EVALUATION OF EXPERIMENTAL GREENS-TYPE HYBRID BERMUDAGRASS SELECTIONS BY GENETIC PROFILING, MORPHOLOGICAL MEASUREMENTS, AND FIELD TESTING

By

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Abstract: The lack of genetic diversity among ultradwarf bermudagrasses [*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] used throughout the southern region and transition zone in the United States is well known. To find genotypes with improvements in areas where ultradwarfs are generally lacking, new genetics must be introduced through traditional crosses. Oklahoma State University has developed multiple genotypes from cold-hardy parent materials for use as putting green surfaces. This research was conducted to quantify the genetic and morphological diversity among these new genotypes and ultradwarf cultivars for comparison, as well as evaluate the performance of these genotypes under putting green management. An additional objective of this research was to evaluate the performance of vegetation indices collected by unmanned aerial systems to estimate the percent canopy coverage of advanced phenotyping trials during and after establishment. Results showed there was significant genetic diversity between the new genotypes and the ultradwarf cultivars, while the latter showed very high genetic similarity to one another. Additionally, there was a significant difference among the morphological characteristics measured in this study in both the field and greenhouse trials. While the new genotypes were genetically distinct from the ultradwarfs, some showed similar morphological characteristics. The putting green field trial showed none of the new genotypes demonstrated similar ball roll distance to standard 'TifEagle', but several genotypes including OKC0920, OKC3920, 11x2, 19x19, and MSB1050 demonstrated similar or improved visual characteristics and rooting depth. A significant relationship between leaf blade length and ball roll distance for the genotypes used in this study was found which could assist breeders in making future selections. For the remote sensing methods, there was a significant relationship present between each of the methods and the logit transformed percent green cover. However, there was a significant difference between the two establishment periods. These indices may be best suited for use on their own and not estimation of percent green cover. Information collected in this research may assist in the introduction of new genotypes for use on putting green surfaces.

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CHAPTER I

REVIEW OF LITERATURE

A Review of Bermudagrass

Bermudagrass is a popular name used for a group of perennial warm-season grass species in the genus *Cynodon* C. L. Rich. The *Cynodon* species likely originated in a large geographic range from southern Africa to eastern Africa, to southern Asia along the Indian Ocean (Kole, 2011). Two species commonly used as turfgrasses across the world are common bermudagrass (*Cynodon dactylon* [L.] var. *dactylon*) and African bermudagrass (*Cynodon transvaalensis* Burtt-Davy), as well as their interspecific hybrids (*C. dactylon* x *C. transvaalensis*) (C. Taliaferro et al., 2004). These grasses are well adapted to tropical or subtropical climates, making them common in the southern and lower transition zone in the United States. The majority of *C. dactylon* are tetraploid (2n=4x=36) that can spread through stolons, rhizomes, as well as seed (Burton et al., 1967). Common bermudagrass has an upright growth habit with medium to course leaf texture and long internodes (McCarty & Miller, 2002). *C. transvaalensis* is a diploid that also spreads through stolons, rhizomes, or seed, but has short, fine textured leaves with a lateral growth habit (Harlan, 1970; Harlan et al., 1970; Juska and Hanson, 1964). The resulting interspecific hybrid of these species can result in sterile genotypes with vigorous growth, a high shoot density, and improved turfgrass quality, color, and fine leaf textured compared to their parental species (Beard, 1973). The first documented bermudagrass breeding program in the United States was

initiated by Dr. Glenn Burton in 1946 (Baxter and Schwartz, 2018). Dr. Burton's breeding program developed and released 'Tifgreen' bermudagrass, for use on putting greens (Hein, 1961).

A Review of Ultradwarf Bermudagrass

Original Ultradwarf Breeding Program

Prior to Dr. Burton's work on developing bermudagrass for use on putting greens, golf courses in the southern United States would rely on closely mown pasture grasses, local bermudagrass strains, sand, or bentgrass (*Agrostis stolonifera* L.) (Morris, 2003). These options were not the ideal solution as scalping, disease, and heat pressures damaged the grass as superintendents tried to lower the mowing height to meet the expectations of the players (Morris, 2003). Dr. Glenn Burton was working with the US Department of Agriculture – Division of Forage Crops and Diseases in cooperation with University of Georgia (UGA) at the Georgia Coastal Plain Experiment Station located in Tifton, Georgia in the late 1940's to develop new interspecific bermudagrass cultivars (Forbes Jr. and Burton, 1963; Hein, 1961). In 1949, Dr. Burton released his first interspecific hybrid, 'Tiffine', which showed improved performance on putting greens compared to then common bermudagrasses used (Hein, 1961). 'Tiffine' was developed by crossing the common bermudagrass cultivar 'Tiflawn' with an African bermudagrass accession, to create a hybrid showing improved color, finer texture, and a more compact growth habit and was released in 1953 (Forbes Jr and Burton, 1963; Hein, 1961).

Continuing his crosses in 1951, Dr. Burton selected a common bermudagrass accession from a putting green in North Carolina and crossed it with an African bermudagrass breeding line. One of the resulting progenies of the cross was released in 1956 as 'Tifgreen' (Burton, 1964; Forbes Jr and Burton, 1963; Hein, 1961). This cultivar showed improvements over 'Tiffine' with a finer texture, increased density, and rapid growth and establishment (Burton, 1964; Hein, 1961). When tested against some seeded common bermudagrass varieties, 'Tifgreen' had better sod

density and weed resistance, finer texture, and comparable color (Hein, 1961). When released this cultivar was recommended for use in the southern United States where bermudagrass was commonly grown (Burton, 1964; Hein, 1961). Off-type grasses were soon discovered in putting greens established with 'Tifgreen', due to genetic instability resulting in mutations (Caetano-Anollés, 1998; Caetano-Anollés et al., 1997). These off-types could be unsightly due to different morphological characteristics. But some mutations had desirable traits better than 'Tifgreen' and were released as a new commercial cultivar. 'Tifgreen' acted as the foundation of bermudagrass cultivars used on putting greens across the southern United States.

'Tifgreen' Derived Varieties

The first of these off-types to be selected and later released occurred in the mid 1960's with the cultivar 'Tifdwarf'. Two off-types were selected from 'Tifgreen' putting greens in Georgia and South Carolina by James Moncrief; he found one of them to have improved performance on putting greens due to its lower growth habit and could be cut at 4.76 mm along with fewer seedheads and a darker green color (Burton, 1965; Burton, 1966; O'Brien, 2012). However, 'Tifdwarf' also showed similar genetic instability like 'Tifgreen' and off-types appeared in established stands of 'Tifdwarf' (Burton, 1965; Burton, 1966; Caetano-Anollés, 1998; Caetano-Anollés et al., 1997).

Another cultivar selected from a mutation from 'Tifgreen' is 'Pee Dee-102', selected from the Pee Dee Experimental Station in Florence, South Carolina. This cultivar had shorter internode lengths and smaller leaves, which helped to improve the putting surface quality (Alderson and Sharp, 1994). 'FloraDwarf' was released in 1995 by the Florida Agricultural Experiment Station from an off-type selected from a 'Tifgreen' putting green in Hawaii (Dudeck and Murdoch, 1998). This off-type had higher canopy density compared to 'Tifgreen', which led

to rapid thatch development, and needed vertical mowing and topdressing often (Dudeck and Murdoch, 1998).

The Mississippi Agricultural and Forestry Experiment Station released 'MS-Supreme' in 1997. This cultivar was selected in 1991 as an off-type in a 'Tifgreen' putting green at Gulf Shores Golf Club (Gulf Shores, AL, USA) that was originally planted in 1964. Like 'FloraDwarf', this grass had a higher density canopy and required intensive care to mitigate thatch issues (Krans et al., 1999). 'MS-Supreme' was tolerant to lower mowing heights than 'Tifgreen' in addition to its increased density and had shorter internodes compared to 'Tifgreen' (Krans et al., 1999).

'Tifdwarf' Derived Varieties

An off-type was selected from a 'Tifdwarf' putting green in Walker County, TX that was planted in 1969; it was selected in 1987 and subsequently released as 'Champion Dwarf' (further referred to as 'Champion') (Brown et al., 1997). 'Champion' has slower vertical growth with higher canopy density and finer leaves compared to 'Tifdwarf' (Brown et al., 1997). Another cultivar known as 'P-18', or 'MiniVerde', was selected from a 'Tifdwarf' line from a greenhouse owned by H&H Seed Company in Yuma, AZ. This cultivar was selected in 1992 due to its high canopy density, rapid growth, and uniform green color compared to 'Tifdwarf' (Kaerwer and Kaerwer, 2001). 'Emerald Dwarf' was selected in 1992 from a 20-year-old 'Tifdwarf' green. This grass was selected for its increased rooting depth and more rhizome production compared to both 'Tifgreen' and 'Tifdwarf' which improved its appearance during transition periods (Brown et al., 2009).

Other Cultivars

The USDA-ARS and UGA Coastal Plain Experimental Station released 'TifEagle' in 1997. This grass was selected in 1990 as TW-72 for its superior quality, fine texture, and ability

to tolerate low mowing. The plant patent for 'TifEagle' describes it as a mutant of irradiated 'Tifway II' using 7000 rads of cobalt-60 gamma radiation (Hanna and Elsner, 1999). However, research into the genetic makeup of 'TifEagle' since its release points to it being a relative of either 'Tifgreen', 'Tifdwarf', or a descendent thereof. Wang et al. (2010) used 11 simple sequence repeat (SSR) markers to evaluate the genetic similarity of 'Champion', 'FloraDwarf', 'MiniVerde', 'Tifdwarf', 'TifEagle', 'Tifgreen', 'Baby', and 'MS-Supreme' and found these cultivars to have a similarity coefficient of one. Additionally, Zhang et al. (1999) used amplified fragment length polymorphism (AFLP) methods to evaluate the relationship between 'TifEagle' and 'Tifway II' and found a high dissimilarity coefficient between the two. These, along with other studies, indicate 'TifEagle' is an off type of 'Tifgreen' or 'Tifdwarf'. 'TifEagle' was shown to have shorter and narrower leaves, fewer seedheads, and produced more stolons compared to 'Tifdwarf' and had an overall superior putting surface when maintained at 4 mm or less (Hanna and Elsner, 1999).

'Champion', 'MiniVerde', and 'FloraDwarf' were originally referred to as "ultradwarf" bermudagrasses. This term was coined by Dr. Philip Busey at the University of Florida in 1995 to describe grasses with more dwarfed morphology than 'Tifdwarf' (Reasor et al., 2016). This term is now used broadly in the turfgrass industry to describe grasses related to 'Tifgreen' or 'Tifdwarf' used for putting green surfaces.

Ultradwarf Bermudagrass Management Practices

Light Requirements

A constant battle on golf courses across the world is to balance the inclusion of trees on the course to increase the difficulty of play and the light requirements of the turfgrass on the course, particularly the putting greens. Determining the light requirements of the grasses used is critical in informing superintendents on the grasses best suited for their location or if tree removal

is needed. In an ideal environment, the greens would have access to at least eight hours of full sunlight, but trees cause a reduced light environment at varying times of the day which can reduce the quality of the underlying turfgrass and cause increased vertical growth (McCarty, 2011). Bunnell et al. (2005) conducted an experiment to quantify the daily light integral (DLI) requirements for ultradwarf bermudagrass greens by exposing them to various shades during morning and afternoon hours. In the two years the trial was conducted, the DLI ranged from 41.6 to 22.1 mol m^{-2} d^{-1} depending on the shade application. When maintained at 3.2 mm, the DLI model estimated a 32.6 mol m^{-2} d^{-1} requirement to maintain a turfgrass quality of 7 or greater. When evaluating the lateral regrowth, total shoot chlorophyll, and total nitrogen concentration, a significant difference from the no shade environment was seen when the DLI fell below 33 mol m^{-2} d^{-1}. Additionally, their evaluations found a more extreme detrimental effect from afternoon shade compared to morning shade. High afternoon shade applications reduced the turfgrass quality, lateral regrowth, shoot chlorophyll, and total nitrogen concentration by 39, 17, 39, and 27%, respectively, compared to high morning shade, which reduce turfgrass quality, lateral regrowth, and shoot chlorophyll by 21, 11, and 16%, respectively. These results can help superintendents in prioritizing the afternoon sunlight on the putting green surfaces.

Thatch Management

The high canopy density and rapid lateral growth make managing the thatch accumulation on ultradwarf putting greens a top priority for superintendents, with an emphasis on prevention rather than control (Hanna, 1998). There are several methods used by superintendents to assist in reducing the organic matter content in the putting green such as topdressing, aerification, and vertical mowing. A Better Management Practices Plan published by the Carolinas Golf Course Superintendents Association (GCSAA) in 2016 recommends keeping the organic matter below 3.5 to 4.5 % by weight (McCarty and Kerns, 2016).

Topdressing refers to adding clean sand to the turfgrass canopy and then incorporating it through brushing. McCarty and Kerns (2016) explains the benefits of topdressing including increasing thatch decomposition, smoothing the playing surface, enhancing turfgrass recovery, and encouraging a denser and finer-textured turf. However, to get the most out of a topdressing program the correct material must be chosen as well as frequency and rates. If a topdressing material is chosen that differs from the underlying soil profile, the formation of soil stratification can occur and the differences in soil characteristics can cause poor root growth due to the impeded movement of water and gases through the soil profile (McCarty and Kerns, 2016). The rate and frequency of topdressing events depend on the superintendent's overall goal, but a moderate to heavy topdressing is used after core aerification to help fill the holes and smooth the surface. During the growing season, research has shown topdressing every 7 to 14 days, in addition to aerification practices twice a year, had lower organic matter concentrations than those with longer intervals between topdressing events (Schmid et al., 2014). Lowe (2013) and O'Brien and Hartwiger (2014) also found this interval in successful ultradwarf management programs across the United States. Using this interval, the USGA (United States Golf Association) recommends using between 0.67 and 1.23 cubic feet of sand per 1000 square feet of turfgrass (Whitlark and Thompson, 2019).

Turfgrass surfaces cannot till the soil to help ease soil issues like in row crops. Aerification, or coring, refers to the removal of soil cores from the turfgrass surface. These holes can range in diameter from 6.4 to 19 mm and depth ranging from 5 to 10 cm. The spacing between the holes is determined by the speed at which the machine is moving and the spacing of the holes on the machine and is determined by the amount of turfgrass surface the superintendent wants to displace with a single aerification event. McCarty and Kerns (2016) provide several benefits for the implementation of aerification including decreasing surface and subsurface compaction, allows for easier flow of water and gases in and out of the root zone, promotes

healthy soil microorganism activity which can assist in breakdown of organic materials, and creates pathways through stratified soil to assist in water flow. There are also a few disadvantages to this practice, the disruption of the playing surface which can cause issues with golfers, but also the possible root desiccation as more soil surface is exposed. Despite these concerns for the practice, it is considered an essential practice to maintain a healthy putting green surface throughout the year. To balance the benefits and concerns with aerification, a common practice for bermudagrass greens is to aerify twice a year during the growing season after the risk of frost is over in the spring, and at least six to eight weeks before the risk of frost in the fall. A percentage basis is also used to determine how much a superintendent should aerify with the general recommendation of disrupting 15 to 20 percent of the surface each year (McCarty and Kerns, 2016). Atkinson et al. (2012) found turfgrass quality decreased around 4.5% as the surface impacted increased from 15 to 25% and was below acceptable levels for around 4 weeks after the event. Their research also found impacting the same surface area but reducing the aerification frequency increases the overall turfgrass quality during the year, but not provide the same improvement in the soil physical properties which is one of the key reasons for this practice. Newer practices like core aerification involve using solid tines instead of hollow tines to create similar holes but does not remove any soil. This can be used to help create similar water and gas exchange improvement to core aerification with minor disruptions to the soil surface. Topdressing can be performed afterward to add clean sand to dilute organic matter in the soil surface or left open and allowed to close overtime while allowing more gas exchange in the soil profile (McCarty and Kerns, 2016).

Vertical mowing, also referred to as verticutting, is a practice which uses vertically mount knives on a reel to slice through the turfgrass and rip out thatch and stolons. While this practice can be done across the golf course, the benefits for performing this on putting greens include removing excess leaf growth causing spongy surfaces, improving mowing quality,

promoting an upright growth habit, and assisting in the incorporation of topdressing sand. For putting greens, the depth of the blades is set between 0.4 to 3.2 mm below the turfgrass surface (Gross, 2013). When performed during the actively growing months, this practice can also help thin the canopy of aggressively growing ultradwarfs to promote a healthier turfgrass canopy.

Fertility

Proper fertilization is key to maintain healthy turfgrass across the golf course. Healthy turfgrass can better withstand abiotic and biotic pressures while providing an aesthetically pleasing playing surface. While the use of sand for putting greens provide less compaction and improved water infiltration rates compared to soils with more clay and silt, it does hold onto nutrients as well in the soil due to its low cation exchange capacity (Bigelow et al., 2001; Brockhoff et al., 2010). This issue makes managing the nutrient levels on putting greens more difficult, but the use and tracking of soil and tissue analysis can assist in understanding what nutrients are needed and reduce nutrient leaching. Nutrients are broken into macro and micronutrients, with macronutrients including primary nutrients (nitrogen, potassium and phosphorous) and secondary nutrients (calcium, magnesium, and sulfur) and micronutrients (boron, zinc, manganese, iron, copper, molybdenum, and chlorine).

Nitrogen is considered one of the most important plant nutrients as it is used in many essential cellular molecules such as amino acids, proteins, and nucleic acids (Torres-Olivar et al., 2014). However, sand-based root zones are not efficient at retaining nitrogen added, particularly when added in large quantities (Bigelow et al., 2001). Large nitrogen applications can also create a spike in plant growth with excess leaf tissue causing an increase in clippings collected and decrease green speed (Massey, 2007). Therefore, it is common for superintendents to use a method called “spoon-feeding” to apply nitrogen to the greens. Recommendations for rates of

nitrogen per week can range from 0.048 – 0.18 kilograms/100 m^2, with yearly rates between 3.9 – 8.8 kilograms/100 m^2 (Massey, 2007; McCarty and Canegallo, 2005; Patrick et al., 2006).

