Effect	DF	TRL	TRSA	ARD	FRL	MRD	RP[£]
CB							
Year	1	4.41	0.50	15.58*	66.54***	-[Ŧ]	0.04
Day	3	3.09*	4.23***	1.55	2.87*	-	0.06
Year*Day	3	0.40	0.76	0.31	0.73	-	0.10
Genotype	3	5.29*	7.8*	4.65*	2.58	-	1.28
Year*Genotype	3	1.47	0.67	2.12	1.05	-	25.34***
Day*Genotype	9	0.11	0.31	0.35	0.39	-	0.14
ST							
Year	1	57.03***	1.92	3.85	24.89*	1.16	53.07***
Day	3	1.00	0.37	0.45	0.82	NA	0.03
Year*Day	3	0.54	0.38	0.95	0.69	NA	0.04
Genotype	3	1.59	1.36	0.13	4.12	13.16***	2.91
Year*Genotype	3	11.64***	10.89***	2.92	0.16	0.33	34.41***
Day*Genotype	9	0.05	0.11	0.78	0.38	NA	0.07
ZJ							
Year	1	0.00	6.71	19.27***	7.98*	0.51	1.55
Day	3	1.27	0.96	0.61	0.39	NA	0.00
Year*Day	3	0.12	0.20	0.29	0.69	NA	0.05
Genotype	3	2.63	2.99	0.08	0.16	2.16	1.08
Year*Genotype	3	0.94	0.85	0.23	0.12	0.39	3.78*
Day*Genotype	9	0.19	0.14	0.98	0.98	NA	0.01
ZM							
Year	1	0.10	3.44	20.18*	9.38	0.07	17.03*
Day	3	0.91	0.80	1.48	0.65	NA	0.02
Year*Day	3	0.13	0.05	0.15	0.38	NA	0.16
Genotype	3	0.45	0.49	0.94	0.78	6.6***	0.94
Year*Genotype	3	1.50	1.32	3.82*	0.71	0.01	1.05
Day*Genotype	9	0.10	0.05	0.39	0.13	NA	0.08

NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

[£] RP is a ratio calculated as TRL at 0-30 cm/ 0-90 cm.

[Ŧ] Genotypes roots growth reached the bottom of the tubes.

Table 3-7: Whole tube (0-90cm) average for total root length (TRL), total root surface are (TRSA), and average root diameter (ARD) of CB[₮] genotypes during period of DD (day 1, 7, 14, 21).

Genotypes	TRL (mm)	TRSA (mm^2)	ARD (mm)
289922	2355 ab[‡]	1867 a	5.04 a
Celebration	2820 a	1885 a	4.28 ab
UFCD347	2870 a	1985 a	4.42 ab
UFCD12	1778 b	1159 b	4.16 b

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05; these traits are reflective of characteristics across the entire root system.

[₮] CB=bermudagrass.

Table 3-8: Average for day for genotype within CB[₮] species at each day at whole tube (0-90 cm) during period of DD (day 1, 7, 14, 21).

Day	TRL (mm)	TRSA (mm^2)	FRL (MM)
1	2299 b[‡]	1629 b	856 b
7	2427 ab	1721 ab	891 ab
14	2492 a	1742 a	942 ab
21	2524 a	1735 ab	1001 a

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05; these traits are reflective of characteristics across the entire root system.

[₮] CB=bermudagrass.

Table 3-9: Whole tube (0-90cm) average for maximum root depth (MRD) of ST genotypes during period of DD (day 1, 7, 14, 21).

Genotypes	MRD (cm)
ST[₮]	
Palmetto	59 bc[‡]
Captiva	68 ab
Sapphire	75 a
Floratam	51 c
ZM[₮]	
BA336	50 ab
Zeon	42 b
BA374	60 a
ToccoaGreen	50 ab

[‡] Means followed by same letters within each column were not significantly different, $P \leq 0.05$; these traits are reflective of characteristics across the entire root system.

[₮] ST=St. augustinegrass, ZM= *Zoysia matrella*.

Table 3-10: Multivariate analysis of variance for across species, across genotypes, genotypes within CB, genotypes within ST, genotypes within ZJ, and genotypes within ZM species during end of DD (day 21).

Variables	Wilks' Lambda
Across Species	10.05***
Across Genotypes	3.92***
Genotypes within CB[ǂ]	2.43**
Genotypes within ST	3.83***
Genotypes within ZJ	4.16***
Genotypes within ZM	2.82**

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

[ǂ] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table 3-11: Within class standardized canonical coefficient for root and shoot parameters across species end of DD (day 21).

Variables	CAN1	CAN2
TRL	0.03372994	-0.443737167
TRSA	-0.358260115	0.810473154
ARD	-0.039571721	0.604024066
FRL	-0.014583941	0.032082602
LFday21	-0.326440586	0.41938295
MRD	0.586687704	0.424053407
TQday21	0.897673971	-0.579167537
RP	-0.590079145	0.450567792
[ƚ]Can R^2	0.90***	0.45*
[‡]PR	0.93	0.05
[≠]Cu PR	0.93	0.98

[ƚ] Canonical Correlation

[‡] Proportion

[≠] Cumulative proportion

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table 3-12: Class mean for canonical variables across species end of DD (day 21).

Species	CAN1	CAN2
CB[ƚ]	3.222078	-0.317283633
ST	0.315722	0.753064699
ZJ	-1.788581	-0.565952322
ZM	-1.749219	0.130171255

[ƚ] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table 3-13: Within class standardized canonical coefficient for root and shoot parameters within CB genotypes end of DD (day 21).

Variables	CAN1	CAN2
TRL	-1.855940467	6.078950363
TRSA	2.530377471	-5.000537395
ARD	-0.060732265	2.213135509
FRL	1.125810291	-1.617521173
LFday21	-0.991268945	0.760577002
MRD	0	0
TQday21	0.06024744	-0.301301104
RP	-0.14418981	0.467602389
ǂCan R^2	0.86**	0.67^{NS}
‡PR	0.70	0.19
≠Cu PR	0.70	0.89

ǂCanonical Correlation

‡Proportion

≠Cumulative proportion

NS, *, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table 3-14: Class mean for canonical variables within CB genotypes end of DD (day 21).

Genotypes	CAN1	CAN2
289922	1.278550361	-1.226818494
Celebration	0.090814052	0.887804942
UFCD12	-2.603735435	-0.278641453
UFCD347	1.234371022	0.617655005

Table 3-15: Within class standardized canonical coefficient for root and shoot parameters within ST genotypes end of DD (day 21).

Variables	CAN1	CAN2
TRL	1.558343026	-0.827321854
TRSA	-0.990268204	-0.278174953
ARD	-0.678558587	0.367138308
FRL	-1.37939388	0.312442849
LFday21	-1.611228349	-1.498227447
MRD	1.91606332	0.313948798
TQday21	0.429413739	1.645649632
RP	1.038106121	-1.126058679
ƗCan R^2	0.95***	0.78^{NS}
‡PR	0.83	0.14
≠Cu PR	0.83	0.97

ƗCanonical Correlation
‡Proportion
≠Cumulative proportion
*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table 3-16: Class mean for canonical variables within STgenotypes end of DD (day 21).

Genotypes	CAN1	CAN2
Captiva	3.561619901	-1.329766623
Floratam	-3.64191039	-0.926363882
Palmeto	-1.756749679	0.774172519
Sapphire	1.837040168	1.481957986

Table 3-17: Within class standardized canonical coefficient for root and shoot parameters within ZJ genotypes end of DD (day 21).

Variables	CAN1	CAN2
TRL	-5.545614761	-0.622545336
TRSA	2.792017128	1.025225528
ARD	0.02753539	-0.329521251
FRL	2.689625268	1.368567015
LFday21	4.13613646	-3.045366338
MRD	1.581441036	0.251642356
TQday21	-2.790758747	3.887858919
RP	-1.081864202	-0.234640589
[ᵻ]Can R^2	0.94***	0.85^{NS}
[‡]PR	0.76	0.23
[≠]Cu PR	0.76	0.99

[ᵻ]Canonical Correlation

[‡]Proportion

[≠]Cumulative proportion

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table 3-18: Class mean for canonical variables within ZJ genotypes end of DD (day 21).

Genotype	CAN1	CAN2
5269-24	-0.036808942	-0.994161092
BA182	-2.247332768	2.339544553
4360	-2.238802735	-1.672087919
JaMur	4.522944445	0.326704458

Table 3-19: Within class standardized canonical coefficient for root and shoot parameters within ZM genotypes end of DD (day 21).

Variables	CAN1	CAN2
TRL	-3.167633224	-6.287495173
TRSA	2.572522483	2.715958916
ARD	0.205854818	1.199022552
FRL	1.548870765	4.722210645
LFday21	-0.87140079	-3.502862057
MRD	1.28584495	-0.286257816
TQday21	0.366222361	4.40706453
RP	1.604435014	0.370420291
ƚCan R^2	0.88**	0.75^{NS}
‡PR	0.64	0.22
≠Cu PR	0.64	0.86

ƚCanonical Correlation

‡Proportion

≠Cumulative proportion

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table 3-20: Class mean for canonical variables within ZM genotypes end of DD (day 21).

Genotype	CAN1	CAN2
BA336	0.908612923	1.402977377
BA374	1.393961901	0.052950704
ToccoaGreen	0.726937146	-1.533183602
Zeon	-3.02951197	0.077255522

Table 3-21: [Ψ]Stepwise regression analysis for root traits that were significant at 0.05 levels that could be associated with species/genotypes leaf firing (LF21) response during DD (day 21).

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	Ln_RP	1	0.3246	0.3246	24.1669	45.18	<.0001
2	LN_FRL	2	0.0888	0.4135	10.8876	14.08	0.0003
3	LN_MRD	3	0.0541	0.4675	3.5880	9.34	0.0029

[ϕ] Significant at P ≤ 0.05 as identified by stepwise regression model.

[Ψ]Stepwise regression model: Ln_LF21 = Ln_TRL Ln_TRSA Ln_ARD Ln_FRL Ln_MRD Ln_RP.

Table 3-22: [Ψ]Stepwise regression analysis for root traits that were significant at 0.05 levels that could be associated with turf quality (TQ1) during field DD (day 21).

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	Ln_RP	1	0.2939	0.2939	32.8399	39.13	<.0001
2	LN_FRL	2	0.1007	0.3946	17.0341	15.47	0.0002
3	LN_MRD	3	0.0739	0.4686	5.9623	12.80	0.0006

[ϕ] Significant at P ≤ 0.05 as identified by stepwise regression model.

[Ψ]Stepwise regression model: Ln_TQ21 = Ln_TRL Ln_TRSA Ln_ARD Ln_FRL Ln_MRD Ln_RP.

Table 3-23: [Ψ]Stepwise regression analysis for root traits that were significant at 0.05 levels that could be associated with species/genotypes leaf firing (LF1) WW (day 1).

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	LN_MRD	1	0.2000	0.2000	1.2616	23.49	<.0001

[ϕ] Significant at P ≤ 0.05 as identified by stepwise regression model.

[Ψ]Stepwise regression model: Ln_LF1 = Ln_TRL Ln_TRSA Ln_ARD Ln_FRL Ln_MRD Ln_RP.

Table 3-24: [Ψ]Stepwise regression analysis for root traits that were significant at 0.05 levels that could be associated with species/genotypes turf quality (TQ1) during WW (day 1).

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	Ln_ARD	1	0.1315	0.1315	8.7899	14.23	0.0003
2	LN_RP	2	0.0564	0.1879	4.2501	6.45	0.0127

[ϕ] Significant at P ≤ 0.05 as identified by stepwise regression model.

[Ψ]Stepwise regression model: Ln_TQ1 = Ln_TRL Ln_TRSA Ln_ARD Ln_FRL Ln_MRD Ln_RP.

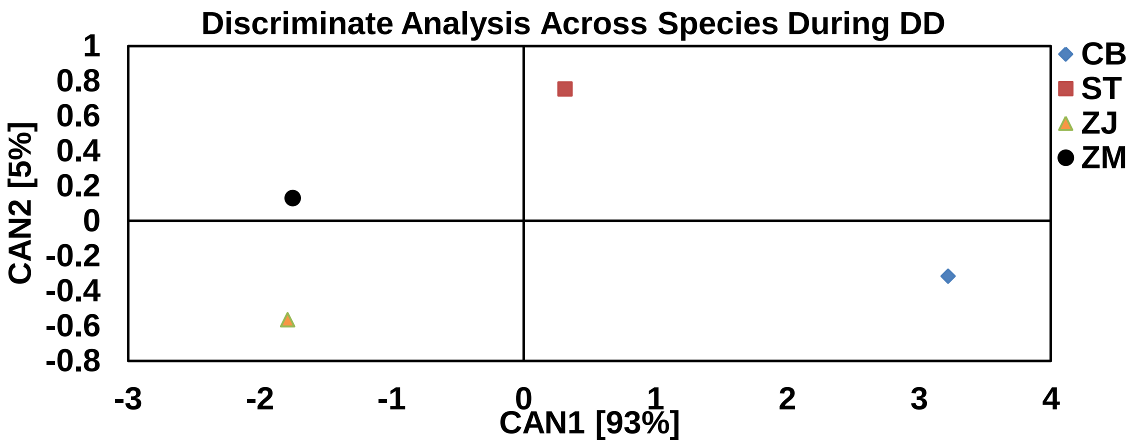

Figure 3-1: Canonical discriminate analysis class means plotted across four warm season turf species.

