EVALUATION OF TURF-TYPE BERMUDAGRASS CULTIVARS AND EXPERIMENTAL GENOTYPES FOR ROOTING CHARACTERISTICS AND DROUGHT PERFORMANCE

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Abstract: Bermudagrass (Cynodon spp.) is one of the most widely used warm-season turfgrasses in the southern and transition zone areas of the United States. Due to the scarcity of water for turfgrass irrigation, it is necessary to identify and use droughtresistant cultivars. The purpose of this research was to evaluate commercially available bermudagrass cultivars and experimental genotypes for rooting traits and drought resistance. Three separate greenhouse studies were conducted to evaluate different bermudagrass genotypes for rooting characteristics when grown in clear polyethylene growth tubes under non-limiting soil moisture conditions. Genotypes were examined for shoot dry weight and root traits [rate of root depth development (RRDD), total root length (TRL), root surface area (RSA), average root diameter (ARD), root volume (RV), root dry weight (RDW), root length density (RLD), and root to shoot ratio (R/S)]. Significant differences were observed among the genotypes for all of the parameters examined. This research will help in identifying genotypes with superior rooting traits and prescreening a large number of genotypes prior to field drought evaluation. Drought performance of ten bermudagrasses was also investigated in the greenhouse when grown in 120 cm deep pots. 'TifTuf' was the top performing genotype for drought resistance. The high correlation among drought response parameters turf quality (TQ), leaf firing (LF), and normalized difference vegetative index (NDVI) indicates their usefulness to assess relative drought resistance. A field study was conducted to characterize the turf performance of 19 experimental and 2 commercially available bermudagrasses TifTuf and 'Tahoma 31'. Genotypes varied significantly for the evaluated parameters such as percent establishment (PE), turf quality (TQ), seedhead prolificacy (SH), fall color retention (FCR), spring green up (SG), and winterkill (WK). TifTuf and OSU1876 had maximum FCR. Winterkill ranged from 5.3 to 97.3 percent among the genotypes. Tahoma 31 had a less winterkill than TifTuf and 80% of the experimental genotypes. The results indicate that significant differences are present in the turf performance of the new genotypes evaluated in this study.

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CHAPTER I

LITERATURE REVIEW

Bermudagrass

Bermudagrasses (*Cynodon* spp.) are warm-season perennial grasses belonging to the *Poaceae* family. Out of the 14 warm-season species commonly used as turfgrass throughout the world, bermudagrasses are among the most important and widely adapted (Beard, 1973). Bermudagrasses are known by different common names such as couchgrass, quickgrass, wiregrass, and devilgrass (Beard, 1973; Emmons, 1995). *Cynodon* has nine species, out of which common bermudagrass [*C. dactylon* (L.) Pers. var. *dactylon*] and African bermudagrass (*C. transvaalensis* Burtt-Davy) are the most important species used for turf production (Taliaferro, 2003). Common bermudagrass is mainly tetraploid (2n = 4x = 36), but hexaploidy has also been reported (Harlan et al., 1970; Wu et al., 2005). African bermudagrass is diploid (2n = 2x = 18) with short stature, fine texture, high shoot density, and a yellow-green color (Hanna, 1986; Harlan et al., 1970).

Bermudagrass: distribution, biology, and adaptation

The center of origin for bermudagrass is southeast Africa, and it was introduced into the United States in the mid-1700s. South Africa, India, Afghanistan, China, and Australia are the

secondary centers of bermudagrass origin (Harlan and de Wet, 1969). Bermudagrass is extensively found between 45° N and 45° S latitudes (Taliaferro, 1995). Bermudagrass is widely used in lawns, parks, athletic fields, golf courses, cemeteries, and along roadsides (Beard, 1973). Propagation can be done by seeds, stolons, or rhizomes. Bermudagrass has a ligule with 1-3 mm long fringe of hairs and a continuous, narrow to medium broad collar. Leaf blades are generally 1.5 to 3 mm wide and inflorescence has four to five digitate spikes. Bermudagrass root systems are deep and fibrous and roots emerge at nodes of the stolons, and the lateral buds formed at nodes produce new stems. New roots are initially white and may turn yellow or brown when mature (Beard, 1973). Bermudagrass flowering is influenced by day length and varies with genotypes within *C. transvaalensis* and *C. dactylon*. Generally, flowering begins during April in African bermudagrass, and seedhead production lasts for 4 to 8 weeks. The second flush of seedheads is observed in the fall in African bermudagrass. *C. dactylon* flowers continuously due to indeterminate growth habit with maximum seedhead densities during the spring and fall (Hanna, 2013). Seedheads reduce the aesthetic quality of turf (Morris, 2000).

Bermudagrass has many important characteristics such as excellent turfgrass quality, high salinity tolerance, high heat tolerance, high drought resistance, good disease resistance, rapid establishment rate, and faster recuperative rate from damage (Taliaferro et al., 2004). Bermudagrasses generally show superior drought resistance than the other warm-season turfgrasses (Carrow, 1995; Qian and Fry, 1997). Warm-season grasses differ from cool-season grasses in their photosynthetic pathways (Moser et al., 2004). Warm-season grasses such as bermudagrass have the C₄ cycle, which suppresses photorespiration and the saturation of C₄ photosynthesis when ambient CO₂ is lower (Moser et al., 2004). Under drought conditions, the CO₂ concentrating mechanism in C₄ plants help mitigate moisture stress by reducing stomatal conductance and leaf transpiration (Ghannoum, 2009). In general, warm-season grasses use water more efficiently than cool-season grasses (Moser et al., 2004). The water requirement of bermudagrass varies with cultivars (Christians and Engelke, 1994). Selected off-types from 'Tifdwarf' having higher shoot densities, shorter internodes, and the ability to be mowed at lower height were released as cultivars and are called ultradwarfs. Ultradwarf bermudagrass cultivars are relatively less drought resistant due to the presence of a shallow root system (McCarty and Canegallo, 2005).

Well-drained soils with a pH ranging from 6.0–6.5 are the best for bermudagrass growth (Higgins, 1998). The species is nitrogen responsive and requires nitrogen fertilizer to maintain higher quality turf. Excellent wear and compaction tolerance is observed in bermudagrass (Christians and Engelke, 1994). It has good tolerance to close mowing because of a prostrate growth habit. A mowing height of 1.27 cm to 2.54 cm is suitable for general purpose turf, with 1.9 cm being more preferred (Beard, 1973). Hybrid bermudagrasses (*C. dactylon* × *C. transvaalensis*) such as 'Tifgreen', Tifdwarf, 'Everglades', and 'Bayshore'' can tolerate daily mowing at 0.63 cm (Beard, 1973). Ultradwarf bermudagrasses can tolerate a mowing height of 3.2 mm or less over a long period (McCullough et al., 2007).

Optimum growth of bermudagrass occurs between 24 to 37°C, which makes them suitable for growth in the southern United States through the transition zone (Beard, 1973). The transition zone (Figure 1) is the area between the northern and southern regions where coolseason and warm-season grasses are well adapted, respectively (Dunn and Diesburg, 2004). Bermudagrasses have a low tolerance to freezing temperature (Beard, 1973) and are susceptible to winter when grown in the transition zone (Fry, 1990). The shoot growth ceases followed by loss of chlorophyll and a change in color to brown when the soil temperature drops below 10°C (Christians and Engelke, 1994). Winter discoloration occurs in bermudagrass because of the physiological process of dormancy, which limits their use in transitional zones (Schiavon et al., 2011). Fall color retention ratings measure the ability of the turfgrasses to retain color during the winter months (Morris, 2000). Turfgrasses accumulate carbohydrates during reduced activity in

the fall, and the energy produced by the metabolization of these stored carbohydrates is used to initiate spring green-up (Rogers et al., 1975). Early spring green-up may encourage earlier sports field use and allow the harvest of green bermudagrass to start earlier on sod farms.



Figure 1. Turfgrass zones of adaptation in the United States.

Turfgrass water use

Approximately nine billion gallons of water is used daily for residential landscape irrigation in the U.S. (EPA, 2017). Over 163,800 km² of the area is occupied by cultivated turfgrass in the U.S, which is three times larger than any other irrigated crop (Milesi, 2005). Various government agencies, municipalities, and nonprofit organizations consider turfgrass as a luxury, and restrictions are being imposed on irrigation. One possible approach to decrease turfgrass water needs is to identify and use drought resistant species and cultivars (Carrow et al., 1990). The use of drought resistant cultivars reduces the cost of production and conserve water resources. Though warm-season turfgrass species are comparatively more drought resistant than cool-season species (Huang, 2008), further improvement is needed to survive prolonged moisture stress when grown under rainfed conditions (McCarty and Miller, 2002).

Drought resistance mechanisms

A period of prolonged water deficit stress limiting the turfgrass growth is called drought (Beard, 1973). Drought resistance is the ability of plants to survive extended moisture stress conditions by mechanisms of escape, tolerance, and avoidance (Beard, 1989; Levitt, 1980). Drought escape is the mechanism where plants complete their life cycle before the onset of drought or enter dormancy during extended drought stress and resume growth when water is available (Kramer, 1980). Browning of leaves occurs in dormancy, however meristematic crowns, stolons, and rhizomes remain viable. The drought tolerance mechanism involves the maintenance of metabolism even at low cellular water levels (Huang, 2008). Cell turgor can be maintained through osmotic adjustment and cellular elasticity, while desiccation tolerance can be achieved through protoplasmic resistance to reduced cell water content (Morgan, 1984). Under the drought avoidance mechanism, plants delay tissue dehydration either by reducing the water use or loss from plant canopy or by increasing water uptake from the soil (Huang, 2008). Drought avoidance characteristics exhibited by plants include enhanced root plasticity, deep roots, reduced leaf growth, and stomatal density (Huang et al., 1997). These drought resistance mechanisms are not mutually exclusive, and the same plant can use more than one of these mechanisms to survive drought (Huang, 2008).

Shoot responses to drought

Turfgrass water use is influenced by the amount of water lost due to the combined canopy transpiration and soil evaporation, and the water uptake by roots from the soil. Therefore, differences in shoot and root characteristics such as canopy configuration or leaf arrangement, shoot density, growth habit, rooting depth, and root density are associated with differences in water use rates of turfgrass species (Beard, 1973; Huang and Fry, 1999). Turfgrasses with a higher rate of vertical shoot extension show increased water consumption due to greater leaf area leading to transpiration losses than slower growing, dwarf type turfgrasses (Kim and Beard, 1988; Shearman and Beard, 1973). Stomatal closure under moisture stress reduces photosynthesis and

growth, leading to the accumulation of soluble carbohydrates in leaves, stems, and roots (Youngner, 1985). Shoot growth decreases under water stress, but root growth may be stimulated due to insufficient moisture near the surface and more moisture in a deeper profile, which promotes a higher root mass to shoot mass ratio.

Fuentealba et al. (2016) observed significant differences in evapotranspiration rates (ET) and breakpoints at which transpiration decreases with soil drying among the warm-season turfgrasses. Zhang et al. (2017) examined canopy and physiological responses of warm-season turfgrasses during a controlled water withdrawal experiment in a greenhouse. Genotypes varied in threshold and midpoint for normalized transpiration ratio, relative gas exchange rate, and leaf firing in response to decreasing fraction of transpirable soil water. Common bermudagrass had the lowest threshold for leaf firing compared with other species, indicating delayed initiation of leaf firing. Zhou et al. (2014) studied eighteen bermudagrass genotypes for drought performance. Grasses were evaluated for days to reach 50% green cover, physiological traits, rhizome dry matter, root length density (RLD), average root diameter (ARD), and water extraction. Drought resistance was positively correlated with more soil water extraction, higher leaf relative water content, lower canopy temperature, and higher photosynthetic rate.

Response of bermudagrass root system to drought stress

The study of root systems requires special techniques as roots are hidden in the soil or substrate (Judd et al., 2015). Roots have been studied traditionally using destructive techniques such as coring, trenching, and excavating. The complete expression of genetic potential for rooting is affected by soil physical and chemical properties such as high soil strength, acid soil complex, low soil oxygen, high soil temperature, and salt toxicities (Foy, 1992; Duncan and Shuman, 1993). Roots can be studied under controlled conditions in greenhouses using transparent tubes, through which the whole root system can be viewed without the use of

destructive procedures (Judd et al., 2015). Evaluation of root growth using flexible plastic tubes in the greenhouse is an effective method for screening plants as the technique is less expensive than traditional field coring and excavation techniques. The variability due to localized changes in the field soil temperature, texture, and water content is also reduced. Further, non-destructive monitoring of root extension through time becomes possible (Marcum, 1995b).

The presence of an extensive root system is an important drought avoidance trait in turfgrass as it allows the water uptake from deeper soil depths (Hurd, 1975). Turfgrass root systems with greater water-conducting ability and surface area are significant for regular water uptake under moisture stress (Huang et al., 1997). Pre–stress due to deficit irrigation can promote root growth in some plants (Fu et al., 2007). Higher root production has been positively correlated with higher shoot production in many plant species (Barbour and Murphy, 1984; Pederson et al., 1984; Ekanayake et al., 1985; Palazzo and Brar, 1997). Root characteristics and root: shoot (R/S) ratio are significant parameters for the selection of drought resistant turfgrass cultivars (Bonos et al., 2004). Plants having high R/S generally have reduced transpiration and their root systems can absorb water from relatively larger volumes of soil.

Interspecific differences in the distribution of root systems in the soil under moisture stress have been observed in warm and cool-season turfgrasses (Doss et al., 1960; Evans, 1978; Sheffer et al., 1987). Zhang et al. (2019) evaluated warm-season turfgrasses for above and below ground drought responses and reported higher drought resistance in common bermudagrass mainly due to its deep, extensive roots as compared to the other species evaluated. Intraspecific differences in rooting depth have been studied using polyvinyl chloride (PVC) tubes filled with sand or fritted clay as a rooting medium (Hays et al., 1991). Hays et al. (1991) evaluated seven bermudagrass experimental genotypes and three cultivars 'Midiron', 'Tifgreen', and 'U3'. The genotypes with roots uniformly distributed throughout the soil profile showed superior drought avoidance by maintaining high visual quality.

In a study to evaluate the root responses of 10 Kentucky bluegrass (*Poa pratensis* L.) genotypes under moisture stress, root volumes at both shallow and deeper soil depths were higher for the tolerant entries (Bonos and Murphy, 1999). Kentucky bluegrass cultivars evaluated for root dry weights under different soil drying treatments in a growth chamber study showed lower root dry weights at shallow drying depth (0-20 cm) (DaCosta et al., 2004). Also, root dry weights were higher for soils with moisture at a deeper profile than well-watered control (DaCosta et al., 2004).

Bonos et al. (2004) conducted a greenhouse study for root evaluation of tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort] and perennial ryegrass (*Lolium perenne* L.) populations using clear flexible tubes. The seeds of the populations were germinated in polyethylene glycol (PEG), and most vigorous seedlings were selected and transferred to clear tubes for selection for deep root production. Bonos et al. (2004) found higher root weight and R/S ratio efficient for the selection of drought resistant cultivars.

Karcher et al. (2008) conducted a field drought study using tall fescue entries under a rainout shelter. Twelve entries were evaluated which included four standard cultivars 'Bonsai', 'Kentucky-31', 'Plantation', and 'Southeast'. The cultivars 'Axiom', 'Wyatt', 'Regiment', and 'Tulsa' were the four parent entries used in this study. Two entries used for this study were previously selected for drought resistance from the parent entries based on a high R/S ratio in the greenhouse study and the other two entries were selected based on traditional field drought stress screening in Griffin, GA. The entries previously selected for high R/S ratio were reported to show better drought tolerance than their parents, while the previously field selected entries exhibited less consistency in drought performance under rainout shelter. This study demonstrated that prescreening genotypes for enhanced root characteristics in the greenhouse helped to identify tall fescue cultivars with improved drought resistance.

Root length density has been often used to estimate the extensiveness of the root system (Carrow, 1996a; Miller and McCarty, 1998). Root length density is the total length of roots per unit of soil volume and is used to determine the soil volume explored by the root system (Barber, 1971). Huang (2000) reported a positive correlation between turfgrass RLD and the rate of water uptake when grown under non-limited moisture conditions. However, according to Su et al. (2008), higher RLD near the soil surface leads to an early onset of drought stress due to rapid water depletion.

Qian et al. (1997) conducted experiments to evaluate three warm-season turfgrasses and tall fescue for (i) rooting characteristics in the greenhouse and field and (ii) relationships among rooting parameters, soil water depletion (SWD), and turfgrass wilting in the field. The cultivars evaluated include 'Midlawn' hybrid bermudagrass, 'Prairie' buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], 'Meyer' zoysiagrass (*Zoysia japonica* Steud.), and 'Mustang' tall fescue. Clear polyethylene root tubes (120 cm long and 3.5 cm diameter) were prepared as described by Lehman and Engelke (1985) for the greenhouse root evaluation of these cultivars. Mustang had higher RLD and maximum root extension (MRE) in 0-120 cm profile than the three warm-season turfgrasses. Higher RLD, total root length (TRL), and SWD due to extensive root system led to better drought performance in Mustang, while shallow roots in Meyer led to inferior drought performance (Qian et al., 1997).

Marcum et al. (1995a) evaluated 25 zoysiagrass genotypes for rooting characteristics in the greenhouse using polyethylene root tubes with 2.5 cm diameter and 90 cm long. They reported the average MRE to be positively correlated with total root weight, root number, and weight at increasing depths. Drought performance of 11 of the zoysiagrass genotypes included in this greenhouse root study was evaluated earlier in a field study at the Texas A&M Research and Extension Center, Dallas (Morton et al., 1991; White et al., 1993). Root depth, root weight, and root number at lower depths in the greenhouse root study showed a positive correlation with the field drought resistance for those 11 zoysiagrass genotypes (Marcum et al.,1995a).

Christensen et al. (2017) evaluated zoysiagrass genotypes for rooting capacity in the greenhouse and drought performance in the field. A positive correlation was found between greenhouse study and field dry down. Genotypes with higher RLD and root biomass at lower depths performed better in the field dry down.

Drought responses of bermudagrass and buffalograss cultivars were evaluated at two soil depths under rainout shelter in the field. Cultivars were planted on a restricted 10 cm soil depth and on native soil with unrestricted rooting potential. The cultivars were subjected to a 60 days' drought period followed by a 60 days' recovery period. In this study, all bermudagrass and buffalograss cultivars grown on 10 cm soil depth completely turned brown within 20 days of drought. No cultivar survived the 60 days' drought when grown in the shallow root zone of 10 cm; however, all the cultivars survived when planted on the unrestricted soil depth. The shallow soil depth restricted the root distribution and water uptake from a lower profile leading to an early onset of drought stress (Steinke and Chalmers et al., 2011).

Root screening studies using clear tubes

Lehman and Engelke (1991) developed a screening technique to study root systems and their heritability in creeping bentgrass (*Agrostis stolonifera* L.). Thirteen creeping bentgrass genotypes were grown in 65 cm long flexible clear polyethylene tubes filled with sand mixed with 3.6 g of plastic-coated, slow-release fertilizer. The clear tubes were heat-sealed at the base and perforated for drainage. The tubes were placed in the PVC pipes and maintained at an approximate angle of 30° with the vertical. Turfgrass plugs with roots removed to 5 cm below the soil surface were planted in the tubes. Maximum rooting depth was marked on the face of the clear tubes to determine root extension. Root tillers were counted and weighed at the end of the study. Rooting characteristics of these genotypes were also studied in the field by removing cores (3.2 cm in diameter and 46 cm in length) from the plots. There was a positive correlation between the greenhouse and field studies for rooting depth indicating that genotypes with shallow rooting depth can be eliminated from a breeding program for improved drought avoidance. Lehman and Engelke (1991) suggested that field rooting potential could be depicted by flexible tube rooting studies in the greenhouse.

Marcum (1995b) evaluated rooting characteristics of 22 buffalograss genotypes using 90 cm long, clear polyethylene tubes filled with sand and observed differences among the entries for average maximum root depth, total root weight, root count, and root weight. Acuna (2010) developed a similar screening technique to monitor the rate of root depth development (RRDD) in bahiagrass (*Paspalum notatum* Flugge) germplasm. The RRDD was calculated as the linear increase in depth of the deepest root with time. The genotypes were grown in two different sized acrylic tubes and two different growing media. The acrylic tubes used were 100 cm long acrylic tubes with 3.5-cm and 10-cm diameters to evaluate the effect of soil volume and plant competition on RRDD. These tubes were filled with sandy soil or commercial potting mix to test the potential effect of organic and inorganic soils on RRDD. There were no interactions between genotypes and the growing medium. Variability occurred among the genotypes for the RRDD. Higher RRDD was associated with a higher shoot and root mass, leading to an early vigor (Acuna et al., 2010). The RRDD responses were constant across the two tube sizes indicating that smaller diameter tubes can be used efficiently for testing a large number of genotypes.

Fuentealba et al. (2015) evaluated the root development and root profile characteristics of bermudagrass and zoysiagrass species in clear acrylic tubes grown outdoors. The species evaluated in the study were African bermudagrass, common bermudagrass, Japanese zoysiagrass, and manila zoysiagrass (*Zoysia matrella* L.). Common bermudagrass had higher RRDD than manila zoysiagrass with 20% of its' TRL distribution at 90-120 cm depth. Manila zoysiagrass

roots were mainly accumulated in the upper soil profile indicating rapid water depletion and an early onset of moisture stress in manila zoysiagrass in comparison to common bermudagrass.

Huot et al. (2020) conducted a greenhouse research to evaluate eight perennial grass species for the RRDD, rate of root length development (RRLD), photosynthesis, and morphological traits such as root angle, root length (RL), ARD, root volume (RV), root surface area (RSA), and leaf area. The grasses were grown in rhizotrons made of folded galvanized steel (120 x 20 x 5 cm) having an open vertical dimension fitted with a removable clear polycarbonate sheet for observing root growth. The rhizotrons were held at 45° angle with the vertical with transparent side facing downwards. Variations were observed among the species for the rates of vertical root developments. Higher RRDD was associated with narrow root angle, as large root angles promote root systems with one horizontal growth and shallow root depth. Higher RRDD was also positively correlated to fine roots with lower diameter and higher root length/leaf area ratio.