Potassium is an important nutrient for regulating physiological processes such as building and strengthening the plant, movement of photosynthates throughout the plant, as well as enhancing the plants resistance to biotic stresses and maintaining proper water status and increasing drought tolerance (Pandey and Mahiwal, 2020; Zörb et al., 2014). Additionally, within the plant cells, potassium is critical in maintaining ion homeostasis, assisting in protein synthesis, osmoregulation, activation of enzymes, as well as regulating membrane potential and charge balance (Egilla et al., 2001; Marschner, 2012; Pandey and Mahiwal, 2020; Umar and Moinuddin, 2002). On a practical level, regarding turfgrasses, potassium is important in increasing the resistance of the turfgrass to stressors such as cold, heat, drought, diseases, and wear (McCarty and Canegallo, 2005; McCarty, 2005). Recommendations on potassium rates are often referred to as a potassium/nitrogen ratio as the critical level of potassium in the plant is around the same as nitrogen (McCarty and Kerns, 2016). Snyder and Cisar (2000) found increasing the K/N ration beyond 0.5 to 1 did not influence turfgrass performance, this ratio can often be increased to 2 prior to seasonal stresses (McCarty and Canegallo, 2005; Snyder and Cisar, 2000).

Phosphorous is a key player in many plant processes including photosynthesis, respiration, cell division, cell enlargement, and energy storage and transfer (adenosine triphosphate). Sufficient phosphorous is critical for early root formation and growth in seedlings and sprigs (Mullins, 2009). In the soil, phosphorous is not as prone to leaching like nitrogen and potassium and as such phosphorous applications are not needed as regularly as nitrogen and potassium. Tissue and soil analysis are the best methods to determine what phosphorus is in the plant and soil and guide when applications are needed. Deficiency occurs when tissue levels fall below 0.2% in newly mature leaves, with an excess when levels are above 1% (McCarty and Kerns, 2016). The minimal soil concentration for phosphorus is 34 kg/ha (Turgeon and Kaminski,

2019). Application of too much phosphorus can create issues such as iron deficiencies. At pH levels below 5, iron and aluminum can form an insoluble complex with phosphorus making them unavailable to the plant (McCarty and Kerns, 2016).

Calcium, magnesium, and sulfur are secondary macronutrient required in similar quantities to phosphorous (McCarty and Kerns, 2016; Turgeon and Kaminski, 2019). Calcium is required in large quantities in meristematic tissues and is responsible for strengthening cell walls, enhancing cell division, and assisting in plant growth, protein synthesis, and carbohydrate movement (McCarty and Kerns, 2016; Turgeon and Kaminski, 2019). Plants take up calcium in the ion form Ca^{+2}, and deficiencies can occur in sandy soils with low cation exchange capacity, low pH, or soils with high levels of sodium which can displace the calcium. Additionally, calcium is an immobile nutrient within the plants and must be available for younger leaves; it also does not move far in the plant when applied through a foliar spray (McCarty and Kerns, 2016).

Magnesium is required for chlorophyll production and makes up 7% of a chlorophyll molecule (McCarty and Kerns, 2016). It is also required for the formation of sugars and other energy reactions and assists in the adsorption and movement of phosphorus (McCarty and Kerns, 2016; Turgeon and Kaminski, 2019). Like most of the other macronutrients, deficiencies can occur in sandy soils if not managed properly especially when clippings are frequently removed, with deficiencies occurring when levels decrease below 4.5 g/m^2 of Mehlich-I extractable magnesium (McCarty and Kerns, 2016). In addition to maintaining proper nutrient levels, the soil should have a balance between the three major cations (calcium, magnesium, and potassium). An ideal balance in the total cation exchange capacity is when it is made up of 60 – 80% calcium, 10 – 15% magnesium, and 2 – 5% potassium (McCarty and Kerns, 2016).

Sulfur is needed for the amino acids cystine, cysteine, and methionine in addition to being a building block for proteins and chlorophyll. Sulfur also plays a role in the soil by

decreasing the pH which can help increase the availability of elements such as iron, manganese, zinc, and phosphorus. Typical leaf concentrations can range from 0.15 to 0.5% of the dry weight (McCarty and Kerns, 2016; Turgeon and Kaminski, 2019). The primary form of sulfur in the soil solution is the sulfate anion (SO_4^{-2}) and is susceptible to leaching like the nitrate form of nitrogen. In anaerobic soil conditions, sulfur containing molecules can reduce to hydrogen sulfide (H_2S) by sulfate-reducing bacteria and lead to the formation of a "black layer" after reacting to soil metals like iron and manganese (Berndt et al., 1987; McCarty and Kerns, 2016).

The micro in micronutrients refers not to the importance of these elements but the quantity in which they are needed. Plants need less than 50 ppm of these elements to function properly, and higher amounts can cause severe turfgrass injury as they reach toxic levels (McCarty and Kerns, 2016; Turgeon and Kaminski, 2019). Most soils contain adequate levels of these elements, but deficiencies can occur with sandy soils or improper pH levels with the latter influencing the availability of these nutrients the most (McCarty and Kerns, 2016). At pH levels below 5, the solubility of aluminum, iron, and manganese increase and can potentially have toxic effects on the plants. With high levels of aluminum, the uptake of calcium, phosphorus, and iron is also reduced. Conversely, pH levels above 7 the solubility of iron, manganese, copper, and zinc decrease making them unavailable to the plant causing deficiencies (McCarty and Kerns, 2016; Turgeon and Kaminski, 2019). A pH of 6.0 to 6.5 is recommended to maintain proper micronutrient availability (McCarty and Canegallo, 2005).

Mowing

Proper mowing practices are critical to properly managing a putting green surface. Mowing plays a key role in managing the green speed of the putting green with lower heights of cut (HOC) increasing the distance the ball can travel, and it is commonly manipulated prior to tournament play when more difficult greens are required (Fagerness et al., 2000; Lulis and

Kaminski, 2022; Nikolai, 2005; Salaiz et al., 1995). However, what must also be understood is the fact that at its core, mowing practices are an abiotic stress on the turfgrass (Dowling and Gross, 2019; Lulis and Kaminski, 2022; Turgeon and Kaminski, 2019). Superintendents must find a balance between green speed and turfgrass health, as other stresses can significantly impact the turfgrass if it is already under high stress levels from mowing. As such, it is recommended to raise mowing heights when other stresses such as drought, temperature extremes, biotic pests, or low light conditions to help the turfgrass better tolerate the natural stressors it is facing (McCarty and Canegallo, 2005; Turgeon and Kaminski, 2019). The general rule for mowing is to not remove more than one third of the green tissue to not significantly remove a significant amount of photosynthetic area and carbohydrate storage which is why mowing creates a stress on the plant (McCarty and Kerns, 2016; Turgeon and Kaminski, 2019). As such, putting greens need to be mown daily to avoid removing more than one third of the leaf area since they are cut short.

The HOC for a putting green is impacted by the species and cultivar used; the heights used for ultradwarf bermudagrasses would not be tolerable for creeping bentgrass or annual bluegrass (*Poa annua* L.) greens (Dowling and Gross, 2019; McCarty and Kerns, 2016; Turgeon and Kaminski, 2019). For ultradwarf bermudagrass, the general range for HOC is 3.2 – 4.7 mm; however, some superintendents have gone as low as 2.5 mm, but it should be noted doing so creates higher stress levels and shallower root systems slowing the recovery of the turf (McCarty and Canegallo, 2005; McCarty and Kerns, 2016).

The quality of cut is also a crucial factor to consider when mowing not just putting greens, but all areas of the golf course. Mowing equipment has improved drastically since its development by Edwin Budding in 1830 (Turgeon and Kaminski, 2019). Today, reel mowers are used on putting greens either in the form of a walk-behind or triplex mowers. A reel mower uses a rotating reel cylinder with blades to guide the turf to a stationary bedknife where it is cut by a shearing action like scissors. A walk-behind mower has a singular reel propelled by a gear-driven

rear drum, while a triplex is equipped with three reels arranged on a three wheeled motorized frame (Dowling and Gross, 2019; Turgeon and Kaminski, 2019). There are pros and cons to each of these machines, but what is most important is the proper setup of the machines and ensuring the blades are properly sharpened and adjusted to provide a higher quality of cut (Dowling and Gross, 2019). If the blades are not set up properly or become dull, they will tear and bruise the leaf blades instead of cutting which can result in browned leaf tips and provide more opportunities for pathogens to enter the plant and cause more damage (Turgeon and Kaminski, 2019).

Ultradwarf bermudagrass are especially susceptible to forming a grain, or horizontal shoot growth, when mown in the same pattern repeatedly. Care should be taken to change the mowing direction with each mowing event to discourage the formation of a grain which can negatively affect the putting performance of the green (Turgeon and Kaminski, 2019). If the grain becomes too extreme on the putting surface, the most effective method to remedy this issue to use aggressive (deep) verticutting perpendicular to the grain should be done followed by mowing to help further remove the horizontal shoots (McCarty and Canegallo, 2005). Turfgrass clippings should also be considered and removed with the use of a bucket attached to the front of the mower to avoid interference with play. With the removal of clippings, the removal of nutrients is also included and should be considered into the fertility program to properly replace these nutrients (McCarty and Kerns, 2016; Turgeon and Kaminski, 2019).

Plant Growth Regulators

Plant growth regulators (PGRs) have come a long way since their initial use to reduce mowing needs on utility turfgrass (Turgeon and Kaminski, 2019). Today, they are a valuable tool for superintendents to effectively manage ultradwarf putting greens (McCullough et al., 2006). There are five classes of PGRs (A – E) with some of the most common for use on turfgrass

coming from Classes A and B, which inhibit gibberellin biosynthesis reducing cell elongation and expansive growth (Turgeon and Kaminski, 2019). Class A PGRs, including Trinexapac-ethyl (TE) (Primo Maxx®) and Prohexadione-Ca (PH)(Anuew®), impact the gibberellin pathway in the late stages, while Class B PGRs, including Flurprimidol (Cutless®) and Paclobutrazol (Profile®, TGR®, Trimmit®), impact the gibberellin pathway in the earlier stages (Ervin et al., 2008; Fagerness et al., 2000; Neware, 2019; Reasor and Brosnan, 2020; Turgeon and Kaminski, 2019; Watschke and DiPaola, 1995). According to the USGA, two of the most used PGRs on ultradwarf greens are Primo Maxx® and Anuew® (Jacobs, 2022). The effects of PGRs on green speed of ultradwarfs has been well researched and shown to provide improvement to an extent, as over applications saw diminished improvements and decreased turf performance (McCarty et al., 2011; McCullough et al., 2006, 2007; Miller, 2007). Typical rates for TE during periods of active growth are around 21 g a.i. ha^{-1} wk^{-1} (Jacobs, 2022). Prohexadione-Ca is becoming popular as a tank mix with TE and is applied around 94 g a.i. ha^{-1} wk^{-1} as it does not have as long of a regulation time as TE (Jacobs, 2022). Recent work by Reasor et al. (2018) has shown the use of growing degree days (GDD) allowed researchers to maximize the benefits of PGR to maximize clipping yield suppression. The researchers found using a reapplication interval of 216 – 230 for TE and 120 – 126 GDD_{10C} for PH. Work by Carroll et al. (2022) supported the previous work by showing the use of a 220 GDD_{10C} reapplication interval for TE resulted in better turf quality than the weekly or twice-weekly applications.

Irrigation

The irrigation on the putting green surface should be managed to evenly apply water to the surface in a way which maximizes overall plant health as well as rooting performance. Sand-based putting greens have the advantage of having a well-draining soil profile to limit oversaturated soils during times of heavy rainfall to maintain playability but need close monitoring to provide enough moisture for proper plant function. When these surfaces are

watered a little daily, it encourages the formation of shallow root systems as there is water readily available at the surface. Instead, infrequent, heavier irrigation application should be used to encourage deeper root growth which will better equip the turfgrass to access proper nutrients and better tolerate drought conditions (Miller, 2007; Qian and Fry, 1996; Turgeon and Kaminski, 2019; Wienecke, 2003).

Several methods are available to assist superintendents in determining how much water they want to apply to the putting greens and when. In addition to thinking about plant health, superintendents must also consider the impact of soil moisture and weather on green speed. When the plants are at their full turgor pressure, they have an increased area which adds resistance to the golf ball as it crosses the putting surface. Conversely, dryer plants exhibit wilt which reduces the surface resistance and increases the green speed (Oatis, 2016). Several methods are available to assist superintendents in determining how much water they want to apply to the putting greens and when. A common method is the use of determining how much water the plant has lost due to evapotranspiration due to the weather and adjusted based on a crop coefficient for the specific species of grass. Using information provided by various weather services through the use an evaporative pan and factoring in bermudagrass use 55 – 65% of the given value, superintendents can determine the amount of water lost from the putting green surface each day (McCarty and Kerns, 2016). From there, they can determine how much and when they would like to return to the soil through irrigation to meet their needs. Soil moisture measuring devices have also become common across golf courses, with time domain reflectometry (TDR) being a prominent one (Karcher et al., 2019; Kenna, 2022). The use of TDR to determine volumetric water content (VWC) is not limited to the turfgrass industry and is also used in agricultural research and crop production (Miller, 2007; Zazueta and Xin, 1994). Time domain reflectometry uses electromagnetic waves sent out into the soil and reflected to the sensors; the time it takes for these waves to return to the sensors differs based on soil water content and soil electrical conductivity

(Miller, 2007; Zazueta and Xin, 1994). The TDR determines the dielectric constant which can then be used to determine the VWC of the soil (Dasberg and Dalton, 1985; Miller, 2007). Using these devices, superintendents can take multiple measurements quickly across the green to determine the amount of moisture in the water and supplement by hand or irrigation when necessary.

Winter Management

One of the major concerns of switching from a bentgrass putting green to an ultradwarf one in the transition zone and mid-South regions of the United States is winter survivability. Some superintendents in this region would rather deal with trying to manage bentgrass during the summer months than prevent winterkill, generic term for turfgrass death during winter months, on ultradwarfs (J. Wooten, personal communication, August 15, 2022). But each year, some golf courses convert bentgrass to ultradwarf bermudagrass on putting greens in the regions. Due to the winterkill concerns, methods have been developed to reduce the risk.

When ultradwarf bermudagrass was first introduced, there was little research on the most effective management practices to use throughout the year. As such, superintendents found ways through trial and error to adjust their management practices to best fit the needs of these new grasses. Preparing for the winter months must come in the early fall when the grass is still growing to make the necessary changes.

A common approach for bermudagrass surfaces in the winter is to overseed the area with a cool season turfgrass such as perennial ryegrass (*Lolium perenne* L.) to provide an aesthetically pleasing surface during the winter months when bermudagrass turns off color as it enters winter dormancy. However, superintendents found not overseeding their ultradwarf greens allowed them to provide a better putting surface, less organic matter accumulation, and an easier transition in the spring (O'Brien and Hartwiger, 2007).

As the turfgrass enters winter dormancy, the green speed at any given height will increase. To prevent excessive green speeds, which make play extremely difficult, superintendents will raise their HOC in the early and late fall to accommodate for this (O'Brien and Hartwiger, 2007). The decision of when to raise and how much varies by superintendent and is impacted by their location, what speed they want, and how much the grass is growing.

Even though the turfgrass is not actively growing during the winter months, moisture management is still a critical part of the winter management program. With the high sand content of USGA root zones and warmer, sunny days, it is easier for the upper few inches of the soil profile to dry out where the growing points of the turfgrass are (O'Brien and Hartwiger, 2007; Richardson and Booth, 2021). When the term winterkill is mentioned, people normally associate this with temperature, but it can also refer to the desiccation of the plants during the winter months (Richardson and Booth, 2021). When the turf is dormant, it is difficult to see the normal signs of wilt occurring; this is why prevention strategies are the best method to prevent winter desiccation (O'Brien and Hartwiger, 2007; Richardson and Booth, 2021). The use of wetting agents on ultradwarf putting greens is a common practice during the summer months to provide consistent soil moisture and deal with any localized dry spots (Richardson and Booth, 2021). However, the use of wetting agents in the late fall and winter has been studied and shown to provide beneficial effects in reducing winter desiccation, particularly during drier winters (DeBoer et al., 2019; DeBoer et al., 2020; Richardson and Booth, 2021).

One of the most effective ways to prevent winterkill on ultradwarfs during extreme cold temperatures is the use of turfgrass covers to increase soil temperature (DeBoer et al., 2019; Goatley et al., 2005; McCarty and Canegallo, 2005; Goatley Jr. et al., 2007; O'Brien and Hartwiger, 2007; Richardson and Booth, 2021; Richardson et al., 2021; Roberts, 1986; Shashikumar and Nus, 1993). The USGA recommended a conservative approach to covering ultradwarfs when the air temperature is predicted to fall below -3.9ºC (O'Brien and Hartwiger,

2007). However, the process of applying covers to all 18 putting greens and practice greens and uncovering presents many financial and logistical problems for superintendents (Richardson and Booth, 2021). Research by DeBoer et al. (2019) evaluated different temperature thresholds for applying covers on ultradwarfs to see if the number of coverings required could be reduced. Their work used a black, woven polypropylene cover with thresholds from -3.9 to -9.4 ºC on cultivars 'Champion', 'MiniVerde', and 'TifEagle' with an uncovered control. After three years of the study, the uncovered control experienced complete winterkill damage, but the cover provided adequate protection for all thresholds used. If the lowest threshold was used instead of the USGA recommended threshold, a golf course would have saved an estimated $9,000 in labor costs over the winter (DeBoer et al., 2019).

To further the cover's effectiveness against extremely cold temperatures, research has been done to evaluate the benefits of adding an air gap between the cover and the turfgrass surface (Richardson and Booth, 2021; Richardson et al., 2021). Researchers have tested the effects of using different strategies to create an air gap including straw, synthetic batting, irrigation pipes, and foam "pool noodles" as well as using one or two covers; however, there was no significant differences between the methods in the canopy temperature and turf winterkill (Richardson and Booth, 2021; Richardson et al., 2021). While these methods can provide a slight benefit over covers alone, it is best reserved for putting greens in poor environments or have a history of severe winterkill to preserve labor, material, and storage costs (Richardson and Booth, 2021).