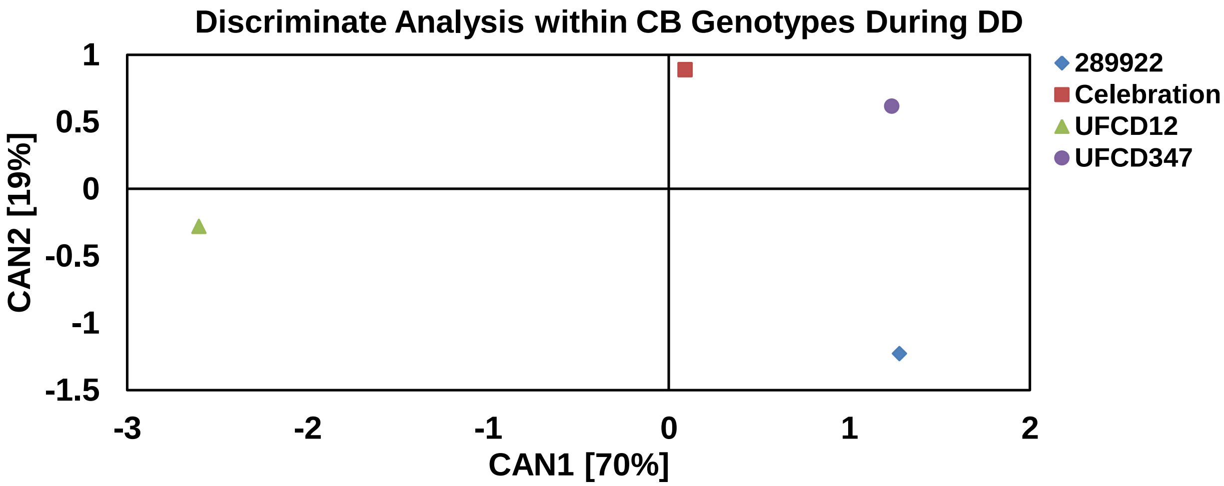

Figure 3-2: Canonical discriminate analysis class means plotted within CB genotypes.

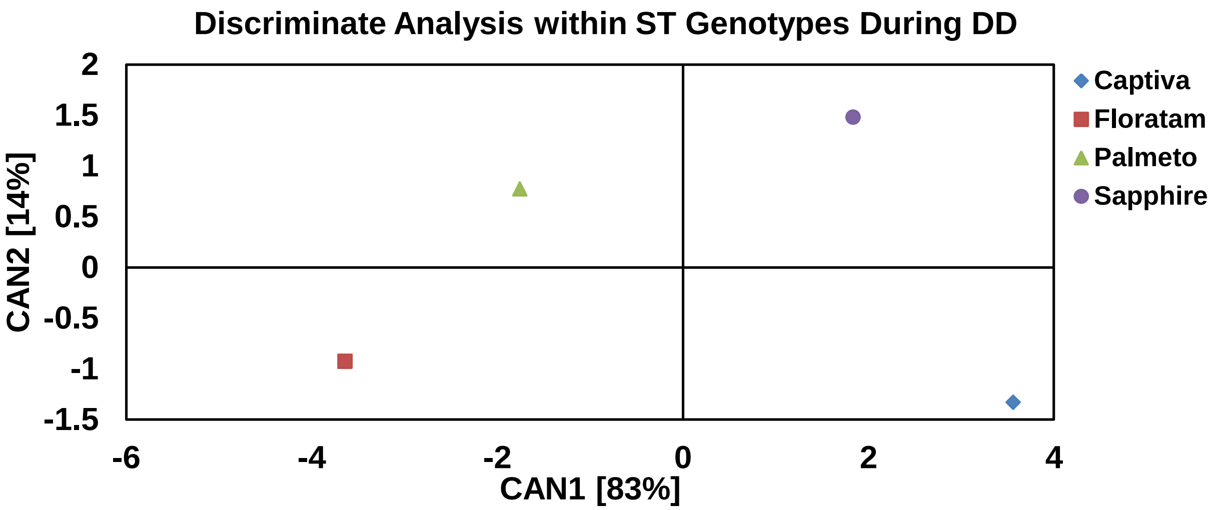

Figure 3-3: Canonical discriminate analysis class means plotted within ST genotypes.

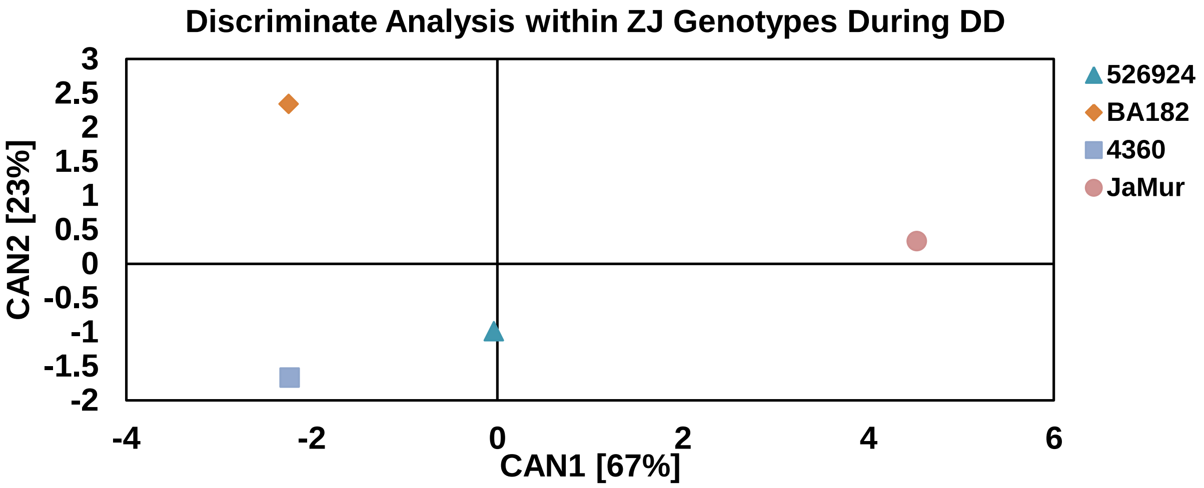

Figure 3-4: Canonical discriminate analysis class means plotted within ZJ genotypes.

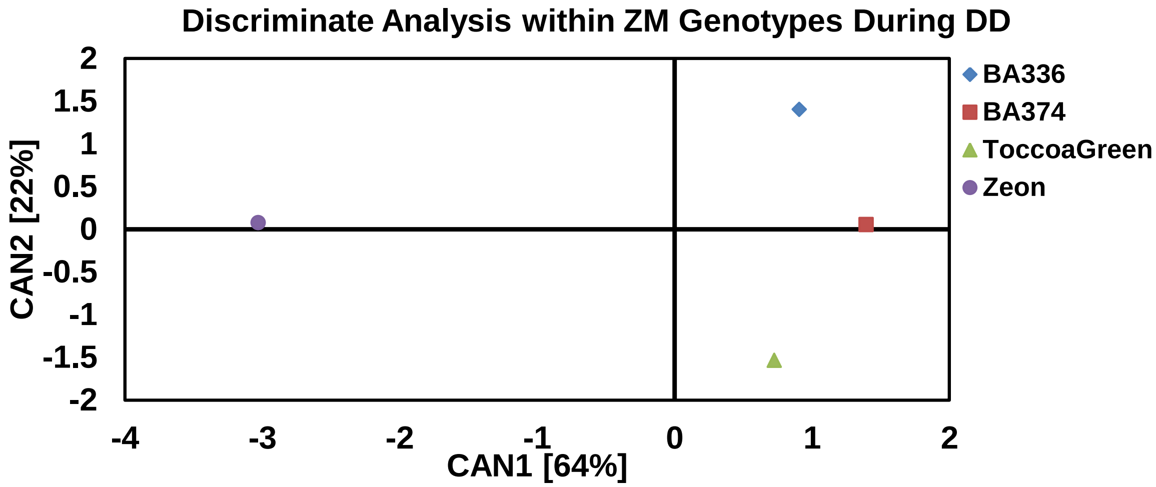

Figure 3-5: Canonical discriminate analysis class means plotted within ZM genotypes.

CHAPTER 4
AN IN SITU STUDY ON ROOTING DYNAMICS OF ST. AUGUSTINE AND BERMUDAGRASS AS AFFECTED BY MOWING HEIGHTS

Introduction

St. Augustinegrass (*Stenotaphrum secundatum* [Waltz.] Kuntze) and bermudagrass (*Cynodon dactylon* [L.] Pers) are the two primary warm season turfgrass species used in the southern U.S. St. Augustingrass is native to coastal regions of the Mediterranean and Gulf of Mexico (Trenholm et al., 2014). The first use for St. Augustinegrass was in Florida home lawns in 1880. ‘Floratam’ was a joint release from the University of Florida and Texas A&M University in 1973 and is the most widely grown turfgrass cultivar in Florida for use in home lawns and landscapes (Busey, 1993). ‘Floratam’ has a dark blue to green color, coarse texture and vigorous growth during spring and summer months (Trenholm et al., 2014).

Bermudagrass is native to Africa and Asia but is well adapted to warmer climates worldwide (de Wet and Harlan, 1970; de Wet and Harlan, 1971). ‘Tifway’ bermudagrass is a hybrid (*Cynodon dactylon* L. × *Cynodon transvalensis* Burtt-Davy) released in 1960 from the University of Georgia (Burton, 1991). ‘Tifway’ has an aggressive rate of growth, dark green color, fine texture, and is mostly grown on golf-course fairways, athletic fields and home lawns (McCarty, 2005).

Mowing is one of the most important management practices performed to enhance turfgrass aesthetic values. Frequent and short mowing is often associated with a shallow root system with decreased production of roots (Liu and Huang, 2002). This shallower root system then leads to reduced drought tolerance in most situations, thus compounding any water scarce or water restriction periods. Worsening drought conditions and strict state laws on water and fertilizer use have become increasingly common across the U.S. as water resources become limited, making management techniques that help improve drought tolerance critically important (Marcum, 2006). Currently in the state of Florida, turfgrass can only be irrigated twice a week (SJRWMD: Saint John River Water Management Districts). Thus, any management techniques that lead to shallow root systems can be detrimental for turfgrass growth and increase irrigation frequency and overall water use (Trenholm et al., 2011).

Understanding the effect of mowing heights on turfgrass root growth and development will help to optimize mowing height practices and encourage more conservative irrigation management strategies. Studies suggest that high or low mowing heights can significantly alter turfgrass root growth and development (Wherley et al., 2011; McCarty et al., 2011; Tucker et al., 2006, Fagerness and Yelverton, 2001). The study by McCarty et al. (2011) evaluated ‘Tifeagle’, an ultra-dwarf bermudagrass (*Cynodon dactylon* L. × *Cynodon transvalensis* Burtt-Davy) used for putting greens, at three different mowing heights (2.5, 3.25 and 4 mm) and found that higher mowing heights increased lateral root growth and overall root length density, and led to better turf quality. Similarly, Wherley et al. (2011) examined ‘Argentine’ bahiagrass (*Paspalum notatum* Flugge.) and Floratam St. Augustinegrass rooting at 10 and 5 cm mowing heights and Tifway bermudagrass and ‘Empire’ zoysiagrass (*Zoysia japonica* Steud.) rooting at 5 and 1.9 cm mowing heights and found that higher mowing heights were associated with more rapid root extension and greater root proliferation in both shallow (0-45 cm) and deep soil (45-90 cm) depths for all species. In addition, raising the clipping height from 3 to 6 cm for a period of 6 weeks was found to cause significant improvements in turf strength, shoot growth rate, and chlorophyll content in 11 warm season turf species (Biran et al., 1980). Henry et al. (2007) reported that higher mowing heights increased rhizome fresh weight (RFW) in comparison to low mowing heights. Rhizome fresh weight reduction was less under high mowing heights (7.6 and 5.2 cm) where there was a 24-33% and 55% reduction in rhizome weight when mowed at 7.6 cm and 5.2 cm mowing height compared to 70-73% reduction at 1.3 cm in two *Paspalum* spp. Similarly, Fagerness and Yelverton (2001) reported reduced turf quality at a mowing height of 3.2 mm compared to 4 mm. They also noted slow autumn recovery and low root biomass (RB) in ‘Pencross’ creeping bentgrass (*Agrostis stolonifera* L.) under the lower cutting height.

Most of the previous research aimed at quantifying the effects of mowing height on rooting utilized destructive root core sampling methods (Yelverton, 1999; Salaiz et al., 1995). There have been a limited number of studies that utilized a non-destructive root sampling method, including minirhizotrons (Beyrouty et al., 1990; Liu and Huang, 2002; Bonin et al., 2013). Studies that utilize minirhizotrons have the advantage of repeated quantification of root growth, expansion, and turnover by imaging the same location within the root system over time. For example, Liu and Huang, (2002) used a minirhizotron camera system to demonstrate that low mowing heights had detrimental effects on new root growth leading to nutrient and water deficiencies in creeping bentgrass. Lower mowing heights were also associated with reduced summer root growth and resulted in overall detrimental effects to root growth, root development and turf quality in creeping bentgrass (Liu and Huang, 2002). Similarly, Bonin et al. (2013) utilized a minirhizotron camera system to study C_4 warm season perennial grassland species' responses to grazing and found that excessive grazing was associated with small root volumes and root diameters. Data generated from growth boxes, consisting of soil filled cylinders, indicated that high mowing heights in turf (Tifway bermudagrass, Empire zoysiagrass, Argentine bahiagrass, and Foratam St. Augustinegrass) were associated with high root extension and root proliferation both within upper and deeper soil depths (Wherley et al., 2011). Important seasonal patterns in warm season turfgrass (bermudagrass and St. Augustinegrass) include high root growth during summer months as well as summer root decline (SRD) during early spring which is due to high soil temperature (Sifers et al., 1985).