OSU turfgrass breeding program

The OSU turfgrass breeding and genetics research program was initiated by Dr. Charles Taliaferro in the 1980s. The goal of the breeding program is multi-fold and focuses on 1) improvement of bermudagrass germplasm for seed production potential, turf performance traits, and stress resistance; 2) development, evaluation, and release of seed- and vegetativelypropagated turf-type bermudagrass varieties for use on fairways, tee boxes, and putting greens; 3) evaluation and maintenance of *Cynodon* germplasm for genetic improvement for turf use (USGA, 2019). The breeding program released seed-propagated turf-type bermudagrass cultivars 'Yukon' and 'Riviera', in 1996 and 2000, respectively. The vegetatively-propagated cultivars developed by OSU are 'Patriot', 'Latitude 36', 'NorthBridge', and 'Tahoma 31'. Latitude 36 bermudagrass is a triploid hybrid developed from a cross of *Cynodon dactylon* (2n=4x=36) with *C*. *transvaalensis* (2n=2x=18) (Wu et al., 2014). Latitude 36 and NorthBridge were both released in 2010 and Tahoma 31 is an interspecific triploid hybrid (2n=3x=27) developed from a cross of *Cynodon dactylon* var. *dactylon* accession A12268 (2n=4x=36) x *C. transvaalensis* OSU selection '2747' (2n=2x=18) (Wu et al., 2020) and was released in 2017. The long-term goal of the program is continuous development and release of new turf-type cultivars with improved quality and resistance to biotic and abiotic stresses (USGA 2019).

Goal and Objectives

The goal of this research was to evaluate and select new turf-type bermudagrass genotypes with extensive root systems and improved drought resistance. The objectives of the research were:

- To evaluate rooting characteristics and drought response of ten bermudagrass genotypes.
- To evaluate differences in rooting characteristics of 21 bermudagrass cultivars and OSU experimental genotypes under non-limiting soil moisture conditions.
- To evaluate differences in rooting characteristics of eight bermudagrass cultivars and experimental genotypes under non-limiting soil moisture conditions.
- To evaluate 21 bermudagrasses for visual characteristics and percent green cover.

Research hypotheses: It was hypothesized that:

- There will be significant differences among bermudagrass genotypes for rooting characteristics and performance under drought.
- There will be significant differences in rooting characteristics among 21 bermudagrass cultivars and OSU experimental genotypes when grown under non-limiting soil moisture conditions.

- There will be significant differences in rooting characteristics among eight bermudagrass genotypes.
- There will be significant differences among bermudagrasses for visual characteristics and percent green cover.

CHAPTER II

EVALUATION OF ROOTING CHARACTERISTICS AND DROUGHT RESPONSE OF TEN BERMUDAGRASS GENOTYPES

Abstract

Drought-resistant turfgrasses have become more important because of the limited availability of water for landscape irrigation. Turfgrasses with extensive root systems can avoid drought by extracting water from deeper soil profiles when moisture levels are low. The objectives of the study were to determine differences in rooting characteristics among ten bermudagrass genotypes and their drought performance. The genotypes evaluated include four commercial standards 'Latitude 36', 'Celebration', 'Tifway' and 'TifTuf' and six experimental genotypes (OSU1337, OSU1403, OSU1439, TifB16107, TifB16113, and TifB16120). For the root study, grasses were grown in 120 cm long, 3.81 cm diameter clear polyethylene tubes under well-watered non stressed greenhouse conditions. Shoot clippings were collected weekly to determine shoot dry weight. Root morphological traits and dry weight at different depths (0–30, 30–60, 60–90, and 90–120 cm) were evaluated at the end of the study. For the drought study, grasses were grown in 120 cm deep polyvinyl chloride (PVC) tubes with 10 cm diameter. Genotypes were assessed for turf quality (TQ), leaf firing (LF), and normalized difference vegetation index (NDVI). Turf quality, LF, and NDVI were all highly significantly correlated, implying that they could be used to characterize turfgrass drought response. The performance of genotypes for all of the testing

parameters decreased as days of drought (DOD) increased. During extreme drought, TifTuf bermudagrass had the highest rating for all parameters (indicating the best drought response) on the majority of dates. Among the experimental genotypes, TifB16113 was the top performing for all the measured parameters.

Introduction

Water is a limited natural resource for which there are no substitutes. Water makes up 75 to 85 percent of the weight of actively growing turfgrass (Beard, 1973). Plants start showing wilting when the water content drops by 10% (Beard, 1973). Water shortages are expected to worsen in the future because of climate change, population growth, and urbanization resulting in more restrictions on turfgrass irrigation. It is critical to identify and use drought resistant turfgrass species and cultivars. A period of prolonged water deficit stress limiting the turfgrass growth is called drought (Beard, 1973). Drought resistance is the ability of plants to survive extended moisture stress conditions by mechanisms of escape, tolerance, and avoidance (Beard, 1989; Levitt, 1980). Drought escape is the mechanism where plants complete their life cycle before the onset of drought or enter dormancy during extended drought stress and resume growth when water is available (Kramer, 1980). The drought tolerance mechanism involves the maintenance of metabolism even at low cellular water levels (Huang, 2008). Under the drought avoidance mechanism, plants delay tissue dehydration either by reducing the water use or loss from plant canopy or by increasing water uptake from the soil (Huang, 2008). Drought avoidance characteristics exhibited by plants include enhanced root plasticity, deep roots, reduced leaf growth, and stomatal density (Huang et al., 1997). Gopinath (2020) conducted bermudagrass drought performance trials in the field with unrestricted root zone and under controlled environment in 17 cm deep pots providing restricted root zone. The results of the two trials revealed that TifTuf and experimental genotypes from University of Georgia performed well in the field but exhibited poor drought performance when grown in 17 cm pots. Based on these

findings, it was hypothesized that extensive rooting could be the primary drought resistance mechanism in those genotypes. Therefore, there was a need to study the root characteristics as well as drought performance in deep pots in the greenhouse conditions. Objectives of this research were (i) to evaluate differences in rooting characteristics of ten bermudagrass genotypes under well-watered non-stressed greenhouse conditions, and (ii) to evaluate drought performance of ten bermudagrass genotypes when grown in 120 cm deep pots in the greenhouse.

Materials and Methods

The research was conducted at the Ridge Road greenhouse facility at Oklahoma State University (OSU), Stillwater, OK. Ten bermudagrass genotypes from the United States Department of Agriculture (USDA) 2016 Specialty Crops Research Initiative (SCRI) were used for the research (Table 1). This study included four industry standards Latitude 36, Celebration, Tifway, and TifTuf, and six experimental genotypes. TifB16107, TifB16113, and TifB16120 bermudagrass genotypes were from the University of Georgia (UGA) breeding program and OSU1337, OSU1403, and OSU1439 were from the OSU turfgrass breeding program.

Flexible root tube study

Procedures were a modification of those described by Lehman and Engelke (1991). Grasses were grown in clear polyethylene tubes, which were 3.81 cm in diameter and 120 cm deep. These tubes were sealed at the bottom with a plastic sealer, and four uniform-sized holes were pricked to facilitate drainage. The clear tubes were held in opaque PVC holding tubes, which were 5.08 cm in diameter and 120 cm deep. These opaque tubes prevented the penetration of light to the roots ensuring a dark root growing zone like that in the soil. The PVC tubes were capped at the bottom with a hole at the center for drainage. The tubes were then placed on a wooden rack held at a 30° angle to the vertical axis to facilitate the visibility of roots along the walls of the clear polyethylene tubes. Calcined clay (Pro's Choice Red Sports Field Conditioner,

Oil-Dri Corporation of America, Alpharetta, GA) sieved to a particle size of 1-2 mm diameter was used as a growing medium as it has relatively low dry bulk density, is non-cohesive, infiltrate easily, good water holding capacity, chemically inert, and can be easily washed from roots (van Bavel et al., 1978). The clear polyethylene tubes were filled with calcined clay and saturated with water a day before planting. Planting was done by sprigging on 27 January 2020. Planting material from the previously grown greenhouse pots were gently washed to remove any adhering growing media and 10 sprigs with 4-5 nodes were planted in each growing tube. The roots of the sprigs were cut at 2.54 cm (1 inch) while planting to facilitate an early establishment of the grasses. Plants were manually irrigated for the first week but low moisture stress symptoms begin to appear and therefore, an overhead sprinkler irrigation system was set up to provide adequate moisture. The system was programmed to water for five minutes every four hours. Fertilization was done using 20-20-20 N-P₂O₅-K₂O water-soluble general purpose fertilizer (J.R. Peters Inc., Allentown, PA) at 250 mg N L^{-1} two times a week. As a precautionary measure, the grasses were treated with bifenthrin (Talstar Insecticide, FMC Agricultural Solutions, Philadelphia, PA) rotated with abamectin (Avid® 0.15EC, Syngenta Crop Protection, Inc. Greensboro, NC) every two weeks at the label rate to prevent infestation of bermudagrass mites (Eriophes cynodoniensis Sayed) and mealybugs (Pseudococcus spp.). A foliar spray of chelated liquid iron (LawnStar® Chelated Liquid Iron, Omega Trade LLC Cheyenne, WY) was done one month after planting. The grasses were trimmed to maintain a height of 5 cm. The data for the average greenhouse conditions such as air temperature, photosynthetically active radiation (PAR, μ mol m⁻² s⁻¹), and relative humidity (RH) was recorded using WatchDog Mini Station 2475 data logger (Spectrum Technologies, Plainfield, IL). Natural light was supplemented with 1000 W overhead lamps in the greenhouse to provide a 14 hours photoperiod. The average temperature during the experiment was 30.5 °C. Relative humidity and PAR for the experiment were 32 % and 612 µmol m⁻² s⁻¹, respectively.

Parameters evaluated include:

Maximum Root Extension (MRE):

MRE is the depth of the deepest visible root in each growing tube. Root extension was marked on the walls of the tubes and measurements were taken weekly from the crown region to the deepest root tip using a ruler The study was ended after 100 days of planting and none of the genotypes reached the 120 cm depth.

Shoot Dry Weight (SDW):

Root Image Analysis:

The root morphological parameters such as total root length (TRL), average root diameter (ARD), root surface area (RSA), and root volume (RV) were determined by root analysis. The clear polyethylene growth tubes were cut into four sections: 0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm and named as zone A, B, C, and D respectively. The roots from each section were washed gently with water to remove any sand particles. A 1-2 mm mesh sieve was placed below to prevent root loss while washing. The roots were placed in plastic bags and stored at 4 \Box until further analysis. Roots were analyzed using WinRHIZO software (Regent Instruments, Nepean, ON, Canada). Prior to scanning, roots were stained using methyl blue at the rate of 5 g L⁻¹ for better visibility of finer root hairs. Roots were then separated on Regent's water-proof trays to

avoid overlapping while scanning. An image acquisition scanner was used to obtain digital images, which were analyzed by WinRHIZO software to obtain the root length, ARD, RSA, and RV for each section. The value obtained for each section for all parameters was added together to obtain the data for the entire 1 to 120 cm growing tube.

<u>Root Dry Weight (RDW):</u>

The root samples were dried in the oven at 80 for 48 hours and weighed using Adam lab PW245 analytical balance (Adam Equipment Co. Ltd., Milton Keynes, United Kingdom) to record RDW.

<u>Root to Shoot ratio (R/S):</u>

Root to shoot ratio was calculated on a dry weight basis using formula -

$$R/S = \frac{Root Dry Weight (RDW)}{Shoot Dry Weight (SDW)}$$

Dry down study

Polyvinyl chloride pipes with 10 cm diameter were cut at 120 cm length to prepare the pots for planting. The PVC tubes were closed at the bottom with the help of rubber caps, which were held tightly to the tubes by stainless steel clamps. Each PVC tube had two holes at the bottom to facilitate drainage. A porous geotextile sheet was placed at the bottom of each tube to prevent the loss of growing medium through the drainage holes.

The growing medium used was a mixture of sand and topsoil (Minick Materials Co. Inc., Oklahoma City, OK) in 1:1 by volume. Both sand and topsoil were sieved with a 2 mm sieve. Particles greater than 2 mm were removed, and particles with a size less than 2 mm were used to fill the PVC tubes. According to the soil test conducted by the Soil, Water, and Forage Analytical Laboratory, the texture was loam with 41.2 % sand, 43.8 % silt, and 15 % clay. The tubes were continuously watered and drained and then again watered to reach complete saturation.

Planting was done by sprigging on 26 February 2020. The sprigs from the previously maintained greenhouse pots were carefully washed to remove any growing material attached. The grasses were allowed to fully establish to obtain uniform turf coverage in all the tubes. The grasses were well-watered throughout the establishment period to avoid any water deficit stress prior to the drought treatment. The grasses were trimmed to maintain a mowing height of 2.54 cm and fertilized weekly at 2 g L⁻¹ using 20-20-20 N-P₂O₅-K₂O water-soluble general purpose fertilizer (J.R. Peters Inc., Allentown, PA).

The grasses were sprayed biweekly with bifenthrin (Talstar Insecticide, FMC Agricultural Solutions, Philadelphia, PA) as per the label rate to prevent infestation of bermudagrass mites (*Eriophes cynodoniensis* Sayed). The data for the average greenhouse conditions such as air temperature, photosynthetically active radiation (PAR), and relative humidity (RH) was recorded using WatchDog Mini Station 2475 data logger (Spectrum Technologies, Plainfield, IL). The average temperature during the drought period was 31.1°C. The average PAR and RH during the drought treatment were 645 µmol m⁻² s⁻¹and 62 % respectively. Natural light was supplemented with 1000 W overhead lamps in the greenhouse to provide a 14 hour photoperiod.

Treatment and measurements:

The planting material was saturated with water to the field capacity of loam soil (35-45 % soil volumetric water content) a day before imposing drought stress treatment. The drought stress was imposed on 27 July 2020. Parameters evaluated in this study include:

Visual evaluations:

Turfgrass performance was visually evaluated using the National Turfgrass Evaluation Program (NTEP) visual rating system (Morris, 2000). Visual ratings for turf quality (TQ) and leaf firing (LF) were recorded by the same human evaluator during the entire study for consistency.

Turf Quality (TQ)

Grasses were visually evaluated daily based on color, density, texture, uniformity, and disease or environmental stress. Turf quality (TQ) ratings were given on a scale of 1-9 where 1 = poorest TQ, 9 = highest TQ, and 6 = minimally acceptable TQ (Morris, 2000). A rating value of 9 is reserved for a perfect or ideal grass but can also represent an exceptional treatment pot. Different grasses may receive a same visual score, but the factors influencing that may differ.

Leaf Firing (LF)

The chlorosis of leaf beginning from the leaf tip and margins, proceeding down the leaf is called leaf firing (LF) (Carrow, 1996). Visual ratings were given every day from 1 to 9 scale where 1 = complete LF and 9 = no wilting and no LF (Morris, 2000).

Normalized Difference Vegetative Index (NDVI)

Turfgrass color was measured by handheld FieldScout TCM 500 NDVI meter (Spectrum Technologies, Plainfield, IL). NDVI color meter value indicates turfgrass reflectance, which measures the relative greenness of the turfgrass pots. An average of three readings was taken for each pot. The equation to calculate NDVI is as follows:

$$NDVI = \frac{(NIR - R)}{(NIR + R)}$$

where NIR = spectral reflectance measurements acquired in near-infrared for a given pixel, R = spectral reflectance measurements acquired in the visible (red) range for a given pixel (Trehholm, 1999).

Statistical Analysis

Flexible root tube study

The experiment was arranged in a completely randomized design with ten bermudagrass entries and four replications. Data was analyzed using the Statistical Analysis System (SAS) 9.4 software (SAS Institute, Inc., Cary, NC). Analysis of variance (ANOVA) was performed using 'PROC GLM'. When ANOVA was significant at P = 0.05 level, means separation were performed using Fisher's Least Significant Difference (LSD) test at P = 0.05 significance level.

Dry down study

The experimental design was a completely randomized design (CRD) with four replications of each genotype. Replications were considered random effects. Genotypes and rating days were the fixed effects and the day of drought was a repeated measure. Generalized linear mixed models (GLIMMIX) methods were used for repeated measure analysis (SAS version 9.4., SAS Institute Inc., Cary, NC, USA). The means of all parameters: TQ, LF, and NDVI were separated within each date using Tukey's Honest Significant Difference (HSD) test at a 0.05 level of significance. The correlation analysis of all the parameters (LF, TQ, and NDVI) was performed using SAS procedure PROC CORR (SAS version 9.4; SAS Institute, Cary, NC).

Results and Discussion

Flexible root tube study

Total Root Length (TRL)

There was a significant difference among genotypes for mean TRL from 0-120 cm (Table 2). Total root length for 0-120 cm depth was highest for Latitude 36 but not statistically different from Celebration, and experimental genotypes TifB16107, TifB16113, and TifB16120. There

was no difference in TRL of TifTuf and Tifway which was in accordance with previously reported by Amgain (2014). All the three OSU experimental genotypes OSU1337, OSU1403, and OSU1439 were in the bottom statistical group for total root length. For zone A, mean root length (RL) was highest for TIFB16107 but was not statistically different from Latitude 36. For zone B, Celebration had higher RL than Latitude 36. Only three genotypes Celebration, Latitude 36, and TifB16120 reached below 60 cm depth, while only Latitude 36 reached below 90 cm depth. Experimental genotype OSU1337 had 96 % of its TRL distributed in zone A (0-30) cm depth indicating presence of shallow roots whereas all the standard cultivars had less than 54 % of their TRL distributed in zone A indicating more root distribution to lower depth and ability to extract water from the deeper profiles (Table 4).

Root Surface Area (RSA)

There were significant differences in mean RSA from 0–120 cm (Table 3). There were no significant differences in mean RSA for zone A. For zone B, there was no significant difference among the standard cultivars. Among the experimental genotypes, TifB16107, TifB16113, and TifB16120 were in the top statistical group while OSU genotypes were in the bottom statistical group. Latitude 36 had the highest RSA below 60 cm depth. There was no difference in total RSA of TifTuf and Tifway which was in accordance with previously reported by Amgain (2014). Similar to percent TRL, percent of RSA at 0-30 cm was also maximum for OSU1337 (Table 4).

Average Root Diameter (ARD)

There were differences in ARD from 0-120 cm ranging from 0.191 mm to 0.223 mm for Latitude 36 and TifTuf, respectively. Among the standard cultivars, TifTuf had the highest ARD while Latitude 36, Celebration, and Tifway had the lower ARD (Table 5). These results were in agreement with those reported by Katuwal et al. (2020) where TifTuf had the highest ARD, higher than Tifway when evaluated in 50 cm deep pots under drought conditions. Among the experimental genotypes, TifB16120, and OSU1439 were in the top statistical group. There were no significant differences among genotypes at 0-30 cm depth. ARD decreased with an increase in depth. For zone B, the mean ARD was lowest for OSU1337 and OSU1439. The trend for ARD for lower depths (60-90 cm) was similar to RSA with most of the genotypes in the bottom statistical category except Latitude 36 and TifB16120.

Root Volume (RV)

There were differences in RV from 0- 120 cm (Table 6). Among the experimental genotypes, OSU1337 had the lowest RV but was not statistically different from OSU1403 and OSU1439. These OSU experimental genotypes were previously reported to have poor drought performance under field trial by Gopinath (2020). Bonos and Murphy (1999) also evaluated the root responses of Kentucky bluegrass genotypes and observed root volumes to be higher for drought resistant entries as compared to the sensitive entries at both shallow and deeper soil depths. This indicates that the lower RV of experimental genotypes OSU1337, OSU1403, and OSU1439 as reported in this research resulted in lower drought performance in previous field trials.

Shoot Dry Weight (SDW) and Root Dry Weight (RDW)

There were significant differences in SDW among the genotypes (Table 8). Shoot dry weight ranged from 1.13 g to 2.7 g among the ten genotypes. Among the standard cultivars, SDW was higher for Latitude 36 while no significant differences in SDW occurred among Celebration, Tifway, and TifTuf. There were no significant differences in total RDW and RDW for zone A (Table 7). For zone B, Latitude 36 had highest RDW while Tifway had the lowest RDW.

Root to Shoot ratio (R/S)

There were significant differences in R/S among the genotypes (Table 9). The R/S ratio ranged from 0.383 to 0.174. Among the standard cultivars, Latitude 36 had the lowest R/S while no significant differences were observed for R/S among Celebration, Tifway, and TifTuf.

Dry down study

The drought period of this experiment lasted for 63 days until the mean TQ of all genotypes was less than the minimum acceptable value of 6. There were highly significant effects of genotype, rating date, and genotype by rating date interactions on TQ, LF, and NDVI (Table 10). Genotype means were separated using Fisher's protected least significant difference test within each rating date for each parameter evaluated. A correlation analysis was performed using the PROC CORR procedure and a highly strong correlation ranging from r = 0.97 to r = 0.95 was observed among all the measured parameters (Table 14).

Turf Quality

Mean TQ for genotypes within each rating date varied significantly (Table 11). All the entries had an acceptable TQ, ranging from 7.3 to 8.0 (Table 11) before the onset of drought treatment. Significant differences for TQ occurred among the genotypes at 0 DOD based on differences in color, texture, density, and uniformity. Turf quality was significantly lower for Celebration on 0 DOD than the rest of the genotypes because of its coarse texture. The differences for TQ increased among the genotypes as the days proceeded in response to the duration of the drought and increasing levels of drought stress. At 14 DOD, the TQ of all bermudagrass genotypes remained acceptable (>6) with means ranging from 6 to 8. OSU1403, OSU1337, and Celebration were the first genotypes to fall below 6 at 21 DOD. At 28 DOD, mean TQ for all the OSU experimental genotypes fell below the acceptable level. OSU1403 remained in the bottom statistical group from 14 DOD untill the end of the dry down period. Among the commercial standards, Celebration was the first to fall below acceptable TQ. TifTuf was the top

performer among all genotypes with TQ rating falling below the minimum acceptable at 49 DOD. These results correspond well with those reported by Jespersen et al. (2019), Katuwal et al. (2020), and Gopinath (2020) with TifTuf consistently being the top performer with highest TQ ratings. In this study, Tifway had a higher TQ than Celebration which was similar to the growth chamber drought study conducted by Katuwal et al. (2020) in 55 cm long tubes. Tifway showed relatively poor drought performance under field conditions in previous drought trials (Katuwal et at., 2020; Gopinath et al., 2020) but intermediate drought performance under controlled environment conditions in our experiment which was similar to growth chamber study results of Katuwal et al., 2020. This emphasizes the role of growing conditions on the bermudagrass drought performance. At the end of the study, TQ ranged from 1.0 for OSU1403 to 5.0 for TifTuf. TifB16113 had significantly higher TQ than all other experimental genotypes at the end of the study.