Another recent method to help combat winterkill issues in the transition zone, is the breeding efforts by Oklahoma State University (OSU) to create high-quality bermudagrasses with enhanced freeze tolerance (Gopinath et al., 2021; Taliaferro et al., 2004). The breeding efforts by OSU has moved away from ultradwarf genotypes and worked on traditional breeding methods to develop interspecific hybrid bermudagrasses capable of tolerating the low mowing heights of

putting greens, but also able to withstand colder temperatures compared to the ultradwarfs. A controlled growth chamber study by (Gopinath et al., 2021) evaluated the performance of select experimental genotypes compared to the 'Champion' and 'TifEagle', as well as 'Tahoma 31' a known cold tolerant hybrid bermudagrass cultivar. 'Champion' has been reported to be a freeze susceptible cultivar by previous work with 'TifEagle' performing slightly better (Anderson et al., 2002), and similar results were found in the work by Gopinath et al. (2021) with 'Champion' having an average LT_{50} value -5.6 ºC and 'TifEagle' was -6.3 ºC. The new genotypes showed significant improvement with LT_{50} values ranging from -7.0 to -8.1 ºC, with one genotype performing statistically the same as 'Tahoma 31' whose values ranged from -7.8 to -9.0 ºC (Gopinath et al., 2021). This work has helped provide the basis for testing of non-ultradwarf genotypes for use on putting green surfaces with improved freeze tolerance.

Simple Sequence Repeat Technology

Marker Assisted Selection

Even though the concept of marker-assisted selection (MAS) was only introduced a little less than 40 years ago by Beckmann and Soller (1983) and Smith and Simpson (1986), the use of phenotypic selection has existed since the early 20th century using observable genes following Mendelian inheritance to act as a marker for the segregation of genes (Ben-Ari and Lavi, 2012; Sax, 1923). The thought process behind this technology was the likelihood that DNA polymorphisms observed were unlikely to be the quantitative trait locus (QTL) but would more likely be linked to the desired QTL and could be used through indirect selection (Ben-Ari and Lavi, 2012). The introduction of MAS into plant breeding has had many advantages including higher efficiency due to decreasing the number of the breeder will eventually test since plants can be tested at an early age (Ben-Ari and Lavi, 2012). However, breeders still need to confirm the presence of the desired gene through field testing. For MAS to work properly, DNA markers

must first be established and go through a linkage analysis to identify the markers that are linked to the genes controlling the desired traits (Ben-Ari and Lavi, 2012). Several methods have been developed to determine and test potential DNA markers. These methods include restriction fragment length polymorphism (RFLP) (Botstein et al., 1980), random amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams et al., 1990), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), simple sequence repeats (SSR) (Edwards et al., 1991; Litt and Luty, 1989), and single nucleotide polymorphism (Collins et al., 1998; Rafalski, 2002).

Development of SSR Technology

Simple sequence repeats (SSRs), also referred to as "microsatellites," are a subcategory of a more general variable number of tandem repeats and contain tandem repeats of short nucleotide motifs (Ben-Ari and Lavi, 2012; Nevo, 2001). These repeats vary from one to five nucleotides and repeat less than a few dozen repeats compared to minisatellites which can contain hundreds of repeated motifs (Ben-Ari and Lavi, 2012; Nevo, 2001). Polymorphisms within SSRs are a result of the number of repeated motifs in the specified loci region, where they experience higher rates of polymorphism compared to nonrepetitive sequences due to errors in replication slippage, sister chromatid exchange, unequal crossing over, and gene conversion (Nevo, 2001). Litt and Luty (1989) first suspected the presence of polymorphism in these microsatellite regions after work identified the presence of repeated sequences of (dT-dG)n, where n = ~10 – 60, across the human genome also displayed elevated levels of polymorphism (Hamada and Kakunaga, 1982; Hamada et al., 1982; Miesfeld et al., 1981; Shen and Rutter, 1984). The use of traditional Southern blotting technique would not be able to detect these polymorphisms as they would differ by only a few base pairs; as such, Litt and Luty (1989) determined using the polymerase chain reaction (PCR) technique (Saiki et al., 1988) would allow researchers to detect these polymorphisms using single-copy primers on either side of the repeats. Additionally, their work

also demonstrated SSRs follow codominant Mendelian inheritance, allowing researchers to distinguish between homozygous and heterozygous loci.

The first reported use of SSR technology in plants was done by Akkaya et al. (1992) in soybeans. Their research identified and investigated ten SSR marker regions containing either dinucleotide or trinucleotide repeats and found the use of SSR technology in plant species would be extremely valuable moving forward. Since then, 250,000 SSRs from almost 4 million genes in 112 different plant species have been identified (Song et al., 2021).

Identification of Bermudagrass SSR Markers

Several researchers have worked to develop a catalog of SSR markers for common bermudagrass which has been beneficial to breeding efforts across the world. Kim et al. (2008) conducted a large-scale comparative genomic analysis of 9414 unigenes from expressed sequence tags (ESTs) and found 1.5% of the ESTs contained SSRs, which were then used to develop 95 EST-SSR primer pairs (PPs). Another 16 EST-SSR markers were developed by Jewell et al. (2010) through characterization of the EST-SSR primer sequences discovered by Kim et al. (2008). Around the same time, work by Harris-Shultz et al. (2010) developed 53 EST-SSR PPs using the sequences of Kim et al. (2008). Using SSRs developed for *Sorghum bicolor*, Tan et al. (2012) transferred 65 for use in C. dactylon and C. transvaalensis. Their work also resulted in 230 EST-SSR PPs by examining 20,327 *Cynodon* ESTs available through the National Center for Biotechnology Information. Kamps et al. (2011) developed another 25 SSR markers using a genomic library which was enriched for the GA/GT repeat motif.

While the development of useful SSR markers and PPs are critical for their extended use to better understand the *Cynodon* genome, it is also critical to develop linkage and QTL mapping resources to better understand how these markers relate to desired genes. With this research goal in mind, Guo et al. (2017) used 1003 SSR PPs to construct a linkage map using a first-generation

selfed population. The researchers were able to use 249 polymorphic SSR PPs to map 18 linkage groups. Combining this knowledge with data collected for ground coverage in replicated field trials, the researchers used QTL mapping to identify five genomic regions related to this trait (Guo et al., 2017). With the development of this and other new understandings of the bermudagrass genome, SSR markers have been able to be used in a number of different applications including identifying different cultivars, off-types, evaluating genetic diversity, and separating selfed and crossed individuals (Fang et al., 2017; Harris-Shultz et al., 2011; Harris-Shultz et al., 2010; Kamps et al., 2011; Khanal et al., 2017; Ling et al., 2012; Reasor et al., 2016; Reasor et al., 2017; Tan et al., 2014). The use of this technology and new continue to broaden the understanding of the *Cynodon* genome and the various locations of critical genes for future work.

Remote Sensing Phenotyping Using Unmanned Aerial Vehicles

Remote Sensing

Remote sensing is a general term referring to the capturing and recording of information without directly contacting the object (Gibson et al., 2013). It represents the process of evaluating physical characteristics by measuring the reflected and emitted radiation from a distance and has a broad application (USGS). Remote sensing today has shifted from the use of satellites to capture images to the use of unmanned aerial vehicles (UAVs) and handheld remote sensors due to their ability to capture fine spatial and high temporal resolution data (Wang et al., 2022; Wang et al., 2020). The use of these UAV methods of remote sensing have been well documented in the agricultural field (Ashapure et al., 2020; Bhandari et al., 2020; Huang et al., 2018; Hunt et al., 2005; Jung et al., 2019; Primicerio et al., 2012; Wang et al., 2013; Wójtowicz et al., 2016; Xiang and Tian, 2011). These methods will use vegetation indices (VIs) to evaluate the physical characteristics of crops unable to be detected with the human eye. One common example of a VI is the normalized vegetation index (NDVI) which measures the difference between near-infrared

and red light (Tarpley et al., 1984). Vegetation indices in general use the relationships between two or more wavelengths to determine various vegetation properties (Jackson and Huete, 1991). These vegetation properties can then be used to determine several aspects of plant health and performance.

Remote Sensing in Turfgrass

The concept of using remote sensing in turfgrass research is not new. Researchers have attempted to find methods to reduce rater bias and fatigue which can occur with traditional visual assessments commonly used in turfgrass studies (Horst et al., 1984; Morris and Shearman, 1998; Trenholm et al., 1999). Several studies have shown the relationships between traditional visual quality methods and ground based VI measurements (Bell et al., 2002; Bremer et al., 2011; Fitz–Rodríguez and Y. Choi, 2002; Jiang and Carrow, 2005, 2007; Lee et al., 2011; Trenholm et al., 1999). These VI-based measurements have also been shifted to UAVs to help more efficiently collect this quantitative data. Xiang and Tian (2011) evaluated turfgrass response to glyphosate applications using an unmanned helicopter and ground survey, finding only 1.5% difference in the estimation of herbicide damage between the two. Other studies have evaluated the nitrogen content and drought status of different turfgrass species. Caturegli et al. (2016) used UAV based NDVI to evaluate differences in nitrogen content based on application gradients for three turfgrass species (*C. dactylon x transvaalensis*, *Zoysia matrella*, and *Paspalum vaginatum*). They found UAV imagery was able to assess the nitrogen content in the turfgrass, as well as its spatial variability making its use for detecting nitrogen deficiencies in large areas like golf courses and sod farms beneficial. Hong et al. (2019a) and Hong et al. (2019b) have used UAV based remote sensing to evaluate drought stress in turfgrass systems. Hong et al. (2019a) used thermal imaging to detect drought stress and were able to detect rises in turfgrass canopy temperature prior to visual symptoms of the stress; however, the data was more strongly correlated with the visual and percent green cover (PGC) than VWC. Hong et al. (2019b) Over the three-year study, near

infrared (NIR, 680 – 780 nm) and GreenBlue VI [(G-B)/(G+B)] showed the most sensitive Vis, detecting the drought stress >5 days before visual decreases in turf quality were observed. Additional work by Zhang et al. (2019) used UAVs to evaluate the performance of different genotypes in a turfgrass selection trial. This study found the use of RGB cameras may provide a benefit in predicting ground-based PGC over multispectral cameras and VIs, as well as the ability to use UAV-based imagery methods to identify top performing genotypes in turfgrass selection trials.

Research Purpose and Objectives

This study's overall objective was to evaluate experimental hybrid bermudagrass genotypes from three universities for use on golf course putting greens. In recent years, the need for more genetically diverse options for bermudagrass putting greens across the southern United States and transition zone has become increasingly more demanding. Breeding work by Oklahoma State University (OSU) and Mississippi State University (MSU) has sought to introduce genetic diversity through traditional breeding methods while maintaining the high-quality performance of the putting greens golfers and superintendents' desire. This study was conducted to provide required knowledge of the genetic background and field performance of these experimental genotypes to determine their viability as a future released cultivar. As these new genetics are introduced into this area of turfgrass breeding, there is a lack of knowledge on the morphological influencers of ball roll distance or green speed of different genotypes. An additional objective of this study was to evaluate the performance of UAV based imagery methods in evaluating turfgrass performance on a replicated field trial as these methods become more popular within the industry. As such, the study was divided into three studies to help answer these questions.

Chapter 2

The ultradwarf genotypes currently used on golf course putting greens have a limited genetic background. These grasses have come about from mutations dating back to a singular cultivar 'Tifgreen'. This has caused issues when superintendents try and select a cultivar best suited for their geographical location. To assist with these issues, OSU and MSU have used traditional breeding methods to introduce new genetics which could be used for golf course putting greens. There is little understood about the genetic and morphological diversity between the ultradwarf genotypes and these new experimental genotypes.

Goal

To quantify the genetic diversity between the included genotypes using SSR markers and morphological characteristics.

Objectives

1. To determine the genetic similarity coefficients of 14 experimental genotypes and 2 commercial standards using 53 SSR PPs and use these to evaluate the genetic diversity among the genotypes.
2. To quantify various morphological characteristics of the included genotypes in a field and greenhouse settings and use these to assist in evaluating the genetic diversity present.

Hypotheses

1. There will exist genetic diversity among the genotypes.
2. The ultradwarf genotypes will group close together.
3. The OSU and MSU genotypes will group separately due to different parent materials.

Chapter 3

It is important to understand how genotypes will perform, particularly under extreme conditions putting greens face, before moving them to release. Important visual factors such as turf quality, as well as performance of the putting surface measure through ball roll distance need to be understood when deciding which genotypes to release. A replicated field study will be used to further evaluate the performance of these genotypes from establishment to gain a better understanding of the diversity among the genotypes.

There is also a lack of knowledge on the factors influencing the ball roll distance for various genotypes. Since extensive morphological characteristics are being measured for the genetic aspect of this study, this data can also be used to determine which characteristics influence the ball roll. This knowledge will allow future breeders to select for these traits early in the breeding process, saving time when large field trials are eventually needed.

Goal

To identify top performing genotypes under putting green management and determine baseline performance of new experimental genotypes.

Objectives

1. To assess the performance of experimental genotypes compared to commercial standards through visual and quantitative measurements.
2. To evaluate the relationship between ball roll distance and select morphological characteristics.

Hypotheses

1. At least one of the experimental genotypes will show promising qualities for use as a putting green cultivar.
2. At least one morphological characteristic will explain variation observed in ball roll distances.

Chapter 4

One common method in determining turfgrass cover and health is using percent green cover through digital analysis. When turfgrass trials become larger, the time and labor required to obtain this data can get large as well. Recent studies have worked to gain a better understanding of the relationships between various VIs using UAVs. There have been promising results showing these indices can be used to determine various aspects of turfgrass health. While the work has shown these relationships under fully established turfgrass trials, few have evaluated the indices throughout the establishment period and the duration of the trial. Additionally, no work has been done to compare how these relationships change as the trials reach full establishment. If the industry moves to one of these UAV methods, it would be ideal for the relationship to be similar between the establishment period and after to be able to easily compare values from each time.

Goal

To evaluate the relationship between various VIs and PGC on a replicated turfgrass trial from establishment through full coverage and evaluate how the relationship changes between the two.

Objectives

1. Evaluate the relationship between select VIs and the PGC during and after establishment.
2. Determine the effectiveness of the indices in predicting PGC.
3. Determine which indices have similar relationships for both periods.

Hypotheses

1. At least one index will be able to effectively predict the PGC.
2. At least one index will show similar relationships during both periods.

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CHAPTER II

MEASUREMENT OF GENETIC DIVERSITY OF GREENS-TYPE HYBRID BERMUDAGRASS SELECTIONS THROUGH SSR MARKERS AND MORPHOLOGICAL ANALYSIS

Abstract

The development of 'Tifgreen' bermudagrass, and the subsequent mutations of this cultivar, has revolutionized the game of golf in the southern United States as superintendents now have access to a high-quality putting surface capable of withstanding hot summers. However, due to the nature of mutations, the genetic makeup of these grasses on the market has not changed much throughout the years. The lack of diversity may make them vulnerable to various natural pests that can impact playability and freeze temperatures that inflict winterkill. In recent years, Oklahoma State University and other universities have begun to create new hybrids between Common (*Cynodon dactylon*) and African bermudagrasses (*C. transvaalensis*) that are able to withstand putting green mowing heights while increasing the genetic diversity within this class of bermudagrass. The objectives of this study were to investigate the genetic diversity and variation among the 14 experimental genotypes and two standard cultivars. Simple sequence repeat (SSR) markers and morphological characteristics were used to evaluate the genetic relationships and variation among the 16 genotypes. The results showed significant variation for the 11 vegetative traits and two reproductive traits. The phenotypic CV was

greatest for the third internode length and the least for the first leaf angle for both the field and greenhouse trials. The other traits ordered differently within each trial. The morphological cluster analysis also showed variation between the two trial environments. The SSR marker cluster analysis showed the 16 genotypes were grouped into four major groups, with a similarity coefficient ranging from 0.44 to 1. The highest level of similarity was found among the two commercial standards and an experimental ultradwarf. This highlights the lack of diversity found within these ultradwarf bermudagrasses but can be introduced through traditional crossing methods. Several new genotypes also grouped with the ultradwarf genotypes in the morphological cluster analysis showing the possibility of achieving similar morphological traits with new genetics.

Introduction

Prior to the development of 'Tifgreen' bermudagrass [*Cynodon dactylon* L. *x C. transvaalensis* Burtt-Davy (2n=3x=27)] in 1956, golf courses in the southern United States relied on either closely mowed pasture grasses, bentgrass (*Agrostis stolonifera* L.), local bermudagrass strains, or sand (Morris, 2003). While these options provided a playing surface, they were not best suited for southern states with scalping, disease, and heat pressures as superintendents lowered the height of cut to meet player expectations (Morris, 2003). Since the development of this cultivar, the use of greens-type bermudagrass in the transition zone and southern United States has increased, but using these grasses comes with their own set of issues (O'Brien and Hartwiger, 2007). These issues include fungal diseases such as spring dead spot (*Ophiosphaerella* spp.), Pythium blight (*Pythium* spp.), and leaf spot (*Drechslera* and *Bipolaris* spp.), and thatch accumulation (Unruh and Davis, 2001).

Dr. Glenn Burton developed 'Tifgreen' from a cross between a common bermudagrass (*C. dactylon*) selected from a green in North Carolina and an African bermudagrass (*C.*

transvaalensis) resulting in a fine textured hybrid bermudagrass superintendents began to use on their greens (Hein, 1961). James Moncrief, a U.S. Golf Association Green Section agronomist, discovered a mutation of Tifgreen in South Carolina. The natural mutant, released as 'Tifdwarf', possessed more dwarfed growth characteristics, which allowed it to tolerate closer mowing heights sought after on golf courses (Baxter and Schwartz, 2018). These two cultivars are the foundation for the ultradwarf cultivars on the market today. Some popular cultivars including 'Champion Dwarf', 'FloraDwarf', 'Mini Verde', and 'TifEagle' appear to be somatic mutants from either 'Tifgreen' or 'Tifdwarf' as revealed using DNA fingerprinting (Harris-Shultz et al., 2011; Wang et al., 2010).