Studies from both destructive and non-destructive techniques suggest that mowing heights can have significant effects on turfgrass root growth and development. In addition, there is need of information in the field on season, species or genotype and mowing height effects on rooting and its importance for management of water and nutrient in central Florida. However, this information is relatively limited in warm season turfgrass species. Because of the importance of warm season turf species to the Florida sod industry and the increasing importance of water and nutrient conservation for the state, there is a critical need for understanding the effect of mowing height practices in Florida's most used turfgrass cultivars such as Floratam St. Augustinegrass and Tifway bermudagrass. The variation between these species in mowing height effects on root growth and development might yield important recommendations for turf management that could be crucial for reducing water and fertilizer use in Florida’s home lawns. Thus, the objectives of this study were to 1) to quantify the effect of three mowing heights on root architecture and root depth 2) to determine if seasonal affects on rooting are also influenced by mowing heights.

Materials and Methods

A field study was conducted from April, 2011 to December, 2013 to quantify the rooting behavior of two commercial warm season turfgrass species, ‘Floratam’ St. Augustinegrass. An additional study was conducted from Jan, 2012 to December, 2013 for ‘Tifway’ hybrid bermudagrass in a sod based system at the University of Florida G. C. Horn turfgrass plots at the Plant Science Research and Education Unit in Citra, Florida. The experimental design was a randomized complete block design (RCBD) with three replications and three mowing height treatments for each species. Mowing heights for Floratam were 5.0, 7.6 and 10 cm and for Tifway were 1.3, 3.8, and 6.4 cm. These mowing heights represent the commonly recommended heights for these two species (Trenholm et al., 2014; Trenholm et al., 2011; Foy, 2014). Mowing was performed weekly using a rotary mower throughout the growing season for heights ≥ 3.8 cm in both species and a reel mower twice a week for the 1.3 cm mowing height in Tifway. Plots were fertilized with 48 kg N per ha per month (64 and 48 kg of P_2O_5 and K_2O respectively) using a 15-20-15 granular fertilizer (Signature brand fertilizer, Greeley, CO). For weed control, the pre-emergent herbicide Ronstar® (Research Triangle Park, NC) along with other selective herbicides such as Celsius^{TM} WG, Dicamba (Research Triangle Park, NC) and Dismiss (Philadellphia, PA) were applied in the spring and summer of each experimental year. Allectus^{TM} (a. i. imidacloprid and bifenthrin; Montvale, NJ) was applied September 2013 to control a mole cricket infestation in Tifway. Both, Floratam and Tifway were maintained healthy throughout the study periods with proper irrigation and fertilizer use.

To study rooting characteristics, transparent 5.10 cm (inner diameter) by 1.83 m (length) minirhizotron tubes were inserted into each field plot at 45° angles in March, 2011 in Floratam and in November, 2011 for Tifway. Root images were collected monthly using a digital camera system (Bartz Technology Corp., Carpinteria, CA). The digital images obtained from the camera system were analyzed with Win-RHIZO Tron software (Regent Instrument Inc., Canada) for specific root parameters including total root length (TRL in mm), total root surface area (TRSA in mm^2), average root diameter (ARD in mm), root proportion (RP in mm/mm) and maximum root depth (MRD in cm). These parameters were selected such as to quantify the differences in root architecture as affected by different mowing heights. RP was calculated as TRL present at 0-30 cm divided by TRL present at 0-90 cm (the length of the whole tube).

Statistical Analysis

Analyses of variance (ANOVA) were conducted to determine differences among mowing heights for TRL, TRSA, ARD, FRL, MRD and RP in both Floratam and Tifway. Proc. GLIMMIX (SAS Institute Inc., Cary, NC) was used to analyze the experiments as mixed models with year, month, mowing height and depth (0-30 and 30-90 cm) as fixed factors. Tubes measured monthly within each mowing height were treated as repeated measures. Depths nested within tubes were also treated as a repeated measures, and replications and years were random factors. Square root transformation was performed for TRL, TRSA, ARD and FRL to conform to the assumption of normality for ANOVA (Bartlett, 1947). Floratam had an unbalanced design for year (only eight months were measured in 2011, versus 12 months in 2012 and 2013); therefore, data from 2011 (months of April-November) were analyzed separately, with 2012 and 2013 analyzed together. For Tifway, 11 months in 2012 and 2013 (data not included for September: due to broken tubes) were analyzed. For each analysis, only two-way interactions were analyzed. When factors were determined as significant at the $p<0.05$ significance level, means were separated using Tukey’s honestly significance differences (HSD) multiple comparison test.

Spider diagrams were plotted for individual traits to visualize the seasonal variation of root growth and development in relation to 7.6 cm for Floratam and 1.3 cm for Tifway and compared to the other mowing heights (5 and 10 cm for Floratam and 3.8 and 6.4 cm for Tifway). For Floratam 7.6 cm mowing was selected as it would represent general home lawn mowing practices (Trenholm et al., 2014). Similarly, as Tifway hybrid bermudagrass is maintained at typically low mowing height (1.3 cm) (Foy, 2014), 1.3 cm mowing height was selected for the analysis. In the analysis, data from both experiment years (year 2012 and 2013) were combined and a two-year average for a given trait was used. To normalize the comparisons, the value of each trait at 7.6 cm for Floratam and 1.3 cm for Tifway mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value. For each trait the value at 7.6 cm mowing height Floratam or 1.3 cm mowing height for Tifway were the denominator function, the ratio thus generated by this denominator function (as a divisor) for each trait were utilized to plot spider diagram. The standardized data for the traits TRL, TRSA, ARD, and FRL at different mowing heights were plotted against months and depth; whereas RP and MRD data, which were representative of the entire tube length, were plotted by month only.

Results

Floratam

For Floratam in 2011, none of the traits were affected by mowing height, except MRD (Table 4-1); in contrast, for 2012 and 2013 the traits TRL, TRSA, ARD, FRL and MRD were affected by mowing height (Table 4-2), with a year by mowing height interaction for MRD (Table 4-2). However, no other interactions with MH occurred. Most interactions present occurred between year and depth, year and month, and month and depth indicating some seasonal changes in rooting by depth (Table 4-2). Average values in 2011 indicated that the high mowing height (10 cm) produced deeper roots compared to the low mowing height (5 cm) (Table 4-3). In 2012 and 2013, TRSA, ARD and FRL were greater when mowed at 10 cm compared to 5 cm and no differences were found between these mowing heights and 7.6 cm (Table 4-4). The MRD showed that both the 10 and 7.6 cm mowing heights produced the deepest roots in 2012 compared to the 5 cm mowing height (Table 4-4). Interestingly, the 7.6 cm height had the deepest roots compared to the 10 and 5 cm mowing heights in 2013 (Table 4-4).

While mowing height significantly affected root traits for Floratam, many traits also exhibited seasonal effects regardless of mowing height as evidenced by the interactions between year and month, year and depth, and month and depth. Within the 0-30 cm depth in 2012, July and August had the highest TRL compared to January and February. For TRSA, January, February and March produced the lowest values compared with May through September (Table 4-5). At the 30-90 cm depth, TRL was lowest in January compared to TRL from June through December (Table 4-5).

In 2013, within 0-30 cm, TRL was lowest in March, September, November and December compared to of its higher value in May (Table 4-5). For TRSA the trend was very similar to that of TRL, with higher values in May and June compared to September, October, November, December and March when TRSA was lower (Table 4-5). Deeper in the soil, 30-90 cm, TRL was highest in October and lowest during March; whereas, TRSA did not differ between months (Table 4-5).

The spider diagram for Floratam TRL illustrates that within 0-30 cm, the average TRL when mowed at 10 cm was greater than the TRL at 7.6 cm in October to May (Figure 4-1), while the 5 cm mowing height had reduced TRL compared to 7.6 cm through all months (Figure 4-1). Interestingly at this depth, 7.6 cm mowing height produced similar amount of TRL and TRSA during June and September, and the values were higher for these traits during July and August compared to the 10 cm mowing height. The spider plots for the 30-90 cm depth show a similar trend, with reduced TRL at 5 cm and enhanced TRL at 10 cm compared to 7.6 cm height of cut throughout the year (Figure 4-1). TRSA followed a similar pattern as that of TRL at these two depths (Figure 4-2).

In 2012, FRL was highest in March and lowest during April (Table 4-5). In 2013, FRL was highest in October and February compared to July and August when FRL values were lower.

Larger diameter roots (ARD) were found within the 0-30 cm soil profile (4.45 mm in 2012 and 3.84 mm in 2013) compared to the diameter of roots (1.63 mm in 2012 and 1.70 mm in 2013) present deeper in the soil (30-90 cm). During May, June and July larger diameter roots were produced compared to those present in March within 0-30 cm; however ARD was similar across months within the deeper (30-90 cm) horizon (Table 4-6).

Spider diagrams show that within the upper 0-30 cm of soil, the 10 cm mowing height produced similar ARD when compared to the 7.6 cm mowing height; whereas, the 5 cm mowing height had decreased ARD values from March through August (Figure 4-3). At the 30-90 cm depth, the ARD for the 10 cm mowing was larger than the ARD values produced under the other two mowing heights from January through June (Figure 4-3). For FRL at the 0-30 cm depth, higher values were observed at the 10 cm mowing height from December through March compared to the 7.6 cm mowing height (Figure 4-4). The months of September, at 0-30 cm, and December and March, at 30-90 cm, had the highest FRL production at 10 and 5 cm mowing heights compared to 7.6 cm mowing height (Figure 4-4). For RP, the 7.6 cm and 10 cm mowing heights had similar values throughout the year. In contrast, RP was higher under the 5 cm mowing height compared to the two taller mowing heights from September to

December (Figure 4-5). For MRD, the 7.6 cm and 10 cm mowing had similar MRD for January, February, March, April, July and August. In contrast, MRD was higher when mowed at 7.6 cm from August through December (Figure 4-5). The lower mowing height was always associated with shorter MRD compared to the two higher mowing heights through the year (Figure 4-5).

Tifway

For Tifway, all traits (TRL, TRSA, ARD, FRL and MRD) except RP were affected by mowing height (Table 4-7). A year by mowing height interaction occurred for all of these traits (Table 4-7). Depth was significant for TRL, TRSA and ARD. In addition interactions between year and month occurred for TRL, TRSA, and ARD (Table 4-7). For FRL, interactions between year and month, and month and depth occurred; while for MRD, interactions occurred between year and mowing height, and year and month (Table 4-7).

The highest mowing height (6.4 cm) produced greater TRL compared to the two low mowing heights (3.8 and 1.3 cm) in both years. TRSA, ARD and FRL were higher when mowed at 6.4 cm compared to 3.8 cm; however, these traits were not different between 6.4 and 1.3 cm mowing heights in both years (Table 4-8). The source of interaction for these traits was due to less root growth and development at the 3.8 cm mowing height in 2012, and 1.3 and 3.8 cm mowing heights in 2013.

Similar to Floratam, Tifway showed seasonal effects across the soil profile as seen by the interactions with year or month with depth. Over both years, TRL, TRSA and ARD were greater at the 0-30 cm than the 30-90 cm depth (data not shown). In 2012 across depth and mowing heights, higher TRL, TRSA and ARD were evident from May through December compared to January through March (Table 4-9). A similar trend was seen for FRL; but by depth, where within the 0-30 cm depth, January through March had the lowest values compared to May through December (except October) (Table 4-9). At the deeper, 30-90 cm depth, FRL was least from January through April compared to December (Table 4-9).

In 2013, the highest TRL and TRSA values occurred during May compared to the lower values from July, and November. In 2013, ARD was not different across months (Table 4-9). Similar to 2012, FRL was different at the two soil depths, with the highest values occurring within 0-30 cm during May and October compared to the lower values from July and December. In contrast, FRL was similar across all months at the 30-90 cm soil depth (Table 4-9).

Similar to Floratam, from spider diagram seasonal differences in root growth at higher mowing heights (3.8 and 6.4 cm) compared to the lowest mowing height (1.3 cm) was evident in Tifway. Within the upper soil profile, 0-30 cm, the 3.8 and 6.4 cm mowing heights had higher TRL values compared to the 1.3 cm height of cut for the majority of the year (Figure 4-6). Deeper, at 30-90 cm, the 6.4 cm mowing height had higher TRL values throughout the year compared to 3.8 and 1.3 cm mowing heights with elevated TRL in May and June and in October, November and December (Figure 4-6). Similar trends were observed for TRSA at these two depths (Figure 4-7).

At the 0-30 cm depth, the highest mowing height (6.4 cm) produced greater ARD during the cooler months from October to March compared to both 1.3 and 3.8 cm mowing heights (Figure 4-8). Within 30-90 cm, the 6.4 cm mowing height produced larger diameter roots in May, June, July, August, September, October, November, December and January compared to the two lower mowing heights (Figure 4-8).