Leaf Firing

None of the genotypes showed leaf firing until 7 DOD (Table 12). Significant differences for LF begin to appear from 14 DOD with OSU1403 and OSU1337 in the lower statistical group. At 21 DOD, all entries were suffering some leaf firing with ratings ranging from 4.8 to 8.3. LF values ranged from 1.0 to 5.5 for OSU1403 and TifTuf, respectively at the end of the study. OSU1403 was the worst-performing genotype and fired completely on 49 DOD.

<u>NDVI</u>

Significant differences in NDVI were found on all rating dates (Table 13). The mean NDVI values ranged from 0.702 to 0.747 and 0.174 to 0.565 at 0 and 63 DOD, respectively. From 0 DOD to 7 DOD, the mean NDVI of few genotypes increased numerically. This may have been due to no mowing stress and the drought stress was also not having a large effect on NDVI of these genotypes for the initial DOD. TifTuf and Latitude 36 were in the top statistical group on all

rating dates. At the end of the study at 63 DOD, TifTuf and Latitude 36 had NDVI values 0.565 and 0.529 respectively. After 63 DOD, OSU1403, OSU1439, TifB16107, and TifB16120 were in the bottom statistical group with mean NDVI ranging from 0.285 to 0.174.

Summary

Flexible root tube study

The results of this study showed differences in root growth characteristics among bermudagrass cultivars. Significant differences were present among the genotypes for each measured root parameter concerning the total 0-120 cm profile except for the parameter total root dry weight. In the entire 120 cm profile, 44% to 95% of TRL and 43% to 96% of RSA were located in the upper 30 cm. Latitude 36 was the only genotype that had roots in the 90-120 cm profile. Celebration and TIFB16120 had roots up to 90 cm profile. These results were in accordance with those reported by Poudel (2010) where Celebration had roots above 90 cm depth while Latitude 36 had root extended below 90 cm depth in the controlled environment root study. Experimental genotypes OSU1403 and OSU1337 had relatively shallow root systems which helped to explain their inferior drought performance in comparison to that of other genotypes. Tiftuf had highest root diameter and R/S ratio. These rooting characteristics are crucial when choosing drought-resistant cultivars. These root parameters could be employed as selection criteria in drought-resistant turfgrass breeding programs. This study was conducted under wellwatered conditions. Rooting patterns in well-watered settings may not be the same as those under drought conditions (Huang, 1999). Further research is needed to gain more information about the root characteristics and the performance of these bermudagrass genotypes under field drought conditions. Although the rooting parameters used in this study indicate extensiveness of the root systems, they do not provide information on root activity, or the ability to absorb water. More research on root activity in bermudagrasses is needed.

Dry down study

Plants adapt to drought stress through various mechanisms in different turfgrass species. Turfgrass shoot system (canopy) response is often used to assess drought resistance (Huang et al., 1997; Chalmers et al., 2008). Drought resistance is often evaluated based on TQ and the corresponding level of leaf hydration or leaf wilting (Fry and Huang, 2004). Turf quality is a commonly used indicator of overall plant performance and encompasses canopy color, uniformity, and density (Turgeon, 1999). Ten bermudagrass entries were evaluated for their drought resistance. Parameters considered for this study were TQ, LF, and NDVI. All the parameters were strongly correlated (> 95%) with each other when Pearson's Correlation coefficient was calculated. A range of drought performance was observed in bermudagrass genotypes. TifTuf was the top-performing commercial standard and similar to field observation. Experimental genotypes OSU 1403 and OSU1337 were the least performing genotypes and fell below the minimum acceptable TQ of 6 at 21 DOD. Among the experimental genotypes, TifB16113 was the top-performing. A significant difference in TQ at 0 DAT is due to the inherent difference in their turf quality (uniformity, texture, and density) under non-stressed conditions. Yurisic (2016) reported TifTuf to have better drought performance compared to Latitude 36 and Tifway.

Conclusion

TifTuf is a highly drought resistant cultivar as observed in the dry down study and previous field trials (Jespersen et al., 2019; Katuwal et al., 2020; Gopinath, 2020) but its performance for rooting characteristics was moderate in the flexible root tube study. TifTuf and Tifway varied significantly for the drought performance but no significant differences were observed for rooting characteristics except root diameter. The results were in accordance with those reported by Katuwal et al., 2020 where mechanisms of drought avoidance and tolerance,

specifically rooting characteristics, osmotic adjustment and antioxidant metabolism were evaluated. TifTuf had superior drought performance in both field and growth chamber studies but no significant differences were reported for total root dry weight partitioned to roots, root distribution in upper 25 cm depth, root depth, root length density, water use efficiency, and electrolyte leakage. However, TifTuf had a higher root diameter, net photosynthesis and relative water content. Greater root diameter can be one of the important root traits contributing to better drought performance of TifTuf than other cultivars. However, further investigation is needed on above-ground and below-grown parameters to get a better understanding on drought avoidance and drought tolerance mechanisms associated with improved drought performance of TifTuf.

Table 1. Bermudagrass cultivars and experimental genotypes tested for rooting characteristics and drought performance.

Bermudagrass genotype ^z	Description
Celebration	Standard cultivar
Latitude 36	Standard cultivar
OSU1337	OSU experimental
OSU1403	OSU experimental
OSU1439	OSU experimental
TifB16107	UGA experimental
TifB16113	UGA experimental
TifB16120	UGA experimental
Tifway	Standard cultivar
TifTuf	Standard cultivar

^zGenotypes with an OSU prefix are from Oklahoma State University and a UGA prefix are from

the University of Georgia.

Table 2. Root length comparisons of bermudagrass genotypes at different zones^z when grown in

polyethylene growth tubes^y.

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Total Root Length
genotype			(cm)		
Celebration	3788.0b ^x	4615.0a	300.4b	0.0b	8704.0ab
Latitude 36	4771.4ab	1433.0с-е	2867.0a	800.7a	9872.0a
OSU1337	3914.2b	200.0e	0.0b	0.0b	4114.0e
OSU1403	3120.1b	1103.0de	0.0b	0.0b	4223.0de
OSU1439	4328.7b	1246.0de	0.0b	0.0b	5575.0de
TIFB16107	6319.2a	2059.0b-e	0.0b	0.0b	8378.0а-с
TIFB16113	4500.9b	4204.0ab	0.0b	0.0b	8705.0ab
TIFB16120	3639.0b	3802.0а-с	676.4b	0.0b	8117.0a-c
TifTuf	3260.4b	2887.0a-d	0.0b	0.0b	6147.0с-е
Tifway	3384.0b	3130.0a-d	0.0b	0.0b	6514.0b-d

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05 significance level. Table 3. Root surface area comparisons of bermudagrass genotypes at different zones^z when

Bermudagrass genotype	Zone A	Zone B	Zone C	Zone D	Total Surface Area
			(cm ²)	
Celebration	268.7 ^x	219.4a	21.1bc	0.0b	509.2b-d
Latitude 36	317.5	108.7а-с	232.9a	72.2a	731.2a
OSU1337	278.6	11.1c	0.0c	0.0b	289.7e
OSU1403	280.5	59.9bc	0.0c	0.0b	340.4de
OSU1439	305.6	53.9bc	0.0c	0.0b	359.5de
TIFB16107	410.8	183.9ab	0.0c	0.0b	594.6ab
TIFB16113	316.4	226.5a	0.0c	0.0b	542.9bc
TIFB16120	295.9	181.5ab	78.1b	0.0b	555.4a-c
TifTuf	255.6	145.2a-c	0.0c	0.0b	398.7с-е
Tifway	242.9	153.4ab	0.0c	0.0b	396.3с-е

grown in polyethylene growth tubes^y.

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^xMeans followed by same letters within each column are not significantly different at the P = 0.05

significance level.

Table 4. Percentage of total root length and root surface area of bermudagrass genotypes in the 0-

30 cm depth.

Bermudagrass Genotype	Total root length	Root surface area		
	%	%		
Celebration	43.5	52.8		
Latitude 36	48.3	43.4		
OSU1337	95.1	96.2		
OSU1403	73.9	82.4		
OSU1439	77.7	85.0		
TIFB16107	75.4	69.1		
TIFB16113	51.7	58.3		
TIFB16120	44.8	53.3		
TifTuf	53.0	64.1		
Tifway	52.0	61.3		

Table 5. Root diameter comparisons of bermudagrass genotypes at different zones^z when grown

in polyethylene growth tubes^y.

Bermudagrass genotype	Zone A	Zone B	Zone C	Zone D	Average root diameter
			(mm)		
Celebration	0.215cd ^x	0.158ab	0.036bc	0.000b	0.192de
Latitude 36	0.219cd	0.182a	0.160a	0.076a	0.191e
OSU1337	0.208cd	0.044c	0.000c	0.000b	0.205b-e
OSU1403	0.195d	0.181a	0.000c	0.000b	0.190e
OSU1439	0.215cd	0.088bc	0.000c	0.000b	0.208a-c
TIFB16107	0.211cd	0.168a	0.000c	0.000b	0.197с-е
TIFB16113	0.220bc	0.180a	0.000c	0.000b	0.207b-d
TIFB16120	0.251a	0.166a	0.075b	0.000b	0.213ab
TifTuf	0.244ab	0.181a	0.000c	0.000b	0.223a
$\frac{\text{Tifway}}{\sqrt{2}}$	0.203cd	0.125ab	0.000c	0.000b	0.194c-e

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^xMeans followed by same letters within each column are not significantly different at the P = 0.05

significance level.

Table 6. Root volume comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

Bermudagrass genotype	Zone A	Zone B	Zone C	Zone D	Total root volume
			(cm ³)		
Celebration	1.314	0.904a ^x	0.070bc	0.000b	2.306ab
Latitude 36	1.495	0.390a-d	1.007a	0.158a	3.050a
OSU1337	0.999	0.049d	0.000c	0.000b	1.048c
OSU1403	1.257	0.270cd	0.000c	0.000b	1.527bc
OSU1439	1.406	0.271cd	0.000c	0.000b	1.677bc
TIFB16107	1.904	0.387a-d	0.000c	0.000b	2.291ab
TIFB16113	1.79	0.818ab	0.000c	0.000b	2.608ab
TIFB16120	1.57	0.509a-d	0.267b	0.000b	2.346ab
TifTuf	1.311	0.729a-c	0.000c	0.000b	2.043a-c
Tifway	1.215	0.340b-d	0.000c	0.000b	1.855bc
^z Zone $A = 0-30$ c	m, zone B =	30-60 cm, zon	e C = 60-90	cm, and zon	e D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^xMeans followed by same letters within each column are not significantly different at the P = 0.05

significance level.

Table 7. Root dry weight comparisons of bermudagrass genotypes at different zones $^{\rm z}$ when grown

in polyethylene growth tubes^y.

Bermudagrass genotype	Zone A	Zone B	Zone B Zone C		Total root dry weight ^x	
				(a)		
0.1.1	0.260	0.0071	0.0001	(g)	0.276	
Celebration	0.369 ^w	0.007bc	0.000b	0.000b	0.376	
Latitude 36	0.426	0.016a	0.004a	0.001a	0.447	
OSU1337	0.479	0.005bc	0.000b	0.000b	0.485	
OSU1403	0.338	0.009a-c	0.000b	0.000b	0.347	
OSU1439	0.44	0.001c	0.000b	0.000b	0.441	
TIFB16107	0.399	0.013ab	0.000b	0.000b	0.412	
TIFB16113	0.307	0.006bc	0.000b	0.000b	0.313	
TIFB16120	0.429	0.008a-c	0.002ab	0.000b	0.438	
TifTuf	0.429	0.006bc	0.000b	0.000b	0.435	
Tifway	0.393	0.002c	0.000b	0.000b	0.396	

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^x Dry weights were recorded after drying at 80°C for 48 hours.

^wMeans followed by same letters within each column are not significantly different at the P =

0.05 significance level.

Bermudagrass genotype	Shoot dry weight ^y	
	(g)	
Celebration	1.200d ^x	
Latitude 36	2.686a	
OSU1337	1.925b	
OSU1403	1.934b	
OSU1439	2.062b	
TIFB16107	1.310cd	
TIFB16113	2.059b	
TIFB16120	1.817bc	
TifTuf	1.132d	
Tifway	1.297cd	

Table 8. Shoot dry weight of ten bermudagrass genotypes grown in polyethylene growth tubes^z.

^zGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^yDry weights were recorded after drying at 80°C for 48 hours.

^xMeans followed by same letters within each column are not significantly different at the P = 0.05

significance level.

Bermudagrass genotype	Root -shoot ratio ^y
Celebration	0.317a-c
Latitude 36	0.174cd
OSU1337	0.253a-d
OSU1403	0.182cd
OSU1439	0.225b-d
TIFB16107	0.316a-c
TIFB16113	0.155d
TIFB16120	0.253a-d
TifTuf	0.383a
Tifway	0.341ab

Table 9. Root to shoot ratio of ten bermudagrass genotypes grown in polyethylene growth tubes^z.

^zGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^yDry weights were recorded after drying at 80°C for 48 hours.

^xMeans followed by same letters within each column are not significantly different at the P = 0.05 significance level.

Table 10. Test of fixed effects for Turf Quality (TQ), Leaf Firing (LF), and Normalized

Difference Vegetation Index (NDVI) using PROC GLIMMIX for dry down study^z.

Source	TQ	LF	NDVI
		p	
Genotype (G)	<.0001	<.0001	<.0001
Rating Days (D)	<.0001	<.0001	<.0001
GXD	<.0001	<.0001	<.0001

^zTQ, LF, and NDVI were recorded on ten weekly rating dates for 63 days' dry down period.

	Turfgrass Quality									
Genotype	0 DOD	7 DOD	14 DOD	21 DOD	28 DOD	35 DOD	42 DOD	49 DOD	56 DOD	63 DOD
Celebration	7.3b ^x	7.3b	6.5bc	4.8cd	4.0cd	3.8ed	2.5de	2.5cd	2.5dc	2.0cd
Latitude 36	8.0a	8.0a	8.0a	7.3a	6.5a	6.0ab	5.5ab	5.3a	4.8ab	4.0ab
OSU1337	8.0a	8.0a	6.5bc	4.3d	3.8cd	3.0ef	2.5de	2.3cd	2.3cd	1.3d
OSU1403	8.0a	8.0a	6.0c	4.3d	2.8d	1.8f	1.3e	1.0e	1.0d	1.0d
OSU1439	8.0a	8.0a	7.3ab	5.5b-d	4.8bc	4.3с-е	3.5cd	3.0bc	2.3cd	2.3cd
TIFB16107	8.0a	8.0a	7.8a	6.5ab	5.5ab	4.3с-е	3.5cd	2.8bc	2.3cd	1.5d
TIFB16113	8.0a	8.0a	8.0a	6.3a-c	5.5ab	4.8b-d	4.3bc	3.8b	3.5bc	3.3bc
TIFB16120	8.0a	8.0a	8.0a	6.3a-c	5.0bc	4.5b-e	2.5de	1.5de	1.0d	1.3d
TifTuf	8.0a	8.0a	8.0a	7.5a	6.5a	6.5a	6.0a	5.5a	5.3a	5.0a
Tifway	8.0a	8.0a	8.0a	6.5ab	6.5a	5.5а-с	5.3ab	5.0a	3.5bc	3.3bc
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	***	***	***	***	***	***	***	***	***	***

Table 11. Mean turfgrass quality^z for ten bermudagrass genotypes from 0 DOD^y to 63 DOD.

^zTurfgrass quality was rated on the scale of 1 to 9, where 1 = dead or dormant turf, 6 = acceptable turf and 9 = excellent turf.

^yDOD = Days of drought treatment.

^xMeans accompanied by the same letter in a column are not significantly different at the P = 0.05 level.

NS non-significant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001, respectively.

	Leaf firing									
Genotype	0 DOD	7 DOD	14 DOD	21 DOD	28 DOD	35 DOD	42 DOD	49 DOD	56 DOD	63 DOD
Celebration	9.0	9.0	8.8a	5.8ed	4.3d-f	4.3cd	3.3b-d	3.0cd	2.5d-f	2.0c
Latitude 36	9.0	9.0	9.0a	8.0ab	7.0ab	7.0a	6.5a	5.8a	5.5ab	4.0ab
OSU1337	9.0	9.0	7.3b	5.8ed	4.0ef	4.0cd	3.0cd	2.3de	2.8de	2.0c
OSU1403	9.0	9.0	6.8b	4.8e	3.3f	2.5d	2.0d	1.0e	1.0f	1.0c
OSU1439	9.0	9.0	8.5a	6.5cd	5.3с-е	5.0bc	4.3bc	3.8b-d	3.3cd	2.5bc
TIFB16107	9.0	9.0	9.0a	7.3а-с	6.3a-c	5.5a-c	4.3bc	3.3b-d	2.8de	1.8c
TIFB16113	9.0	9.0	9.0a	6.8b-d	5.8a-d	5.8a-c	5.0a-c	4.8b-d	4.5a-c	4.0ab
TIFB16120	9.0	9.0	9.0a	7.3а-с	5.5b-e	5.0bc	3.3b-d	2.3de	1.3ef	1.3c
TifTuf	9.0	9.0	9.0a	8.3a	7.3a	7.0a	6.5a	6.0a	6.0a	5.5a
Tifway	9.0	9.0	9.0a	7.3а-с	6.5a-c	6.3ab	5.3ab	5.0ab	4.0b-d	3.8b
i	NS	NS	***	***	***	***	***	***	***	***

Table 12. Mean leaf firing^z for ten bermudagrass genotypes from 0 DOD^y to 63 DOD.

^zLeaf firing was rated on the scale of 1 to 9, where 1 = complete leaf firing and 9 = no wilting or leaf firing.

# ^yDOD = Days of drought treatment

^xMeans accompanied by the same letter in a column are not significantly different at the P = 0.05 level.

NS non-significant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001, respectively.

Tifway	0.725bc	0.72a-e	0.698a-c	0.604ab	0.577ab	0.529bc	0.518bc	0.494a-c	0.429bc	0.427bc
	0.75540	0.75540								
TifTuf	0.733ab	0.735ab	0.706ab	0.682a	0.659a	0.648a	0.633a	0.614a	0.565a	0.565a
TIFB16120	0.713b-d	0.711a-f	0.671a-c	0.571bc	0.478cd	0.442cd	0.362de	0.293ef	0.184e	0.174e
TIFB16113	0.714b-d	0.703a-f	0.669a-d	0.571bc	0.543bc	0.499b-d	0.498bc	0.482a-c	0.447bc	0.421bc
TIFB16107	0.719b-d	0.723a-d	0.670а-с	0.613ab	0.542bc	0.483b-d	0.464cd	0.392с-е	0.311cd	0.230de
OSU1439	0.706cd	0.700a-f	0.656b-d	0.600ab	0.539bc	0.487b-d	0.417cd	0.389de	0.377cd	0.285de
OSU1403	0.719b-d	0.674a-i	0.608d	0.439d	0.332e	0.288e	0.264e	0.194f	0.179e	0.174e
OSU1337	0.716b-d	0.687a-h	0.638cd	0.503cd	0.449cd	0.402d	0.381de	0.370 de	0.340cd	0.332cd
Latitude 36	0.747a	0.754a	0.727a	0.651ab	0.590ab	0.574ab	0.573ab	0.541ab	0.534ab	0.529ab
Celebration	0.702d	0.651a-l	0.607d	0.450d	0.432d	0.385d	0.356de	0.338e	0.337cd	0.324cd
Genotype	0 DOD	7 DOD	14 DOD	21 DOD	28 DOD	35 DOD	42 DOD	49 DOD	56 DOD	63 DOD
					NDVI					

Table 13. Mean NDVI^z for ten bermudagrass genotypes from 0 DOD^y to 63 DOD.

 z NDVI = Normalized Difference Vegetation Index was measured using GreenSeekerTM handheld sensor.

^yDOD = Days of drought treatment.

^xMeans accompanied by the same letter in a column are not significantly different at the P = 0.05 level.

NS non-significant at the 0.05 level.

*, **, *** significant at P = 0.05, 0.01, and 0.001, respectively.

Table 14. Pearson's correlation analysis for turf quality (TQ), leaf firing (LF), and normalized difference vegetation index (NDVI) for dry down study.

Parameter	TQ	LF	NDVI
TQ	1	0.97***	0.96***
LF		1	0.95***
NDVI			1

*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively.

# CHAPTER III

# DIFFERENCES IN ROOTING CHARACTERISTICS OF BERMUDAGRASS GENOTYPES UNDER NON-LIMITING SOIL MOISTURE CONDITIONS

# Abstract

Limited water resources are a major concern for the turfgrass industry. Identification and use of drought resistant cultivars is an important approach to reduce turfgrass water usage. An extensive root system contributes to increased drought resistance by enabling the extraction of water from deeper soil profiles. Therefore, the objectives of this research were i) to evaluate differences in rooting characteristics of two bermudagrass cultivars and 19 OSU experimental genotypes when grown under well-watered controlled environment conditions and ii) to identify and select genotypes with higher root to shoot ratios. 'Tahoma 31' and 'TifTuf' were used as industry standards. Grasses were grown in flexible, clear plastic tubes filled with 100% sand. The experimental design was a completely randomized design with four replications of each genotype. Bermudagrass genotypes showed variation in rooting parameters when evaluated for a 120 cm vertical root profile. The genotype OSU1646 had high rate of root depth development (RRDD) followed by OSU18910, OSU1433, and 17-4200-19X13. The genotype OSU1646 had uniform root length density (RLD) in different zones, while OSU1601 accumulated the majority of its roots in the upper 30 cm and had the lowest RLD in the lower zones (60-120 cm). Genotypes such as OSU1646, OSU18910, OSU1433 have superior root morphological traits, higher RLD, better distribution of RLD in the lower soil depths, and fast RRDD and can be

considered to have better drought avoidance. These findings provide useful information for turfgrass breeders and may contribute to the identification and selection of genotypes with superior rooting characteristics and higher root to shoot ratios which would help in pre-screening genotypes with improved rooting characteristics and drought resistance.