Even though these grasses have been specifically selected because of their ability to tolerate the mowing heights superintendents desire, the stress from this management practice can lead to a decline in quality and increase the possibility of disease damage (Unruh and Davis, 2001). Suboptimal growing conditions can also increase stress and lead to a decline in bermudagrass quality. This stress on the bermudagrass also increases the opportunity for disease to multiply (Unruh and Davis, 2001). With the cultivars on the market coming from mutations from 'Tifgreen' or 'Tifdwarf', the base genetic material is highly similar. Using 11 simple sequence repeat (SSR) markers, Wang et al. (2010) reported the genetic similarity coefficients were one (i.e., 100%) between 'Champion Dwarf', 'FloraDwarf', 'MiniVerde', 'Tifdwarf', 'TifEagle', 'Tifgreen', 'Baby', and 'MS-Supreme'. Additional studies have shown high genetic similarity among ultradwarf cultivars (Capo-chichi et al., 2005; Fang et al., 2017; Harris-Shultz et al., 2010; Harris-Shultz et al., 2011; Reasor et al., 2016; Reasor et al., 2017; Zhang et al., 1999).

The introduction of more genetic diversity from crosses conducted at Oklahoma State University may increase host plant resistance potential to abiotic and biotic stresses. Growing in the transition zone, these grasses can often experience winterkill from sustained below freezing temperatures (Patton, 2012). The use of cold-hardy parent material can also allow for selection of

genotypes that can tolerate colder temperatures without needing to be covered (Taliaferro et al., 2004). Gopinath et al. (2021) demonstrated statistically improved mean lethal temperatures resulting in 50% survival of OSU experimental genotypes compared to commercial cultivars 'Champion Dwarf' and 'TifEagle'. One genotype demonstrating statistically similar tolerance as 'Tahoma 31', a known cold tolerant cultivar (Wu, 2020).

In addition to OSU, Mississippi State University's breeding program also works on traditional hybridization to develop bermudagrass putting green genotypes. These genotypes would potentially have a different genetic background to the OSU genotypes as well as the ultradwarfs. There is little information on the magnitude of genetic diversity among the genotypes mentioned. Also, there is no information regarding the morphological differences between the ultradwarf varieties and the new experimental genotypes to help further reveal genetic differences. As such, the objective of this study was to characterize the genetic diversity among experimental greens-type bermudagrasses compared to two ultradwarf cultivars using SSR markers and morphological characteristics. It is hypothesized there will exist significant diversity among the included genotypes and the ultradwarf cultivars used will cluster together. In addition, DNA marker profiles and morphological characteristics will be established for the new clonal genotypes for potential patent applications.

Materials and Methods

Plant Materials

Sixteen genotypes were used in this study. This included 12 advanced selections from Oklahoma State University's breeding program, one advanced selection from Mississippi State University, and sone selection from the University of Florida's breeding program, respectively, and the commercial standards 'TifEagle' and 'Tifdwarf' (Table 1). One experimental location (F6 block, OSU Turf Center, Stillwater, OK, 36.124210, -97.101974) was used in the field

morphological data collection. For each entry, 48 (16 x 3 reps) conetainers were prepared in the greenhouse by planting one sprig and grown for three months. These plants were then transplanted in the field in a randomized complete block design with three replications and twenty subsamples per replication. The plots were 1.5 m x 1.5 m with 0.6 m alleys, and 16 plugs were transplanted on 0.3 m centers within each plot. The plots were not mown to allow for natural growth of the plant, but alleys were kept clear by spraying glyphosate (Buccaneer Plus, Tenkoz, Inc., Alpharetta, GA, USA) to avoid contamination of neighboring plots. The plots were allowed to mature for three months prior to the first data collection. Prior to spring green up the following year, the plots were mown to 1 cm and allowed to regrow for three months prior to the second data collection. Additionally, one experimental location (OSU Controlled Environmental Research Lab, Stillwater, OK, 36.128957, -97.085345) was used for the greenhouse morphological data collection. For each entry, three (30 cm x 25 cm) pots were prepared by planting approximately ten stolons in each pot. The pots were arranged in a randomized complete block design with three replications, with one table in the greenhouse containing a replication and twenty subsamples per replication. The pots were rearranged weekly within the table to expose the plants to equal light. Only the sides of the pots were trimmed to avoid contamination of neighboring pots and allow natural growth of the plants. The pots were allowed to mature for three months prior to the first data collection, afterwards the pots were trimmed to the soil surface and allowed to regrow for three months prior to the second collection. Fresh leaf tissue used for the genetic testing was collected from the original pots.

Morphological Characteristic Data Collection

Morphological characterization was conducted September 2021 and July 2022 for the field trial and May and August 2022 for the greenhouse trial. The variation and diversity of the morphological characteristics was done by evaluating eleven vegetative and two reproductive traits. Turf canopy height was obtained in in the field or greenhouse, while all others were

evaluated after transferring the material to the laboratory. The materials were stored in a -20 °C freezer to preserve the tissue until they were able to be measured (Guo et al., 2017). Turf height was measured using a ruler in twenty random points in each plot or pot. Additionally, 20 stems were collected randomly from the entire plot or pot area. Leaf length, leaf width, internode length, internode diameter, and inflorescence length were measured using a General Ultratech digital caliper (General Tools and Instruments, Secaucus, NJ, USA). The leaf angle was measured by taking an image of the stem, and using ImageJ software (Schneider et al., 2012) to measure the angle from the leaf sheath and the bottom of the leaf. As such, larger values indicate a more upright leaf growth, with lower values indicating a flatter leaf growth. Leaf length, width, and angle were measured on the two leaves on the second newest node. The internode length and diameter were taken then on the second and third internodes. Spikelet number indicates the number of spikelets on each inflorescence, and total inflorescence length is the sum of all spikelets.

DNA Extractions, Polymerase Chain Reaction (PCR), Amplification, and Electrophoresis

Five to ten fresh leaves were collected from each genotype and placed in a -20 °C freezer prior to DNA extraction. DNA isolation methods followed protocol outlined in Fang et al. (2017). After the leaves were stored for 24 hours, the tissue was ground into a fine powder using a GenoGrind (Spex Sample Prep, Metuchen, NJ, USA). A DNA extraction buffer was added to each sample to lyse the cells and release the DNA from the nucleus. To remove the proteins and other organic materials, a mixture of phenol, chloroform, and isoamyl alcohol was added, and after mixing the solution was centrifuged to separate the DNA from the other materials. To precipitate DNA from the solution, sodium acetate and cold isopropanol was added to the solution and centrifuged again. The DNA pallet was then washed with ethyl alcohol, dried, and resuspended in nuclease free water. The DNA quality and concentration was measured using a

ND 1000 spectrophotometer (Nano Drop Products, Wilmington, DE, USA), and then 1000 µl with a DNA concentration of 10 ng/µl was prepared for PCR amplification.

A total of 54 SSR markers were selected and used for genotyping the sixteen genotypes. These SSR markers were selected to cover the majority of the common bermudagrass whole genome based on the linkage map of 1094.7 cM (Guo et al., 2017). The DNA samples were amplified in 10.5 µl reaction volumes containing 1 µl of 10x standard PCR buffer, 0.2 µl of 10 mM deoxyribonucleotide triphosphate, 2 µl of forward/reverse SSR primer, 0.2 µl of either 1 mM M13 700 or 800 florescence, 0.05 µl of Taq DNA polymerase, 1.5 µl of 10 ng/µl DNA solution, and 5.55 µl nuclease free water. Biosystems 2720 thermal cyclers (Applied Biosystems, Waltham, MA, USA) were used to conduct the PCR using cycling parameters of 94 °C for 5 minutes, the 14 cycles of 94 °C 20 seconds, 58 °C 1 minute, 72 °C 30 seconds; 28 cycles of 94 °C 20 seconds, 55 °C 1 minute, 72 °C 30 seconds; 72 °C for 10 minutes and held at 4 °C. After the initial cycle, 5 µl of Blue Stop solution was added (95% formamide, 20 mM EDTA, and 0.05% bromophenol blue) and spun down before being placed in the thermocycler again for 3 minutes at 94 ºC. To detect for amplified fragments from the SSR markers, a LI-COR 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, NE, USA) was used to separate the PCR products using 6.5% Long Ranger™ Gel. The bands were scored visually twice to ensure accuracy using a “1” to indicate band presence, “0” for absence, and “9” for an ambiguous band in an excel spreadsheet.

Data Analysis

To determine genetic similarity using the SSR marker data, the SIMQUAL module of the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) version 2.2 was used to generate a Jaccard’s genetic similarity coefficient matrix. Using this matrix, a dendrogram was constructed using the unweighted pair group method arithmetic average (UPGMA) to determine genetic relationships between the sixteen genotypes. The data analysis for the morphological

measurements was completed using SAS/STAT® software (Version 9.4 for Windows, Cary, NC, USA). A linear mixed methods analysis was used to determine the least squares means of the entries for each trial location and year. The morphological cluster analysis was conducted using SPSS software (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp).

Results

Phenotypic Variation of Morphological Characteristics

There was significant variation among the morphological characteristics measured in this study, as well as location and year variation among the 16 genotypes (Tables 3 and 4). For the greenhouse study, the highest phenotypic variation values were observed for the 3rd and 2nd internode lengths, with values of 49.00% and 44.70%, respectively. The minimum coefficient of variation (CV) was observed for the 1st and 2nd leaf angles, which were 9.92% and 10.00%, respectively. For this study, the morphological CV was 3rd internode length > 2nd internode length > 1st leaf length > raceme length > 2nd leaf length > canopy height > 2nd internode diameter > 3rd internode diameter > 2nd leaf width > raceme number > 1st leaf width > 2nd leaf angle > 1st leaf angle.

The second greenhouse study had the same characteristic with the highest CV, 3rd internode length – 48.52%. However, the second highest for this year was the 1st leaf length with a CV of 44.53%. The minimum CV observed for this year was raceme number and 2nd leaf angle, which were 6.22% and 11.18%, respectively. The order of morphological CV for this year was 3rd internode length > 1st leaf length > 2nd leaf length > 2nd internode length > canopy height > raceme length > 3rd internode diameter > 2nd internode diameter > 2nd leaf width > 1st leaf width > 1st leaf angle > 2nd leaf angle > raceme number.

The two years of field studies resulted in the same characteristics with the maximum and minimum CV. The maximum values were observed for 2nd and 3rd internode length, with values 75.16% and 72.44% for year one, respectively, and 74.15% and 73.98% for year two, respectively. The minimum values were observed for the 1st and 2nd leaf angles, with values 11.75% and 11.55% for year one, respectively, and 9.70% and 9.46% for year two, respectively. The order of morphological CV for the first year was 2nd internode length > 3rd internode length > canopy height > 2nd leaf length > 1st leaf length > raceme length > 2nd leaf width > raceme number > 3rd internode diameter > 2nd internode diameter > 1st leaf width > 2nd leaf angle > 1st leaf angle. For the second year, the order was 2nd internode length > 3rd internode length > canopy height > raceme length > 2nd leaf length > 1st leaf length > 3rd internode diameter > raceme number > 2nd internode diameter > 2nd leaf width > 1st leaf width > 2nd leaf angle > 1st leaf angle.

An analysis of variance using linear mixed methods was used to evaluate if there was a significant difference between the two years of each trial location and among the traits measured. These tests indicated there was a significant difference between Year 1 and Year 2 of the field trial for all traits ($p<0.001$). Significant differences were also seen among genotypes for all traits within each year ($p<0.001$). For the greenhouse trials, there was also a significant difference between Year 1 and Year 2 for all of the traits ($p<0.01$). There was also a significant difference among genotypes for all traits within each year ($p<0.01$)

Morphological Characteristic Cluster Analysis

The squared Euclidean distance using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used to analyze the 16 genotypes based on the fourteen observed morphological characteristics. To gain a better understanding of how these genotypes grow and incorporate year to year variation, the two years data was averaged together for both years of the field data, and then the two greenhouse trials were also averaged.

For the greenhouse trials, based on a Euclidean distance of 20, all genotypes were separated into three main groups (Figure 1). Group 1 was comprised of 3x23; which had long, slender leaf blades, long internodes, but a tall canopy height. Based on a Euclidean distance of 15, Group 2 was divided into two subgroups A and B. Subgroup A contained OKC0920, which had long, thicker leaf blades with long internodes and a tall canopy. Subgroup B was comprised of OKC0805, OKC1609, and 63x18. This group displayed long, thin leaf blades with long internodes and an intermediate canopy height. Group 3 was divided by a Euclidean distance of 10 into Subgroups C and D. Subgroup C contained genotypes OKC3920, 15x9, 12x3, MSB1050, and 11x2. This subgroup had intermediate leaf lengths with thin leaf blades, as well as intermediate internode length and canopy height. Subgroup D contained standards 'TifEagle' and 'Tifdwarf' as well as FB1901, 5x23, 34x20, and 19x19. This subgroup displayed short leaf lengths with intermediate leaf thickness, short internode lengths with a short canopy height.

The field trials resulted in slightly different clustering based on different growth habits (Figure 2). Based on Euclidean distance of 15, the sixteen genotypes separated into two main groups. Group 1 included OKC0920, 15x9, and MSB1050. This group had long, thick leaf blades and internodes, with a tall canopy height and long raceme lengths. Group 2 was divided into two subgroups A and B based on a Euclidean distance of 10. Subgroup A was subsequently divided into two small groups a and b. Small group a contained OKC1609 and 12x3 which had short leaves with intermediate thickness, and short, thin internodes with a short canopy height and short raceme lengths. Small group b contained 5x3, 34x20, 19x19, OKC3920, FB1901, and 'TifEagle'. This group also had short leaf blades with an intermediate thickness, but had short, thick internodes along with a short canopy height and short raceme length. Subgroup B had entries 11x2, 'Tifdwarf', 3x23, OKC0805, and 63x18. This subgroup displayed intermediate leaf length and thickness, with intermediate internode length and thickness, as well as intermediate canopy height and raceme length.

SSR Polymorphisms

The chosen 54 primer pairs displayed high polymorphisms, generating a total of 311 bands, and 302 bands showed rich polymorphism (97.11%). Among the 53 primer pairs, 46 pairs displayed 100% polymorphism. The number of bands ranged from 3 to 11, with a mean of 5.9 bands per primer pair. The primer pair CDGA8-1807/1808 generated the greatest number of bands (11 bands).

Genetic Diversity and Cluster Analysis using SSR Markers

Three hundred and two polymorphic bands were analyzed for genetic diversity among the 16 genotypes evaluated. The genetic similarity coefficient (GSC) ranged from 0.44 to 1.00. The highest genetic similarity was found to be among the three ultradwarf genotypes ('TifEagle', 'Tifdwarf', and FB1901), with a value of 1.00. The smallest GSC was among the ultradwarfs and OSU genotype 11x2, with a value of 0.44. A wide range of GSCs (0.56 – 0.93) was detected among the OSU developed genotypes. Among the OSU derived genotypes and the MSU genotype, the range in GSC was found to be 0.59 – 0.64. The cluster analysis using unweighted pair group method arithmetic mean (UPGMA) showed the 16 genotypes were grouped into 2 main groups (Figure 3). Group I included all three ultradwarf derived genotypes. Group 2 then divided into three subgroups, A, B, and C. The MSU genotype MSB1050 was the only genotype in Subgroup A. Subgroup B was the largest group and included seven of the OSU genotypes (OKC3920, 15x9, 3x23, 5x23, 34x20, 11x2, and 63x18). The Subgroup C contained the remaining five OSU genotypes (OKC0805, OKC1609, 12x3, OKC0929, and 19x19). Overall, there was a significant genetic difference among the ultradwarf genotypes and those created through traditional breeding.

Comparison of Dendrograms Derived from Morphological Characteristics and SSR Markers

Overall, the dendrograms generated from the two morphological studies and the molecular markers are inconsistent. However, there were some similarities among the three graphs. Genotypes 5x23, 34x20, and 19x19 clustered below a squared Euclidian distance of 5 in both morphology studies. 5x23 and 34x20 also cluster together based on molecular markers, but not 19x19. The greenhouse and molecular marker cluster analysis showed the ultradwarf genotypes cluster together, but 'Tifdwarf' did not cluster with 'TifEagle' and FB1901 in the field study. The greenhouse and molecular marker clusters also agreed on grouping OKC3920 and 15x9 close together. In the two morphology studies, entries OKC0805 and 63x18 clustered close together, but based on the molecular markers they were in different clusters.

Discussion and Conclusion

This study found significant variation among the evaluated genotypes for morphological characteristics. In the four experiments conducted, the internode length displayed the greatest variation, with the maximum coming from the 2nd internode length of the first field trial (75.16%). As expected, the ultradwarf genotypes had some of the shortest internode lengths, but some experimental genotypes such as OKC3920 also displayed short internodes. Likewise, the leaf angles demonstrated the lowest variation, with the minimum coming from the second field trial 1st leaf angle. In both trial locations, the leaf angle for both leaves typically fell between 130 and 140 degrees. The raceme number of the second greenhouse trial had a CV of 6.22%; however, only six entries of the sixteen produced any seedheads to be evaluated.

Through morphological cluster analysis, the sixteen genotypes were divided into 6 and four different groups for the greenhouse and field trials, respectively. It was expected that the ultradwarf varieties would cluster together as they would likely have similar dwarfed characteristics. This was the case for the greenhouse trial as all three clustered together based on

their short but wider leaf blades compared to others and a short canopy height. In the field trial 'TifEagle' and FB1901 cluster together with four OSU genotypes also showing short leaves and a short canopy height, but also a shorter raceme length. In the field, 'Tifdwarf' clustered with another group that displayed longer leaf blades and a longer raceme length. The OSU genotypes formed the other clusters based on similar morphological characteristics. The MSU genotype clustered with OSU 15x9 in the field and greenhouse studies, indicating the two perform similarly in different environments. The two studies showed some differences in the grouping of the evaluated genotypes. As shown by tables 3 and 4 the field trial overall had higher variation among the measured characteristics. This is likely caused by the variation that accompanies field trials, particularly temperature and light quantity and quality. The greenhouse was able to provide a consistent temperature and light profile throughout the study and most likely allowed the plants to grow to their truest potential.