Greater FRL values were observed at both depths when Tifway was mowed at 3.8 and 6.4 cm compared to 1.3 cm (Figure 4-9). July, August and October produced greater FRL values within the 0-30 cm soil depth for both the 3.8 and 6.4 cm mowing heights. Deeper at 30-90 cm, greater FRL values occurred during May, June and October when mowed at 6.4 and 3.8 cm.

The maximum rooting depth (MRD) exhibited different seasonal patterns among the mowing heights. The highest mowing height (6.4 cm) was associated with deeper MRD compared to the intermediate mowing height (3.8 cm) in November and May of 2012 and July, August, October, November and December in 2013 (Table 4-10). Contrary to expectations, the lowest mowing height (1.3 cm) actually had similar MRD to the 6.4 cm mowing height for the two months in 2012 and July in 2013, when the intermediate mowing height showed shallower MRD.

Overall, from spider diagram RP was higher at the 1.3 cm and 3.8 cm mowing heights compared to 6.4 cm (Figure 4-10). Overall root growth was concentrated within the upper soil profile, (0-30 cm) when Tifway was mowed at 1.3 at and 3.8 cm. The higher mowing height (6.4 cm) was associated with deeper MRD compared to the lower mowing heights and across all months (Figure 4-10).

Discussion

The results from this study suggest that low mowing heights affected overall root morphology and development as well as root depth in both Floratam and Tifway. Not only overall root growth (TRL), but also TRSA, FRL, ARD and MRD were negatively affected by low mowing heights. Similarly, the two lower mowing heights for each species (5 cm in Floratam and 3.8 cm in Tifway) were associated with shallower rooting depths when compared to the higher mowing heights in both Floratam and Tifway. However, the real biological impact of this reduction in root growth by low mowing heights may be minor because, in both species, most of the root architecture was found to be concentrated in the upper 0-30 cm of soil depth (in relation to TRL and TRSA in Floratam and FRL in Tifway) no matter what mowing treatment was utilized. The reduction in root growth throughout the growing season at the deep depth (30-90 cm) by low mowing heights would impact only 5-10% (TRL at 30-90/ TRL at 0-90 cm) of the root system (data not shown). While this is certainly a small percentage of the total root system, the deep root system seen at the high mowing heights could still be advantageous for the absorption of water or nutrients (Bonos and Murphy, 2014; Huang et al., 1997a). Although the study do not have data on water uptake to support this hypothesis, this small reduction in root architecture might still represent a critically important impact on water and nutrient uptake in turf. This knowledge gap could be explored in the future.

The impact of mowing height on root architecture found in the current study is similar to results from other studies. High mowing heights increased lateral root regrowth (McCarty et al., 2011) as well as enhanced root extension, and proliferation within both shallow (0-45 cm) and deep soil (45-90 cm) depths (Wherley et al., 2011). Similarly, Guertal and Evans (2006) in a three year study, showed that the root growth of TifEagle ultra-dwarf bermudagrass was decreased at low mowing heights. Low mowing heights were also associated with reduced summer root growth and had detrimental overall effects on root growth and development in creeping bentgrass (Liu and Huang, 2002).

Seasonal variation (January-December) in root growth and development across mowing heights was highly evident in both 2012 and 2013. Overall trends indicated that during summer and fall (July-August in 2012 and May-June in 2013 for Floratam; July-December in 2012 and May in 2013 for Tifway) greater root growth and development occurred than in winter (January-March in 2012) for both Floratam and Tifway. In addition, deeper MRD at the high mowing height (6.4 cm) compared to the 3.8 cm mowing height in May and November in 2012 and July-December in 2013 was also evident in Tifway. The deeper rooting that occurred in May 2012 in Tifway could be an important finding because May is annually one of the driest months in Florida. Besides, in year 2012, TRL, TRSA and FRL did not differ between 1.3 and 3.8 cm mowing heights as expected. This might be due to significant mole cricket (*Scapteriscus Borellii* Giglio-Tos) infestation in Tifway during the months of July-September. Allectus^{TM} (a. i. imidacloprid and bifenthrin; Montvale, NJ) was applied during these months (Zhang, 2014) to control the mole cricket infestation. Similar to the results in this study, seasonal variation in root growth and development in cool season turfgrass species such as Penncross creeping bentgrass and annual bluegrass were reported by Murphy et al. (1994). The decline in root growth (TRL and TRSA) during the winter months in 2012 (January, February and March) in the current study, could be similar to the phenomenon of summer root decline (SRD) in cool season turfgrass as reported by Murphy et al. (1994) and Liu and Huang

(2002). Summer root decline is a unique root decline process initiated due to rapid soil warming and high soil temperatures that cause root death in cool season turf during the summer (Murphy et al., 1994). A root decline during the winter months in Floratam and Tifway showed patterns similar to that described for cool-season turf SRD (Liu and Huang, 2002). However, this trend of root decline during winter was not as distinct in year 2013, possibly due to increased root growth during the summer and fall in 2012 that might have helped to sustain the root system during the 2013 winter. Besides, overall root growth (TRL) was relatively consistent in 2013 compared to 2012, this could be due to 2012 as a root establishment year in Tifway. In 2013, seasonal root growth (TRL) was almost similar for most of the months with exception of May that had higher root growth and development and decreased root growth during July, November and December. This information on root development by season could thus be utilized in managing water and nutrient in central Florida.

Beyrouty et al. (1990), also described seasonal variation in root growth and development in bermudagrass that showed two stages of root growth: minimal root growth and development in combination with stolon proliferation and soil surface establishment in the early growing season (spring and summer), followed by increased TRL over two succeeding forage production seasons. In addition, Sifers et al. (1985) assessed root decline during the winter months in 11 warm season turf species (Common, ‘Tifgreen’, and Tifway bermudagrass; ‘Adalayd’ seasore Paspalum; ‘Pensacola’ bahiagrass; Floratam and Texas Common St. Augustinegrass; ‘Texoka’ buffalograss [*Bouteloua dactyloides* (Nutt.)]; common centipedegrass [*Eremochloa ophiuroides* (Munro) Hack], and

‘Meyer’ zoysiagrass) and reported that the mechanisms behind reduced root growth during winter months can be linked to high temperatures and favorable growth environments in the spring, such that shoot growth accelerates faster than the roots, causing reserved carbohydrates to be preferentially allocated towards shoot growth over root growth (Sifers et al., 1985). Although carbohydrate utilization was not accessed in this study, the phenomenon of high root growth during the summer months in Floratam and Tifway in this study could be supported by future work in this direction by quantifying the utilization of carbohydrate reserves with increasing temperatures and photosynthetic activity as reported in Chalmers and Schmidt, (1979) and Schmidt and Blaser, (1969) studies.

Spider diagrams further illustrated these seasonal patterns in rooting behavior at low and high mowing heights for both Floratam and Tifway. Spider plots are useful tools to access the performance of multifunctional systems, where traits of interest share a common origin and standardized scale, and the obtained polygons help explain the performance of multiple factors without the need for interpretation of larger data sets and tables (Gareau et al., 2010). The spider plots in this study showed the reduced TRL and TRSA at lower mowing heights for both species at both soil depths. The impact of low mowing height on root growth was significant within the deeper soil depth (30-90 cm) throughout the year with overall reduced root growth. In contrast, at this depth increased mowing heights were associated with improved root growth and development that was greater during June, July, August, October, and December for Floratam and May, June, October, and December for Tifway compared to the 7.6 cm height in Floratam St. Augustinegrass and 1.3 cm in Tifway bermudagrass. Although photosynthetic activity was not assessed in our study, this rooting pattern might be due to increased photosynthetic activity at high mowing heights during these months compared to low mowing. Interestingly, in a similar study, greater chlorophyll absorption occurred under higher mowing heights compared to low mowing heights for both Floratam and Tifway (Zhang, 2014). Fine root production at the 0-30 cm depth was also enhanced with higher mowing heights compared to 5 cm in Floratam and 1.3 cm in Tifway with peaks in September, January and March for Floratam, and in April and July for Tifway. At the 30-90 cm depth, high FRL production was observed in December for Floratam, and October for Tifway. Higher root partitioning at the deeper depth in Tifway was associated with the high mowing height compared to the two lower mowing heights. Moreover, a deeper overall root system was produced throughout the year in both species when mowed at their respective highest mowing heights. The patterns from the spider diagrams suggest that lower mowing is associated with reduced root growth and development, and that utilizing higher mowing compared to lower mowing heights could enhance overall root growth and development throughout the year. Similar to the results of our study, Tucker et al., (2006) reported greater root growth and development in TifEagle ultra-dwarf bermudagrass in July and August; and reported an 11% increase in TRSA and root length density (TRL/volume) when mowed at 4 mm compared to 3.2 mm. These results are consistent with those reported by Fagerness and Yelverton, (2001) and Liu and Huang, (2002) where overall reductions in root growth were reported when creeping bentgrass was mowed shorter compared to higher heights of cut during two growing seasons.

Summary

The result from our study suggests that in overall, the root growth and development of Floratam and Tifway were less affected by different mowing height treatments. Although some variation were observed between mowing height treatments in Tifway where 6.4 cm mowing produced higher TRL compared to 1.3 and 3.8 cm mowing heights in both the years however the result were not consistence for other traits such as TRSA, ARD and FRL in 2012 and 2013. Similarly, in Floratam although 10 cm mowing produced higher root growth and development compared to 5 m mowing, in most 10 cm mowing height had similar root growth and development as in 7.6 cm mowing height. Similar was the result between 7.6 and 5 cm mowing heights. This implies that the overall root growth and development in central Florida is less affected by different mowing heights. However, the current finding also reports the seasonal variation in root growth and development that could be utilized to regulate and manage fertilizer, water or other turf management practices and utilize the information of root growth during months of spring, summer or fall in central Florida. Moreover, the study emphasizes the knowledge gap that exists in understanding species variation in partitioning of reserved carbohydrate between above- and belowground systems, especially during early spring or late fall. Lastly, this study illustrates that the minirhizotron technique was effective for the seasonal evaluation of rooting dynamics by depth of warm season turfgrass species, where the fate of individual root segments or portions of root systems at different depths can be followed and quantified over time.

Table 4-1: Analysis of variance in 2011 for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL), maximum root depth (MRD) and root proportion (RP) of Floratam.

Effect	DF	TRL	TRSA	ARD	FRL	MRD	RP
Months	7	15.35***	16.82***	13.47***	5.55***	3.72**	0.58
Depth	1	43.12***	43.22***	70.58***	14.76**	NA	NA
Month*Depth	7	5.14***	6.16***	3.56***	1.66	NA	NA
MH	2	1.92	2.01	1.42	0.97	4.04*	0.19
Month*MH	14	0.8	0.87	0.47	0.66	0.43	0.86
MH*Depth	2	0.96	0.41	0.02	1.67	NA	NA

*, **, *** significant at P ≤ 0.05, 0.01 or at 0.001 respectively.

Table 4-2: Analysis of variance for Floratam with twelve months in year 2012 and 2013 for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL), maximum root depth (MRD) and root proportion (RP).

Effect	DF	TRL	TRSA	ARD	FRL	MRD	RP
Year	1	0.12	0.21	1.68	0.01	1.09	3.51
Months	11	4.99***	8.88***	5.38***	1.97*	1.07	2.59**
Year*Months	11	4.39***	4.62***	1.43	5.03***	0.42	1.27
Depth	1	145.85***	134.49***	379.11***	163.65***	NA	NA
Year*Depth	1	5.68*	7.29**	7.64**	8.09**	NA	NA
Month*Depth	11	2.24*	3.52***	2.07*	0.96	NA	NA
MH	2	6.5**	5.72**	7.44**	5.3**	15.85***	0.99
Year*MH	2	0.13	0.32	0.66	0.36	3.27*	1.49
Month*MH	22	0.82	0.87	0.77	0.89	0.16	2.26**
MH*Depth	2	0.10	0.04	0.16	1.38	NA	NA

*, **, *** significant at P ≤ 0.05, 0.01 or at 0.001 respectively.

Table 4-3: The effect of mowing height on mean values for Floratam maximum root depth (MRD) for year 2011 at 10, 7.6 and 5 cm mowing heights

MH (cm)	MRD (cm)
10	49 a
7.6	48 ab
5	44 b

‡Means followed by same letters are not significantly different.

Table 4-4: The effect of mowing height on mean values for Floratam total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL) for pooled years 2012 and 2013, and maximum root depth (MRD) pooled for year 2012 and 2013 at 10, 7.6 and 5 cm mowing heights.

MH (cm)	TRL (mm)	TRSA (mm)	ARD (mm)	FRL (mm)	MRD (cm)	
					2012	2013
10	550 a	652 a	3.06 a	56 a	58 a	59 b
7.6	449 ab	495 ab	2.79 ab	47 ab	57 a	69 a
5	268 b	309 b	2.46 b	31 b	49 b	54 b

‡Means followed by same letters are not significantly different.

Table 4-5: The effect of month by depth on mean values of Floratam total root length (TRL) and total root surface area (TRSA); and fine root length (FRL) averaged between soil depths for each month in 2012 and 2013. Average values were taken across the mowing heights (5, 7.6 and 10 cm).