#### Introduction

Turfgrasses have significant environmental and social benefits such as reduced erosion, pollution, and heat, as well as provide functioning greenspaces. More than 20 million hectares are occupied by maintained turfgrass in the United States (National Turfgrass Federation, 2017). Furthermore, the Economic Research Service estimates that the turfgrass industry is worth \$40 billion (National Turfgrass Federation, 2017) and therefore demands turfgrasses that match consumer expectations, conserve water by lowering irrigation, and fulfill the needs of existing and future landscapes.

Water availability for irrigating landscapes is becoming increasingly limited and various restrictions are being imposed on turfgrass water use. As a result, water conservation is a major priority for the turfgrass industry, and breeding efforts are focused to develop drought-resistant turfgrasses. Drought resistance is the ability of plants to survive extended moisture stress conditions by mechanisms of escape, tolerance, and avoidance (Beard, 1989; Levitt, 1980). Deep rooting has been proposed as a drought-resistance mechanism in a variety of plants (Levitt, 1980) and used as a selection criterion in drought avoidance breeding programs.

Since roots are hidden in the soil or substrate, root system research necessitates specialized techniques (Judd et al., 2015). The complete expression of genetic potential for rooting is affected by soil physical and chemical properties such as high soil strength, acid soil complex, low soil oxygen, high soil temperature, and salt toxicities (Foy, 1992; Duncan and Shuman, 1993). Greenhouse screening procedures are useful for evaluating a large number of

genotypes in a small amount of time and space. Evaluating root growth using flexible plastic tubes in the greenhouse is an efficient approach for screening plants as it is less costly than conventional field coring and excavation methods and non-destructive root extension tracking over time becomes possible. Further, variability due to localized changes in soil temperature, texture, and moisture level in the field is minimized (Marcum, 1995b).

The presence of a large root system in turfgrass is an essential drought-resistant trait because it allows water absorption from deeper soil depths and wider volumes (Hurd, 1975). Under moisture stress, turfgrass root systems with greater water-conducting capacity and surface area are essential for regular water uptake (Huang et al., 1997). Root characteristics and root-toshoot (R/S) ratio are important factors to consider when selecting drought resistant turfgrass cultivars (Bonos et al., 2004). Generally, plants that have a high R/S can effectively adapt to dry soil conditions. In addition, plants having high R/S generally have reduced transpiration and their root systems can absorb water from relatively larger volumes of soil.

Intraspecific variations in rooting depth have been studied using PVC tubes filled with sand or fritted clay as a rooting medium. Hays et al. (1991) tested seven bermudagrass genotypes and three cultivars, Midiron, Tifgreen, and U3. The genotypes with roots uniformly distributed across the soil profile showed superior drought avoidance by maintaining high visual turf quality. Root volumes were higher for the resistant entries at both shallow and deeper soil depths when the root responses of ten Kentucky bluegrass (*Poa pratensis* L.) genotypes were evaluated under moisture stress (Bonos and Murphy, 1999). Root length density is a measure for estimating the root system's extensiveness (Carrow, 1996a; Miller and McCarty, 1998).

Root length density is the total length of roots per unit of soil volume and is used to calculate the amount of soil volume explored by the root system (Barber, 1971). Huang (2000) found a positive association between RLD and water uptake rate when turfgrass was grown under

non-limited moisture conditions. However, according to Su et al. (2008), higher RLD near the soil surface causes early onset of drought stress due to rapid water depletion. Marcum et al. (1995a) evaluated 25 zoysiagrass genotypes for rooting characteristics in the greenhouse using polyethylene root tubes with a diameter of 2.5 cm and a length of 90 cm. They reported the average MRE to be positively correlated with total root weight, root number, and weight at increasing depths. Drought performance of 11 of the zoysiagrass genotypes included in this greenhouse root study was assessed earlier in a field study at the Texas A&M Research and Extension Center, Dallas (Morton et al., 1991; White et al., 1993). Root depth, root weight, and root number at lower depths in the greenhouse root study showed a positive correlation with the field drought resistance for those 11 zoysiagrass genotypes (Marcum et al., 1995a). The greenhouse and field studies for rooting depth showed a positive association, suggesting that genotypes with shallow rooting depth should be eliminated from breeding programs for better drought resistance.

Lehman and Engelke (1991) reported greenhouse flexible tube rooting experiments to well represent field rooting potential. Acuna (2010) used a similar screening technique to monitor bahiagrass (*Paspalum notatum* Flugge) germplasm's rate of root depth growth. The RRDD was measured as the increase in the depth of the deepest visible root with time. Higher RRDD was linked to more shoot and root mass, indicating early vigor (Acuna et al., 2010). Huot et al. (2020) conducted a greenhouse study to assess the RRDD, rate of root length development (RRLD), photosynthesis, and morphological traits such as root angle, root length (RL), ARD, root volume (RV), root surface area (RSA), and leaf area in eight perennial grass species. Higher RRDD was linked to a narrow root angle since broad root angles encourage horizontal root growth and shallow root depth. Fine roots with a smaller diameter and a higher root length/leaf area ratio were also positively associated with higher RRDD. Emphasis is being placed on identifying and selecting bermudagrass genotypes with extensive root systems.

New bermudagrass cultivars are regularly being developed by the breeders with a major focus on improving drought resistance. These new cultivars may show different drought resistance mechanisms than the older cultivars. Therefore, a study comparing the rooting properties of older and newer bermudagrass cultivars as well as experimental lines may aid in improving our understanding of the underlying drought avoidance mechanisms that lead to increased drought performance. Furthermore, only a few researchers have investigated root to shoot ratio in turfgrasses for screening drought-resistant genotypes, and these studies have primarily focused on cool-season turfgrasses such as tall fescue (Bonos et al., 2004; Karcher et al., 2008). The objective of this study was to evaluate differences in rooting characteristics and root to shoot ratio of 21 bermudagrass genotypes under well-watered non stressed greenhouse conditions.

#### **Materials and Methods**

The study was conducted in the Controlled Environment Research Lab greenhouse facility at Oklahoma State University (OSU) Stillwater, OK. Twenty-one bermudagrass genotypes, including 19 experimental entries developed by the OSU turfgrass breeding program and two standard cultivars, TifTuf and Tahoma 31 were evaluated in this study (Table 15). The rooting characteristics of these bermudagrass genotypes were evaluated using a similar flexible root tube study protocol as in Chapter 2. However, the growing media for this study was sand as it drains easily and can be easily washed off the roots for analysis. Root growth in the calcined clay in study one (Chapter 2) did not completely mimic the typical root growth in native soil or sand as the roots were more fibrous and weak. Sports fields and golf greens are often constructed using sand-based root zones, consisting primarily of medium to coarse sand (0.25–1.00 mm). The highly permeable sands resist compaction and have adequate aeration, infiltration, and percolation. Further, the use of sand helped to maintain uniformity of the growing medium as the

genotypes will be further evaluated for drought performance on sand-based systems in various field trials.

Grasses were planted in clear polyethylene tubes with a diameter of 3.5 cm and a depth of 120 cm. Polyethylene tubes were heat-sealed at the base and four holes were pricked for the drainage. These tubes were than held in place by polyvinyl chloride (PVC) tubes with a diameter of 5.08 cm and a depth of 120 cm. The bottoms of the holding tubes were closed with PVC plugs, and a small hole was drilled for drainage. Holding PVC tubes were opaque and inhibited the light from reaching the root zone. The holding tubes were positioned at 30 angle as described by Qian et al. (1997) on wooden racks to facilitate the visibility of the roots along the wall of the clear tubes for data measurements. The first trial was planted in August 2020 and the second trial was planted in November 2020 and had 65 and 57 days' duration, respectively.

Planting was done by sprigging using 10 sprigs with 4-5 nodes in each growth tube. The growth tubes were placed under a mist system with an automatic irrigation timer. The mist system was set to water 5 minutes every 2 hours for the first two weeks. The mist system was configured to water 5 minutes every 4 hours after a two-week establishment period. A solution of 20-20-20 N-P2O5-K2O (20-8.6-16.6 NPK) general purpose fertilizer (J.R Peters Inc., Allentown, PA) was administered twice a week at 250 mg N L⁻¹. Every week, the grasses were cut to a height of 5 cm. Clippings were gathered in a paper envelope and dried at 80°C for 48 hours, and the shoot dry weight was recorded throughout the trial. Root depth measurements to estimate the maximum root extension, based on the single deepest visible root, were initiated the first week after planting and subsequently recorded every week.

The study was ended when the MRE in one of the tubes reached 120 cm depth. Four sections of clear polyethylene tubes were cut: 0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm and named as zone A, zone B, zone C, and zone D, respectively. Roots were separated by cutting

from the base of the crown and rhizomes. Above-ground shoots were clipped, dried at 80°C for 48 hours, and the final SDW was recorded. Roots were washed to remove the sand, and then collected in plastic bags and stored at 4°C until further analysis. Roots were further cleaned to remove any remaining sand particles and then stained with methyl blue (5 g L⁻¹ water) for a clear image of finer roots. Roots were then scanned and analyzed using WinRHIZO (Regent Instruments Nepean, ON, Canada) to compute total root length (TRL), average root diameter (ARD), root surface area (RSA), and root volume. Following the analysis, the roots were dried for 48 hours at 80°C, weighed separately, and the root dry weight (RDW) was recorded. The root to shoot ratio (R/S) was computed using RDW and SDW. Root length density (RLD, cm root cm⁻³ soil), has been widely used to quantify the extensiveness of the roots and was measured as root length per volume of growing medium. Rate of root depth development (RRDD) was determined as the depth of the deepest visible root as a function of time.

Natural light was supplemented with 1000 W overhead lamps in the greenhouse to provide a 14 hours' photoperiod. Temperature, photosynthetically active radiation (PAR), and relative humidity (RH) were measured at 15-minute intervals using weather station (Mini WatchDog, 2475 Plant growth station, Spectrum Technologies, Plainfield, IL). The average temperatures were 31 $\square$  and 29 $\square$  for trial one and trial two, respectively. Photosynthetically active radiation (PAR) at the canopy level and RH were 482 µmol m⁻² s⁻¹ and 48%, respectively for trial one. For trial two, PAR and RH were 410 µmol m⁻² s⁻¹ and 54%, respectively. For study two, PAR was reduced because of lower solar radiation during November and December.

# Statistical analysis

The experiment was arranged in a completely randomized design with four replications of each genotype and was replicated in time. Data was analyzed using the Statistical Analysis System (SAS) 9.4 software (SAS Institute, Inc., Cary, NC). Analysis of variance (ANOVA) was performed using 'PROC GLIMMIX'. When ANOVA was significant at P = 0.05 level, means separation tests were performed using Tukey's Honest Significant Difference (HSD) at P = 0.05significance level. Regression analysis was used to estimate the rate of root depth development (RRDD) corresponding to the slope of the linear function between time and depth of the deepest visible root.

# **Results and discussion**

# Rate of Root Depth Development (RRDD)

The RRDD measures the rate of daily root growth for each genotype. Bermudagrass genotypes varied for RRDD when grown in unrestricted growing depth (Table 16). These results indicate that genetic variability for RRDD is present in the bermudagrass germplasm and it will be a good opportunity for breeders to select genotypes with rapid RRDD for better water and nutrient uptake from deep soil layers. Further, clear polyethylene columns can be effective in screening bermudagrass germplasm in the greenhouse for genetic potential in RRDD. The daily growth rates ranged from 0.48 to 1.76 cm day⁻¹ (Table 16). The RRDD values obtained in this study were generally lower compared to those observed by Fuentealba et al. (2015) which were 4.02 cm day⁻¹ and 3.46 cm day⁻¹ for common bermudagrass and African bermudagrass, respectively. This was possibly due to the use of plugs with 1.5 cm long pre-established roots and external growing conditions with high irradiance in their research as compared to the use of stolons with no initial roots and greenhouse conditions in our research. Grasses with faster RRDD have a better chance to survive any soil surface dryness immediately after established (Huot et al., 2020).

Among the twenty-one bermudagrass genotypes, the highest daily rate of root depth development was of experimental genotype OSU1646, followed by OSU18910, OSU1433 and the lowest was for OSU1896. Experimental genotype OSU1646 had the maximum root extension

and was the first to reach 120 cm depth. Turfgrass with a higher RRDD can extract moisture from deeper soil layers, which may lower the quantity of supplemental irrigation required to sustain acceptable turfgrass quality (Qian and Engelke, 1999). Under soil drying, a rapid RRDD would delay the onset of water stress under soil drying (Huang, 2000). This suggests that genotypes such as OSU1646, OSU18910, OSU1433 would be better able to avoid water stress during surface drying by accessing profile moisture.

#### **Total Root Length (TRL)**

There was significant difference among genotypes for mean TRL for 0-120 cm depth, zone A (0-30 cm), zone B (30-60 cm), and zone C (60-90 cm) (Table 17). TRL for zone D (90-120 cm) did not show any significant differences among the genotypes. The mean TRL for the entire profile ranged from 1550.2 cm to 4376.4 cm for OSU1601 and OSU1646, respectively. The mean TRL for each zone A-D were 1319.5 cm to 3633.4 cm, 27.6 cm to 731.2 cm, 0 to 193.5 cm, and 0 to 62.4 cm, respectively. All genotypes had greater TRL in zone A, which then dropped significantly for deeper zones.

The roots of all the genotypes reached 30 cm depth. 71 % of the genotypes reached beyond 60 cm depth while only 52 % of the genotypes reached beyond 90 cm depth. OSU1646 was in the top statistical group for mean TRL for all the zones. Other genotypes in the higher statistical group were OSU1156, OSU1433, OSU1893, OSU18910, OSU18718, and Tahoma 31. The percentages of roots distributed within zone A to the total root length (Table 17) indicate differing root distribution abilities among these bermudagrass genotypes. Genotypes such as OSU1646, OSU18910, OSU1433, OSU1101 had the more uniform root distribution between the four zones in comparison with OSU1896, OSU1601, OSU1657 and OSU1892 which distributed > 95 % of total root length in the top zone (0-30 cm) (Table 18). For 0-120 cm depth, Tahoma 31 had significantly higher TRL than TifTuf. For individual zones, Tahoma 31 had significantly higher TRL for zone A, whereas no significant differences occurred among these standards for the lower depths. The results were different from the 2013 NTEP mean root length data where no significant differences occurred among Tahoma 31, TifTuf, Latitude 36, Celebration, and Tifway. In our study, experimental genotypes 2008-4X16, OSU1601, OSU1651, and OSU1657 were in the bottom statistical group for mean TRL for the entire profile. Experimental genotypes OSU1601, OSU1651, OSU1657, OSU1892, OSU1893, and OSU18718 had no roots beyond 60 cm depth. Thus, these genotypes accumulated the majority of their roots in the upper soil profiles, and this would likely lead to rapid water depletion and result in the early onset of water stress.

# **Root Surface Area (RSA)**

There were differences in mean RSA from 0–120 cm (Table 19). RSA for zone B, zone C, and zone D did not show any significant differences among the genotypes. The mean RSA for the entire profile ranged from 139.6 cm to 380.1 cm for OSU1601 and OSU1646, respectively. For zone A, the mean RSA was highest for OSU1646 and Tahoma 31 and was the lowest for OSU1601 (Table 19). Tahoma 31 had a higher RSA than TifTuf at 0-30 cm depth while total RSA for the entire profile had no differences. Cultivars with higher root surface area can absorb water from larger soil areas.

# Average Root Diameter (ARD)

There were no differences in ARD for 0-120 cm depth as well as zone B and D (Table 20). For zone A (0-30 cm depth), mean ARD was highest for 2008-4X16. Other genotypes in higher statistical groups were OSU1156, OSU1873, and OSU1876. Genotype OSU1893 had the lowest ARD for 0-30 cm depth. For zone C, ARD was highest for OSU18910 while many genotypes did not reach this depth. Root diameter of turfgrasses decrease as primary elongation advances (Beard 1973, Taiz and Zeiger 1998). Rimi et al., 2012 reported a negative correlation between root diameter and RLD indicating that root length density is higher when the roots are

finer with lower diameter. Su et al. (2008) reported that although tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort] had lower RLD, up-took more soil water and exhibited better drought performance compared to a hybrid bluegrass due to greater root diameter and root length. Thicker roots can take up a larger amount of water and are better in withstanding soil drying due to the larger capacity of water storage and hydraulic lift for water from deeper soil profile to the surface (Caldwell et al., 1998; Huang 1999).

#### Root Volume (RV)

There were differences in TRV from 0–120 cm (Table 21). Mean RV for zone B, zone C, and zone D did not show any significant differences among the genotypes. The mean RV for the entire profile ranged from 1.147 cm³ to 3.151 cm³ for OSU1601 and OSU1873, respectively. These findings are consistent with those of Doss et al. (1960), who reported that root concentrations of warm-season grasses such as bermudagrass were highest near the surface and reduced as root depth increased. For zone A, mean RV was highest for OSU1156 followed by OSU1873 and was lowest for OSU1601 (Table 21). Total root volume was associated with drought resistance in rice (Zuno-Altoveros et al., 1990).

#### **Root Length Density (RLD)**

Genotypic differences were also found for the distribution of RLD (Table 22). Root length density for each zone A-D ranged from 10.629 to 4.423 cm cm-³, 0.081 to 2.139 cm cm-³, 0 to 0.972 cm cm⁻³, and 0 to 0.972 cm cm-³, respectively. Total RLD ranged from 3.2 to 1.134 for OSU1646 and OSU1601, respectively. These RLDs illustrate that increasing root depth is associated with a decrease in RLD. Both standard cultivars had greater RLDs in the A zone, which then dropped significantly for deeper zones. For zone A, RLD was highest for OSU1646 followed by OSU1893 and Tahoma 31 and the lowest RLD was for 2008-4X16. Other genotypes having lower RLD at shallow depth were OSU1601 and OSU1651. For zone B, RLD was highest

for OSU18718 and lowest for OSU1601, OSU1896, and OSU1657. For zones C and D, RLD was highest for OSU1646 but was not statistically different from OSU18910.

Root length density is the total length of roots per unit of soil volume and has been used to calculate the amount of soil volume explored by the root system (Barber, 1971). Huang (2000) found a positive association between RLD and water uptake rate when turfgrass was grown under non-limited moisture conditions. However, high RLD alone does not translate to good performance during drought. Su et al. (2008) reported that high RLD in the surface soil would result in faster depletion of water and early onset of drought stress. Carrow (1996b), reported that high RLD close to the soil surface was related to greater leaf firing, while high RLD in the 20– 60-cm zone was associated with less leaf firing and wilting in tall fescue cultivars during drought. Higher RLD in the lower profile is therefore desirable. OSU1646 and OSU18910 distributed more RLD in the deeper zones (60–120 cm) than other genotypes. OSU1601 and OSU 1651 had a greater percentage of total RLD within upper profile showing shallow rooting. This is likely a disadvantage for these genotypes during periodic droughts. Improvement of root depth distribution in bermudagrass genotypes, especially at lower zones, could result in gains at delaying drought stress

# Shoot Dry Weight (SDW) and Root Dry Weight (RDW)

There were no significant differences among the genotypes for total SDW (Table 24). However, significant differences occurred among the genotypes for total RDW as well for RDW for zones A, B, and C (Table 23). The genotypes did not differ significantly for RDW for the zone D (90-120 cm). Total RDW was highest for OSU18910 and OSU1156 followed by Tahoma 31, Tahoma 31 and OSU18718 had higher RDW in the upper soil profile (0-30 cm). OSU18910 had higher RDW than all the genotypes for zones B and C.

# **Root Shoot Ratio (R/S)**

There were differences in R/S among the genotypes (Table 25). Root to shoot ratio for the twenty-one bermudagrass genotypes ranged from 0.4012 to 1.459. There were no differences for R/S between TifTuf and Tahoma 31. OSU1156 had the highest R/S while OSU1601 and OSU1876 had lower R/S ratios. All other genotypes did not show any difference in their R/S ratio. The results indicate that that variability among the bermudagrass genotypes for R/S is relatively low and other root traits need to be considered along with the R/S ratio for selecting for drought avoidance. Xu et al., 2015 reported an increase in R/S in response to drought stress. This increase in R/S was associated with the higher proportion of dry matter and soluble sugar in roots, and this occurred via an increase in leaf sucrose-phosphate synthase and root invertase activity, and thus more sucrose was available for transport from leaves to roots. This indicates rate R/S ratio may vary under drought stress relative to when the plant is under non-limited soil moisture conditions.

# Conclusion

The rooting characteristics of bermudagrass genotypes were not evaluated under drought stress in this study. However, a quick RRDD, would delay the start of water stress in the event of soil dryness (Huang, 2000). Rooting patterns under well-watered conditions may not translate to rooting patterns under drought (Huang, 1999); however, the ability to develop deep and extensive root systems under well-watered conditions may ensure access to moisture deeper in the soil profile at the onset of drought. In this study, differences were observed in RRDD, R/S ratio, and root profile characteristics among the bermudagrass genotypes. Experimental genotype OSU1646 was the first to reach 120 cm depth in both runs and had statistically higher RRDD followed by OSU18910 and OSU1433. Genotypes OSU1646 and OSU18718 were in the top statistical group for most of the rooting parameters while OSU1601, OSU1651, and OSU1657 were in a lower statistical group for all the rooting parameters. Tahoma 31 had the higher RLD than TifTuf. A deep, broad root system and a high R/S ratio are two important ways by which

turfgrasses maximize water absorption and avoid drought (Kramer, 1980). R/S may vary for the genotypes under stressed conditions due to differences in carbon allocation to roots and shoots under moisture deficit conditions. Uniform root distribution throughout the soil profile, as shown by genotypes such as OSU1646, might be a beneficial characteristic to identify. While genotypes showing shallow rooting with slow RRDD and poor performance for most of these rooting parameters, such as OSU1601, can be eliminated. Genotypes with desired rooting properties such as high RRDD and uniform rooting distribution, as well as high RLD at deeper soil depths in comparison to commercial standards can be forwarded for further evaluation under drought conditions. Zhou et al., 2014 reported no relationship between drought resistance and RLD before or after drought, or between drought resistance and ARD before drought however it was associated with ARD after drought. Genotypes with superior performance for rooting characteristics can be further evaluated under drought conditions. Root viability during drought needs to be investigated more, since it would add to our knowledge of these genotypes

Table 15. Bermudagrass cultivars and experimental genotypes tested for rooting characteristics

under non-limiting soil moisture conditions.