Simple sequence repeat markers developed for common bermudagrass (*C. dactylon*) have been shown to be an effective method for identifying genetic differences among hybrid bermudagrass genotypes (Fang et al., 2017; Guo et al., 2017; Harris-Shultz et al., 2011; Reasor et al., 2017; Wang et al., 2010). In this study, the 53 selected SSR primer pairs resulted in 311 clear bands, including 302 polymorphic bands. Using these polymorphic bands, cluster analysis by UPGMA showed the sixteen genotypes grouped into two main groups based on a similarity coefficient of 0.60. These results differed from those obtained from the clusters created based on the morphological characteristics. The molecular markers used were unable to distinguish between the three ultradwarf genotypes, and separated the MSU genotype from the OSU genotypes, which formed one and two subgroups, respectively.

This comparison of ultradwarf genotypes and greens-type hybrid bermudagrass developed by traditional crosses shows significant genetic variation can be introduced to this market of hybrid bermudagrass through traditional breeding. Breeders can select genotypes

exhibiting improved traits compared to the ultradwarf genotypes, such as cold tolerance (Gopinath et al., 2021), by introducing a more diverse genetic background. This work also demonstrates even though genetic variation has been introduced, some genotypes possess similar morphological characteristics to the ultradwarf genotypes as indicated by the morphological clusters. Similar morphology could indicate their ability to perform adequately under low mowing conditions while also introducing genetic diversity which can be utilized by golf course superintendents. More work is needed to evaluate these genotypes' performance under low mowing heights to further understand this relationship; however, this work provides a basis as more programs look towards traditional breeding methods to develop greens-type hybrid bermudagrass genotypes to provide solutions to challenges facing the golf course industry.

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Table 1. Experimental genotype/variety name and origin/reference of the 16 *C. dactylon* x *C. transvaalensis* hybrids included in this study.

Entry Number	Entry Name	Origin/Reference
1	OKC0805	Oklahoma State University
2	OKC0920	Oklahoma State University
3	OKC3920	Oklahoma State University
4	OKC1609	Oklahoma State University
5	3x23	Oklahoma State University
6	5x23	Oklahoma State University
7	11x2	Oklahoma State University
8	12x3	Oklahoma State University
9	15x9	Oklahoma State University
10	19x19	Oklahoma State University
11	34x20	Oklahoma State University
12	63x18	Oklahoma State University
13	MSB1050	Mississippi State University
14	FB1901	University of Florida
15	'TifEagle'	Hanna and Elsner (1999)
16	'Tifdwarf'	Burton (1966)

Table 2. List of Simple Sequence Repeat marker primer pairs used in this study

No	SSR PP	Linkage Group	Forward Primer Sequences (5'-3')	Reverse Primer Sequences (5'-3')
1	CDCA1-11/12	2	AGGAACTCCAAGATGATGCC	TCAAGTCGCTTGGATTCTTG
2	CDCA1-21/22	5	GGGCCTCCCCTTTTATACAT	GGTAACCAATCAAGGCCACT
3	CDCA1-25/26	10	CCTGTGTTAGCCTGCACTGT	ATTAGTTTGTAGTGGCGGGC
4	CDCA1-27/28	18	GCTCTGACGACTCACCAAAA	GAGCCTAAGTGGCTGAGGAC
5	CDCA1-81/82	18	CCATATAACGGGTTCAGCCT	TCAAGAGTTCAGCCTCATGC
6	CDCA2-125/126	16	AAATTGACCCTCCACAAAGC	AGTCAGGGGTTTCCATTTTG
7	CDCA2-227/228	11	CTTCTGAAATGCATGGGATG	GGGTGAACACTGCTGATGAC
8	CDCA3-245/246	3	GTGTGAAACGGCATACATGA	TAACACACGCCCTTTCAATG
9	CDCA3-299/300	1	CTTTTGTGAGCCAGAAGCAA	GGGTCATGAGTCAAATGTGC
10	CDCA4-319/320	4	CATGTTCCAGACAAGGATGG	GCAACAAACAGCCACAGAAT
11	CDCA4-325/326	17	CGTACGACCGAGTTCTCTGA	GAAGATGTCATCACGATGGG
12	CDCA5-431/432	12	GCGCACGTAGTAGTAGCAGC	TCTTTTGTAATCAGGCGTCG
13	CDCA5-501/502	6	ATACACCCATCCATCGCTTT	GCTGAAGAAGGATGCAGACA
14	CDCA6-535/536	13	GTTTCAGAGTGCAGAGCCAA	GAATAATGCGATGCTGTTCC
15	CDCA6-559/560	9	CATTTCCAGCAACTCTGCAT	CGGTGCAAAGAAACACTTGA
16	CDCA7-651/652	18	CTGGAATTAGGATCCGGTGT	CTTTCTTGTTCATGCGTCGT
17	CDCA8-709/710	4	CATGTTCCAGACAAGGATGG	GCAACAAACAGCCACAGAAT
18	CDCA8-725/726	7	AAAATATCTGGCGGATGAGG	AGAGAAGGATCGGACGAATG
19	CDGA1-791/792	7	CAAATCCTCGACATTTCCCT	GTGAACGAATGAACTGGGTG
20	CDGA1-805/806	9	ACCGGTAAAGCCATGCTATC	GCATTCAAACAGAACGCAAC
21	CDGA1-827/828	14	CTAGGAAGGAGCACGGAGAG	CGCGACTCTAACAACACGAT
22	CDGA1-829/830	4	TAGGGCCTGTCATCTCAATG	TACACTCTTGCCTTGCATCC
23	CDGA1-875/876	11	GCTGCTGCTGCTGTATTTGT	CACAGATGCTGCCAAGCTAT
24	CDGA1-877/878	2	AGCGACACTCCAAGGAGAAG	AAATCGGTTAGTGGAGGTGC
25	CDGA1-899/900	10	TTCGTCTCTTGCGAAATCAC	CCGTCCTTCTCCTTCAAAAG
26	CDGA1-915/916	14	AAGACGAGCAGAGAAGAGCC	CGTTCTGGATAGGTGGGAGT
27	CDGA2-999/1000	16	ATATATACCCCCTCTGCCCC	TAGAGCCAACATGAGCCAAG
28	CDGA2-1003/1004	15	TGCCTCTGCTCTTTGAATTG	TTGTAAGGCAGGCAGAACAG
29	CDGA2-1011/1012	8	CCTCGCGAATACAAAGATCA	AATCGCAATTGACAGAGGTG
30	CDGA2-1015/1016	12	AGCAGAGTAGCAGACCCGAT	CGATGGACCAGTGAAGAGAC
31	CDGA3-1103/1104	5	AAGAATAATGCCCAAGGCAC	ACCATCACTCGACACCACAT
32	CDGA3-1133/1134	3	CGCTACAGCAATTCTCTTCG	GCATGACGAAAAGGTGACAG
33	CDGA3-1177/1178	11	GGGTGAACACTGCTGATGAC	CTTCTGAAATGCATGGGATG
34	CDGA3-1187/1188	17	TCACATGGCCGTGTTACTCT	ATTGTGCTAGTGACGGGGTT
35	CDGA3-1197/1198	17	TTGCTGGTCTTTACCTGTGC	TAAGCATCTGGGTGCTTGAG
36	CDGA3-1215/1216	15	TGATGGTCTTGCGATAGAGG	TTTAACGGATGGGAGTAGCC
37	CDGA4-1269/1270	9	ATTCTTGCTGGTTTCCATCC	TCTTGAGGAGATGAGGGAGG
38	CDGA4-1301/1302	6	TGACACAACAGCCACCTTCT	TGCTTTACAAAGGTCAGCCA
39	CDGA2-1307/1308	8	GACATGAGAAGTGCCTTTGC	AGGTGGAAAAGAGGATGGTG
40	CDGA4-1331/1332	5	CTCCCTCTATAAACCGTGAGA	AATGCATTCTAGAGTCGGGG
41	CDGA5-1369/1370	10	ATTTCCTCTGCTGCCATTTC	AAGAACCATCTCCCCTTCCT
42	CDGA5-1381/1382	12	AAACCTGTTCGGACTTGGAC	TTCCTTCCAAGGGAGAGAGA
43	CDCA1-1391/1392	13	CAGGTTCAATGCTCTGCATC	TCTCTCGAGTGTCTGCCATC
44	CDGA5-1399/1400	7	CACATCTCGATCCTGGAAGA	CAGGCCATGACATGTACGA
45	CDAG5-1425/1426	1	GGTTTTCTTCGTCCAATCGT	CTCCCTCTCCATCTCCATGT
46	CDGA5-1439/1440	8	GCACGTTTGAAAGTCGGTAA	CAGGTGCTTATGGGGATTTT
47	CDGA7-1665/1666	13	TTCCATACCCGTGAGAAATG	ACCTACGTGGCCCTATTTTG
48	CDGA8-1807/1808	1	CCTCAACTCCAGTGCTGAAA	TGTTAACCGGGGTTCAGATT
49	CDATG1-1875/1876	15	CCAGGGTGCTACTGGATTCT	ACTTTGATCGCAGCAATCTG
50	CDATG1-1889/1890	3	AAACGTGAGAGGCTCTTGCT	GTATGACACACGGAAGGACG
51	CDATG3-2001/2002	14	GGTTGTTCCGGAAGAGATGT	TGGAACAGTTGGACGACATT
52	CDATG4-2059/2060	6	ATTAGTGGTTTTTGGGCAGG	TCCAGTCATTCGAGGAATTG
53	CDAAC3-2391/2392	16	TTGGGGGTTGGGTTAGAATA	GGCAGTCAGTTTTGGCTACA
54	CDAAC4-2463/2464	2	TGTCATCCCTGAAGATGTGC	TCAAGGAAGTTGGGATAGGC

Table 3. Morphological variation of the 16 *C. dactylon* x *C. transvaalensis* hybrids from the two greenhouse trials with mean value, minimum value, maximum value, SD, and CV for each characteristic.

Statistical Parameter	Mean		Minimum		Maximum		SD		CV	
Year	1	2	1	2	1	2	1	2	1	2
1st Leaf Length (mm)	14.00	10.60	2.10	1.98	41.60	32.89	5.46	4.72	38.99	44.53
1st Leaf Width (mm)	1.14	1.11	0.66	0.66	1.75	1.64	0.16	0.14	13.66	13.00
1st Leaf Angle (deg)	138.81	134.03	88.65	74.98	167.84	171.77	13.77	15.62	9.92	11.65
2nd Leaf Length (mm)	12.79	10.12	2.86	2.30	33.15	29.99	4.61	4.28	36.01	42.26
2nd Leaf Width (mm)	1.14	1.11	0.60	0.65	1.74	1.60	0.16	0.15	14.02	13.23
2nd Leaf Angle (deg)	135.26	132.41	82.36	68.52	168.07	165.65	13.52	14.81	10.00	11.18
2nd Internode Length (mm)	5.58	4.63	1.17	1.28	19.69	16.86	2.49	1.93	44.70	41.76
2nd Internode Diameter (mm)	0.51	0.53	0.25	0.22	0.83	0.84	0.10	0.10	20.37	18.94
3rd Internode Length (mm)	5.77	5.10	1.04	0.11	21.55	24.71	2.83	2.47	49.00	48.52
3rd Internode Diameter (mm)	0.47	0.49	0.20	0.21	0.77	0.88	0.09	0.10	20.09	20.10
Raceme Number[z]	2.09	2.02	2.00	2.00	3.00	3.00	0.29	0.13	13.89	6.22
Raceme Length (mm)	26.35	18.38	11.35	2.66	57.50	29.11	10.11	4.27	38.36	23.25
Canopy Height (mm)	73.72	37.79	35.00	3.00	160.00	88.00	19.67	15.23	26.68	40.31

[z]Number of spikelets for each inflorescence.

Table 4. Morphological variation of the 16 *C. dactylon* x *C. transvaalensis* hybrids from the two field trials with mean value, minimum value, maximum value, SD, and CV for each characteristic

Statistical Parameter	Mean		Minimum		Maximum		SD		CV	
Year	1	2	1	2	1	2	1	2	1	2
1st Leaf Length (mm)	23.21	32.15	3.52	5.00	69.72	89.86	12.72	17.08	54.79	53.12
1st Leaf Width (mm)	1.37	1.65	0.69	0.50	2.29	2.50	0.24	0.24	17.75	14.87
1st Leaf Angle (deg)	124.24	131.44	62.99	85.20	161.29	166.09	14.35	12.43	11.55	9.46
2nd Leaf Length (mm)	22.22	29.14	1.67	2.72	75.46	87.00	12.60	15.56	56.71	53.39
2nd Leaf Width (mm)	1.36	1.62	0.15	0.97	11.51	2.50	0.41	0.25	30.05	15.39
2nd Leaf Angle (deg)	121.58	133.85	78.69	89.54	156.79	165.08	14.29	12.99	11.75	9.70
2nd Internode Length (mm)	7.70	13.74	1.53	2.14	43.41	64.00	5.79	10.18	75.16	74.15
2nd Internode Diameter (mm)	0.64	0.73	0.30	0.28	1.10	1.52	0.11	0.17	17.83	23.19
3rd Internode Length (mm)	10.24	14.79	1.44	0.83	58.45	72.00	7.41	10.94	72.44	73.98
3rd Internode Diameter (mm)	0.64	0.69	0.16	0.20	0.96	1.52	0.12	0.17	19.03	25.24
Raceme Number[z]	2.73	2.85	2.00	2.00	4.00	5.00	0.64	0.68	23.25	24.00
Raceme Length (mm)	51.90	60.07	14.02	2.70	125.14	197.73	22.74	32.82	43.81	54.64
Canopy Height (mm)	75.51	115.24	5.00	14.00	295.00	346.00	53.14	73.18	70.37	63.51

[z]Number of spikelets for each inflorescence.

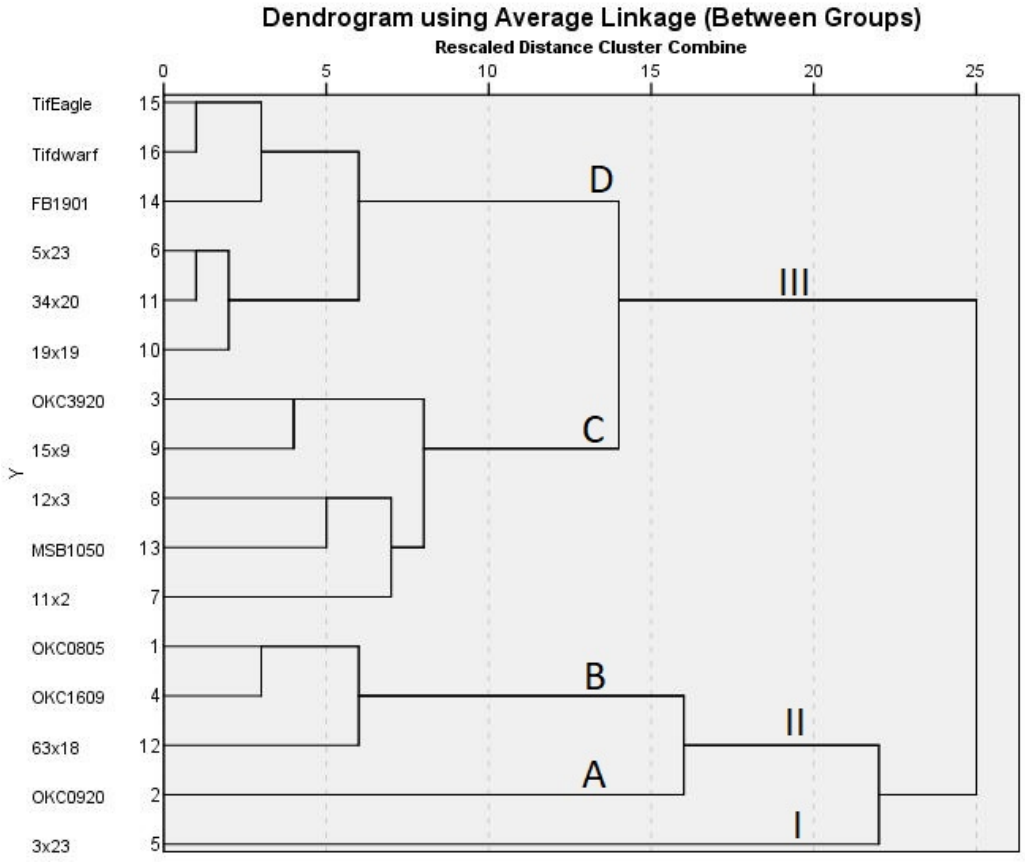

Fig. 1. An unweighted pair group method arithmetic mean dendrogram generated for the 16 *C. dactylon* x *C. transvaalensis* hybrids based on their greenhouse morphological characteristics. The scale bar represents rescaled Euclidean morphological distance. The letter designations indicate main and subgrouping.