Effect	Depth (cm)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2012													
TRL	0-30	761 b	787 b	873 ab	913 ab	996 ab	1088 ab	1190 a	1259 a	1167 ab	937 ab	983 ab	1060 ab
mm	30-90	30 c	34 bc	43 bc	63 abc	69 abc	74 ab	95 ab	101 ab	119 a	108 ab	99 ab	96 ab
TRSA	0-30	823 c	830 c	861 bc	1297 ab	1378 a	1521 a	1637 a	1682 a	1394 a	1172 abc	1205 abc	1294 ab
mm^2	30-90	29 c	33 c	39 bc	78 abc	82 abc	89 abc	108 abc	115 a	114 abc	133 a	113 abc	108 abc
FRL	0-30,	50 abc	56 abc	81 a	26 c	35 bc	39 bc	51 abc	60 ab	55 abc	29 bc	28 bc	32 bc
mm	30-90												
2013													
TRL	0-30	997 abc	967 abc	737 bc	893 abc	1211 a	1117 ab	774 abc	823 abc	695 bc	827 abc	601 c	666 c
mm	30-90	105 ab	104 ab	96 b	102 ab	129 ab	181 ab	141 ab	184 ab	167 ab	220 a	144 ab	192 ab
TRSA	0-30	1209 ab	1122 ab	837 bc	1036 abc	1379 a	1306 a	949 abc	1030 abc	777 bc	719 bc	589 c	719 bc
mm^2	30-90	112 a	105 a	98 a	103 a	128 a	188 a	149 a	192 a	172 a	164 a	108 a	182 a
FRL	0-30,	44 bcd	58 ab	46 bcd	43 bcd	56 bc	36 bcd	17 d	20 cd	35 bcd	111 a	50 bcd	49 bcd
mm	30-90												

‡Means followed by same letters within each row are not significantly different.

Table 4-6: The effect of month on average root diameter (ARD) for Floratam pooled across experiment years and mowing heights (5, 7.6 and 10 cm).

Effect	Depth (cm)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
ARD mm	0-30	2.02 ab	1.98 ab	1.91 b	2.02 ab	2.11 a	2.14 a	2.12 a	2.10 ab	2.05 ab	1.99 ab	1.98 ab	1.99 ab
	30-90	1.23 a	1.24 a	1.23 a	1.32 a	1.28 a	1.30 a	1.30 a	1.33 a	1.38 a	1.33 a	1.27 a	1.32 a

‡Means followed by same letters within a depth are not significantly different.

Table 4-7: Analysis of variance for Tifway root traits with eleven months in each year 2012 and 2013 for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL), maximum root depth (MRD) and root proportion (RP).

Effect	DF	TRL	TRSA	ARD	FRL	MRD	RP
Year	1	7.5	6.06***	0.1	2.31	2.6	1.32
Months	10	4.52***	4.11***	2.62**	3.29***	0.45	0.33
Year*Months	10	11.38***	12.98***	4.3***	4.26***	1.91*	0.67
Depth	1	172.38***	198.78***	291.74***	176.2***	NA	NA
Year*Depth	1	0.28	0.21	0.25	0.26	NA	NA
Month*Depth	10	1.45	0.57	0.56	3.25***	NA	NA
MH	2	8.06**	9.67***	12.66***	3.52*	67.16*	5.35
Year*MH	2	3.29*	3.8*	5.96**	4.93***	5.79**	2.91
Month*MH	20	1.21	1.6	1.6	0.5	0.65	2.45**
MH*Depth	2	2.66	2.53	0.63	0.91	NA	NA

*, **, *** significant at P ≤ 0.05, 0.01 or at 0.001 respectively.

Table 4-8: Mean Tifway total root length (TRL), total root surface area (TRSA), average root diameter (ARD), and fine root length (FRL), in 2012 and 2013, for 1.3, 3.8 and 6.4 cm heights of cut.

MH (cm)	TRL (mm)	TRSA (mm)	ARD (mm)	FRL (mm)
2012				
6.4	241 a	203 a	1.96 a	55 a
3.8	113 b	96 b	1.60 b	23 b
1.3	153 b	142 ab	2.00 a	39 ab
2013				
6.4	464 a	365 a	2.23 a	83 a
3.8	314 b	224 b	1.75 b	86 a
1.3	164 c	127 c	1.71 b	37 b

‡Means followed by same letters are not significantly different.

Table 4-9: The effect of month and year on mean Tifway total root length (TRL), total root surface area (TRSA), and root diameter (ARD) across depth and mowing heights; and the effect of month by depth on mean FRL across mowing heights (1.3, 3.8 and 6.4 cm). Means are shown for both 2012 and 2013.

Effect	Depth (cm)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Oct	Nov	Dec
2012												
TRL mm	(0-30,30-90 cm)	49 d	23 d	37 d	161 c	212 abc	171 bc	304 ab	258 abc	229 abc	284 abc	318 a
TRSA mm^2	(0-30,30-90 cm)	52 de	32 e	43 e	111 cd	167 abc	142 bc	248 ab	226 ab	214 ab	239 ab	268 a
ARD mm	(0-30,30-90 cm)	1.62 b	1.54 b	1.51 b	1.78 ab	2.01 a	1.77 ab	2.07 a	2.06 a	2.07 a	2.01 a	1.97 a
FRL	0-30	13 b	12 b	13 b	216 a	212 a	221 a	235 a	180 a	88 ab	126 a	145 a
mm	30-90	2 b	1 b	1 b	2 b	3 ab	3 ab	6 ab	6 ab	5 ab	5 ab	11 a
2013												
TRL mm	(0-30,30-90 cm)	314 ab	379 ab	345 ab	376 ab	422 a	372 ab	204 b	262 ab	296 ab	204 b	205 b
TRSA mm^2	(0-30,30-90 cm)	252 abc	293 ab	273 abc	296 ab	317 a	293 ab	153 bc	201 abc	186 abc	136 c	166 abc
ARD mm	(0-30,30-90 cm)	1.38 a	1.97 a	2.01 a	1.97 a	1.94 a	1.85 a	1.80 a	1.86 a	1.77 a	1.78 a	1.95 a
FRL	0-30	163 ab	170 ab	159 abc	171 ab	284 a	177 ab	108 bc	136 abc	277 a	174 ab	54 c
mm	30-90	12 a	15 a	12 a	11 a	11 a	12 a	9 a	11 a	13 a	16 a	14 a

[‡] Means followed by same letters within each row are not significantly different.

Table 4-10: Tifway maximum root depth (MRD, cm) by month for three mowing heights (1.3, 3.8 and 6.4 cm) in year 2012 and 2013.

MH (cm)	Jan cm	Feb cm	Mar cm	Apr cm	May cm	Jun cm	Jul cm	Aug cm	Oct cm	Nov cm	Dec cm
2012											
6.4	54 a	49 a	37 a	57 a	72 a	69 a	69 a	69 a	66 a	83 a	79 a
3.8	55 a	52 a	38 a	36 a	36 b	36 a	31 a	36 a	38 a	38 b	50 a
1.3	42 a	43 a	42 a	43 a	53 ab	53 a	62 a	54 a	54 a	52 ab	52 a
2013											
6.4	83 a	82 a	82 a	82 a	82 a	82 a	72 a	79 a	78 a	79 a	80 a
3.8	41 a	42 a	57 a	56 a	55 a	53 a	36 b	36 b	36 b	38 b	37 b
1.3	52 a	49 a	50 a	54 a	54 a	52 a	45 ab	44 b	45 b	41 b	41 b

‡Means followed by same letters within each row are not significantly different.

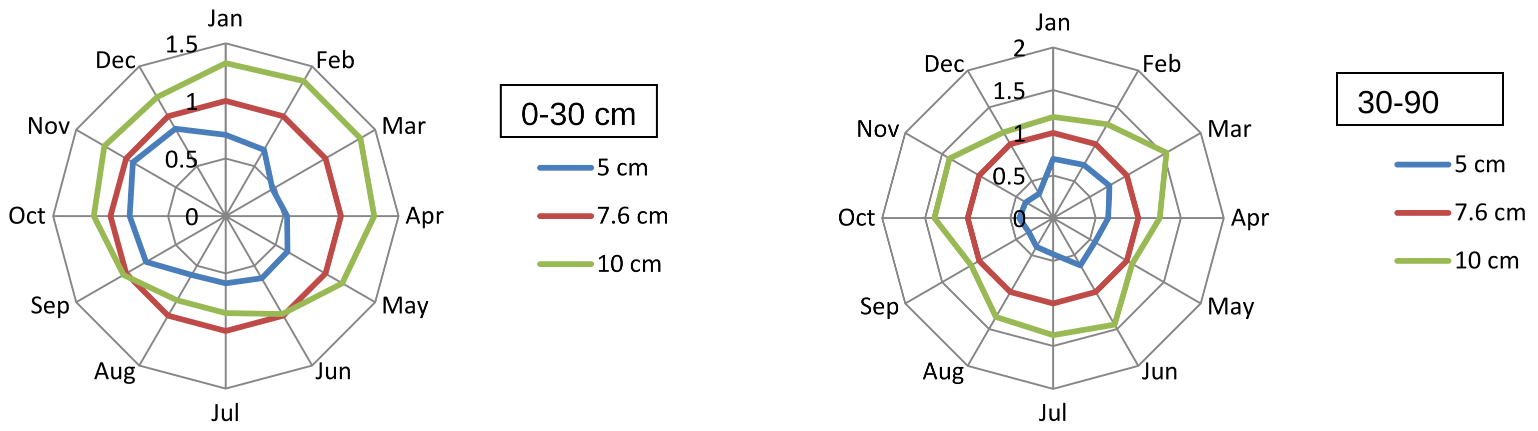

Figure 4-1: Total root length (TRL) at 0-30 cm and 30-90 cm depth for Floratam by months. The value of TRL at 7.6 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

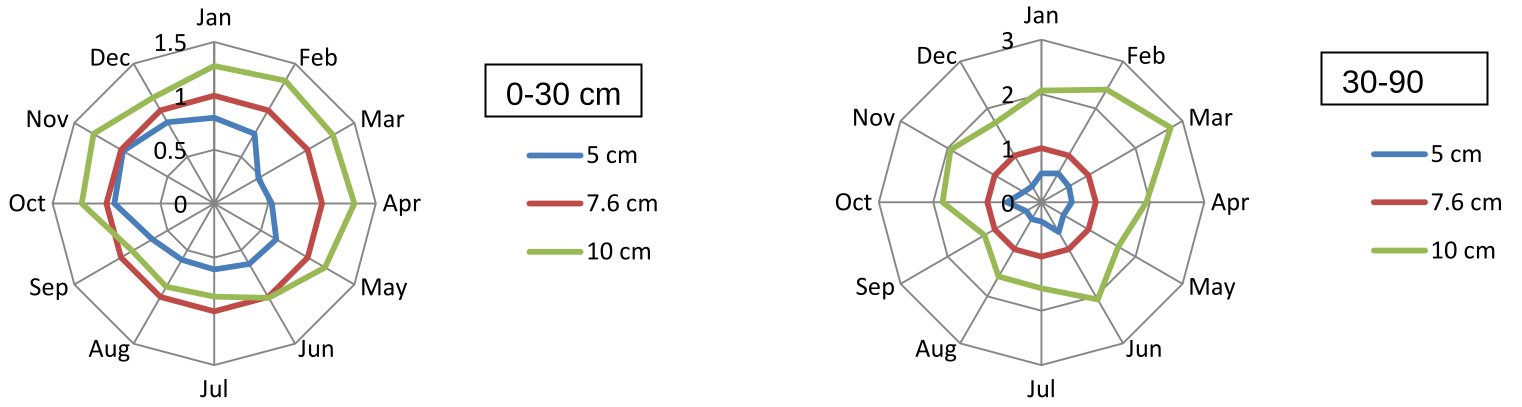

Figure 4-2: Total root surface area (TRSA) at 0-30 cm and 30-90 cm depth for Floratam by months. The value of TRL at 7.6 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

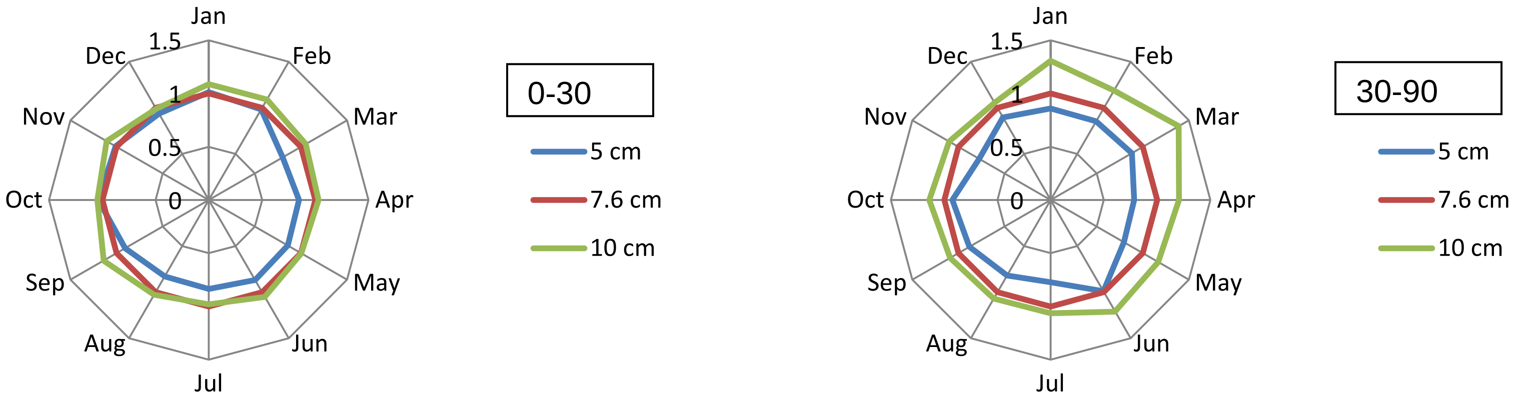

Figure 4-3: Average root diameter (ARD) at 0-30 cm and 30-90 cm depth for Floratam by months. The value of TRL at 7.6 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