Bermudagrass genotype ^z	Description
2008-4X16	OSU experimental
OSU1101	OSU experimental
OSU1156 (OSC103)	OSU experimental
OSU1408	OSU experimental
OSU1433	OSU experimental
OSU1601	OSU experimental
OSU1646	OSU experimental
OSU1651	OSU experimental
OSU1657	OSU experimental
17-4200-19X13	OSU experimental
17-4200-19X21	OSU experimental
OSU1873	OSU experimental
OSU1876	OSU experimental
OSU1892	OSU experimental
OSU1893	OSU experimental
OSU1896	OSU experimental
OSU1898	OSU experimental
OSU18910	OSU experimental
OSU18718	OSU experimental
TifTuf	Standard cultivar
Tahoma 31	Standard cultivar

²Genotypes with an OSU prefix are the experimental lines from Oklahoma State University.

Table 16. Comparison of twenty-one bermudagrass genotypes for their rate of root depth

Bermudagrass genotype	RRDD cm day ⁻¹		
2008-4X16	0.78d-h ^y		
OSU1101	1.18a-f		
OSU1156 (OSC103)	1.20а-е		
OSU1408	1.27а-е		
OSU1433	1.40a-c		
OSU1601	0.51gh		
OSU1646	1.76a		
OSU1651	0.53gh		
OSU1657	0.61f-h		
17-4200-19X13	1.35a-d		
17-4200-19X21	1.03b-h		
OSU1873	1.01b-h		
OSU1876	1.14b-f		
OSU1892	0.73e-h		
OSU1893	0.73e-h		
OSU1896	0.48h		
OSU1898	1.22а-е		
OSU18910	1.57ab		
OSU18718	0.88c-h		
TifTuf	0.90c-h		
Tahoma 31	1.09b-g		

development (RRDD)^z (cm day⁻¹).

^zRegression analysis was used to estimate the rate of root depth development (RRDD)

corresponding to the slope of the linear function between time and depth of the deepest visible

root.

^yMeans followed by the same letter within a column are not significantly different ( $P \le 0.05$ ).

Table 17. Root length comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Total Root Length	
genotype			(cm)	(cm)		
2008-4X16	1319.5h	278.6bc	15.4b	0.0	1613.5 j	
OSU1101	2001.7ef	417.4a-c	103.5b	5.9	2528.5 e-i	
OSU1156	2888.8bc	468.6ab	69.6b	1.3	3428.2 bc	
OSU1408	2206.0de	290.6bc	89.9b	29.7	2616.1 d-h	
OSU1433	2788.5bc	372.6а-с	99.2b	17.5	3277.8 b-f	
OSU1601	1522.6gh	27.6c	0.0b	0.0	1550.2 j	
OSU1646	3633.4a	348.2a-c	332.4a	62.4	4376.4 a	
OSU1651	1511.9gh	98.7bc	0.0b	0.0	1610.6 j	
OSU1657	1726.7f-h	69.4c	0.0b	0.0	1796.0 ij	
17-4200-19X13	1974.5ef	309.0bc	51.4b	18.7	2353.6 g-j	
17-4200-19X21	2216.2de	231.9bc	45.0b	2.1	2495.1 f-i	
OSU1873	2049.9ef	329.1bc	99.7b	2.0	2480.6 f-i	
OSU1876	1890.5e-g	199.8bc	86.2b	0.6	2177.1 g-j	
OSU1892	2637.6cd	113.1bc	0.0b	0.0	2750.7 c-h	
OSU1893	3105.6b	238.6bc	0.0b	0.0	3344.1 b-d	
OSU1896	2885.4bc	30.3c	5.9b	0.0	2921.6 b-g	
OSU1898	2038.5ef	334.7а-с	69.1b	1.5	2443.7 g-i	
OSU18910	2927.5bc	480.3ab	193.5ab	31.5	3325.8 b-e	
OSU18718	2620.4cd	731.2a	0.0b	0.0	3658.8 ab	
TifTuf	1895.9e-g	196.7bc	1.7b	0.0	2094.3 h-j	
Tahoma 31	3090.1b	225.1bc	97.9b	0.0	3413.0 b-d	

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05significance level.

Bermudagrass genotype	Percent TRL in zone A		
	%		
2008-4X16	82e-g		
OSU1101	79g		
OSU1156 (OSC103)	85d-g		
OSU1408	85d-g		
OSU1433	85d-g		
OSU1601	98a		
OSU1646	83e-g		
OSU1651	94a-c		
OSU1657	96ab		
17-4200-19X13	84d-g		
17-4200-19X21	89b-f		
OSU1873	84e-g		
OSU1876	87c-g		
OSU1892	96ab		
OSU1893	93a-d		
OSU1896	99a		
OSU1898	84e-g		
OSU18910	79g		
OSU18718	80fg		
TifTuf	89b-е		
Tahoma 31	88b-e		

Table 18. Percentage of total root length of bermudagrass genotypes in the 0-30 cm depth.

Table 19. Root surface area comparisons of bermudagrass genotypes at different zones^z when

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Total surface area
genotype			(cm ² )		
2008-4X16	173.2b-d	42.1	1.8	0.0	217.1 а-с
OSU1101	220.4a-d	33.8	6.6	0.7	261.5 а-с
OSU1156	267.8ab	59.9	5.8	5.0	338.4 ab
OSU1408	252.6а-с	32.0	10.2	5.6	300.4 а-с
OSU1433	203.0b-d	64.2	46.6	2.1	315.8 ab
OSU1601	133.3d	6.3.0	0.0	0.0	139.6 c
OSU1646	308.9a	44.8	21.6	4.8	380.1 a
OSU1651	182.8b-d	8.5	0.0	0.0	191.3 bc
OSU1657	194.9b-d	6.0	0.0	0.0	200.9 bc
17-4200-19X13	253.7а-с	33.2	5.2	2.0	294.1 а-с
17-4200-19X21	223.6a-d	42.8	2.9	0.3	269.6 а-с
OSU1873	251.7а-с	45.4	9.4	0.1	306.7 а-с
OSU1876	158.7cd	28.4	7.1	0.0	194.3 bc
OSU1892	265.1ab	10.7	0.0	0.0	275.8 а-с
OSU1893	274.2ab	24.7	0.0	0.0	298.9 а-с
OSU1896	245.9а-с	6.6	0.5	0.0	253 а-с
OSU1898	206.6a-d	35.5	7.0	0.3	249.3 а-с
OSU18910	244.7а-с	52.7	25.4	12.0	345.7 ab
OSU18718	255.7а-с	65.8	0.0	0.0	310.4 а-с
TIFTUF	185.8b-d	31.9	0.1	0.0	217.8 а-с
TAHOMA 31	307.3a	32.3	5.0	0.0	344.6 ab

grown in polyethylene growth tubes^y.

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05significance level.

Table 20. Root diameter comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Average root diameter
genotype			(mm)		
2008-4X16	0.359a	0.269	0.080a-c	0.000	0.314
OSU1101	0.332а-с	0.278	0.133a-c	0.089	0.266
OSU1156	0.340ab	0.276	0.114a-c	0.074	0.284
OSU1408	0.331a-d	0.270	0.163a-c	0.054	0.285
OSU1433	0.331a-c	0.287	0.257ab	0.135	0.310
OSU1601	0.251hi	0.139	0.000c	0.000	0.195
OSU1646	0.271g-i	0.229	0.195a-c	0.214	0.235
OSU1651	0.276d-i	0.202	0.000c	0.000	0.240
OSU1657	0.291b-h	0.140	0.000c	0.000	0.215
17-4200-19X13	0.323a-f	0.302	0.199a-c	0.101	0.304
17-4200-19X21	0.298b-h	0.279	0.055bc	0.059	0.290
OSU1873	0.341ab	0.364	0.038bc	0.023	0.344
OSU1876	0.343ab	0.358	0.165a-c	0.022	0.314
OSU1892	0.273e-i	0.207	0.000c	0.000	0.240
OSU1893	0.233i	0.216	0.000c	0.000	0.224
OSU1896	0.285c-i	0.110	0.032c	0.000	0.197
OSU1898	0.311a-g	0.228	0.200a-c	0.028	0.258
OSU18910	0.261g-i	0.290	0.285a	0.098	0.286
OSU18718	0.293b-h	0.203	0.000c	0.000	0.232
TIFTUF	0.327а-е	0.326	0.067a-c	0.000	0.321
TAHOMA 31	0.301b-h	0.223	0.168a-c	0.000	0.241

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05significance level.

Table 21. Root volume comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Total root volume
genotype			(cm ³ )		
2008-4X16	1.598d-f	0.332	0.017	0.000	1.947 a-c
OSU1101	2.010а-е	0.270	0.034	0.007	2.320 a-c
OSU1156	2.581a	0.347	0.039	0.030	2.997 ab
OSU1408	2.199a-d	0.243	0.113	0.025	2.580 a-c
OSU1433	1.985a-e	0.684	0.036	0.022	2.728 ab
OSU1601	1.069f	0.078	0.000	0.000	1.147 c
OSU1646	2.043а-е	0.663	0.098	0.049	2.853 ab
OSU1651	1.475ef	0.064	0.000	0.000	1.537 bc
OSU1657	1.530ef	0.042	0.000	0.000	1.571 bc
17-4200-19X13	2.052а-е	0.291	0.042	0.018	2.403 a-c
17-4200-19X21	1.888b-e	0.192	0.016	0.004	2.099 a-c
OSU1873	2.336ab	0.746	0.071	0.001	3.151 a
OSU1876	1.627c-f	0.145	0.047	0.000	1.82 a-c
OSU1892	1.765b-e	0.051	0.000	0.000	1.816 a-c
OSU1893	2.242a-d	0.207	0.000	0.000	2.448 a-c
OSU1896	1.944a-e	0.040	0.003	0.000	1.987 a-c
OSU1898	1.619c-f	0.231	0.061	0.005	1.915 a-c
OSU18910	2.259а-с	0.251	0.757	0.078	3.094 a
OSU18718	2.006а-е	0.267	0.000	0.000	2.525 а-с
TifTuf	1.634c-f	0.104	0.001	0.000	1.739 a-c
Tahoma 31	2.250а-с	0.078	0.025	0.000	2.353 а-с

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05significance level.

Table 22. Root length density comparisons of bermudagrass genotypes at different zones^z when

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Root length density
genotype			$(\mathrm{cm}  \mathrm{cm}^{-3})$		
2008-4X16	3.860h	0.815bc	0.045b	0.045b	1.180 j
OSU1101	5.855ef	1.221a-c	0.303b	0.303b	1.849 e-i
OSU1156	8.451bc	1.371ab	0.204b	0.204b	2.507 bc
OSU1408	6.453de	0.850bc	0.263b	0.263b	1.913 e-i
OSU1433	8.157bc	1.090a-c	0.290b	0.290b	2.397 c-f
OSU1601	4.454gh	0.081c	0.000b	0.000b	1.134 j
OSU1646	10.629a	1.019a-c	0.972a	0.972a	3.200 a
OSU1651	4.423gh	0.289bc	0.000b	0.000b	1.178 j
OSU1657	5.051f-h	0.203c	0.000b	0.000b	1.313 ij
17-4200-19X13	5.776ef	0.904bc	0.150b	0.150b	1.721 g-i
17-4200-19X21	6.483de	0.678bc	0.132b	0.132b	1.825 f-i
OSU1873	5.996ef	0.963bc	0.292b	0.292b	1.814 f-i
OSU1876	5.530e-g	0.584bc	0.252b	0.252b	1.592 g-j
OSU1892	7.716cd	0.331bc	0.000b	0.000b	2.012 c-h
OSU1893	9.085b	0.698bc	0.000b	0.000b	2.446 b-d
OSU1896	8.441bc	0.089c	0.017b	0.017b	2.137 b-g
OSU1898	5.963ef	0.979a-c	0.202b	0.202b	1.787 g-i
OSU18910	8.564bc	1.405ab	0.566ab	0.566ab	2.432 b-e
OSU18718	7.665cd	2.139a	0.000b	0.000b	2.676 ab
TifTuf	5.546e-g	0.575bc	0.005b	0.005b	1.531 h-j
Tahoma 31	9.039b	0.658bc	0.286b	0.286b	2.496 b-d

grown in polyethylene growth tubes^y.

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05 significance level. Table 23. Root dry weight comparisons of bermudagrass genotypes at different zones^z when

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Total root dry weight
genotype			$(cm^3)$		
2008-4X16	0.460d-f	0.023b	0.001b	0.000	0.485 b-e
OSU1101	0.584b-e	0.045b	0.002b	0.001	0.633 b-d
OSU1156	1.022a-d	0.028b	0.003b	0.000	1.052 a
OSU1408	0.751a-d	0.023b	0.003b	0.009	0.785 a-c
OSU1433	0.769a-c	0.030b	0.003b	0.001	0.802 a-c
OSU1601	0.164f	0.009b	0.000b	0.000	0.173 e
OSU1646	0.617b-e	0.035b	0.007b	0.004	0.663 b-d
OSU1651	0.458d-f	0.006b	0.000b	0.000	0.464 b-e
OSU1657	0.561b-e	0.004b	0.000b	0.000	0.564 b-d
17-4200-19X13	0.685b-d	0.029b	0.003b	0.002	0.719 a-d
17-4200-19X21	0.462de	0.025b	0.002b	0.000	0.489 b-e
OSU1873	0.515с-е	0.033b	0.001b	0.000	0.549 b-d
OSU1876	0.378ef	0.023b	0.002b	0.000	0.403 de
OSU1892	0.502с-е	0.005b	0.000b	0.000	0.507 b-e
OSU1893	0.601c-e	0.029b	0.000b	0.000	0.630 b-d
OSU1896	0.599b-e	0.006b	0.000b	0.000	0.605 b-d
OSU1898	0.569b-e	0.032b	0.003b	0.000	0.604 b-d
OSU18910	0.459d-f	0.197a	0.046a	0.012	1.075 a
OSU18718	0.820ab	0.029b	0.000b	0.000	0.488 с-е
TifTuf	0.559b-e	0.025b	0.000b	0.000	0.584 b-d
Tahoma 31	0.820ab	0.021b	0.003b	0.000	0.844 ab

grown in polyethylene growth tubes^y.

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^xDry weights were recorded after drying at 80°C for 48 hours.

^wMeans followed by same letters within each column are not significantly different at the P =

Bermudagrass genotype	Shoot dry weight
	(g)
2008-4X16	0.4834
OSU1101	1.074
OSU1156 (OSC103)	0.7447
OSU1408	0.836
OSU1433	0.755
OSU1601	0.4554
OSU1646	0.8303
OSU1651	0.6724
OSU1657	0.9555
17-4200-19X13	0.6724
17-4200-19X21	0.5906
OSU1873	0.7968
OSU1876	0.7698
OSU1892	0.5551
OSU1893	0.7739
OSU1896	0.5304
OSU1898	0.5758
OSU18910	0.9335
OSU18718	0.5078
TifTuf	0.6709
Tahoma 31	0.8315

Table 24. Shoot dry weight of 21 bermudagrass genotypes grown in polyethylene growth tubes^z.

^zGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^yDry weights were recorded after drying at 80°C for 48 hours.

^xMeans followed by same letters within each column are not significantly different at the P = 0.05

Table 25. Root to shoot ratio of ten bermudagrass genotypes grown in polyethylene growth tubes^z.

Bermudagrass genotype	Root to shoot ratio
2008-4X16	1.065ab
OSU1101	0.6031ab
OSU1156 (OSC103)	1.4593a
OSU1408	1.0303ab
OSU1433	1.0771ab
OSU1601	0.4012b
OSU1646	0.8105ab
OSU1651	0.7209ab
OSU1657	0.6111ab
17-4200-19X13	1.178ab
17-4200-19X21	1.0023ab
OSU1873	0.6953ab
OSU1876	0.5342b
OSU1892	0.9445ab
OSU1893	0.8454ab
OSU1896	1.2879ab
OSU1898	1.1198ab
OSU18910	1.2331ab
OSU18718	1.0146ab
TifTuf	0.8907ab
Tahoma 31	1.0454ab

^zGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^yDry weights were recorded after drying at 80°C for 48 hours.

^xMeans followed by same letters within each column are not significantly different at the P = 0.05

# CHAPTER IV

# EVALUATION OF EIGHT BERMUDAGRASS GENOTYPES FOR ROOTING CHARACTERISTICS

## Abstract

Identifying drought-resistant cultivars for commercial use is a crucial step in reducing turfgrass water demand. The presence of an extensive root system enhances drought resistance by assessing water from deeper soil layers. A controlled environment greenhouse study was conducted at Oklahoma State University, Stillwater, OK, USA, i) to evaluate differences in rooting characteristics of eight bermudagrass cultivars and experimental genotypes when grown under non-limiting soil moisture conditions and ii) to identify and select genotypes with higher root to shoot ratios. For this study, four industry standards 'Latitude 36,' 'Tahoma 31,' 'TifTuf,' and 'Tifway' and four experimental genotypes were used. The experiment was arranged as a completely randomized design. Grasses were grown in clear polyethylene tubes plastic filled with Profile's field & fairway natural®. Grass shoot dry weight and root traits (rate of root depth development [RRDD], total root length [TRL], root surface area [RSA], average root diameter [ARD], root volume [RV], root dry weight [RDW], root length density [RLD], and root to shoot ratio [R/S] were examined in two repeated trials. Bermudagrass genotypes showed differences in rooting traits when examined for a 120 cm vertical root profile. The genotypes with the highest RRDD were OSU2094 and TifB16117 while the genotype OSU1682 had the lowest RRDD.

The genotypes with the highest RRDD were OSU2094 and TifB16117 and genotypes with the lowest RRDD were OSU1682. The genotype OSU2094 and TifB16117 showed uniform RLD across all zones, whereas OSU1862 accumulated the majority of its roots in the upper 30 cm. These findings assist in the selection of genotypes with superior rooting traits and greater root to shoot ratios, that would aid in improving drought resistance.

# Introduction

Bermudagrasses (*Cynodon* spp.) is one of the most widely used warm-season turfgrass. It is one of the most drought resistant turfgrass species and outperforms other warm season turfgrasses such as zoysiagrass (*Zoysia* spp.), St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze), centipedegrass [*Eremochloa ophiuroides* (Munro.) Hack.] (Carrow, 1996; Huang et al.,1997; Qian and Fry, 1997). Bermudagrass also has excellent turfgrass quality, high salinity tolerance, high heat tolerance, good disease resistance, rapid establishment rate, and faster recuperative rate from damage (Taliaferro et al., 2004) and is therefore widely used in lawns, parks, athletic fields, golf courses, cemeteries, and along roadsides (Beard, 1973).

Water availability for irrigating landscapes is becoming increasingly limited and various restrictions are being imposed on turfgrass water use. As a result, water conservation is a major priority for the turfgrass industry, and breeding efforts are focused to develop drought-resistant turfgrasses. Drought resistance is the ability of plants to survive extended moisture stress conditions by mechanisms of escape, tolerance, and avoidance (Beard, 1989; Levitt, 1980). Deep rooting has been proposed as a drought-resistance mechanism in a variety of plants (Levitt, 1980) and used as a selection criterion in drought avoidance breeding programs. Since roots are hidden in the soil or substrate, root system research necessitates specialized techniques (Judd et al., 2015). Evaluating root growth using flexible plastic tubes in the greenhouse is an efficient approach for screening plants as it is less costly than conventional field coring and excavation

methods and non-destructive root extension tracking over time becomes possible. Further, variability due to localized changes in field soil temperature, texture, and water content is minimized (Marcum, 1995b).

The presence of a large root system in turfgrass is an essential drought-resistant trait because it allows water absorption from deeper soil depths (Hurd, 1975). Under moisture stress, turfgrass root systems with greater water-conducting capacity and surface area are essential for regular water uptake (Huang et al., 1997). Root characteristics and root-to-shoot (R/S) ratio are important factors to consider when selecting drought resistant turfgrass cultivars (Bonos et al., 2004). Intraspecific variations in rooting depth have been studied using PVC tubes filled with sand or fritted clay as a rooting medium. Hays et al. (1991) tested seven bermudagrass genotypes and three cultivars, Midiron, Tifgreen, and U3 and drought avoidance was better in genotypes with roots evenly distributed across the soil profile as the visual quality was maintained. Root volumes were higher for the resistant entries at both shallow and deeper soil depths in a study evaluating the root responses of ten Kentucky bluegrass (Poa pratensis L.) genotypes under moisture stress (Bonos and Murphy, 1999). Root length density is a measure for estimating the root system's extensiveness (Carrow, 1996a; Miller and McCarty, 1998). Root length density is the total length of roots per unit of soil volume and is used to calculate the amount of soil volume explored by the root system (Barber, 1971). Huang (2000) found a positive association between RLD and water uptake rate when turfgrass was grown under non-limited moisture conditions. However, according to Su et al. (2008), higher RLD near the soil surface causes early onset of drought stress due to rapid water depletion.

Marcum et al. (1995a) used polyethylene root tubes with a diameter of 2.5 cm and a length of 90 cm to test 25 zoysiagrass genotypes in the greenhouse for rooting characteristics. Researchers found average MRE to be positively associated with total root weight and root number at rising depths. Drought tolerance of 11 zoysiagrass genotypes studied in this

greenhouse root study was assessed in a field study at the Texas A&M Research and Extension Center in Dallas (Morton et al., 1991; White et al., 1993). The greenhouse and field studies for rooting depth showed a positive association, suggesting that genotypes with deeper rooting depth should be selected from breeding programs for better drought resistance.