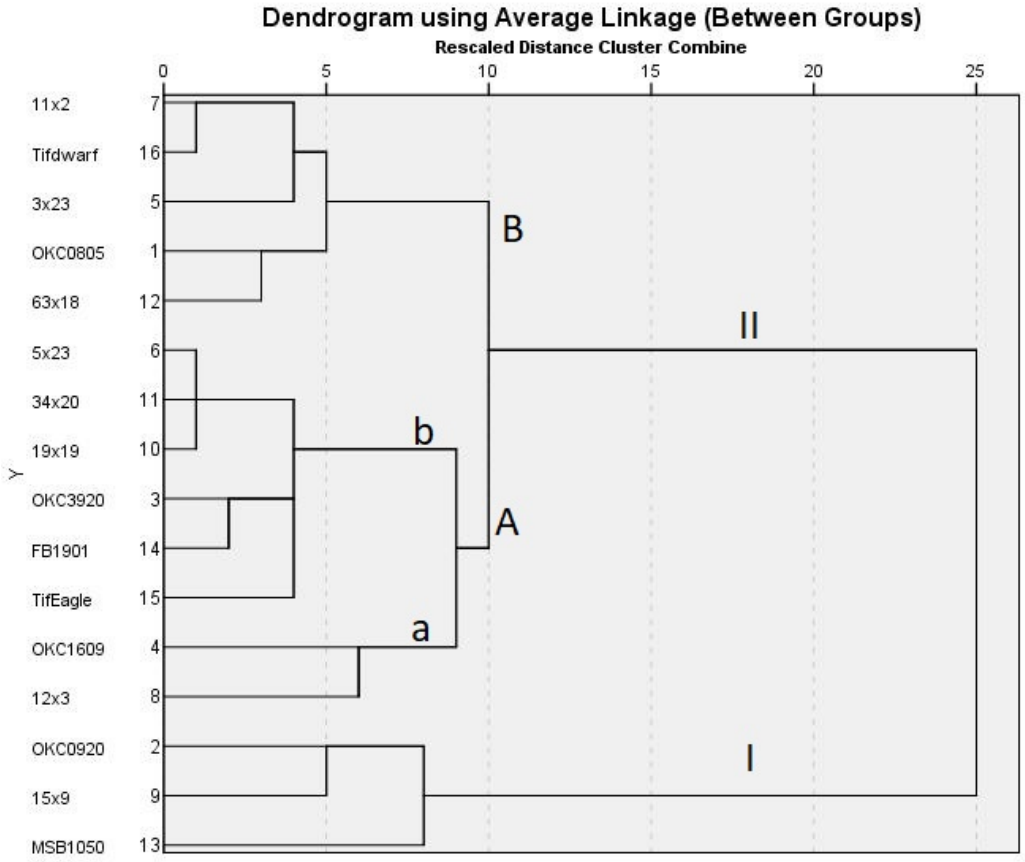

Fig. 2. An unweighted pair group method arithmetic mean dendrogram generated for the 16 *C. dactylon* x *C. transvaalensis* hybrids based on their field morphological characteristics. The scale bar represents rescaled Euclidean morphological distance. The letter designations indicate main and subgrouping.

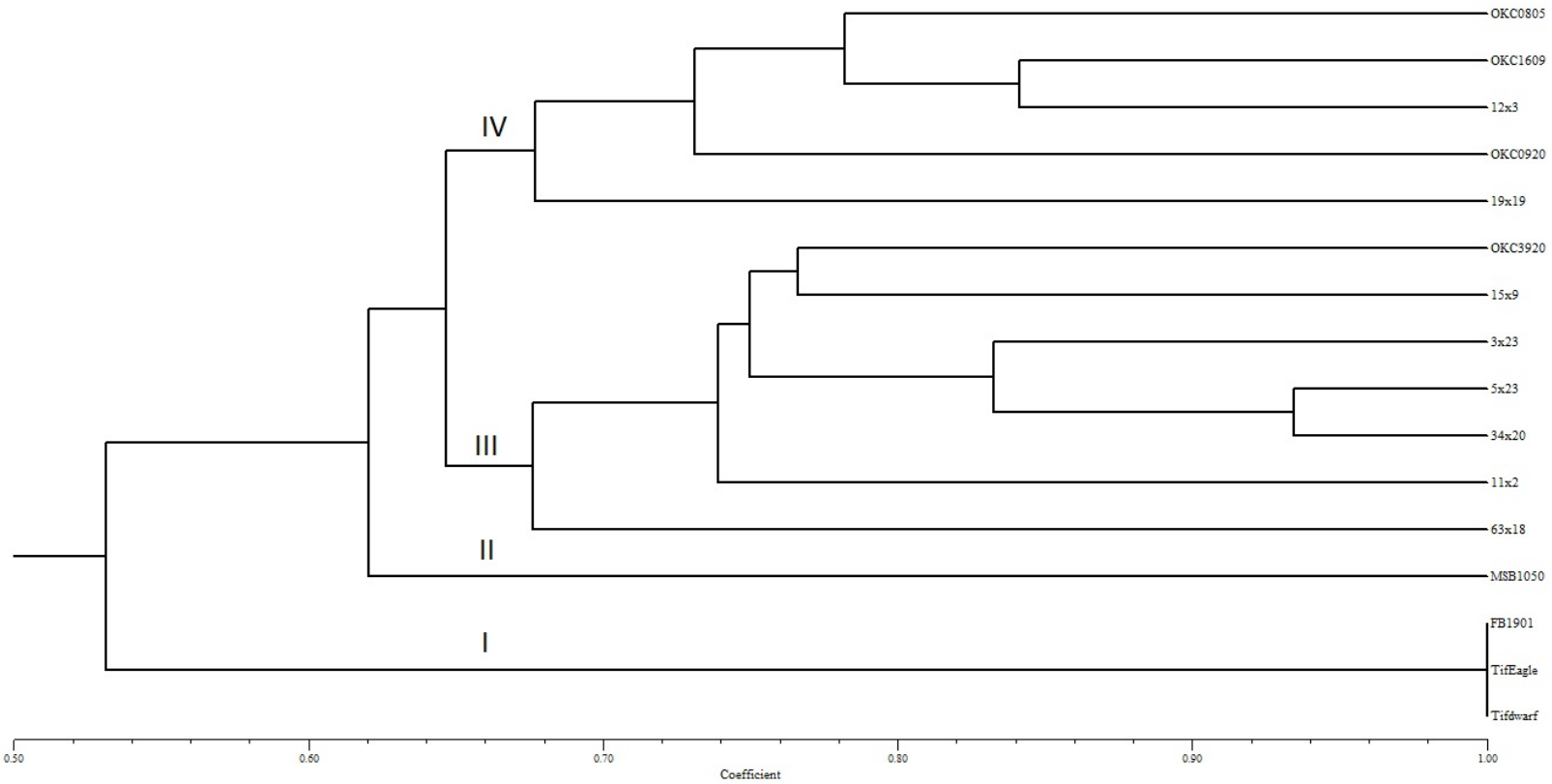

Fig. 3. An unweighted pair group method arithmetic mean dendrogram based on genetic relationships among the 16 *C. dactylon* x *C. transvaalensis* hybrids based on genetic similarity coefficients. The four clusters are indicated by roman numerals.

Supplemental Table 1. Phenotypic characteristics of the 16 *C. dactylon* x *C. transvaalensis* hybrids for both years of the greenhouse trial.

Entry	1st Leaf Length (mm)		1st Leaf Width (mm)		1st Leaf Angle (deg)		2nd Leaf Length (mm)		2nd Leaf Width (mm)		2nd Leaf Angle (deg)		2nd Internode Length (mm)		2nd Internode Diameter (mm)		3rd Internode Length (mm)		3rd Internode Diameter (mm)		Raceme Number		Raceme Length (mm)		Canopy Height (mm)	
Year	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
OKC0805	16.93	13.34	1.13	1.12	147.35	146.62	15.93	12.51	1.14	1.14	143.28	141.01	6.98	5.70	0.44	0.50	5.83	6.30	0.43	0.48	-	-	-	-	88.90	43.67
OKC0920	19.05	11.87	1.27	1.30	141.79	136.21	17.31	11.27	1.28	1.31	141.36	135.27	7.62	6.21	0.55	0.59	7.87	6.78	0.48	0.53	2.13	2.00	37.97	21.14	101.93	41.92
OKC3920	16.78	13.37	1.12	1.12	135.48	128.78	16.21	12.63	1.10	1.12	135.30	129.76	4.84	4.14	0.46	0.49	4.60	4.68	0.43	0.45	-	-	-	-	71.75	42.77
OKC1609	16.08	11.45	1.11	1.08	141.97	140.42	15.09	10.63	1.14	1.11	136.04	132.61	8.02	5.29	0.44	0.44	7.61	5.43	0.40	0.41	2.00	2.00	24.04	19.61	90.17	43.60
3x23	17.00	15.66	1.08	1.07	143.99	146.25	15.47	15.17	1.05	1.06	141.90	147.16	5.47	5.19	0.46	1.20	5.49	5.33	0.44	0.44	-	-	-	-	80.92	64.27
5x23	13.70	10.36	1.19	1.13	137.92	134.71	12.42	10.00	1.20	1.14	136.93	133.91	3.86	2.89	0.60	0.60	3.91	3.35	0.53	0.57	-	-	-	-	68.28	35.40
11x2	14.25	9.16	1.10	1.02	147.28	138.63	12.79	8.95	1.11	1.02	142.67	135.47	4.95	4.15	0.49	0.50	4.69	4.45	0.46	0.44	-	-	-	-	70.70	40.08
12x3	12.50	8.94	1.11	1.01	131.21	131.09	11.29	8.84	1.07	1.02	126.62	132.72	6.04	4.74	0.52	0.51	6.12	4.67	0.49	0.48	2.00	2.00	16.30	10.63	84.08	38.83
15x9	15.08	13.86	1.02	1.06	129.55	131.32	13.47	13.46	1.00	1.05	129.91	127.52	4.32	4.84	0.40	0.44	5.26	5.73	0.38	0.41	2.00	2.00	23.23	23.24	73.68	47.98
19x19	12.21	9.05	1.13	1.11	136.11	129.78	11.66	9.18	1.12	1.13	131.98	125.24	4.07	3.89	0.56	0.58	4.33	4.38	0.53	0.55	-	-	-	-	65.67	28.28
34x20	12.23	8.92	1.18	1.15	138.98	125.43	11.45	8.37	1.17	1.16	132.89	128.44	3.39	2.99	0.59	0.66	3.62	3.20	0.54	0.61	-	-	-	-	67.42	31.60
63x18	17.43	17.21	1.14	1.12	145.22	138.61	14.51	15.34	1.12	1.15	139.34	137.83	7.01	7.06	0.52	0.52	9.00	9.43	0.47	0.45	2.00	2.02	18.37	17.25	81.92	48.88
MSB1050	11.42	7.28	1.24	1.04	137.01	134.45	11.09	7.05	1.20	1.04	135.12	133.20	6.51	5.07	0.45	0.44	6.45	5.26	0.43	0.43	-	-	-	-	67.08	35.43
FB1901	8.20	6.27	1.09	1.13	134.14	120.45	7.32	6.24	1.11	1.12	127.40	122.78	5.20	4.81	0.53	0.55	5.53	4.65	0.48	0.49	-	-	-	-	53.83	21.13
TifEagle	8.56	6.65	1.22	1.10	130.67	135.32	8.04	6.37	1.23	1.13	126.04	129.65	5.74	3.80	0.60	0.59	6.10	4.33	0.53	0.54	-	-	-	-	52.50	18.72
Tifdwarf	12.52	6.30	1.16	1.12	142.33	126.50	10.58	5.92	1.17	1.15	137.31	125.93	5.27	3.28	0.56	0.61	5.99	3.56	0.50	0.56	3.00	2.50	41.76	22.69	60.67	22.10
LSD†	1.63	1.23	0.05	0.05	4.54	5.05	1.33	1.11	0.05	0.05	4.45	4.88	0.76	0.57	0.03	0.03	0.88	0.72	0.03	0.03	§	§	§	§	5.31	3.65
CV(%)¶	38.99	44.53	13.66	13.00	9.92	11.65	36.01	42.26	14.02	13.23	10.00	11.18	44.70	41.76	20.37	18.94	49.00	48.52	20.09	20.10	13.89	6.22	38.36	23.25	26.68	40.31

†Fisher's Protected Least Significant Difference test: difference in means less than value is not significantly different at p = 0.05

§ Not enough data available to conduct appropriate pairwise tests

¶ Coefficient of variation expressing the dispersion of genotype means around the total morphological mean expressed as a percent

Supplemental Table 2. Phenotypic characteristics of the 16 *C. dactylon* x *C. transvaalensis* hybrids for both years of the field trial.

Entry	1st Leaf Length (mm)		1st Leaf Width (mm)		1st Leaf Angle (deg)		2nd Leaf Length (mm)		2nd Leaf Width (mm)		2nd Leaf Angle (deg)		2nd Internode Length (mm)		2nd Internode Diameter (mm)		3rd Internode Length (mm)		3rd Internode Diameter (mm)		Raceme Number		Raceme Length (mm)		Canopy Height (mm)	
Year	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
OKC0805	24.48	38.26	1.36	1.61	126.84	135.62	23.03	33.37	1.36	1.57	126.38	136.65	7.70	16.62	0.59	0.61	13.11	18.96	0.58	0.56	3.17	3.38	71.43	35.57	86.98	146.10
OKC0920	30.81	53.33	1.58	1.88	124.20	133.87	29.70	44.98	1.51	1.87	122.33	131.05	10.00	22.28	0.62	0.70	15.16	26.54	0.59	0.68	2.58	3.12	71.72	100.35	107.07	205.18
OKC3920	16.96	20.09	1.45	1.52	123.80	119.16	16.11	18.90	1.45	1.50	114.50	122.00	4.60	4.70	0.70	0.74	6.71	5.78	0.71	0.69	2.92	2.28	41.62	54.50	38.40	40.13
OKC1609	16.93	21.81	1.20	1.52	129.17	129.28	15.83	19.06	1.16	1.49	123.90	129.74	5.55	9.60	0.55	0.62	8.86	10.28	0.56	0.58	2.67	2.63	45.34	42.73	54.67	58.82
3x23	31.40	36.49	1.31	1.61	134.63	138.40	30.18	32.57	1.30	1.58	134.02	142.53	9.45	12.91	0.64	0.74	10.11	14.01	0.62	0.71	2.72	2.83	75.33	82.12	118.38	159.93
5x23	17.33	28.09	1.37	1.66	124.82	130.30	17.34	26.99	1.35	1.64	120.93	134.63	5.03	13.28	0.62	0.72	6.41	13.50	0.61	0.71	2.15	2.42	38.26	55.16	54.42	114.93
11x2	15.14	39.07	1.31	1.69	122.98	139.68	14.34	37.97	1.31	1.67	120.43	144.70	3.78	16.33	0.67	0.77	5.22	15.31	0.68	0.77	2.95	3.17	41.83	66.51	30.82	162.10
12x3	20.61	16.78	1.31	1.38	112.38	119.01	19.24	15.01	1.26	1.37	109.80	117.98	6.22	7.19	0.57	0.65	8.06	8.08	0.54	0.65	2.13	2.03	35.31	30.53	83.93	54.47
15x9	45.94	53.77	1.46	1.88	131.01	136.29	45.32	49.84	1.39	1.82	130.81	138.86	14.15	20.87	0.69	0.84	16.42	24.25	0.69	0.80	3.48	3.57	84.49	113.71	113.25	207.32
19x19	23.15	31.64	1.39	1.68	118.35	128.01	22.21	29.82	1.50	1.64	116.53	133.30	5.42	8.61	0.64	0.80	7.82	9.01	0.66	0.78	2.13	2.20	29.03	35.88	60.35	80.13
34x20	15.52	26.53	1.24	1.60	119.05	126.73	14.87	25.63	1.25	1.58	116.51	131.28	4.27	11.43	0.67	0.74	5.39	10.60	0.67	0.73	2.25	2.42	37.70	50.77	48.78	88.23
63x18	38.52	36.75	1.36	1.69	123.37	128.58	37.09	34.38	1.37	1.65	122.30	135.11	16.87	18.50	0.62	0.70	19.05	18.70	0.62	0.66	2.87	2.63	51.41	47.42	131.37	124.75
MSB1050	38.75	50.92	1.77	1.84	136.06	142.40	37.41	44.63	1.76	1.81	132.92	146.66	18.43	32.84	0.74	0.67	25.51	33.49	0.71	0.57	-§	3.02	-§	78.84	188.35	201.77
FB1901	10.69	17.07	1.15	1.57	116.19	128.74	9.74	15.02	1.11	1.53	114.69	128.30	3.75	9.06	0.62	0.74	4.86	9.83	0.62	0.71	3.17	3.22	36.71	53.20	27.28	68.88
TifEagle	8.45	15.21	1.34	1.56	122.93	128.71	8.17	13.61	1.32	1.55	119.77	130.87	3.08	5.20	0.66	0.88	4.21	5.91	0.69	0.80	2.88	3.30	30.58	47.96	20.25	37.95
Tifdwarf	16.68	28.61	1.32	1.66	122.06	138.27	14.99	24.52	1.29	1.63	119.45	137.95	4.95	10.35	0.71	0.68	6.87	12.39	0.69	0.65	3.23	3.32	54.74	65.89	43.87	93.10
LSD†	2.56	4.24	0.07	0.08	4.69	3.80	2.56	3.97	0.14	0.08	4.58	3.86	1.23	2.64	0.04	0.06	1.65	2.85	0.04	0.06	0.39	0.18	12.90	8.55	10.39	16.47
CV(%)¶	54.79	53.12	17.75	14.87	11.55	9.46	56.74	53.39	30.05	15.39	11.75	9.7	75.16	74.15	17.83	23.19	72.44	73.98	19.03	25.24	23.25	24.00	43.81	54.64	70.37	63.51

†Fisher's Protected Least Significant Difference test: difference in means less than value is not significantly different at p = 0.05

§ Missing data excluded from Fisher's LSD test

¶ Coefficient of variation expressing the dispersion of genotype means around the total morphological mean expressed as a percent

CHAPTER III

THE EVALUATION OF GREENS-TYPE HYBRID BERMUDAGRASS SELECTIONS FOR FIELD PERFORMANCE AND UNDERSTANDING MORPHOLOGICAL RELATIONSHIP TO BALL ROLL DISTANCE

Abstract

Ultradwarf bermudagrasses (*Cynodon dactylon* x *C. transvaalensis*) generally face issues with disease, rooting depth, and winter survivability. The lack of genetic diversity among the cultivars does not give superintendents the opportunity to select a cultivar best suited for their location. The introduction of new genetic material through traditional crosses could provide the opportunity to select genotypes possessing superior traits to the ultradwarfs. To achieve this goal, the turfgrass breeding program at Oklahoma State University (OSU) developed new interspecific hybrids with short internodes and fine leaves using cold-hardy materials. These experimental genotypes have been shown to tolerate mowing at 3.2 mm, but their performance as a putting surface, particularly their ball roll distance (BRD), has not been evaluated. The objective of this study was to screen experimental genotypes from OSU, Mississippi State University (MSU), and the University of Florida (UF) against two commercial ultradwarfs: 'TifEagle' and 'Tifdwarf' as well as evaluate the relationships present between several morphological traits and BRD. Several parameters were evaluated to determine the performance of the genotypes including visual ratings, ball roll distance measurements, and root lengths. Results showed only the experimental

genotype from UF showed similar or improved BRD compared to 'TifEagle', the top performing standard. However, genotypes OKC0920, OKC3920, 11x2, 19x19, and MSB1050 showed similar or improved visual characteristics compared to both standard entries. Additionally, OKC0920 and OKC3920 showed significantly improved rooting depth compared to both standards. These genotypes demonstrated adequate performance under putting green management and can help introduce new genetics to the bermudagrasses used for putting surfaces. This data also showed a significant relationship between leaf length and BRD with an adjusted R^2 value of 0.91. There were also significant relationships between leaf length and other morphological characteristics to better assist breeders in selecting genotypes which will show adequate BRD performance.