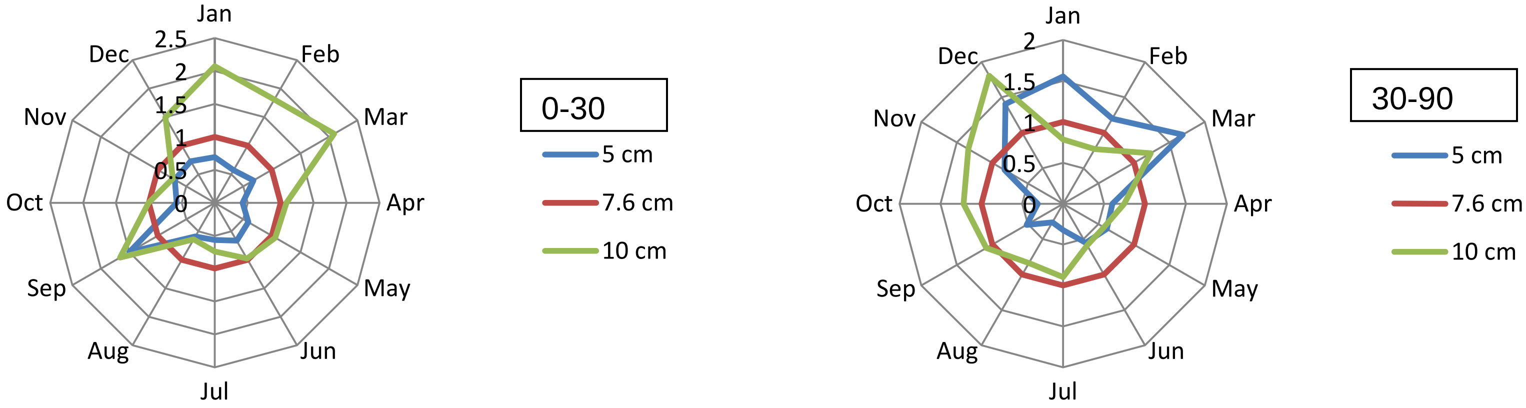

Figure 4-4: Fine root length (FRL) at 0-30 cm and 30-90 cm depth for Floratam by months. The value of TRL at 7.6 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

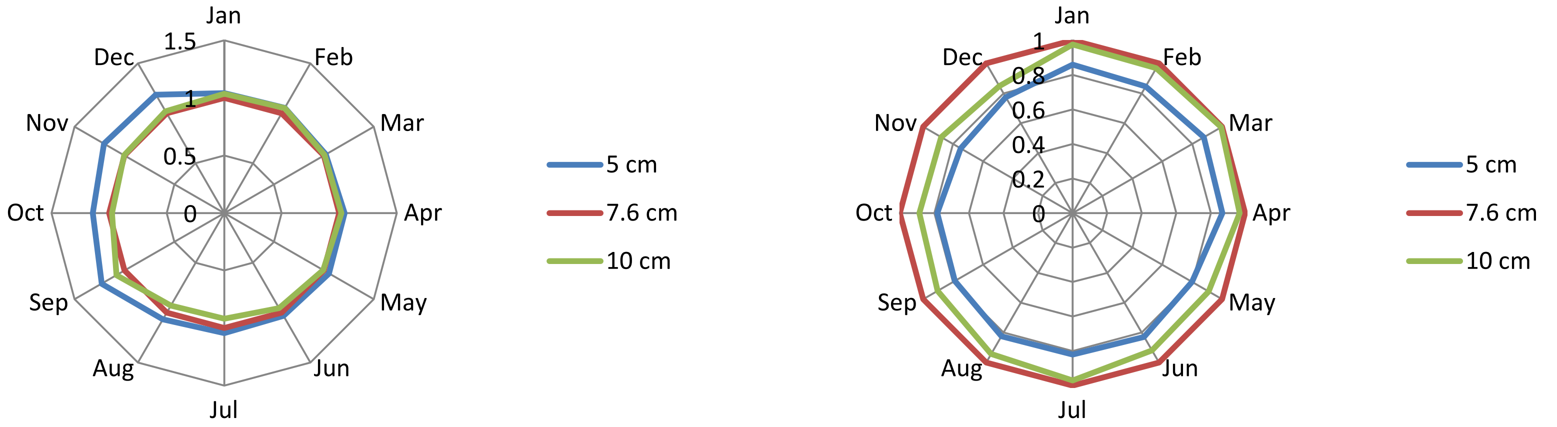

Figure 4-5: Root proportion (RP) and maximum root depth (MRD) respectively for Floratam by months. The value of TRL at 7.6 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

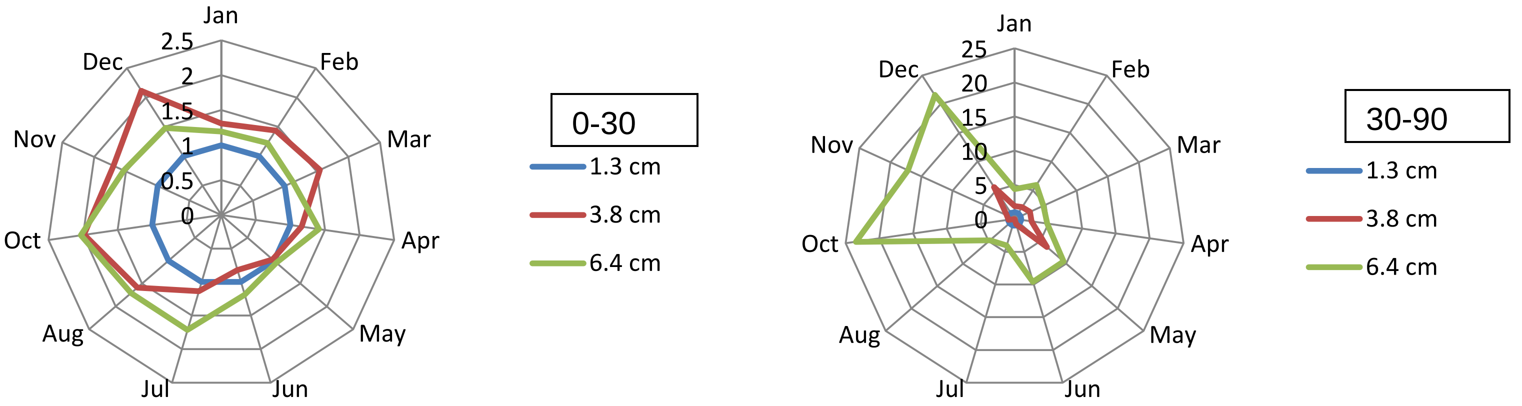

Figure 4-6: Total root length (TRL) by months at 0-30 cm and 30-90 cm depth for Tifway. The value of TRL at 1.3 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

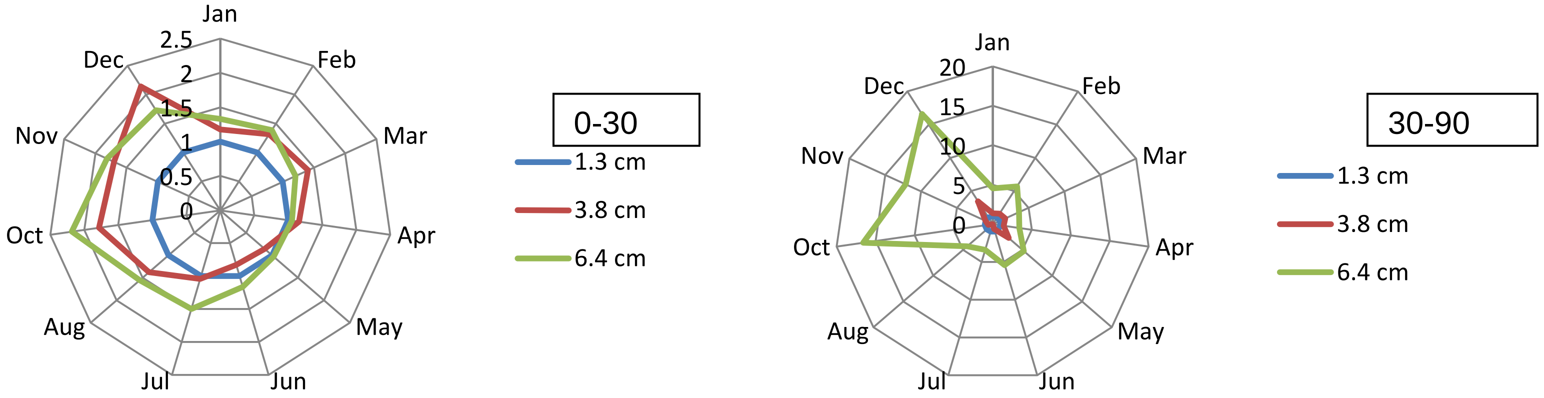

Figure 4-7: Total root surface area (TRSA) at 0-30 cm and 30-90 cm depth for Tifway by months. The value of TRL at 1.3 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

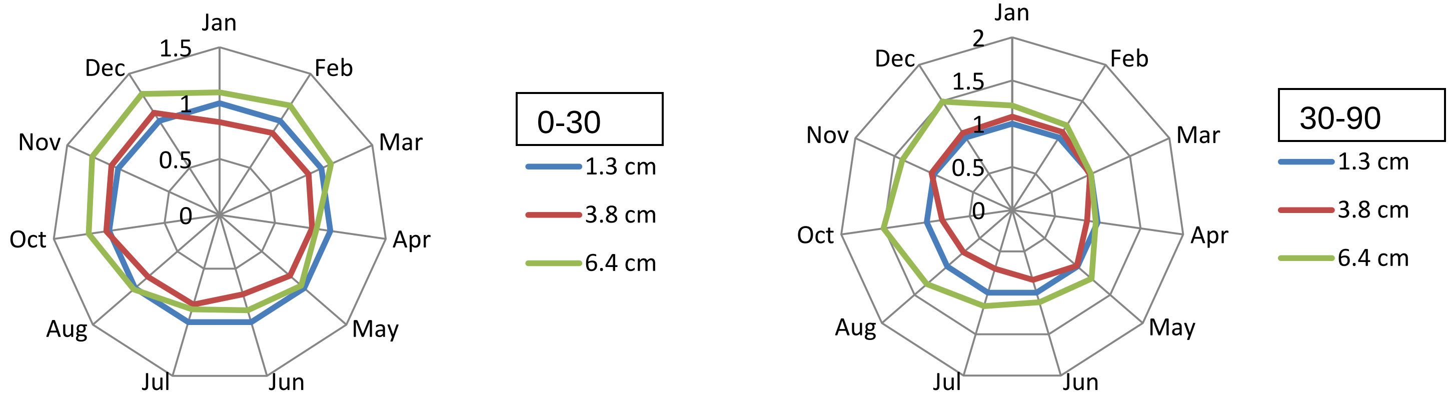

Figure 4-8: Average root diameter (ARD) at 0-30 cm and 30-90 cm depth for Tifway by months. The value of TRL at 1.3 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

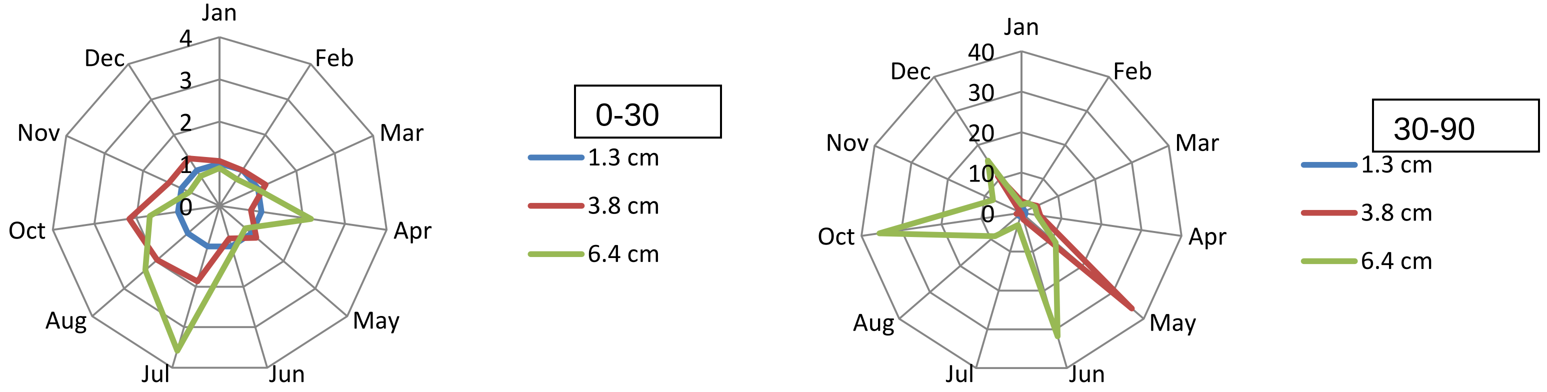

Figure 4-9: Fine root length (FRL) at 0-30 cm and 30-90 cm depth for Tifway by months. The value of TRL at 1.3 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