Flexible tube rooting experiments in the greenhouse have been reported to represent field rooting potential by Lehman and Engelke (1991). Acuna (2010) used a similar screening technique to monitor bahiagrass (*Paspalum notatum* Flugge) germplasm's rate of root depth growth. The RRDD was measured as the time-dependent increase in the depth of the deepest visible root. Higher RRDD was linked to more shoot and root mass, indicating early vigor (Acuna et al., 2010). Huot et al. (2020) conducted a greenhouse study to assess the RRDD, rate of root length growth (RRLD), photosynthesis, and morphological traits such as root angle, root length (RL), ARD, root volume (RV), root surface area (RSA), and Leaf area in eight perennial grass species. Higher RRDD was linked to a narrow root angle since broad root angles encourage horizontal root growth and shallow root depth. Fine roots with a smaller diameter and a higher root length/leaf area ratio were also positively associated with higher RRDD. Emphasis is being placed on identifying and selecting bermudagrass genotypes with extensive root systems. The objective of this study was to evaluate differences in rooting characteristics of 21 bermudagrass genotypes under well-watered non stressed greenhouse conditions.

#### **Materials and Methods**

The study was conducted in the Controlled Environment Research Lab greenhouse facility at Oklahoma State University (OSU) Stillwater, OK. Eight bermudagrass genotypes included four standard cultivars 'Latitude 36', 'Tahoma 31', 'TifTuf', and 'Tifway' and four experimental genotypes (Table 26). 'Latitude 36' bermudagrass is a triploid hybrid developed from a cross of *Cynodon dactylon* (2n=4x=36) with *C. transvaalensis* (2n=2x=18) (Wu et al.,

2014) and was released in 2017. Tahoma 31 is an interspecific triploid hybrid (2n=3x=27) developed from a cross of *Cynodon dactylon* var. *dactylon* accession A12268  $(2n=4x=36) \times C$ . *transvaalensis* OSU selection '2747' (2n=2x=18) (Wu et al., 2020) and was released in 2017.

Experimental genotype TifB16117 was from the United States Department of Agriculture (USDA) 2020 Specialty Crops Research Initiative (SCRI) and developed by UGA turfgrass breeding program. Experimental genotypes OSU1682, OSU2082, and OSU2094 were developed by the OSU turfgrass breeding program. Turfgrass breeders, extension workers, and researchers in the Southern United States are collaborating to produce new turfgrass genotypes with increased drought and salinity tolerance through USDA Specialty Crop Research Initiative (SCRI) funding. New bermudagrass experimental genotypes developed by UGA and OSU turfgrass breeding programs are tested at multiple locations in the Southern US. The information gained from these trials will help turfgrass developers to take a decision whether the experimental bermudagrass lines tested have the potential to significantly reduce water use in future landscapes and should be further pursued for possible commercial release or not.

The rooting characteristics of these bermudagrass genotypes were evaluated using a similar flexible root tube study protocol as in Chapter 2. However, the growing media for this study was Profile's field & fairway natural® (Profile Products, LLC, Buffalo Grove, IL). It is a heat-treated montmorillonite clay mineral, contains 3-5% crystalline silica, and has a bulk density of 0.56 g cm⁻³. It is chemically inert and can be easily washed away from roots. Before planting, the growing tubes were saturated with water.

Grasses were planted in clear polyethylene tubes with a diameter of 3.5 cm and a depth of 120 cm. Polyethylene tubes were placed in PVC pipes with a diameter of 5.08 cm and a depth of 120 cm to maintain the roots in dark conditions similar to inside soil. The bottoms of the holding tubes were closed with PVC plugs, and a small hole was drilled for drainage. Holding tubes were

prepared and positioned at 30 angle as described by Qian et al. (1997) on wooden racks to facilitate the visibility of the roots along the wall of the clear tubes for data measurements. The first trial was planted in February 2021 and the second trial was planted in mid-March, 2021 and had 54 and 51 days' duration, respectively. Planting was done by sprigging using 10 sprigs with 4-5 nodes in each growth tube. The growth tubes were placed under a mist system with an automatic irrigation timer. The mist system was set to water 5 minutes every 2 hours for the first two weeks. The mist system was configured to water 5 minutes every 4 hours after a two-week setup period. A solution of 20-20-20 N-P2O5-K2O (20-8.6-16.6 NPK) general purpose fertilizer (J.R Peters Inc., Allentown, PA) was administered twice a week at 250 mg N ^{L-1}.

The data for the average greenhouse conditions such as air temperature, photosynthetically active radiation (PAR,  $\mu$ mol m⁻² s⁻¹), and relative humidity (RH) was recorded every 15 minutes using WatchDog Mini Station 2475 data logger (Spectrum Technologies, Plainfield, IL). Natural light was supplemented with 1000 W overhead lamps in the greenhouse to provide a 14 hours' photoperiod. The average temperatures were 30 and 31 for trial one and trial two, respectively. Photosynthetically active radiation (PAR) at the canopy level and RH were 558 µmol m⁻² s⁻¹ and 30 %, respectively for trial one. For trial two, PAR and RH were 615 µmol m⁻² s⁻¹ and 48 %, respectively.

The grasses were clipped to a height of 5 cm every week. Clippings were gathered in a paper envelope and dried at 80°C for 48 hours, and the shoot dry weight was recorded throughout the trial. Root depth measurements to estimate the maximum root extension, based on the single deepest visible root, were initiated the first week after planting and subsequently recorded every week by marking the outside of the clear tube for the duration of the study.

When the MRE of one of the tubes reached 120 cm depth, the study was ended. Four sections of clear polyethylene tubes were cut: 0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm and

named as zone A, zone B, zone C, and zone D, respectively. Roots were separated by cutting from the base of the crown and rhizomes. Above-ground shoots were clipped, dried at 80°C for 48 hours, and the final SDW was recorded. Roots were collected in plastic bags and stored at 4°C until further analysis after calcined clay was removed from the roots. Roots were cleaned to remove sand, and then stained with methyl blue (5 g L⁻¹ water) for a clear image of finer roots. To compute total root length (TDR), average root diameter (ARD), root surface area (RSA), and root volume, roots were scanned and analyzed using WinRHIZO (Regent Instruments Nepean, ON, Canada).

Following the analysis, the roots were dried for 48 hours at 80°C, weighed separately, and the root dry weight (RDW) was recorded. The root to shoot ratio (R/S) was computed using RDW and SDW. Root length density (RLD, cm root cm⁻³ soil), has been widely used to quantify the extensiveness of the roots. The root length density was calculated by dividing the root length data obtained from WinRHIZO by the volume of soil for each zone (RLD). The volume of each zone was calculated based on the diameter and length of each zone. Rate of root depth development (RRDD) was determined as the slope of maximum root extension with time.

# Statistical analysis

The experiment was arranged in a completely randomized design with four replications of each entry and replicated in time. Data was analyzed using the Statistical Analysis System (SAS) 9.4 software (SAS Institute, Inc., Cary, NC). Analysis of variance (ANOVA) was performed using 'PROC GLIMMIX'. When ANOVA was significant at P = 0.05 level, means separation tests were performed using Tukey's Honest Significant Difference (HSD) at P = 0.05significance level. Regression analysis was used to estimate the rate of root depth development (RRDD) corresponding to the slope of the linear function between observations of the depth of the deepest visible root and time.

#### **Results and discussion**

#### **Rate of Root Depth Development (RRDD)**

The RRDD measures the rate of daily root growth for each genotype. According to Kramer and Boyer (1995), grasses have an average RRDD of 0.1 cm day⁻¹ to 1.2 cm day⁻¹. The average RRDD in our study ranged from 0.42 to 2.19 cm day⁻¹ (Table 27). Environmental factors such as temperature, soil accessible water, and nutrient concentration in the growing medium might influence the expression of genetic potential in the field. Among the twenty-one bermudagrass genotypes, OSU2094 was the first to reach 120 cm depth and had maximum root extension followed by TifB16117. The highest daily rate of root depth development was of experimental genotype OSU2094 but not statistically different fromTifB16117 and the lowest RRDD was for OSU1682. Tahoma 31 had the highest RRDD among the standard cultivars. No significant differences were observed among TifTuf and Tifway for RRDD.

# **Total Root Length (TRL)**

There were significant differences among genotypes for mean TRL for 0-120 cm depth as well as for zones A, B, C, and D (Table 28). The mean TRL for the entire profile ranged from 1140.2 cm to 5654.6 cm for OSU1682 and OSU2094, respectively. The mean TRL for each zone A-D were 1140.2 cm to 3450.0 cm, 0 cm to 1648.5 cm, 0 to 391.5 cm, and 0 to 163.9 cm, respectively. All genotypes had greater TRL in zone A, which then dropped significantly for deeper zones. The roots of all the genotypes reached 30 cm depth. 50 % of the genotypes reached beyond 60 cm depth while only 38 % of the genotypes reached beyond 90 cm depth. OSU2094 was in the top statistical group for mean TRL for all the zones. Among the commercial standards, Tahoma 31 had the highest TRL while there were no significant differences among Latitude 36, TifTuf, and Tifway. These results were in accordance with those previously reported by Amgain (2014) when bermudagrass genotypes were grown in similar polyethylene tubes under non-

limited moisture conditions. Katuwal et al, in 2020 also reported no difference in root depth among TifTuf and Tifway when tested for rooting characteristics and drought performance in a controlled environment growth chamber trial. Yurisic (2016) also found no significant difference in root length among TifTuf, Tifway, and Latitude 36 when grown in 45 cm deep polyvinyl chloride pots, but the grasses were subjected to 28 days drought treatment. Experimental genotypes OSU1682 was in the bottom statistical group for mean TRL for the entire profile and had no roots beyond 30 cm depth. Thus, this genotype accumulated the majority of its roots in the upper soil profiles, and this would likely lead to rapid water depletion and result in the early onset of water stress. Experimental genotype OSU2082, Tifway, and TifTuf had no roots beyond 60 cm depth.

# **Root Surface Area (RSA)**

There were differences in mean RSA among the genotypes from 0–120 cm (Table 29) as well as for all the individual zones. The mean RSA for the entire profile ranged from 99.1 cm² to 527.5 cm² for OSU1682 and OSU2094, respectively. For each zone, the mean RSA was highest for OSU2094. Cultivars with higher root surface area can absorb water from larger soil areas. There was no difference in total RSA of TifTuf and Tifway which was in accordance with previously reported by Amgain (2014) and in chapter 2.

## Average Root Diameter (ARD)

There were differences in ARD for 0-120 cm depth as well as for all zones A to D (Table 30). For all the zones, mean ARD was highest for experimental genotype TIFB16117. Among the standards, TifTuf had the highest ARD while Tifway had the lowest ARD. These results were in agreement with those reported by Katuwal et al (2020) where TifTuf had higher ARD than Tifway when evaluated in 50 cm deep pots under drought conditions. Among the standards, TifTuf did to 30 cm depth while ARD of Latitude 36, Tahoma 31, and TifTuf did

not differ at 0-30 cm depth. Genotype OSU1682 had the lowest ARD for 0-30 cm depth. Thicker roots can take up a larger amount of water and are better in withstanding soil drying due to larger capacity of water storage and hydraulic lift for water from deeper soil profile to the surface (Caldwell et al., 1998; Huang 1999).

#### Root Volume (RV)

There were differences in TRV from 0 - 120 cm as well as for all the zones (Table 31). The mean RV for the entire profile ranged from 0.787 cm³ to 4.646 cm³ (Table 31). Total root volume was highest for TifB16117 and was statistically similar to OSU2094 followed by Tahoma 31. Experimental genotype OSU1682 was in the bottom statistical group. TifTuf and Tifway were in the same statistical group for the root volume distribution in individual sections as well as for the TRV for 0-120 cm depth. Bonos and Murphy (1999) also evaluated the root responses of Kentucky bluegrass genotypes and observed root volumes to be higher for drought resistant entries as compared to the sensitive entries at both shallow and deeper soil depths. This indicates higher RV of experimental genotypes TifB16117 and OSU2094 could contribute to better drought resistance.

#### **Root Length Density (RLD)**

Genotypic differences among the genotypes were also found for the distribution of RLD (Table 32). Root length density for each zone A-D ranged from 3.335 to 10.091, 0 to 4.857 cm, 0 to 1.145 cm, and 0 to 0.229 cm, respectively. Total RLD ranged from 0.834 to 4.135 for OSU1682 and OSU2094, respectively. All genotypes had greater RLDs in the A zone, which then dropped significantly for deeper zones. These RLDs illustrate that increasing root depth is associated with decrease in RLD. These results agree with those from Qian et al. (1997) who reported that the highest RLD for all species (bermudagrass, buffalograss, zoysiagrass, and tall fescue) was in the upper 30 cm of roots.

For zone A, RLD was highest for OSU2094 followed by Tahoma 31 and Latitude 36 and the lowest RLD was for OSU1682. Root length density is the total length of roots per unit of soil volume and has been used to calculate the amount of soil volume explored by the root system (Barber, 1971). Huang (2000) found a positive association between RLD and water uptake rate when turfgrass was grown under non-limited moisture conditions. However, high RLD alone does not translate to good performance during drought. Su et al. (2008) reported that high RLD in the surface soil would result in faster depletion of water and early onset of drought stress. Carrow (1996b), reported that high RLD close to the soil surface was related to greater leaf firing, while high RLD in the 20–60 cm zone was associated with less leaf firing and wilting in tall fescue cultivars during drought. Higher RLD in the lower profile is therefore desirable. Other studies suggest that high RLD in the upper soil depths may be a desirable trait when combined with an even distribution of roots (Christensen et al., 2017). Among the experimental genotypes OSU2094 and TifB16117 distributed more RLD in the deeper zones (60–120 cm) than other genotypes. OSU1682 had a greater percentage of total RLD within the upper profile. This is likely a disadvantage for these genotypes during periodic droughts.

Improvement of root depth distribution in bermudagrass genotypes, especially at lower zones, could result in gains at delaying drought stress. High RLD near the soil surface has been classified as a negative drought-responsive trait (Su et al., 2008). Grasses with this characteristic tend to use water faster and experienced drought stress sooner (Su et al., 2008). Other studies suggest that high RLD in the upper soil depths may be a desirable trait when combined with an even distribution of roots (Christensen et al., 2017). In this study, genotypes OSU2094, TifB16117, and Tahoma 31 had these characteristics with high RLD in the 0–30 cm soil depth and even distribution of roots throughout the 120 cm deep profile.

## Shoot Dry Weight (SDW) and Root Dry Weight (RDW)

There were significant differences among the genotypes for total SDW (Table 34). Among the experimental genotypes, SDW was maximum for OSU2094 and lowest for OSU1682. Significant differences occurred among the genotypes for total RDW as well for RDW for zone A, B and C (Table 33). The genotypes did not differ significantly for RDW for the zone D (90-120 cm). Total RDW was highest for OSU2094 followed by Tahoma 31. TifTuf had higher total RDW than Latitude 36 and Tifway.

#### **Root Shoot Ratio (R/S)**

There were differences in R/S among the genotypes (Table 35). Root shoot ratio for the eight bermudagrass genotypes ranged from 0236 to 0.633. TifTuf had the highest R/S while OSU1682 had the lowest R/S ratio. Cultivars with higher root surface area can absorb water from larger soil areas. The R/S ratio may be an important characteristic for selecting drought resistance cultivars. In turfgrass, high R/S ratio is desirable (Beard, 1973). A high R/S ratio is very effective means for plants to adapt to dry conditions. Plants having a high R/S ratio transpiration surface is reduced while root systems can absorb water from large volumes of soil. Karcher et al. (2008) reported, that tall fescues selected for higher R/S performed better in the field and were first to recover from drought stress after re-watering as compared to lower root to shoot ratio selections.

## Summary

Rooting patterns under well-watered conditions may not translate to rooting patterns under drought (Huang, 1999); however, the ability to develop deep and extensive root systems under well-watered conditions may ensure access to moisture deeper in the soil profile at the onset of drought. In this study, differences were observed in RRDD and root profile characteristics among the bermudagrass genotypes.

OSU2094 had consistently higher TRL, RSA, RV, RDW, and RLD in the entire profile and sub profile whereas OSU1682 had consistently lower TRL, RSA, ARD, RV, RDW and RLD in the entire profile and sub profiles. However, there was variability in rankings of other cultivars. Experimental genotype OSU2094 was the first to reach 120 cm depth in both runs and had statistically higher RRDD. OSU2094, TifB16117, and Tahoma 31 had roots at 90-120 cm profile which suggests that it can absorb water form deep soil profile during drought. Cultivars having greater TRL at lower soil profile can absorb water from deep soil profile. Among the experimental genotypes, OSU2094 and TifB16117 were in top statistical group for most of the rooting parameters while OSU1682 was the worst performing followed by OSU2082. Tahoma 31 had highest RLD among the standards while no differences occurred among the other three standards for total RLD. Drought resistance is believed to be enhanced by these rooting characteristics. Uniform root distribution throughout the soil profile, as shown by genotypes such as OSU2094 and TifB16117, might be a beneficial characteristic to identify. While genotypes showing shallow rooting with slow RRDD and poor performance for most of these rooting parameters, such as OSU1682, can be eliminated. Genotypes with desired rooting properties such high RRDD and uniform rooting distribution, as well as high RLD at deeper soil depths in comparison to commercial standards can be forwarded for further evaluation under drought conditions.

Table 26. Eight bermudagrass cultivars and experimental genotypes^z tested for rooting characteristics under non-limiting soil moisture conditions.

Bermudagrass genotype	Description
Latitude 36	Standard cultivar
OSU1682	OSU experimental
OSU2082	OSU experimental
OSU2094	OSU experimental
Tahoma 31	Standard cultivar
TifB16117	UGA experimental
TifTuf	Standard cultivar
Tifway	Standard cultivar

²Experimental genotypes with an OSU prefix are the experimental lines from Oklahoma State University and UGA prefix is from University of Georgia.

Table 27. Eight bermudagrass cultivars and experimental genotypes tested for rate of root depth

development (RRDD) under non-limiting soil moisture conditions.

Bermudagrass genotype	RRDD
	cm day ⁻¹
Latitude 36	1.15c
OSU1682	0.42e
OSU2082	0.78d
OSU2094	2.19a
Tahoma 31	1.42b
TifB16117	2.06a
TifTuf	0.77d
Tifway	0.84d

^zRegression analysis was used to estimate the rate of root depth development (RRDD)

corresponding to the slope of the linear function between time and depth of the deepest visible

root

^yMeans followed by the same letter within a column are not significantly different ( $P \le 0.05$ ).

Table 28. Root length comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Total root length
genotype			(cm)		
Latitude 36	2617.0bc	461.7c	120.7bc	0.0c	3199.3d
OSU1682	1140.2e	0.0d	0.0c	0.0c	1140.2f
OSU2082	2008.7d	488.3c	0.0c	0.0c	2491.0e
OSU2094	3450.0a	1649.5a	391.5a	163.9a	5654.6a
Tahoma 31	2963.9b	1067.1b	326.0a	78.2b	4435.0b
TifB16117	2595.5c	930.0b	209.7bc	67.5b	3802.6c
TifTuf	2540.0c	208.6d	0.0c	0.0c	2748.6e
Tifway	2545.8c	156.2d	0.0c	0.0c	2702.1e

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05

Table 29. Root surface area comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Total surface area
genotype			(cm ² )		
Latitude 36	283.5b	49.9cd	9.5bc	0.0c	343.0cd
OSU1682	99.1e	0.0e	0.0c	0.0c	99.1f
OSU2082	183.8d	36.0d	0.0c	0.0c	219.8e
OSU2094	344.1a	138.2a	27.8a	17.4a	527.5a
Tahoma 31	304.9ab	75.4bc	31.1a	8.1b	149.6b
TifB16117	282.8b	85.1b	18.5ab	7.2b	393.7bc
TifTuf	249.2bc	26.3de	0.0c	0.0c	276.8de
Tifway	262.7bc	23.8de	0.0c	0.0c	286.5de

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^xMeans followed by same letters within each column are not significantly different at the P = 0.05 significance level.

Table 30. Root diameter comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Average root diameter
genotype			(mm)		
Latitude 36	0.342b	0.244d	0.195c	0.000c	0.260de
OSU1682	0.265d	0.000f	0.000d	0.000c	0.265de
OSU2082	0.302c	0.286b	0.000d	0.000c	0.294c
OSU2094	0.362b	0.247d	0.227b	0.265a	0.275cd
Tahoma 31	0.350b	0.259cd	0.239b	0.243b	0.273de
TifB16117	0.473a	0.321a	0.304a	0.284a	0.346a
TifTuf	0.373b	0.275bc	0.000d	0.000c	0.323b
Tifway	0.308c	0.196e	0.000d	0.000c	0.252e

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05

Table 31. Root volume comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Total root volume
OSU16820.787e0.000d0.000c0.000dOSU20821.814d0.421b0.000c0.000dOSU20943.380a0.799a0.269a0.199aTahoma 312.945b0.492b0.247a0.102cTifB161173.608a0.689a0.234a0.121b	
OSU20821.814d0.421b0.000c0.000dOSU20943.380a0.799a0.269a0.199aTahoma 312.945b0.492b0.247a0.102cTifB161173.608a0.689a0.234a0.121b	3.373c
OSU20943.380a0.799a0.269a0.199aTahoma 312.945b0.492b0.247a0.102cTifB161173.608a0.689a0.234a0.121b	0.787f
Tahoma 312.945b0.492b0.247a0.102cTifB161173.608a0.689a0.234a0.121b	2.236e
TifB16117 3.608a 0.689a 0.234a 0.121b	4.646a
	3.790b
TifTuf 2.336c 0.261c 0.000c 0.000d	4.661a
1111ui 2.550c 0.201c 0.000c 0.000d	2.595de
Tifway         2.673bc         0.262c         0.000c         0.000d	2.935d

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05

Table 32. Root length density comparisons of bermudagrass genotypes at different zones^z when grown in polyethylene growth tubes^y.