Introduction

The development of 'Tifgreen' bermudagrass, an interspecific hybrid (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy), by Dr. Glenn Burton ignited a revolution in the quality of putting green surfaces in the southern United States (Hein, 1961). 'Tifgreen' is known to produce somatic (vegetative) mutations hypothesized to be caused by aneuploidy (Reasor et al., 2016). Some of these mutations have exhibited superior performance as a putting green surface compared to the parent material and have led to a class of hybrid bermudagrass called 'ultradwarfs'. This type of bermudagrass has become the predominate putting green surface in the southern agronomic region, comprising 80% of putting green surfaces in 2007 (Lyman et al., 2007). These grasses can provide superior putting green surfaces in the region compared to creeping bentgrass (*Agrostis stolonifera* L.) (Patton, 2012; Unruh and Davis, 2001). However, before some of these mutations become new cultivars, they are classified as "off-types", which have different morphological characteristics and performance compared to the desired cultivar (Caetano-Anollés, 1998; Caetano-Anollés et al., 1997). These off-types can decrease playability and visual appeal of the putting green surface. Very few of the total off-types appearing on ultradwarf putting greens end up becoming a new cultivar, and the majority of the

time exist as a contamination superintendents must deal with. Additionally, as a result of the methods of exclusively selecting new cultivars from mutations, there exists very little genetic diversity among these grasses. Previous studies have demonstrated the lack of genetic diversity among these ultradwarf mutations (Capo-chichi et al., 2005; Fang et al., 2017; Harris-Shultz et al., 2010; Harris-Shultz et al., 2011; Kamps et al., 2011; Reasor et al., 2016; Reasor et al., 2017; Wang et al., 2010; Zhang et al., 1999).

Due to the lack of genetic diversity, many of the current cultivars on the market suffer from similar issues; however, some cultivars have been able to be selected that show a slight improvement over older cultivars. For example, 'TifEagle' was released as an improvement to 'Tifdwarf' showing fewer seedhead production and a greater tolerance to tawny mole crickets (*Scapteriscus vicinus* Scudder) (Hanna and Elsner, 1999).

However, three major issues of ultradwarfs as a whole face are disease (Unruh and Davis, 2001), rooting depth (Martin, 2016), and winter survivability (Richardson et al., 2014). Three fungal diseases Unruh and Davis (2001) found causing the most damage on ultradwarfs are bermudagrass decline (*Gaeumannomyces graminis* Saccardo), spring dead spot (*Ophisosphaerella herptricha* Walker), and curvularia blight (*Curvularia* species). Controlling these diseases is best done by raising the mowing height to allow the turf to have a more competitive advantage over the disease, but this option is not usually available to superintendents who want to keep the greens at the height for the best playability (Unruh and Davis, 2001). The high genetic similarity among these grasses presents a risk of a pathogen arising with devastating damage, like what is happening with 'Floratam' (*Stenotaphrum secundatum* [Walt.] Kuntze) and the sugarcane mosaic virus (family: *Potyviridae*, genus: *Potyvirus*) (Harmon et al., 2015). An outbreak of this virus is moving across the state of Florida, affecting only this cultivar of St. Augustine grass, and causing severe mosaic and necrosis of the turf (Harmon et al., 2015).

As the cultivars are selected for more dwarfed shoot characteristics (i.e., shorter internodes and leaf lengths), this has also affected the root performance with cultivars such as 'TifEagle' and 'Champion Dwarf' generally having a shallower root system compared to 'Tifdwarf' (Martin, 2016). When factoring in the typical USGA root zone allowing for better water percolation and the addition of hydrophobic zones, this makes managing the root zone moisture a critical and time intensive affair (Martin, 2016).

Additionally, winter survivability of these grasses, particularly as they become more prevalent in the mid-south to the transition zone, is an important concern for superintendents. Even with proper management practices, these cultivars can still experience winterkill (Richardson et al., 2014). Current cultivars on the market have T_{mid} (the temperature killing 50% of the plants in test) values ranging from -4.8 to 6.5 °C (Anderson et al., 2002). The USGA recommends covering greens with a turf cover when ambient temperatures are going to be below -3.8 °C which follows the data found by Anderson et al. (2002) (O'Brien and Hartwiger, 2013). These covers can be made of multiple types of materials and work to retain the heat of the soil in, keeping the temperature at the crown of the plant above the point where the plant would die, but take labor and time some courses do not have available during the winter months (O'Brien and Hartwiger, 2013). A freeze chamber study conducted by Gopinath et al. (2021) evaluating 'Champion Dwarf', 'TifEagle', and experimental genotypes developed by Oklahoma State University (OSU) for use on putting greens, demonstrated similar T_{mid} values for the two ultradwarfs found by (Anderson et al., 2002). Additionally, they found the experimental genotypes had statistically improved T_{mid} values ranging from -7 to -8.1 °C. This improved tolerance to low temperatures could reduce the frequency of covering needs or remove the need altogether depending on the location.

The turfgrass breeding program at OSU has developed cold-hardy hybrid bermudagrass genotypes exhibiting short internodes and fine leaves which could be used for putting green

surfaces. These genotypes have been evaluated in internal mowing trials and showed to tolerate mowing heights of 3.2 mm, which is commonly done on golf course greens. However, there has not been extensive evaluations to determine the performance of these genotypes regarding ball roll distance (BRD) and root length in addition to turf quality. Therefore, the goal of this experiment was to evaluate the performance of 12 advanced experimental hybrid bermudagrass genotypes developed by OSU for use on putting green surfaces. In addition to these, one experimental genotype was included from Mississippi State University and one from the University of Florida. 'TifEagle' and 'Tifdwarf' were used as standard entries, and 'Tahoma 31' was included to evaluate its potential for use as a putting surface. Additionally, there have been studies to evaluate the relationship between bermudagrass morphology and wear tolerance (Kowalewski et al., 2015), but no studies were found to evaluate the relationship between morphological characteristics and BRD performance on putting greens. As such, the performance of these entries was evaluated against morphological measurements collected by Earp et al. (2022) to determine if there exists a relationship which could be used by breeders to assist in the selection process. In addition, data collected can be used to make important decisions on which genotypes to pursue further for release as a commercial cultivar.

Materials and Methods

Study Site

One experimental location was used for the putting green trial (Block 8, OSU Turf Center, Stillwater, OK, 36.121713, -97.103271) using a randomized complete block design with 5 replicates with a plot size of 4.57 m by 1.83 m using a one-way treatment structure with seventeen levels. The research putting green was built in 1982 using a 90:10 (V:V) sand and rice hull construction profile with no intermediate layer component. A total of 25.4 centimeters of this top mix was placed over a geotextile mat which was placed over a coarse layer of gravel (Liu,

2014). Prior to establishment of the putting green, renovation was done to remove the previous turf cover, as well as level the surface with a 0.5% slope to assist with drainage and incorporate peat moss into the upper 15.24 cm of the soil to increase organic matter from 0.55% to 1% to assist with water holding during the establishment period.

Plant Material

Twelve advanced genotypes with promising performance in selection and evaluation nurseries were used, along with one experimental genotype from Mississippi State University (MSU) and one experimental genotype from the University of Florida (UF) in the 2019 National Turfgrass Evaluation Program (NTEP) warm season putting green trial, and three commercially available cultivars 'Tahoma 31', 'Tifdwarf', and 'TifEagle'. 'Tahoma 31' was initially released as an interspecific hybrid bermudagrass for used on athletic fields, golf course fairways and tees, and home lawns. Internal experiments revealed its ability to tolerate mowing heights down to 3.2 mm and was subsequently included in this experiment to further evaluate its performance under such conditions. Previously, 15.24 cm pots had been established of the seventeen plant materials from their original location (Table 1).

Expansion of the starting material began in November 2020. A root mixture of 80% sand and 20% peat moss (Premier Tech Horticulture, Quakertown, PA, USA) was used to emulate the root zone mixture of the field trial to avoid contamination issues when planting. To prepare enough material for a sprigging rate of 1:10 for each plot, thirty trays (52 cm x 25.4 cm x 6 cm) were prepared for each genotype and grown in greenhouses set with a 16-hour light schedule at 32 °C and 24 °C the eight hours the lights are off. The trays were watered daily to prevent drought stress and fertilized bi-weekly with 12-4-8 (Scotts Miracle-Gro, Marysville, OH, USA) at 3 grams of nitrogen per m^2. To prevent contamination between entries, the edges of the trays were trimmed.

Establishment

Prior to planting, the field corners were set, and string used to outline each plot to assist in planting. To plant each plot, six trays of the respective genotype were torn apart by hand into 10-15 cm sprigs and placed in a plastic tub, with the soil from the trays placed in another plastic tub. A rake was used to pull back the top 2.5 cm of soil of the plot area, and the sprigs were spread evenly in this area. Afterwards, the soil was spread back across the sprigs and the soil from the trays was used to help even out the surface. After planting each plot, they were hand-watered to prevent desiccation of the sprigs. Once the plots were sprigged, a water-filled roller was pulled across the surface to assist in smoothing the surface and ensuring proper soil to root contact.

Site Maintenance

During May and June of 2021, the plots were watered four times a day to apply a total of a half inch of water to prevent the sprigs from drying out. After the plots are established, the plots were irrigated to prevent stress.

Before planting, the field was fertilized with 18-24-14 (Harrells, Lakeland, FL, USA) and 13-2-13 (The Andersons, Maumee, OH, USA) at a rate of 309 kg/ha each. One week after planting the field was fertilized with 18-24-12 at a rate 145 kg/ha; this was followed the next week with an application of 13-1-13 at a rate of 97 kg/ha. Three weeks after planting the field received 8-16-16 at 152 kg/ha, and the fourth week it received 8-16-16 at 152 kg/ha. The fifth week and following the plot received alternating foliar applications of 30-0-0, 4-0-0, and 0-0-30 at 9.5 L/ha (Steve Batton and Associates, 2020). The rotation of foliar fertilizers was also used the second year after spring greenup. The third week of September the field received an application of 0-0-50 at 117 kg/ha to assist the plants in transitioning to winter dormancy. Fertilizer was obtained through Harrell's LLC. (Lakeland, FL, USA) for this trial.

Once the roots established and it was safe to mow, the field was maintained at a height of cut (HOC) of 6.4 mm. During the establishment, the borders of the plots were hand maintained to prevent entries from crossing over to each other until the plots were filled in. As plots were near full establishment, the height was be lowered to 5 mm. When all plots were established, the mowing height was lowered every third mowing by 0.25 mm until the HOC was 3.2 mm.

Additionally, after all plots were established, the field was sprayed with Primo Maxx© (Syngenta, Greensboro, NC, USA) at 21 g a.i. ha^{-1} every 220 growing degree days (Carroll et al., 2022). The field was top dressed every other week and brushed into the turf canopy. During the winter, turf covers were used when air temperatures are predicted to fall below -3.89 °C to prevent winterkill injury that could negatively affect other measurements and removed when the temperature was expected to exceed 7.22 °C following USGA guidelines (O'Brien and Hartwiger, 2013).

Data Collection

Establishment Rate

Establishment rate is a visual estimate of percent green cover (PGC) reflecting the speed of coverage (Morris and Shearman, 1998). In addition to a visual estimate, digital image analysis (DIA) was used as an objective method to determine PGC by capturing digital images using a Canon G9X camera (Canon Inc., Ōta, Tokyo, Japan) (Shutter: 1/160, F-stop: 2.2, ISO: 200) mounted to a lightbox (0.91m x 0.6m x 0.6m) with four lights. Each image was cropped to exclude the sides of the box using FastStone Photo Resizer (FastStone Soft), and then analyzed using TurfAnalyzer (Green Research Services, LLC, Fayetteville, AR, USA) for PGC (0-100%) using a hue range of 70 – 170, saturation range of 10 – 100, and brightness range of 0 – 100 using the TurfAnalyzer recommended values. However, an error in herbicide application to the plots resulted in ununiform damage during the establishment phase, and as such the results will not be

presented. This damage also prevented the full establishment of plots in the establishment year, so only data collected in the second year of data collection will be presented.

Ball Roll Distance

The standard method of determining BRD, or the distance a golf ball travels when rolled at a set speed, is by using a Stimpmeter ® (USGA, 2012). This device is an aluminum bar, 36 inches in length, with a notch 30 inches from the end where a golf ball is placed. The notch and end of the Stimpmeter ® are constructed in a way to release the ball when the Stimpmeter ® is raised to approximately 20 degrees from the putting surface to release the ball at a consistent speed. The BRD was measured weekly from June to August 2022 by averaging three rolls in each direction lengthwise direction of the plot as the standard protocol for measuring BRD (USGA, 2012). Disease pressure increased during September and October to the point of negatively impacting BRD of certain entries above others, and as a result the data will not be evaluated in determining the performance of each genotype. Monthly averages were then taken for each plot and used in the statistical analysis.

Turfgrass Quality

Visual turfgrass quality was evaluated biweekly from June to September 2022 using the standard NTEP 1-9 rating scale, with 9 indicating the best turf and 1 the poorest (Morris and Shearman, 1998). The quality rating is based on a combination of turf color, density, uniformity, texture, and disease/environmental stress (Morris and Shearman, 1998). Monthly averages were calculated and used in the statistical analysis.

Genetic Color

Genetic color ratings indicate the inherent color of the turfgrass genotype not influenced by cold temperature senescence, and was rated using the standard NTEP 1-9 scale, with 9

indicating a dark green color and 1 a light green (Morris and Shearman, 1998). Color ratings were evaluated biweekly from June to September 2022, and monthly averages were calculated and used for statistical analysis.

Turfgrass Density

Turfgrass density is a visual estimate of the number of tillers in a unit area and was rated comparatively among plots using the 1-9 scale with 9 indicating the maximum number of tillers (Morris and Shearman, 1998). The density is also an indication of the genotype's tolerance to the mowing height used. Mowing is generally considered a stress on the turfgrass plant as photosynthetically active material is removed. This is particularly the case when dealing with putting green surfaces, where there is little leaf tissue left on the plant (Lyman, 2015). If the plant is not able to produce enough nutrients, its vigor will decrease and eventually be unable to survive. Density ratings were evaluated biweekly from June to September 2022, and monthly averages were calculated and used for statistical analysis.

Turfgrass Texture

Turfgrass texture is evaluated as an estimate of leaf width, using a 1-9 scale with 9 indicating a very fine leaf blade and 1 indicating a very course leaf blade (Morris and Shearman, 1998). This method is less precise than physical measurements but is less time and labor intensive. Physical measurements were taken of plots that were not mown to understand the natural growth characteristics of these genotypes by (Earp et al., 2022). Texture ratings were evaluated biweekly from June to September 2022, and monthly averages were calculated and used for statistical analysis.

Turfgrass Uniformity

Turfgrass uniformity is a visual determination of how visually consistent the turf within a plot is, using a 1-9 scale with 9 indicating the turf presents identical color, density, and texture within the entire plot and 1 indicating the turf varies in the same parameters (Morris and Shearman, 1998). Uniformity ratings were evaluated biweekly from June to September 2022, and monthly averages were calculated and used for statistical analysis.

Mowing Stress

Some turfgrasses exhibit poor quality after mowing, particularly when mown at putting green height. This may be caused by the inability of the genotype to tolerate these heights, or due to the turfgrass not resulting in a clean cut across the surface (Morris and Shearman, 1998). The ratings scale is 1-9 with 9 being the cleanest cut with no mowing stress and 1 the poorest cut quality and highest mowing stress. Mowing stress ratings were evaluated biweekly from June to September 2022, and monthly averages were calculated and used for statistical analysis.

Seedhead Production

Seedhead production on putting green surfaces can negatively impact the smoothness of the surface and subsequently the BRD performance. This is a common issue golf course superintendents face when managing annual bluegrass (*Poa annua*) or zoysiagrass putting greens (Dowling, 2019; Patton et al., 2018). One benefit of the ultradwarf cultivars, particularly the newer ones, is they do not produce many seedheads when maintained at putting green height (Reasor et al., 2016). This is an important piece of information golf course superintendents need to know if switching to a non-ultradwarf genotype for their course, as more work is required if the genotype has increased seedhead production. To measure this, monthly ratings were taken during the summer of 2022 using the NTEP 1-9 scale, with 9 indicating no seedheads present and 1 indicating an abundance of seedheads (Morris and Shearman, 1998). The monthly ratings were

analyzed using repeated measures to see if there was an interaction between the month and genotype.

Fall Color Retention

Fall color retention is a visual assessment of the overall plot color beginning after the first hard frost in the fall (Morris and Shearman, 1998). This was done using a 1-9 scale with 9 indicating completely green and 1 is straw brown with no green color present. There were five weeks of consecutive data collections in the fall of 2021 and 2022.