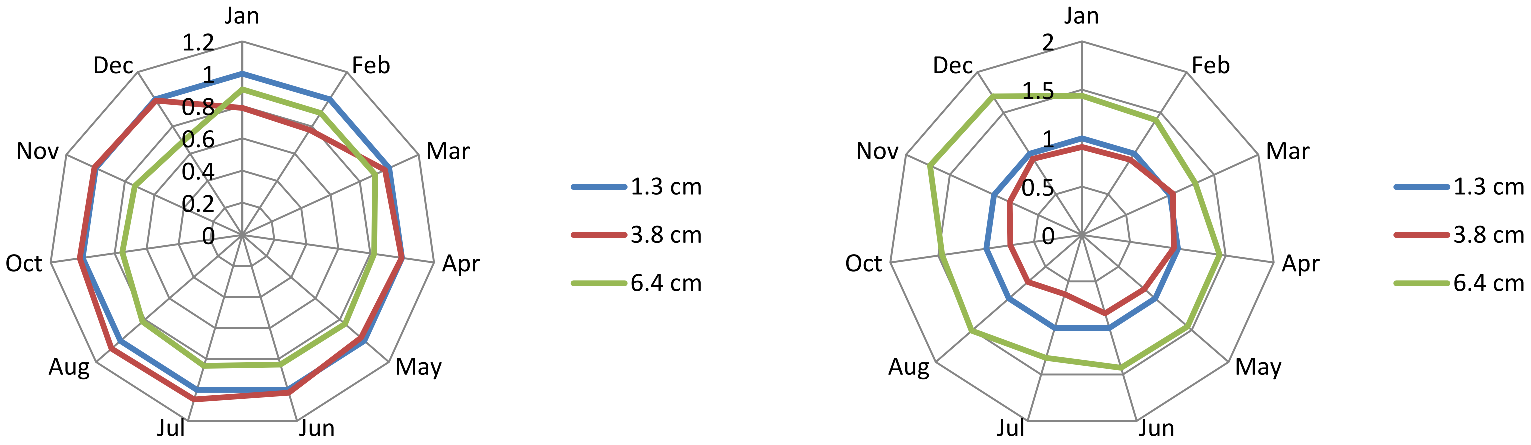

Figure 4-10: Root proportion (RP) and maximum root depth (MRD) respectively for Tifway by months. The value of TRL at 1.3 cm mowing heights were assumed a value of 1.0 (ratio), and values for the trait at the other mowing dates were presented as a proportion relative to that value.

CHAPTER 5
CONCLUSION

Our greenhouse study results suggest that understanding species and genotype variation for root morphology is very important for screening for drought resistance. However, this study also clearly shows that the historical and current concentration of efforts on determining differences in TRL alone is not adequate for effective genotype selection. This study also showed that one particularly important difference among genotypes was in the overall arrangement of roots between the two depth zones studied; with some utilizing a fairly uniform distribution across the 90 cm examined, while others preferentially concentrated roots in the shallow soil profile. This root architecture alone has important implications about the ability to access adequate soil water under drought conditions. Differences between species or genotypes for these root parameters mentioned above has provided important information that can be utilized (information on root trait and their association with improved canopy response of species during GH or field DD) in selecting of improved drought tolerant genotypes. Based on this study, it is clear that the inclusion of species such as ST and CB and specific genotypes identified in other species such as BA336 (ZM), and 5269-24 (ZJ) that have deep and extensive root systems (TRL, TRSA and FRL) are important contributors for root architectural improvements related to drought avoidance.

Similarly, results from the field drought suggests that FRL, MRD and RP were also important root traits for separating drought performance. Screening species just on the basis of TRL alone, therefore, might not always adequately represent true drought avoidance characteristics; thus drought screening program should include other parameters including FRL, MRD and RP. Moreover, while assessing turf for drought resistance, greenhouse and field studies must be employed. In our study, turf species showed different drought responses in the field experiment compared to the greenhouse experiment. Importantly, root traits at the relatively shallow depth of 0-30 cm showed little variability among species or genotypes. Therefore, it is important to assess deep root traits as in this study at 30-90 cm. In addition, separation among species or genotypes may be more apparent during longer drought periods. In this study, not every genotype with a shallow root system (or the majority of TRL confined to the 0-30 cm) showed poor drought response. Exceptions included Floratam (ST) and Zeon (ZM) that had shallow root systems but were better drought performers by exhibiting delayed leaf firing and a relatively green canopy during the dry down. Therefore, it is equally important to explore root and shoot responses simultaneously to truly understand turfgrass performance under drought.

Future work should be focused on variation that considers root physiological attributes, including root water uptake in field conditions, along with root morphological characteristics among species and genotypes to improve breeding efforts for drought resistance in turfgrass.

In addition, the current finding also reports the seasonal variation in root growth and development that could be utilized to regulate and manage fertilizer, water or other turf management practices and utilize the information of root development during months of spring, summer or fall in central Florida. Moreover, the study emphasizes the knowledge gap that exists in understanding species variation in partitioning of reserved carbohydrate between above- and belowground systems, especially during early spring or late fall. Lastly, this study illustrates that the minirhizotron could be one of the technique that would be effective to study the seasonal evaluation of rooting dynamics of warm season turfgrass species, where the fate of individual root segments or portions of root systems at different depths can be followed and quantified over time.

APPENDIX A
GREENHOUSE GENOTYPE COMPARISON

Table A-1**:** Analysis of variance F-statistics at (0-30, 30-90 cm) for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL) and root proportion (RP) across genotypes for DD tubes.

Effect	DF	TRL	TRSA	ARD	FRL	RP[£] (0-30 cm)
Year	1	2.53	10.49*	2.96	1.72	16.65***
Genotype	15	63.73***	50.54***	2.20***	62.30***	35.72***
Year*Genotype	15	5.63***	5.37***	2.64***	5.41***	3.71***
Depth	1	172.01***	256.96***	46.14***	155.00***	NA[£]
Year* Depth	1	5.55*	0.00	1.20	6.85***	NA
Genotype*Depth	15	10.83***	6.42***	2.00*	11.12***	NA

NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

[£] Root proportion (RP) parameter is a ratio, the parameter was analyzed at (0-30) cm depth.

Table A-2: Average for total root length (TRL), total root surface are (TRSA), and root proportion (RP) at 0-30, 30-90 cm depth across genotypes for DD tubes.

		2012					2013				
		TRL (cm)		TRSA (cm^2)		RP (cm/cm)	TRL (cm)		TRSA (cm^2)		RP (cm/cm)
Genotypes	Species	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm
Sapphire	ST[¥]	6001 a[‡]	6859 a	774 a	758 a	0.46 e	4837 a-b	6238 a	724 a-b	789 a	0.44 f-g[£]
Palmetto	ST	6494 a	5163 b-c	835 a	592 a-b	0.55 d-e	5087 a	7021 a	751 a	877 a	0.42 g
Floratam	ST	5836 a	7181 a	709 a-b	837 a	0.44 e	4434 a-d	5228 a-b	641 a-c	627 a-b	0.46 e-g
Captiva	ST	6031 a	3906 c-d	770 a	407 b-c	0.60 c-e	5086 a	7424 a	747 a	916 a	0.41 g
289922	CB	3329 b-c	2275 d-g	528 b-c	288 c-e	0.59 d-e	3756 a-e	3104 b-c	575 a-d	373 b-c	0.55 d-g
Celebration	CB	3553 b-c	1623 e-h	489 b-d	199 c-f	0.70 b-d	3471 c-e	2203 c-e	513 b-d	271 c-d	0.61 d-e
UFCD347	CB	3105 c	1818 e-h	394 c-e	200 c-f	0.63 c-e	3023 e	2195 c-f	479 c-d	275 c-d	0.59 d-f
UFCD12	CB	2726 c	808 f-h	263 e	66 d-f	0.79 a-d	3896 a-e	2214 c-e	593 a-d	279 c-d	0.64 d
5269-24	ZJ	3748 b-c	2456 d-f	399 c-e	288 c-e	0.64 c-e	3640 b-e	3642 c-d	479 c-d	312 b-d	0.58 d-f
4360	ZJ	2254 c	217 h	247 e	17 f	0.95 a	4749 a-c	2194 c-f	749 a	262 c-d	0.70 c-d
BA182	ZJ	3010 c	196 h	403 c-e	31 e-f	0.94 a	2812 e	233 e-f	394 d	38 d	0.91 a-b
JaMur	ZJ	2591 c	335 g-h	356 c-e	49 e-f	0.89 a-b	2739 e	0 f	393 d	0 d	1.00 a
BA336	ZM	5118 a-b	2815 d-e	685 a-b	319 c-d	0.66 b-e	4783 a-c	2177 c-f	724 a-b	278 c-d	0.70 c-d
BA374	ZM	3369 b-c	621 f-h	391 c-e	73 d-f	0.84 a-c	4928 a-b	2194 c-f	680 a-c	286 c-d	0.69 c-d
ToccoaGreen	ZM	2807 c	337 g-h	319 c-e	42 e-f	0.89 a-b	3340 d-e	786 d-f	512 b-d	111 c-d	0.82 b-c
Zeon	ZM	2695 c	824 e-h	306 e	98 d-f	0.78 a-d	2966 e	149 e-f	397 d	26 d	0.95 a-b

[‡] Means followed by same letters within each column were not significantly different, $P \le 0.05$.

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

[£] Root proportion (RP) parameter is a ratio of total root at 0-30/30-90 cm soil depth classes; therefore, depth is not a parameter in the analysis of this characteristic.

Table A-3: Average for average root diameter (ARD) and fine root length (FRL) at 0-30, 30-90 cm depth across genotypes for DD tubes

		2012				2013			
		ARD (mm)		FRL (cm)		ARD (mm)		FRL (cm)	
Genotypes	Species	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm
Sapphire	ST[¥]	0.4267 a-b[‡]	0.3566 a	5739 a	6690 a	0.4803 a-c	0.4013 a	6690 a	6047 a-b
Palmetto	ST	0.4089 a-b	0.3634 a	6290 a	5059 a-b	0.4711 a-c	0.3951 a	5059 a-b	6089 a-b
Floratam	ST	0.4031 a-b	0.3762 a	5617 a-b	6956 a	0.4581 a-c	0.3818 a	6956 a	5114 b
Captiva	ST	0.4094 a-b	0.3342 a	5857 a	3859 b-c	0.4678 a-c	0.3901 a	3859 b-c	7276 a
289922	CB	0.5093 a	0.3985 a	3103 c-d	2192 c-f	0.4871 a-c	0.3849 a	2192 c-f	3006 c
Celebration	CB	0.4393 a-b	0.4226 a	3416 c-d	1573 d-g	0.4701 a-c	0.3952 a	1573 d-g	2137 c-e
UFCD347	CB	0.4088 a-b	0.3418 a	3022 c-d	1781 d-g	0.5048 a	0.3970 a	1781 d-g	2125 c-e
UFCD12	CB	0.3445 b	0.3717 a	2666 d	794 d-g	0.4812 a-c	0.4049 a	794 d-g	2142 c-e
5269-24	ZJ	0.3421 b	0.3533 a	3677 b-d	2417 c-e	0.4212 c	0.3840 a	2417 c-e	2575 c-d
4360	ZJ	0.3731 a-b	0.0642 a	2207 d	214 f-g	0.5007 a-b	0.3810 a	214 f-g	2145 c-e
BA182	ZJ	0.4224 a-b	0.2544 a	2897 d	186 g	0.4417 a-c	0.3923 a	186 g	220 e-f
JaMur	ZJ	0.4367 a-b	0.3535 a	2488 d	319 f-g	0.4657 a-c	0.0000 b	319 f-g	0 f
BA336	ZM	0.4316 a-b	0.3741 a	4946 a-c	2755 c–d	0.4785 a-c	0.3892 a	2755 c-d	2093 c-f
BA374	ZM	0.3671 a-b	0.3819 a	3285 c-d	605 e-g	0.4390 a-c	0.4129 a	605 g-f	2097 c-f
ToccoaGreen	ZM	0.3675 a-b	0.2821 a	2744 d	325 f-g	0.4878 a-c	0.5010 a	325 g-f	756 d-f
Zeon	ZM	0.3525 b	0.2735 a	2642 d	799 d-g	0.4265 b-c	0.2890 a-b	799 d-g	41 e-f

[‡] Means followed by same letters within each column were not significantly different, P ≤ 0.05.

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

APPENDIX B
FIELD STUDY GENOTYPE COMPARISON

Table B-1: Analysis of variance at (0-30, 30-90 cm) depths for total root length (TRL), total root surface area (TRSA), average root diameter (ARD), fine root length (FRL) and root proportion (RP) across genotypes during period of DD (day 1, 7, 14, 21).

Effect	DF	TRL	TRSA	ARD	FRL	RP[£]
Year	1	29.41***	1.87	10.94*	28.95***	0.55
Day	3	6.47***	4.55***	1.31	4.58***	0.02
Year*Day	3	0.97	1.06	0.54	2.41	0.07
Genotype	15	3.57***	3.59***	0.61	3.21***	10.33***
Year*Genotype	15	11.55***	11.48***	1.76*	6.67***	26.40***
Genotype*Day	45	0.18	0.18	0.48	0.35	0.07
Depth	1	107.00***	103.55***	4.83*	108.26***	NA
Year*Depth	1	0.52	1.44	4.89*	15.22***	NA
Day*Depth	3	0.72	0.30	1.56	0.43	NA
Genotype*Depth	15	3.03***	2.55***	0.76	2.42***	NA

NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

[£] Root proportion (RP) parameter is a ratio of the TRL at 0-30/30-90 cm depths.