Bermudagrass	Zone A	Zone B	Zone C	Zone D	Total length density
genotypes			$(\mathrm{cm}\mathrm{cm}^{-3})$		
Latitude 36	8.240b	1.352c	0.353bc	0.000c	1.826e
OSU1682	3.335e	0.000d	0.000c	0.000c	0.834f
OSU2082	5.876d	1.428c	0.000c	0.000c	1.826e
OSU2094	10.091a	4.825a	1.145a	0.0479a	4.135a
Tahoma 31	8.670b	3.121b	0.954a	0.229b	3.243b
TifB16117	7.593c	2.720b	0.613ab	0.197b	2.781c
TifTuf	7.430c	0.610d	0.000c	0.000c	2.010e
Tifway	7.447c	0.457d	0.000c	0.000c	1.976e

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05

Table 33. Root dry weight comparisons of bermudagrass genotypes at different zones^z when

Bermudagrass genotype	Zone A	Zone B	Zone C	Zone D	Total root dry weight
			(g)		
Latitude 36	0.528e	0.038d	0.025c	0.000	0.591d
OSU1682	0.240h	0.000g	0.000e	0.000	0.240h
OSU2082	0.560d	0.019f	0.000e	0.000	0.579e
OSU2094	0.685a	0.0827a	0.0325b	0.017	0.816a
Tahoma 31	0.629b	0.058c	0.039a	0.003	0.730b
TifB16117	0.479g	0.067b	0.014d	0.016	0.573f
TifTuf	0.608c	0.022e	0.000e	0.000	0.630c
Tifway	0.479f	0.021e	0.000e	0.000	0.500g

grown in polyethylene growth tubes^y.

^zZone A = 0-30 cm, zone B = 30-60 cm, zone C = 60-90 cm, and zone D = 90-120 cm.

^yGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length). ^xMeans followed by same letters within each column are not significantly different at the P = 0.05 significance level.

Table 34. Shoot dry weight of eight bermudagrass genotypes grown in polyethylene growth

tubes^z

Bermudagrass genotype	Shoot dry weight		
	(g)		
Latitude 36	1.273c		
OSU1682	1.016g		
OSU2082	1.082e		
OSU2094	1.601b		
Tahoma 31	1.609a		
TifB16117	1.265d		
TifTuf	1.015g		
Tifway	1.053f		

^zGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^yDry weights were recorded after drying at 80°C for 48 hours.

^xMeans followed by same letters within each column are not significantly different at the P = 0.05 significance level.

Table 35. Root to shoot ratio of eight bermudagrass genotypes grown in polyethylene growth tubes^z.

Bermudagrass	Root to shoot ratio	
genotypes		
Latitude 36	0.467e	
OSU1682	0.236g	
OSU2082	0.537b	
OSU2094	0.511c	
Tahoma 31	0.460f	
TifB16117	0.460f	
TifTuf	0.633a	
Tifway	0.479d	

^zGrowth tubes were made from clear polyethylene tubing (3.81 cm diameter x 120 cm length).

^yDry weights were recorded after drying at 80°C for 48 hours.

^xMeans followed by same letters within each column are not significantly different at the P = 0.05

# CHAPTER V

# EVALUATION OF 21 BERMUDAGRASSES FOR VISUAL CHARACTERISTICS AND PERCENT GREEN COVER

## Abstract

Bermudagrasses (*Cynodon* spp.) are warm-season perennial grasses widely grown throughout the humid and semi-arid regions of the southern and western United States. Bermudagrasses are used on home lawns, parks, athletic fields, golf courses, industrial sites, and other recreational areas. Along with superior drought performance, genotypes with improved turfgrass quality, and other important traits such as improved tolerance to cold temperature, better fall color retention, early post dormancy regrowth are required. The objective of this research was to assess the turf performance of new bermudagrass genotypes developed by the OSU turfgrass breeding program under replicated field trial for use in the transition zone. A field study was conducted to characterize the turf performance of 19 experimental and 2 commercially available bermudagrasses. The two commercial standards were 'TifTuf' and 'Tahoma 31'. Genotypes were evaluated for visual turf performance parameters such as establishment rate, turf quality, seedhead prolificacy, fall color retention, spring green up, and winterkill. Percent green cover was evaluated through digital image analysis (DIA). Bermudagrass genotypes varied significantly for all these parameters. TifTuf and Tahoma 31 had similar seedhead counts. Experimental genotype

for most of the genotypes because of the winterkill incidence, especially in the north end plots of the nursery which were not covered by snow during the period of freezing temperatures. The mean winterkill percentage ranged from 5.3 to 97.3 % among the genotypes. Tahoma 31 had low winterkill than TifTuf and 80% of the experimental genotypes. Experimental genotypes OSU1433, 17-4200-19X21, OSU1408, and OSU1646 had low winterkill. These results demonstrate the variability among bermudagrass genotypes for these visual parameters. The findings of this research are important in selecting genotypes for use as advanced lines in further research trials to identify genotypes suitable for the transitional climatic zone.

# Introduction

Bermudagrasses (*Cynodon* spp.) are warm-season perennial grasses belonging to the *Poaceae* family. These are widely used on home lawns, parks, athletic fields, golf courses, industrial sites, and other recreational areas. It has high recuperative ability and wear tolerance (Turgeon, 2005). Water is a limited renewable resource and an important strategy to maintain turfgrass in limited water environments is to identify and use drought resistant species and cultivars. Along with superior drought performance, genotypes with improved turfgrass quality, and other important ancillary traits such as improved tolerance to cold temperature, better fall color retention, early post dormancy regrowth are required. The researchers at Oklahoma State University have been consistently working on breeding, screening, and identifying genotypes with improved drought resistance as well as cold tolerance (Wu et al., 2013).

Optimum growth of bermudagrass occurs between 24 to 37°C, which makes them suitable for growth in transition zones (Beard, 1973). Bermudagrasses have excellent tolerance to heat and drought, but low tolerance to freezing temperature (Beard, 1973). Bermudagrasses are susceptible to winter when grown in the transition zone (Fry, 1990). The shoot growth ceases followed by loss of chlorophyll and a change in color to brown when the soil temperature drops

below 10°C (Christians and Engelke, 1994). Winter discoloration occurs in bermudagrass because of the physiological process of dormancy, which limits their use in transitional zones (Schiavon et al., 2011). Fall color retention ratings measure the ability of the turfgrasses to retain color during the winter months (Morris, 2000). Turfgrasses accumulate carbohydrates during reduced activity in the fall, and the energy produced by the metabolization of these stored carbohydrates is used to initiate spring green-up (Rogers et al., 1975). Early spring green-up may encourage earlier sports field use and allow the harvest of green bermudagrass to start earlier on sod farms.

Bermudagrasses are susceptible to winter injury when grown in the transition zone (Fry, 1990). The shoot growth ceases followed by loss of chlorophyll and a change in color to brown when the soil temperature drops below 10°C (Christians and Engelke, 1994). Lower cold hardiness, lower fall color retention, and tendency to enter dormancy during the winter months reduce the utility of bermudagrass on sports fields in the transition zone. Genotypes with improved cold hardiness will help in expanding utility of bermudagrass in the northern parts of the United States.

The overall goal of the study was to assess turf performance of new bermudagrass genotypes developed by the OSU turfgrass breeding program under replicated field trial for use in the transition zone. The preliminary data from this trial will assist in screening genotypes for further research trials. The objective of this study was to evaluate OSU experimental genotypes and standard cultivars for visual characteristics and percent green cover from the replicated field trials. It was hypothesized that there were significant differences among the bermudagrasses for visual characteristics and percent green cover.

## Description of research site and entries

This study was conducted as a replicated field trial from 2019 to 2021. The experiment site was located at the Oklahoma State University (OSU) Turfgrass Research Center in Stillwater, OK (latitude 36°07'15.2472" N longitude and 97°06'15.138" W). The field area was 148.64 m² (24.4 m long and 6.1 m wide) with 0.91 m borders around the perimeter. According to the soil test results, the texture was 90 % sand, 6.3 % silt, and 3.8 % clay, and the soil pH was 7.1. The phosphorous test index was 107 and the potassium test index was 180. No additional phosphorus or potassium applications were made because the levels were higher than the sufficiency indices of 65 and 250, respectively. There were 21 bermudagrass entries in total out of which two were industry standards TifTuf and Tahoma 31, and the remaining 19 were the experimental lines provided by the OSU turfgrass breeding program (Table 36). All the experimental lines were interspecific hybrids.

#### **Cultural management**

The planting was done by sprigging on August 6, 2019. An automatic sprinkler irrigation system was used and plots were irrigated to prevent moisture stress. The plots were mowed three times a week with a reel mower to maintain a height of 2.54 cm, and the clippings were removed from the plots. The trial was fertilized with a total of 195 kg N ha⁻¹ in 2020 from April to September. The herbicide glyphosate (Roundup Pro, Monsanto, St. Louis, MO) was applied to the border areas as needed to prevent bermudagrass growth into the adjacent plots of other cultivars. Weeds were manually removed from the plots by hand and contamination into adjacent plots was also prevented by using a gas-powered lawn edger weekly.

## **Data Collection**

## **Percent Establishment (PE)**

Establishment is a visual estimate of root and shoot growth rate to reach a mature, stable turf after planting (Morris, 2013). Factors such as genetics, seed/sod quality, environment, and

management practices influence turfgrass establishment rate. Faster establishment of bermudagrass after planting makes the turf denser and reduces erosion and therefore, cultivars with improved establishment rates are demanded by the sod growers, turfgrass managers, and consumers. The establishment data reflects the relative speed to develop into mature sod and ranges from 0 to 100, with 100 as completely established (Morris, 2007). This index was evaluated based on visual ratings on a percentage basis. The data was taken every two weeks in May and June in 2020.

## **Turfgrass Quality (TQ)**

The term 'turfgrass quality' was adopted by turfgrass scientists in the 1950's to assess the turfgrass performance (Beard, 2005). The turfgrass quality term includes six components, which are uniformity, shoot density, leaf texture, leaf orientation, smoothness, and color (Morris, 2000). Ratings were taken on a 1 to 9 scale, with 1 representing poorest, and 9 representing excellent turf. A rating of 6 was considered as the minimum acceptable (Morris, 2000). Ratings were taken every two weeks in 2020 from August to the first week of November.

# Seedhead Prolificacy (SH)

Seedheads reduce the aesthetic quality of the turf. Seedhead production reduces vegetative quality by diverting the stored energy towards reproductive growth. The ratings were taken on September 9, 2020 on a scale of 1 to 9, with 1 representing complete seedhead coverage of canopy and 9 representing no seedhead (Morris, 2007).

# Fall Color Retention (FCR)

Fall color retention ratings measure the ability of the turfgrasses to retain color during the winter months (Morris, 2000). The scale used for visual evaluation ranges from 1 to 9, with 1

representing straw brown or no color retention and 9 representing dark green (Morris, 2000). Ratings were taken every week from mid-October to December 2020.

# Spring Green-Up (SG)

Spring green-up measures the transition of grasses to post dormancy spring growth after the cessation of winter. The ratings take into account the plot color and not the genetic color. The scale ranges from 1 to 9, 1, indicating straw brown color and 9 indicating dark green (Morris, 2000). Ratings were taken every week from mid-March to the first week of May in 2021.

# **Percent Green Cover (PGC)**

Percent green cover defines the area of the green canopy on the ground. It may fluctuate with variation in management practices such as top dressing, weeding, aerification, mowing height, fertilizer, and pesticide applications. Percent green cover was assessed via digital image analysis (DIA). Digital image analysis is an objective measure of percent green cover or average color (Richardson, 2001). The images were taken with Canon PowerShot G16 12.1 MP CMOS (Canon, Melville, NY). A lightbox having four fluorescent bulbs fixed inside it was used to maintain uniform light conditions while taking images. The images were analyzed using Turf analyzer software. Turf analyzer is a java-based application that run on Windows, Mac, and Linux operating systems. Camera settings included a shutter speed of 1/60 s, an aperture of F2.2, and International Organization for Standardization (ISO) set at 200. The hue and saturation threshold settings ranged from 70 to 170 and 10 to 100, respectively. The brightness threshold settings ranged from 0 to 100.

# Winterkill (WK)

Winterkill refers to the loss of turf during the winter (Beard, 1973). It is generally measured on the basis of the percentage of live above-ground shoots during spring (Martin et al.,

2001). Percentage of winterkill evaluated after spring green-up has been used to measure the ability of turfgrass species to survive low temperature severities (Anderson et al., 1988). Winterkill was calculated based on the percent live cover data. Percent live cover was measured visually on 0 to 100 scale with 0= complete injury and = complete live cover.

Winterkill was calculated using the formula,

$$Percent winterkill = 100 X \left[ 1 - \left( \frac{Percent spring live cover}{Percent last live cover} \right) \right]$$

where percent spring live cover was recorded on April 15, 2021 and percent last live cover was recorded on October 15, 2020.

Winterkill reduces the aesthetic quality as well as playability of the recreational turf sites. Installation of protective covers on the turf sites is expensive and labor intensive. Further, there is additional cost of re-establishment of any loss turf sites due to winterkill. Winterkill data can be useful in selecting genotypes with relatively higher freeze tolerance.

# **Statistical Analysis**

The experimental design was a randomized complete block with 21 bermudagrass entries and three replications. The cultivar, block, and evaluation date were the independent variables. Generalized linear mixed models (GLIMMIX) methods were used for repeated measure analysis (SAS version 9.4., SAS Institute Inc., Cary, NC, USA). The means separation test was performed for cultivar performance using Tukey's HSD test at P=0.05 significance level.

# **Results and discussion**

## **Percent Establishment (PE)**

There were significant genotype and rating date effects, but no genotype by rating date interaction effect (Table 37). Tukey's HSD was used to separate genotype means within each

rating date. There were significant differences among the bermudagrass genotypes for percent establishment on only one out of the four rating dates (Table 38). On one rating date, OSU1893 had a significantly lower percent establishment than Tahoma 31. Slower establishment rate increases the sod production cost, making the establishment of new turfgrass sites more expensive (White, 2001). All the genotypes reached more than 90% establishment by June 17, 2020 and complete coverage was achieved on July 2, 2020 (11 months after planting). There were no significant differences among Tahoma 31 and TifTuf for percent establishment performance which is in accordance with the 2017 NTEP mean percent establishment data collected in Stillwater, OK (NTEP, 2017).

## **Turfgrass Quality (TQ)**

TQ ratings were taken on seven rating dates (August to November) in 2020. Significant genotype, date, and genotype by rating date interaction effects were found in TQ data (Table 36). There were significant differences among the genotypes on five out of the seven rating dates (Table 40). All the genotypes maintained an acceptable TQ of 6 or more in the months of August, September, and October. There was a decline in TQ for all the genotypes in November which was due to the decrease in temperature and day length duration. TQ decreased below the minimal acceptable (>6) in November for all the genotypes except 17-4200-19X21, OSU1876, and TifTuf. 17-4200-19X13, 17-4200-19X21, OSU1873, OSU1876, and TifTuf were in top statistical group for TQ on all the rating dates. Tahoma 31 was in the top statistical group for 6 out of the 7 rating dates and the decline in TQ on the last rating date was due to the initiation of fall dormancy. These results were in accordance with the 2019 NTEP mean turfgrass quality performance where Tahoma 31, TifTuf, and OSU1876 were in the top statistical group (NTEP, 2019). Genotype OSU1873 also performed similar for the 2019 NTEP turfgrass quality and had TQ statistically similar to TifTuf and OSU1876 (NTEP, 2019). Genotypes OSU18910 and OSU1898 were the

least performing based on coarse texture, lower density, and uniformity and appeared in the top statistical group on only 2 out of the 7 rating dates.

# Seedhead prolificacy (SH)

Seedhead ratings were taken in the fall of 2020 (Table 39). Mowing was halted for a week prior to the rating date to allow the development of seedheads. There were significant differences among the 21 bermudagrass genotypes for seedhead production with mean seedhead proficiency ratings ranging from 2.7 to 8.0 (Table 39). TifTuf, Tahoma 31 and 11 experimental genotypes were in the top statistical group showing least seedhead production. These results were in accordance with previous national trials where both Tahoma 31 and TifTuf had lower and statistically similar number of seedheads (NTEP, 2014). Experimental genotypes OSU1873 and OSU1876 also showed a similar performance for 2020 NTEP mean seedhead ratings and were in the top statistical group (NTEP, 2020). Experimental genotypes OSU1101, OSU1898, OSU18910, OSU1601, OSU1896, OSU18718, OSU1893, and OSU1892 were the poor performing with maximum seedhead production.

# Fall Color Retention (FCR)

There was significant genotype, rating date, and genotype by rating date interaction effect. (Table 36.). Significant differences occurred among the 21 genotypes on only three out of the six rating dates (Table 41). TifTuf and OSU1876 were in the top statistical group for all the rating dates with significant differences among the genotypes. These results are in accordance with those reported by Gopinath (2016) where TifTuf was in the top statistical group for FCR. Genotypes that maintain their green color longer into the fall could reduce the amount of overseeding required to meet the aesthetic demands of late-season sporting events. The ability to maintain the integrity of the plant cell and plastid membrane in response to fall chilling stress is recognized to be important for fall color retention or chilling tolerance (Fontanier et al., 2020;

Kimball and Salisbury, 1973). A sharp decline was observed in FCR ratings for all the genotypes on November 1, 2020. This was possibly due to the sudden decline in temperature during the end of October 2020 (Figure 2). However, an increase in FCR ratings was observed for some genotypes on the following rating date due to a rise in the average soil temperature above 15  $\Box$ . 2008-4X16, OSU1408, OSU1433, OSU1601, OSU1646, OSU1651, and OSU1657 had lower FCR and were in the lowest statistical group on all the rating dates having significant differences among the genotypes. The experimental genotype OSU1408 showed a change in color of its stolons and leaves to purple making it unfit for turfgrass sites with high aesthetic values.

# Spring Green-Up (SG)

For SG in 2021, there was significant genotype effect (Table 36). Significant differences occurred among the genotypes on seven out of the eight rating dates (Table 42). Genotypes 17-4200-19X21, OSU1646, and Tahoma 31 were the first to begin greening up. Tahoma 31 has previously been reported to show superior spring green up performance (NTEP, 2017). Tahoma 31 had early spring green up than TifTuf which is in accordance with the results of the previous national bermudagrass trials (NTEP, 2017). Under controlled environment study, Tahoma 31 showed early green up when chilling stress was removed (Fontanier et al., 2020). The genotypes 17-4200-19X21 and OSU1433 were the first to reach minimum acceptable spring green up rating of 6. However, only 52 % of the bermudagrass genotypes showed mean spring green up ratings equal to or above the minimum acceptable value of 6. The lower performance of genotypes for spring green up was due to the winterkill incidence exhibited by these genotypes.

## Percent Green Cover via DIA

## 2020 (May – December)

There were significant genotype, rating date, and genotype by rating date interaction effects for PGC in 2020. Significant differences among genotypes were present on 7 out of the 10 rating dates (Table 43). PGC was less than 2% on the first rating date, due to the initial stage of establishment and reached more than 95% for all the genotypes in July (Table 43). A decline in PGC was observed in November and December due to the initiation of fall dormancy. TifTuf was the top performer as it appeared in the top statistical group on all the 10 rating dates followed by Tahoma 31 and experimental genotypes OSU1101, 17-4200-19X13, 17-4200-19X21, OSU1876, and OSU1892 which appeared in top statistical group on nine rating dates. TifTuf and Tahoma 31 were in the same statistical group except on one rating date. These results were in accordance with 2020 NTEP mean percent live cover data where TifTuf, Tahoma 31, and OSU1876 were in the same statistical group (NTEP, 2020). OSU1651 was lower performing as it appeared in top statistical group on only four rating dates.

### Spring 2021 (March – May)

Percent green cover data was taken in Spring 2021 on three rating dates during the months of March, April, and May. Significant genotype, rating date, and genotype by rating date interaction effects were present. Significant differences were present for spring PGC on two out of the three rating dates (Table 44). All the genotypes had less than 1% green cover on the first rating date. PGC is generally lower in early spring due to post dormancy regrowth phase. An overall lower PGC was reported for the genotypes even in the late spring due to the winterkill incidence.

### Winterkill (WK)

Due to prolonged snow and low temperature incidence in February 2021 (Figure 3,4), some bermudagrass genotypes showed winterkill. Data was collected in mid-April, 2021. According to Oklahoma Mesonet data, the average soil temperature under sod at 5 cm and 10 cm soil depths was 0.5°C and -0.7°C, respectively (Stillwater Site, Oklahoma Mesonet). There were significant differences among the genotypes for winterkill (Table 45). The mean winterkill percentage ranged from 5.4 to 97.3 for OSU1433 and OSU1156, respectively. Other experimental genotypes with superior winter survival were 17-4200-19X21, OSU1408, and OSU1646. TifTuf and Tahoma 31 showed mean winterkill of 70.8 % and 30.9 %, respectively. Winterkill was more for TifTuf than Tahoma 31 which is in agreement with the national bermudagrass research trials (NTEP, 2017). Tahoma 31 has previously shown superior winterkill performance under field conditions with an average of 14.5 % winterkill across Indiana and Kentucky (NTEP, 2014). Along with field observations, Tahoma 31 has also been reported as the top performing genotype with an LT50 value ranging from -7.8 to - 9.0 when tested for freeze tolerance under controlled environment conditions (Gopinath, 2020).

Snow cover act as a natural insulator against low temperature and minimize the winter injury. Insulation capacity of ice is relatively lower than the snow (Leep et al., 2001). Snow traps air between the multiple snowflakes and has a relatively high solar radiation reflectance which results in better insulation capacity of snow (Roebber et al., 2003). Insulation and reflectance of solar radiation vary with the depth, density, morphology, and patterns of snow distribution (Namias, 1985). The insulating capacity of snow decreases with time and the snowflakes bond together to form large ice masses releasing the entrapped air (Takei and Maeno, 2001). In this trial, the distribution of snow cover over the plots was not uniform. A thinner snow cover was observed on the northern side of the field as compared to the southern side. As expected, the winterkill incidence was higher on the northern side of the field in comparison to rest of the field. The blocks ran from North to South, so blocking could not help in controlling the variability due to uneven snow depth and distribution.

#### Summary

In this study, 19 experimental bermudagrass genotypes were compared to two commercially available bermudagrass genotypes for multiple turf performance parameters.

Individual comparisons among genotypes for specific performance parameters can now be made because people have varying expectations and some value one parameter more over another. The data collected for turf performance from this replicated field trial would help in screening genotypes for further research trials. TifTuf and Tahoma 31 had a lower but similar number of seedheads which is highly desirable as seedheads reduce the aesthetic quality of turf. The maximum fall color retention was found in OSU1876 and TifTuf. These genotypes retain green color longer into the fall and could reduce the amount of over-seeding required to meet the aesthetic demands of late-season sporting events. The worst performing genotype for PGC was OSU1651. Tahoma 31 and two experimental genotypes 17-4200-19X21 and OSU1646 were the first to begin greening up. Winterkill ranged from 5.3 to 97.3 percent among the genotypes. Tahoma 31 had lower winterkill than TifTuf as well as less winterkill than 80% of the experimental genotypes. The tolerance of Tahoma 31 to lower winter temperatures resulted in it having less winter injury compared to less winter hardy varieties.