Table B-2: Average for total root length (TRL), total root surface are (TRSA), and root proportion (RP) at 0-30, 30-90 cm depth across genotypes during period of DD (day 1, 7, 14, 21).

		2013				2014			
		TRL (mm)		TRSA (mm^2)		TRL (mm)		TRSA (mm^2)	
Genotypes	Species	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm	0-30 cm	30-90 cm
Sapphire	ST[¥]	1533 a[‡]	853 a-c	1362 a	862 a-b	1761 a	1235 a-b	1223 a	871 a
Palmetto	ST	825 a	332 a-c	759 a	301 a-b	1623 a	887 a-d	1260 a	681 a-b
Floratam	ST	629 a	2 c	666 a	3 b	1278 a	497 a-e	953 a	406 a-d
Captiva	ST	2205 a	343 a-c	1997 a	314 a-b	2497 a	333 a-e	1623 a	225 a-d
289922	CB	1233 a	1112 a-b	1029 a	997 a	1404 a	949 a-c	1113 a	621 a-c
Celebration	CB	1351 a	1107 a-b	974 a	842 a-b	1344 a	1679 a	919 a	890 a
UFCD347	CB	1140 a	1572 a	854 a	1206 a	1416 a	1612 a	917 a	978 a
UFCD12	CB	927 a	560 a-c	747 a	397 a-b	1195 a	796 a-e	698 a	402 a-d
5269-24	ZJ	983 a	57 b-c	812 a	55 a-b	845 a	29 d-e	492 a	17 d
4360	ZJ	998 a	64 b-c	781 a	64 a-b	1262 a	12 e	675 a	9 d
BA182	ZJ	1634 a	146 a-c	1260 a	155 a-b	1812 a	133 b-e	1178 a	85 b-d
JaMur	ZJ	954 a	128 a-c	897 a	85 a-b	927 a	94 c-e	597 a	40 c-d
BA336	ZM	1359 a	113 a-c	1210 a	101 a-b	1808 a	113 b-e	1147 a	79 b-d
BA374	ZM	1850 a	229 a-c	1361 a	263 a-b	1732 a	143 b-e	919 a	94 b-d
ToccoaGreen	ZM	1707 a	87 a-c	1407 a	85 a-b	1496 a	19 e	950 a	11 d
Zeon	ZM	1062 a	129 a-c	821 a	100 a-b	973 a	76 c-e	588 a	51 b-d

[‡] Means followed by same letters within each column were not significantly different, $P \leq 0.05$.

[¥] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table B-3: 0-30 cm and 30-90 cm average for total root length (TRL), total root surface are (TRSA), and fine root length (FRL) across sixteen (16) genotypes during period of DD (day 1, 7, 14, 21).

		2013			2014		
		FRL (mm)		RP (mm/ mm)	FRL (mm)		RP (mm)
Genotypes	Species	(0-30 cm)	(30-90 cm)	(0-30 cm)	(0-30 cm)	(30-90 cm)	(0-30 cm)
Sapphire	ST[¥]	260 a[‡]	55 a-b	0.68 a-d	705 a	504 a-c	0.60 c-e
Palmetto	ST	75 a	32 a-b	0.72 a-d	643 a	256 a-d	0.65 b-e
Floratam	ST	29 a	0 b	0.99 a	484 a	125 a-d	0.72 a-e
Captiva	ST	251 a	17 a-b	0.84 a-c	1098 a	162 a-d	0.88 a-d
289922	CB	328 a	171 a-b	0.52 c-d	578 a	407 a-d	0.59 d-e
Celebration	CB	609 a	155 a-b	0.57 b-d	665 a	984 a	0.44 e
UFCD347	CB	312 a	236 a	0.41 d	747 a	694 a-b	0.46 e
UFCD12	CB	327 a	179 a-b	0.60 b-d	730 a	471 a-d	0.60 c-e
5269-24	ZJ	278 a	7 a-b	0.93 a-b	459 a	14 c-d	0.95 a
4360	ZJ	214 a	3 a-b	0.92 a-b	686 a	2 d	0.98 a
BA182	ZJ	472 a	2 a-b	0.90 a-b	911 a	60 b-d	0.89 a-c
JaMur	ZJ	181 a	62 a-b	0.83 a-c	477 a	67 b-d	0.88 a-d
BA336	ZM	422 a	38 a-b	0.92 a-b	950 a	59 b-d	0.94 a-b
BA374	ZM	606 a	47 a-b	0.87 a-c	1101 a	65 b-d	0.92 a-b
ToccoaGreen	ZM	472 a	13 a-b	0.93 a-b	678 a	7 c-d	0.97 a
Zeon	ZM	181 a	25 a-b	0.88 a-c	478 a	29 c-d	0.91 a-b

[‡] Means followed by same letters within each column were not significantly different, $P \leq 0.05$; these traits are reflective of characteristics across the entire root system.

[¥] STA: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

APPENDIX C
MULTIVARIATE ANALYSIS OF VARIANCE ACROSS GENOTYPE FOR GREENHOUSE AND FIELD STUDIES

Table C-1: Multivariate analysis of variance for across genotypes, during end of DD experiment (greenhouse).

Variables	Wilks' Lambda
Across Genotypes	4.27 ***

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

ƗST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table C-2: Within class standardized canonical coefficient for root and shoot parameters across genotypes end of **DD** experiment (greenhouse).

Variables	CAN1	CAN2
TRL	-7.871605	10.1288
TRSA	1.1338226	-2.235
ARD	-0.245211	0.3158
MRD	-0.50183	0.54494
RDW	0.2100808	0.09886
TRL	7.873806	-9.4305
TQ15	-0.271552	-0.5863
LF15	0.2488212	0.51158
RP	-0.439067	-1.0145
ƗCan R^2	0.92***	0.78***
‡PR	0.64	0.18
≠Cu PR	0.64	0.82

ƗCanonical Correlation

‡Proportion

≠Cumulative proportion

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table C-3: Class mean for canonical variables across sixteen genotypes end of DD experiment (greenhouse).

Genotypes	Species	CAN1	CAN2
289922	CB[ǂ]	-0.581206	1.8931
Celebration	CB	-0.770796	1.17789
UFCD12	CB	-0.989266	1.39878
UFCD347	CB	-0.643394	2.2187
Captiva	ST	3.6848837	-0.9697
Floratam	ST	3.4339397	0.55422
Palmeto	ST	3.8317971	-0.9519
Sapphire	ST	3.6102115	-0.3143
4360	ZJ	-0.33525	1.44635
5269-24	ZJ	-2.453116	-1.392
BA182	ZJ	-1.147295	-1.0332
JaMur	ZJ	-2.514904	-1.5481
BA336	ZM	0.1551096	-0.5656
BA374	ZM	-1.059764	-0.6858
ToccoaGreen	ZM	-2.019233	-0.4419
Zeon	ZM	-2.201718	-0.7866

[ǂ] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table C-4: Multivariate analysis of variance for across genotypes, during end of DD (day 21) in the field.

Variables	Wilks' Lambda
Across Genotypes	3.92***

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

ǂST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

Table C-5: Within class standardized canonical coefficient for root and shoot parameters across genotypes end of DD (day 21) in the field.

Variables	CAN1	CAN2
TRL	-0.455354408	0.838223502
TRSA	-0.138611067	-0.011770785
ARD	0.021658457	-0.103412782
FRL	0.230535754	-0.534966571
LFday21	-0.80642026	-0.059972101
MRD	0.578810012	0.863723404
TQday21	1.399477348	-0.588315567
RP	-0.696562302	0.794509104
ǂCan R^2	0.94***	0.81***
‡PR	0.72	0.16
≠Cu PR	0.72	0.88

ǂCanonical Correlation

‡Proportion

≠Cumulative proportion

*, **, *** Significant at P ≤ 0.05, 0.01 or at 0.0001 respectively.

Table C-6: Class mean for canonical variables across sixteen genotypes end of DD (day 21) in the field.

Genotypes	Species	CAN1	CAN2
289922	CB[ǂ]	3.73768025	0.02301
Celebration	CB	4.35475363	-0.700577
UFCD12	CB	4.3371897	-0.790026
UFCD347	CB	3.57578653	0.072823
Captiva	ST	-0.6181561	3.310646
Floratam	ST	-0.8476883	-1.470379
Palmeto	ST	0.71018615	-0.650343
Sapphire	ST	2.49502659	1.588599
4360	ZJ	-2.4666223	-0.789025
5269-24	ZJ	-2.3253421	-0.273273
BA182	ZJ	-3.1958064	-0.60484
JaMur	ZJ	-1.0974165	-0.67238
BA336	ZM	-2.4781326	0.345729
BA374	ZM	-1.3633684	1.732901
ToccoaGreen	ZM	-2.8694546	0.860048
Zeon	ZM	-1.9486354	-1.982912

[ǂ] ST: St. Augustinegrass; CB: Common Bermudagrass; ZJ: *Zoysia japonica*; ZM: *Zoysia matrella*.

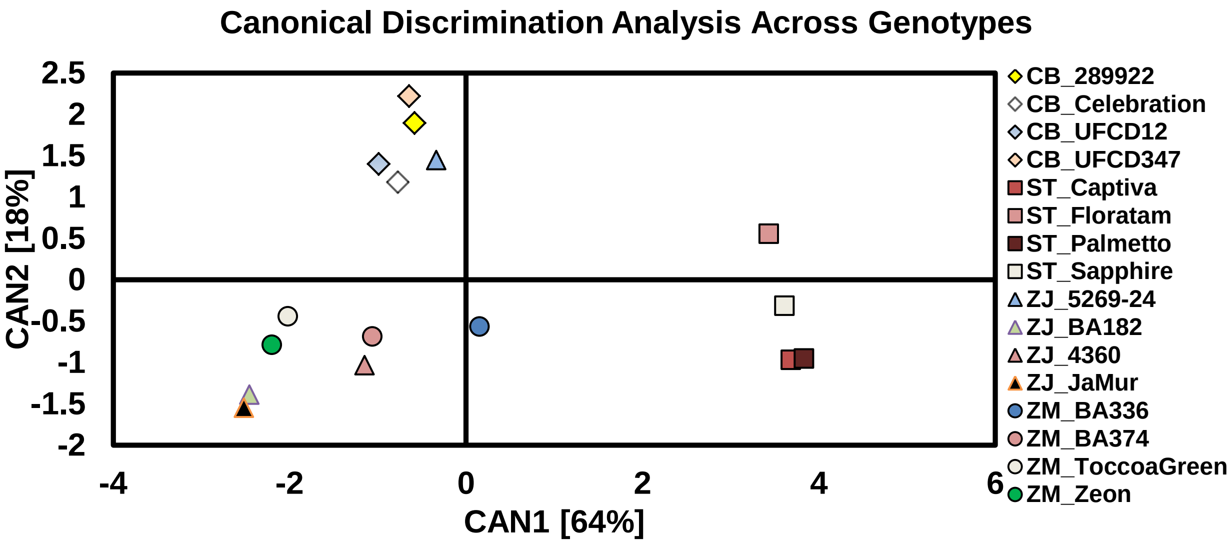

Figure C-1: Greenhouse study canonical discriminate analysis class means plotted across across sixteen warm season turf genotypes.

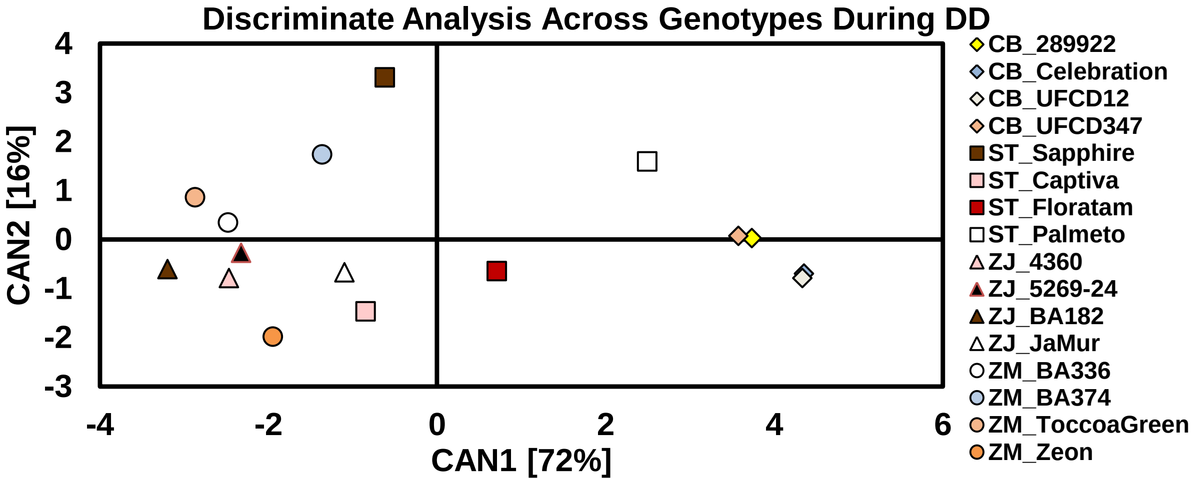

Figure C-2: Field drought study canonical discriminate analysis class means plotted across across sixteen warm season turf genotypes.

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BIOGRAPHICAL SKETCH

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