### Conclusion

The genotypes varied significantly for their visual parameters. The results of this study indicate that significant improvements have been made by the breeders in the turf performance of the new genotypes evaluated in this study. The findings of this field study when paired with information gained from other trials will help turfgrass developers to more effectively select experimental lines with potential for commercial release. Experimental genotypes OSU1876 and OSU1873 have improved density, darker green color, higher fall color retention, higher percent green cover and fewer seedheads compared to their parental lines, however, their drought resistance remains unknown. Genotypes evaluated in the trial which had satisfactory ratings for all or most of the parameters would be considered suitable for use in the further drought trials. Parents of experimental genotypes performing superior to the commercial standards for one or a few traits can be used as parents for future breeding purposes. Future research work will focus on

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evaluation of these 21 bermudagrass genotypes for their drought performance under rainout shelter. The field will be re-established due to the loss of some turfgrass plots because of winterkill incidence. After complete establishment, the experimental area will be saturated to field capacity prior to drought treatment. Drought performance will be assessed by measuring TQ, LF, NDVI, green cover using DIA, and canopy temperature (CT). TQ and LF will be evaluated on a scale of 1-9 based on the NTEP visual rating system (Morris, 2000). The NDVI and CT will be measured weekly using a FieldScout TCM 500 NDVI Turf Color Meter (Spectrum Technologies, Aurora, IL) and a handheld Fluke 561 infrared thermometer, respectively.

Table 36. Bermudagrass cultivars and experimental genotypes evaluated for field turf

performance.

Bermudagrass genotype ^z	Description
2008-4X16	OSU experimental
OSU1101	OSU experimental
OSU1156 (OSC103)	OSU experimental
OSU1408	OSU experimental
OSU1433	OSU experimental
OSU1601	OSU experimental
OSU1646	OSU experimental
OSU1651	OSU experimental
OSU1657	OSU experimental
17-4200-19X13	OSU experimental
17-4200-19X21	OSU experimental
OSU1873	OSU experimental
OSU1876	OSU experimental
OSU1892	OSU experimental
OSU1893	OSU experimental
OSU1896	OSU experimental
OSU1898	OSU experimental
OSU18910	OSU experimental
OSU18718	OSU experimental
TifTuf	Standard cultivar
Tahoma 31	Standard cultivar

^zGenotypes with an OSU prefix are the experimental lines from Oklahoma State University.

Table 37 Repeated measures analysis of twenty-one	e bermudagrass genotypes using SAS software
feature PROC GLIMMIX.	

Source	PE (2020)	TQ (2020)	PGC (2020)	PGC (2021)	FCR (2020)	SG (2021)
			p	-value		
Entry	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Date	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Entry*Date	0.7719	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 38. Mean visual percent establishment of 21 genotypes in the Block 16 Bermudagrass Trial during 2020.

Percent establishment‡

Permudagrass genetune	1-May	15-May	1-Jun	17-Jun
Bermudagrass genotype				
2008-4X16	71.33ab†	88.33	99.00	99.67
OSU1101	73.00ab	92.33	99.33	100.00
OSU1156 (OSC103)	70.33ab	89.67	99.00	99.33
OSU1408	71.00ab	84.67	96.67	99.00
OSU1433	61.67ab	77.33	95.67	99.67
OSU1601	64.67ab	83.33	98.00	100.00
OSU1646	70.00ab	85.67	96.33	99.33
OSU1651	53.67ab	69.67	96.00	98.33
OSU1657	47.67ab	71.33	87.67	91.67
17-4200-19X13	70.00ab	88.33	100.00	100.00
17-4200-19X21	74.33ab	87.67	96.00	98.33
OSU1873	61.67ab	76.67	92.00	98.33
OSU1876	68.00ab	86.33	97.67	98.00
OSU1892	68.00ab	80.33	95.00	98.33
OSU1893	40.00b	62.00	85.33	95.00
OSU1896	69.67ab	81.33	97.00	100.00
OSU1898	53.00ab	71.33	86.33	93.33
OSU18910	59.00ab	75.00	96.00	98.67
OSU18718	69.33ab	88.67	98.67	100.00
TifTuf	73.67ab	88.00	99.00	100.00
Tahoma 31	76.00a	87.00	98.00	100.00

†Means within columns followed by the same letters are not statistically different at P =0.05

based on Tukey's HSD test.

 $\ddagger$  Ratings are based on a scale of 1–100 (100 = complete establishment).

Table 39. Mean visual seed head ratings for 21 bermudagrasses in Fal1, 2020.

Visual Seed Head Rating‡

Bermudagrass genotype	Fall 2020
2008-4X16	7.3ab†
OSU1101	2.7e
OSU1156 (OSC103)	7.7a
OSU1408	8.0a
OSU1433	6.7a-d
OSU1601	3.7e
OSU1646	8.0a
OSU1651	7.7a
OSU1657	8.0a
17-4200-19X13	7.3ab
17-4200-19X21	7.0a-c
OSU1873	8.0a
OSU1876	7.3ab
OSU1892	4.7с-е
OSU1893	5.0b-e
OSU1896	4.0e
OSU1898	3.0e
OSU18910	3.3e
OSU18718	4.3de
TifTuf	7.7a
Tahoma 31	7.7a

† Means within columns followed by the same letters are not statistically different at  $P \le 0.05$ 

level based on Tukey's HSD test.

 $\ddagger$  Ratings are based on a scale of 1–9 (1 = no seed head).

Table 40. Mean visual turf quality ratings of 21 genotypes in the Block 16 Bermudagrass Trial during 2020.

Turf Quality‡

Bermudagrass genotype	1-Aug	14-Aug	1-Sep	14-Sep	1-Oct	15-Oct	4-Nov
2008-4X16	8.0a†	8.0a	8.0a	7.7	8.0a	7.0	3.7e-g
OSU1101	8.0a	8.0a	8.0a	7.0	7.0b	6.0	5.0с-е
OSU1156 (OSC103)	7.7ab	7.7ab	8.0a	7.3	8.0a	7.0	5.0с-е
OSU1408	7.7ab	8.0a	8.0a	7.7	7.7ab	6.7	3.0g
OSU1433	8.0a	8.0a	8.0a	8.0	7.3ab	6.3	3.3fg
OSU1601	8.0a	8.0a	8.0a	7.3	7.0b	6.0	3.7e-g
OSU1646	8.0a	8.0a	8.0a	7.7	8.0a	6.7	3.7e-g
OSU1651	7.0b	7.3ab	8.0a	7.3	7.0b	6.0	2.7g
OSU1657	8.0a	8.0a	8.0a	7.7	7.7ab	6.0	3.7e-g
17-4200-19X13	8.0a	8.0a	8.0a	7.7	8.0a	7.0	5.7a-d
17-4200-19X21	8.0a	8.0a	8.0a	7.7	7.7ab	7.0	6.0a-d
OSU1873	8.0a	8.0a	8.0a	8.0	8.0a	7.0	6.3a-c
OSU1876	8.0a	8.0a	8.0a	8.0	8.0a	7.0	7.0a
OSU1892	7.3ab	7.3ab	7.3ab	8.0	8.0a	6.7	5.0с-е
OSU1893	7.3ab	7.3ab	7.3ab	7.3	7.3ab	7.0	5.3b-d
OSU1896	7.3ab	7.3ab	7.3ab	7.7	7.0b	6.0	4.7d-f
OSU1898	7.0b	7.0b	7.0b	7.3	7.0b	6.3	4.7d-f
OSU18910	7.0b	7.0b	7.0b	7.0	7.0b	6.0	5.0c-e
OSU18718	7.7ab	7.7ab	7.7ab	7.3	7.0b	6.0	5.0c-e
TifTuf	8.0a	8.0a	8.0a	8.0	8.0a	6.7	6.7ab
Tahoma 31	8.0a	8.0a	8.0a	8.0	8.0a	6.7	5.0с-е

[†] Means within columns followed by the same letters are not statistically different at P  $\leq 0.05$ 

level based on Tukey's HSD test.

 $\ddagger$  Ratings are based on a scale of 1–9 (1 = poorest TQ).

Table 41. Mean visual fall color retention ratings of 21 genotypes in the Block 16 Bermudagrass Trial during 2020.

Fall color retention[‡]

Bermudagrass genotype	1-Oct	15-Oct	1-Nov	16-Nov	30-Nov	11-Dec
2008-4X16	8.7	8.0	3.7	1.7f†	1.3ef	1.0f
OSU1101	8.7	7.0	5.7	4.7b-d	3.3de	2.0de
OSU1156 (OSC103)	9.0	8.0	4.7	6.0a-c	4.3a-d	2.0de
OSU1408	8.7	7.7	3.7	1.0f	1.0f	1.0f
OSU1433	8.3	7.3	4.0	1.7f	1.0f	1.0f
OSU1601	8.7	7.0	3.0	1.3f	1.3ef	1.0f
OSU1646	8.3	7.7	3.7	1.0f	1.0f	1.0f
OSU1651	8.0	7.0	3.0	1.0f	1.0f	1.0f
OSU1657	8.7	7.0	3.0	2.3ef	1.3ef	1.0f
17-4200-19X13	9.0	8.0	4.3	5.0a-d	4.0b-d	2.3cd
17-4200-19X21	9.0	8.0	4.7	4.3cd	3.3de	2.0de
OSU1873	9.0	8.0	4.7	6.3ab	5.7a-c	3.3b
OSU1876	9.0	8.0	5.7	7.0a	6.0ab	4.3a
OSU1892	9.0	7.7	5.0	5.0a-d	4.0b-d	3.0bc
OSU1893	8.7	8.0	4.3	5.3a-d	4.0b-d	2.7b-d
OSU1896	8.0	7.0	3.7	4.0de	3.0d-f	1.0f
OSU1898	9.0	7.3	5.0	4.3cd	3.0d-f	2.0de
OSU18910	8.7	7.0	4.7	5.0a-d	3.7d	2.0de
OSU18718	8.7	7.0	5.3	4.0de	3.0d-f	1.3ef
TifTuf	8.7	7.7	5.7	6.7a	6.3a	4.7a
Tahoma 31	9.0	7.7	3.7	4.0de	2.7d-f	1.0f

† Means within columns followed by the same letters are not statistically different at  $P \le 0.05$ 

level based on Tukey's HSD test.

 $\ddagger$  Ratings are based on a scale of 1–9 (1 = no color retention).

Table 42. Mean visual spring green up ratings of 21 genotypes in the block 16 bermudagrass trial

during 2021.

Bermuadgrass genotype	15-Mar	22-Mar	29-Mar	5-Apr	12-Apr	19-Apr	26-Apr	3-May
2008-4X16	1.0c†	1.3bc	1.7bc	2.0c	2.0cd	2.0c	2.3ab	4.0
OSU1101	1.0c	1.3bc	2.0bc	2.7bc	2.7cd	2.7bc	3.7ab	5.3
OSU1156 (OSC103)	1.0c	1.0c	1.0c	1.3c	1.3d	1.3c	1.7b	4.7
OSU1408	1.0c	2.0a-c	3.7ab	5.3a-c	6.0a-c	6.3a-c	8.0ab	7.7
OSU1433	1.0c	3.3ab	5.0a	6.7ab	7.7a	8.0a	8.3a	8.7
OSU1601	1.0c	1.7bc	2.3bc	2.7bc	3.0b-d	3.7а-с	5.7ab	6.0
OSU1646	1.7ab	3.0а-с	3.7ab	5.3a-c	5.7a-d	5.7a-c	7.3ab	8.7
OSU1651	1.0c	1.7bc	2.7a-c	4.7a-c	5.7a-d	5.7a-c	6.3ab	7.0
OSU1657	1.0c	1.7bc	2.3bc	4.0a-c	4.3a-d	4.7a-c	5.3ab	4.0
17-4200-19X13	1.0c	2.0a-c	1.7bc	2.0c	2.0cd	2.0c	2.7ab	6.0
17-4200-19X21	2.0a	4.0a	5.0a	7.0a	7.3ab	7.3ab	8.3a	5.3
OSU1873	1.0c	1.0c	1.0c	1.3c	1.7cd	1.7c	3.3ab	3.0
OSU1876	1.0c	1.7bc	2.0bc	3.0a-c	3.0b-d	3.3a-c	4.0ab	5.7
OSU1892	1.0c	1.0c	1.7bc	2.3c	2.7cd	2.7bc	3.7ab	4.3
OSU1893	1.0c	2.0a-c	1.7bc	2.3c	2.3cd	2.3bc	3.0ab	4.3
OSU1896	1.0c	1.0c	1.3bc	1.7c	2.0cd	2.0c	2.3ab	4.7
OSU1898	1.0c	2.0a-c	3.3a-c	4.0a-c	4.7a-d	4.7a-c	5.0ab	7.3
OSU18910	1.0c	1.7bc	1.7bc	2.3c	2.3cd	2.3bc	2.7ab	6.3
OSU18718	1.0c	1.3bc	1.7bc	2.3c	3.0b-d	4.3a-c	3.3ab	6.0
TifTuf	1.0c	1.3bc	1.7bc	2.7bc	3.0b-d	3.3a-c	4.3ab	6.7
Tahoma 31	1.3bc	2.3a-c	3.0a-c	4.0a-c	4.3a-d	4.3a-c	5.3ab	6.7

† Means within columns followed by the same letters are not statistically different at  $P \le 0.05$ 

level based on Tukey's HSD test.

 $\ddagger$  Ratings are based on a scale of 1–9 (1 = Straw brown color).

Table 43. Mean percent green cover of 21 genotypes in the block 16 bermudagrass trial during 2020.

Percent green cover‡

Bermudagrass genotype	5-Mar	30-Mar	1-May	29-May	6-Jul	3-Aug	1-Sep	1-Oct	30-Oct	4-Dec
2008-4X16	0.84a†	31.37b-d	68.75a-d	92.57ab	98.11a	95.95a	97.69a-c	98.69ab	89.76ab	1.52hi
OSU1101	0.57a	38.51bc	75.69a-d	97.41a	99.44a	99.02a	99.33a	99.08a	94.77ab	46.60a-d
OSU1156 (OSC103)	0.92a	36.35bc	78.10a-d	72.27b	93.79a	92.37a	94.62bc	99.13a	92.49ab	36.90b-е
OSU1408	0.82a	33.16b-d	59.57с-е	98.88a	98.69a	93.46a	97.86a-c	97.44a-c	88.15a-c	0.23i
OSU1433	0.92a	31.75b-d	66.15a-d	98.65a	98.97a	95.10a	98.87a	95.16bc	78.81b-d	3.73g-i
OSU1601	0.39a	37.35bc	65.16a-e	98.88a	99.40a	93.17a	99.16a	97.04a-c	84.65a-d	6.85f-h
OSU1646	0.86a	34.17bc	78.42a-d	99.62a	99.38a	93.23a	98.68a	95.97a-c	69.22cd	5.04g-i
OSU1651	0.96a	30.17b-d	60.19с-е	98.35a	95.48a	82.47b	94.33c	94.39c	67.13d	2.84hi
OSU1657	0.42a	18.48cd	39.95ef	98.40a	98.52a	96.89a	99.18a	97.06a-c	80.39a-d	8.67e-i
17-4200-19X13	1.19a	45.03ab	86.09ab	99.36a	99.62a	93.46a	99.16a	98.87ab	93.01ab	30.02c-h
17-4200-19X21	1.28a	40.40a-c	89.02a	97.54a	97.11a	95.52a	99.00a	98.66ab	91.22ab	10.59e-i
OSU1873	1.41a	27.00b-d	53.44d-f	97.37a	94.46a	96.22a	97.99ab	99.64a	96.64ab	61.19ab
OSU1876	1.24a	31.59b-d	70.91a-d	97.52a	97.79a	94.44a	97.74a-c	99.51a	98.38a	73.23a
OSU1892	1.08a	32.13b-d	67.67a-d	99.39a	98.92a	98.14a	99.13a	99.27a	98.82a	56.24а-с
OSU1893	0.99a	11.06d	29.45f	97.78a	98.20a	97.59a	98.64a	98.29ab	97.12ab	52.63a-d
OSU1896	1.64a	33.75bc	76.93a-d	98.72a	98.98a	96.61a	98.41a	98.71ab	78.50b-d	12.83e-i
OSU1898	0.94a	27.02b-d	62.03b-e	98.44a	98.53a	97.51a	99.08a	98.02a-c	92.50ab	26.04d-i
OSU18910	1.44a	27.42b-d	60.28с-е	98.36a	96.20a	97.01a	98.20a	98.43ab	94.04ab	32.78b-g
OSU18718	1.01a	33.90bc	71.76a-d	98.96a	92.81a	95.99a	96.90a-c	99.01ab	96.39ab	34.33b-f
TifTuf	1.21a	39.43а-с	79.72а-с	97.94a	98.60a	97.47a	98.03ab	99.60a	84.18a-d	54.57a-d
Tahoma 31	0.79a	61.63a	88.00a	92.71ab	99.71a	97.46a	97.85а-с	98.93ab	91.24ab	15.42e-i

[†] Means within columns followed by the same letters are not statistically different at  $P \le 0.05$ level based on Tukey's HSD test.

 $\ddagger$  Ratings are based on a scale of 1–100 (100 = complete green cover).

Table 44. Mean percent green cover of 21 genotypes in the block 16 bermudagrass trial during

spring 2021.

Percent g	reen cover‡	
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Bermudagrass genotype	1-Mar	5-Apr	1-May
2008-4X16	0.20ab†	3.70c	14.87
OSU1101	0.08b	5.38c	36.79
OSU1156 (OSC103)	0.34ab	1.60c	22.25
OSU1408	0.63ab	29.21а-с	52.68
OSU1433	0.25ab	52.33a	88.15
OSU1601	0.09b	22.48а-с	50.43
OSU1646	0.24ab	35.96а-с	62.02
OSU1651	0.37ab	29.37а-с	75.86
OSU1657	0.11ab	7.18c	44.33
17-4200-19X13	0.09b	1.66c	8.06
17-4200-19X21	0.45ab	50.52ab	84.87
OSU1873	0.38ab	0.93c	22.56
OSU1876	0.46ab	9.82bc	43.55
OSU1892	0.06b	1.54c	21.85
OSU1893	0.23ab	6.02c	12.56
OSU1896	0.72ab	1.78c	5.02
OSU1898	0.30ab	32.77а-с	64.62
OSU18910	0.14ab	5.25c	5.80
OSU18718	0.22ab	4.47c	17.81
TifTuf	0.83a	12.01a-c	31.56
Tahoma 31	0.37ab	23.58а-с	40.69

[†] Means within columns followed by the same letters are not statistically different at  $P \le 0.05$ 

level based on Tukey's HSD test.

 $\ddagger$  Ratings are based on a scale of 1–100 (100 = complete green cover).

Table 45. Mean visual	winterkill ratings for 2	1 bermudagrasses	during Spring 2021

Winterkill Rating‡

Bermudagrass genotype	Percentage Winterkill Rating (%)		
2008-4X16	89.9ab†		
OSU1101	78.1a-c		
OSU1156 (OSC103)	97.3a		
OSU1408	14.4de		
OSU1433	5.4e		
OSU1601	54.2а-е		
OSU1646	23.6с-е		
OSU1651	43.1а-е		
OSU1657	58.7а-е		
17-4200-19X13	86.5ab		
17-4200-19X21	9.1e		
OSU1873	81.6a-c		
OSU1876	69.9a-d		
OSU1892	88.2ab		
OSU1893	85.6ab		
OSU1896	88.2ab		
OSU1898	59.4а-е		
OSU18910	86.2ab		
OSU18718	76.4а-с		
TifTuf	70.8a-d		
Tahoma 31	30.9b-е		

† Means within columns followed by the same letters are not statistically different at  $P \le 0.05$ 

level based on Tukey's HSD test.

 $\ddagger$  Ratings are based on a scale of 1–9 (1 = complete winterkill).

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# APPENDICES

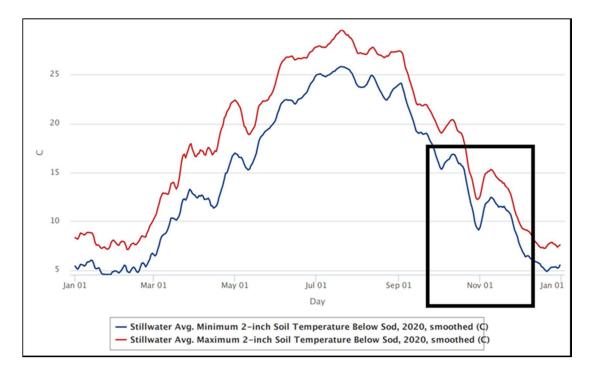


Figure 2. Block 16 field trial Mesonet data for average maximum and minimum soil temperature (°C) at 5 cm depth during 2020.

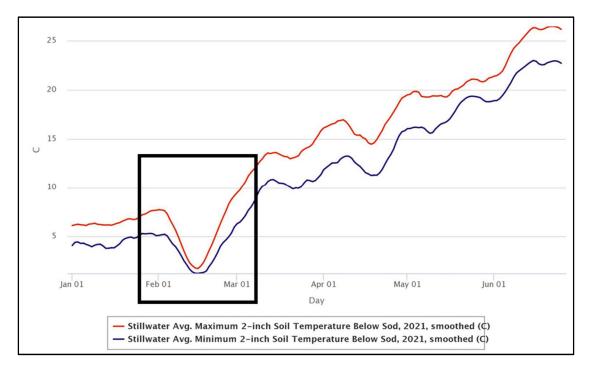


Figure 3. Block 16 field trial Mesonet data for average maximum and minimum soil temperature (°C) at 5 cm depth during 2021.



Figure 4. Block 16 field trial a) live cover on October 15, 2020, b) uneven snow cover over plots on February 15, 2021, and c) winterkill observed on plots on April 19, 2021.

## VITA

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