Spring Green Up

Spring green up is a similar measurement evaluating the transition from winter dormancy to active summer growth (Morris and Shearman, 1998). This is done based on the overall plot color, with 1 indicating straw brown and 9 a completely green plot. This was collected for five consecutive weeks in the spring of 2022.

Rooting Depth

To evaluate the rooting performance of these genotypes, three 11 cm wide plugs were pulled randomly from each plot and averaged. The plugs were lifted and the loose soil at the bottom was allowed to fall off. The longest root was then measured from the soil surface. The traditional method of root length is to divide the samples into soil segments, washed and evaluated using a program such as WinRHIZO (Regent Instruments, Nepean, ON, Canada) (Kaur, 2021). This can evaluate various measurements such as total root length and root area. This experiment looked at a more practical measurements superintendents might use to check the rooting performance of their greens. Genotypes exhibiting strong rooting characteristics at putting green height can be further evaluated in a typical root length study to further understand how

these genotypes perform. Data was collected once in the spring, summer, and fall to also evaluate any seasonal variability in root length.

Disease Ratings

Whenever disease symptoms occurred during the trial, visual ratings were collected to evaluate the severity of the disease for each of the genotypes. NTEP ratings were collected on a 1-9 scale with 9 denoting no disease presence and 1 denoting complete disease coverage of the plot (Morris and Shearman, 1998). Ratings were collected weekly during the disease episode to track the progression of the disease for the genotypes. During the trial there were a total of two disease episodes with one during Summer 2021 (late July and early August) and one during Summer 2022 (September and October). The data was analyzed within each episode using repeated measures.

Statistical Analysis

The experiment was conducted as a randomized complete block design with five blocks, and a one-way treatment structure (genotype) with 17 levels. The data was analyzed using linear mixed methods with repeated measures, where genotype was treated as a fixed effect and block as a random effect. When the date by genotype interaction was not significant, the results were run together to get overall least squares mean; this was only the case for the root length data, all others were analyzed with repeated measures by month. Tukey's honestly significant difference (referred to as Tukey's test) was used for all pairwise comparisons, and all tests were performed at 0.05 level of significance (Tukey, 1977). The data analysis for the BRD and visual ratings was completed using SAS/STAT® software, Version 9.4 for Windows Copyright © 2014 SAS Institute Inc. and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute, Cary, NC, USA. To examine the relationship between BRD and the observed morphological characteristics, R (Version 4.2.2) was used (R Core Team, 2022)

using the packages “tidyverse” (Wickham et al., 2019), “readxl” (Wickham and Bryan, 2022), “ggplot2” (Wickham, 2016), “lubridate” (Grolemund and Wickham, 2011) and “GGally” (Schloerke et al., 2021).

Results

Ball Roll Distance

There was a significant month by genotype interaction (Table 2), with BRDs increasing as the summer went on (Table 3). For the three months evaluated, the top performing entries were FB1901, ‘TifEagle’, and ‘Tifdwarf’ with FB1901 having the highest overall BRD of 3.23 m during August. OSU genotype OKC3920 was consistently towards the bottom of the three months, with the slowest BRD of 2.28 m occurring in June. Additionally, the MSU genotype MSB1050, was not statistically different than ‘Tifdwarf’ for all three months. OSU genotype 19x19 also showed statistically similar BRDs to ‘Tifdwarf’ in June and July, and similar speeds to MSB1050 in August. Similarly, genotype 12x3 also showed similar ball roll to 19x19 in June, and similar ball roll to ‘Tifdwarf’ in July and August. Throughout the three months, OSU genotypes averaged under 2.75 meters while the ultradwarf and MSU genotypes were able to exceed this average speed during the study.

Turfgrass Quality

There was a significant month by genotype interaction for turfgrass quality for the five months (Table 4). ‘TifEagle’ and 11x2 were among some of the top performers across the study (Table 5). OKC0805 had the highest overall rating of 6.7 for the month of June. ‘Tifdwarf’, 63x18, and OKC1609 were among some of the poorest performers for most of the trial. At the beginning of September, disease pressure increased across the trial and impacted the overall quality of some genotypes. This will be discussed further in the disease section, but some of the impacts are observed in the overall quality. This was seen in the drastic overall quality of

OKC0805, which decreased from the first and second overall in June and July, to the worst in October.

Genetic Color

There was also a significant month by genotype interaction for the genetic color during this time (Table 4). Darker green colors are typically preferred in golf course settings as it is aesthetically appealing for most people, but some individuals may prefer a lighter green color. Several genotypes performed well in this regard, with the best overall being 11x2 which had a color rating of 7.7 for July and was in the top two values four out of the five months (Table 6). MSB1050 also performed well with a rating of at least 6 for four of the five months. The ultradwarf genotypes also performed acceptably overall towards the top of the rankings each month. On the other side, OKC0920 consistently had a lighter green color throughout the trial along with OKC1609 and 12x3.

Turfgrass Density

There was a significant month by genotype interaction for density (Table 4). Several genotypes performed consistently well throughout the summer. 'TifEagle', FB1901, MSB1050, OKC0805, OKC0920, and OKC3920 were consistently the top performing entries across all five months (Table 7). During the study, 'TifEagle', FB1901, and MSB1050 all achieved a rating of 8 at one point indicating a high number of tillers present. 63x18 and 3x23 were among the poorer performers during the study, with 63x18 having a rating in the bottom three for four of the five months.

Turfgrass Texture

A significant month by genotype interaction was found for this measurement (Table 4). In general, the leaf texture of hybrid bermudagrass genotypes is much improved over the

generally courser common types used in the past for putting green surfaces. This is seen in this trial as well with all genotypes except FB1901, 'Tifdwarf', and 'Tahoma 31' having an acceptable rating (6) for the entirety of the trial (Table 8). This was to be expected as 'Tifdwarf' is the oldest grass used in the trial, coming from a time when leaf thickness was generally courser, and 'Tahoma 31' was developed for use on athletic fields where extremely fine leaf blades are not required. However, although the numerical value for 'Tahoma 31' was generally lower, they were not significantly different than entries rated 6.5. Like the density ratings, MSB1050, OKC0805, OKC0920, and OKC3920 performed numerically better throughout the trial and were among the top statistical grouping with OKC0920 having the highest rating of 7.8 in September and October.

Turfgrass Uniformity

There was a significant month by genotype interaction for the turfgrass uniformity rating (Table 4). There was a general decrease in uniformity across all genotypes as the summer progressed with various influences such as heat, mowing, and disease stressors impacting the uniformity of the plots. This was shown most genotypes being in the top overall statistical group for June, but only 4 and 1 in September and October, respectively (Table 9). In general, 11x2 performed numerically well throughout the trial along with 19x19 and 15x9. 63x18 and OKC0920 showed poorer uniformity during the trial.

Mowing Stress

There was a significant interaction for month by genotype for the mowing stress rating; (Table 4) however, when looking at within month, there was not as much statistical differences between genotypes (Table 10). Numerically, 'TifEagle' and FB1901 performed well throughout the study. 'Tifdwarf' was impacted by disease at the beginning of the summer which may have confounded with the mowing stress but improved in the later season. This rating also showed a

trend downward as the summer went on, likely due to the same influences as the uniformity. However, 11x2 showed good tolerance to the mowing treatment along with OKC0805 and OKC3920 for the first three months. MSB1050 showed generally a lower mowing stress rating due to the quality of cut when mowed.

Seedhead Production

Most genotypes showed acceptable seedhead production during the trial, but there was a month by genotype interaction (Table 11). The data showed some genotypes went through a floral production period where they produced seedheads, and after a time went back to primarily vegetative growth. During June, OKC3920 and 15x9 had seedhead ratings of 5.8 and 5.6, respectively, which was statistically different than the other genotypes during the month (Table 12). From July on, 63x18 was in the bottom statistical group ranging from 4.4 to 6.6, with 15.9 and OKC0805 also showing some seedhead production but not statistically different.

Disease Ratings

Turf samples were sent to Turfgrass Diagnostics labs for each of the disease episodes, but definitive results were not obtained for the first disease event. For the second event, DNA from *Culvularia* spp. and *Exerohilum* ssp. were found in the samples. Based on visual assessments, the disease followed the pattern of leaf spot on putting greens for each of the episodes.

During the Summer 2021 disease episode, two data collections were taken. There was a significant date by genotype interaction (Table 13). For this episode, the ultradwarf genotypes were most effected followed by OKC3920. On the first date, ‘TifEagle’ had a rating of 5.4, followed by FB1901 with a rating of 5, and ‘Tifdwarf’ with 4.4 (Table 14a). OKC3920 had a rating of 6.6 which was not significantly different from ‘TifEagle’ but was only different from the top two genotypes. All genotypes showed significant recovery by the second date with no significant difference among the genotypes, with the lowest rating of 8.2 for ‘Tifdwarf’.

During the Summer 2022 disease episode, seven total data collections were taken from September 9th to October 18th. As with the previous disease episode, there was a significant date by genotype interaction (Table 13). Disease recovery during this time was limited by poor growing conditions as the day length shortened and temperatures cooled. All genotypes were affected during this episode, with some performing slightly better across the dates collected. Genotype OKC0805 showed the first signs of disease and had the lowest rating on the first data of 4.2 but was not significantly different than half the total genotypes ranging from 6.4 to 5.4 (Table 14b). By the second date, OKC0805 had a rating of 3 and was not different from the two genotypes above it at 4. 15x9, MSB1050, 11x2, OKC3920 had the top ratings ranging from 6.4 to 6. The bottom three (OKC0805, OKC1609, and 12x3) stayed the same the following week, with the top genotypes staying with 15x9 and 11x2 followed by the ultradwarf genotypes.

Fall Color Retention

Fall 2021

Data collection in the Fall of 2021 was collected weekly from October 25th to November 29th, and there was a significant date by genotype interaction during this time (Table 15). OKC1609 was the first genotype to begin entering winter dormancy with a rating of 6 on October 25th with all other entries ranging from 9 to 7.2 (Table 16a). This continued the following week with OKC1609 being statistically different than all other genotypes with a rating of 4. Among the top numerical values for this date were 11x2, MSB1050, and the ultradwarf genotypes ranging from 8.8 to 8.4. All of these were significantly different than the bottom three, 5x23, 34x20, and OKC1609. For the 8th of November, the bottom two genotypes, 5x23 and OKC1609, were significantly different than all other entries with ratings of 3.4 each. The top entry for the same week was MSB1050 with a rating of 8 but was not significantly different than the ultradwarf genotypes and 11x2 as before. The following week MSB1050 and the ultradwarfs remained at the

top, but was not different than ‘Tahoma 31’, OKC3920, and 19x19. The last week of data, MSB1050 was significantly different than all other genotypes with a rating of 5.8. The next highest was OKC0920 with a rating of 3. At this point, MSB1050 was not significantly different than the lowest three genotypes on the first collection date.

Fall 2022

Data collection for the Fall of 2022 was done weekly from October 26th to November 24th, and there was a significant date by genotype interaction during this time (Table 15). The disease pressure in late summer of 2022 caused issues in determining the fall color retention. Before the time of the first hard frost when fall color data would begin, four genotypes (OKC0805, 5x23, 34x20, and ‘Tahoma 31’) were unable to recover from the disease pressure and displayed symptoms of winter dormancy prior and confounded the ratings to assess fall color retention. As a result of this, these genotypes were excluded from data collection to avoid drawing incorrect conclusions on their performance. The other genotypes did show some effects of the disease pressure faced as they began to turn off color much quicker than the previous year, and with different orders. On the first date, the color retention ratings ranged from 6.2 to 3 with ‘TifEagle’ having the highest and OKC3920 and 11x2 at the lowest (Table 16b). For the rest of the dates, ‘TifEagle’ continued to retain the best color along with ‘Tifdwarf’ and FB1901. OSU genotypes 19x19 and OKC0920 also showed color retention that was not significantly different than the ultradwarf genotypes.

Spring Greenup

Data in the spring of 2022 was collected weekly from March 28th to April 25th, and there was also a significant interaction between the date and genotype (Table 17). The ultradwarf genotypes showed earlier spring green up along with OKC0920 and OKC1609. This continued the following week, but then other genotypes showed a quick return of color as temperatures

increased. From April 5th to 11th, OKC3920 went from 5.6 to 7.2, and 5x23 went from 3.6 to 6.4 compared to 'TifEagle' increasing from 6.6 to 7.4 (Table 18). MSB1050 displayed a delayed green up start, but quickly reached full color once started. Some OSU genotypes were slower to return to full green cover, such as 19x19 only rating a 3.2 on the 11th and 6.4 on the 18th.

Rooting Depth

The data was analyzed to detect any seasonal variability using repeated measures analysis. There was no significant interaction between the season and genotype (Table 19), and as such the three data collections were analyzed together for a more powerful test (Table 20). This analysis determined genotypes OKC0920 and OKC3920 were in the top statistical group with root lengths of 21.78 and 20.39 cm, respectively (Table 21). Forming the bottom statistical group were 'Tahoma 31', 12x3, 'TifEagle', and OKC1609, with 'TifEagle' having an average root depth of 13.83 cm.

Ball Roll Distance and Morphological Relationship

To evaluate the relationship between BRD and certain morphological characteristics, the average BRD collected for the three months was calculated to determine an overall average speed for each genotype. For the morphological characteristics, there were a total of eleven measurements taken: 1st and 2nd leaf on the second node length, width, angle, as well as 2nd and 3rd internode length and diameter, and canopy height. Three replications of each genotype were allowed to grow untrimmed for three months to reach full maturity and their natural growth habit. Twenty random stems were collected from each replication of each genotype grown in the greenhouse, and the listed characteristics were evaluated. The canopy height was measured in 60 random locations. After the first data collection, the pots were trimmed to the soil and allowed to grow for another three months, and the data collection was repeated as before. This data was then analyzed to determine the least squares means for each characteristic over both trials, and then the

two leaves and internodes were averaged to determine an overall average leaf length, width, angle, and internode length and diameter which were then used to evaluate the relationship with BRD.

A pairs plot was made to evaluate the relationship between the BRD and observed morphological characteristics (Figure 1). Upon observation, a few morphological characteristics had significant correlations to BRD and appeared to have a curvilinear relationship to the BRD; these are leaf length, canopy height, and leaf angle. These selected characteristics were then further evaluated to determine the best fit model to understand the impact of morphology on BRD.

Based on the curvilinear trends observed for each of the characteristics, a cubic regression model was fitted for each relationship. Of the three models, the leaf length model ($F_{3,13}$ = 55.55, p = <0.001) resulted in the highest adjusted R^2 value and lowest AIC, 0.91 and -43.10, respectively (Table 22). The canopy height model ($F_{3,13}$ = 39.47, p = <0.001) was slightly behind with values of 0.85 and -34.54, respectively. The leaf angle model ($F_{3,13}$ = 3.8, p = 0.037) had the worst fit with values of 0.34 and -9.16, respectively. After the leaf length model was chosen, the other variables were added to the model to determine if they would be able to improve the model fit, but they were unable to add any meaningful explanation to the model.

The leaf length model was chosen as the best fit model to explain the impact of morphology on BRD ($BRD = 7.67 - 1.12LL + 0.0804LL^2 – 0.00191LL^3$) (Figure 2). The negative coefficient of the linear and cubic terms indicates an overall downward trend of BRD as the average leaf length increases. Additionally, the small coefficient values indicate a flatter relationship, with BRD leveling off from 12 – 18 mm. The adjusted R^2 of 0.91 indicates this model can explain most of the variability observed in the data.

Discussion

Putting Green Trial

'TifEagle' was the top-performing commercial standard ranking towards the top of most measured traits, which follows the reports of its improved characteristics over the older 'Tifdwarf' cultivar (Hanna and Elsner, 1999; Hein, 1961). 'TifEagle' ranked consistently in the top statistical group for two of the more important traits for golfers: BRD and turf quality. However, 'TifEagle' displayed one of the shortest root length measurements across the data collections, which is one of the downfalls of these ultradwarf genetics and a characteristic superintendents would like to see improved according to USGA agronomist John Daniels (2020). Improved performance under limited water resources has been seen with increased rooting depth in warm-season grasses (Hays et al., 1991; Huang et al., 1997). Increased rooting depth would also allow for greater nutrient absorption and uptake as nutrients move through the soil profile (Thorup-Kristensen et al., 2020). 'TifEagle' was also among the genotypes showing more damage during the disease episode during 2021, but slight improvement during the 2022 episode.

The performance of the fourteen experimental genotypes and 'Tahoma 31' showed significant variation across the observed measurements. FB1901 was among one of the top-performing experimental genotypes and was the only genotype to show improved BRD compared to 'TifEagle'. This experimental genotype ranked similarly to 'TifEagle' in all other characteristics. FB1901 showed a numerically deeper rooting length of 16.4 cm compared to 13.83 of 'TifEagle', but the two were not significantly different. Genetic analysis of the genotypes evaluated in this study done by Earp et al. (2022), revealed FB1901 to have a genetic similarity coefficient of 1 compared to 'TifEagle' and 'Tifdwarf' indicating they have the same genetic background, which revealed FB1901 as an ultradwarf mutation as well. This explains the

similar or improved performance to ‘TifEagle’; however, this work indicated all other genotypes were from distinctly different genetic backgrounds compared to the ultradwarfs.

The remaining genotypes evaluated showed variable performance under the 3.2 mm HOC implemented in this study. While none of the experimental genotypes were able to reach the same BRD as ‘TifEagle’ and FB1901, some were able to perform similar to ‘Tifdwarf’ which some courses still use today. Additionally, it should be noted there are many management practices which can increase the BRD for any given grass that were not implemented in this study. These practices include mowing height and frequency, rolling, fertility, verticutting, brushing, topdressing, irrigation, and PGRs (Dowling and Gross, 2019; Fagerness et al., 2000; Lulis and Kaminski, 2022; McCarty et al., 2011; Nikolai, 2005; Oatis, 2016; Patrick et al., 2006; Reasor and Brosnan, 2020; Richards et al., 2008). This field trial was managed to promote healthy turf and limit stressors. The grass was cut once daily and was not verticut during the trial to limit any cross contamination between plots. The recommended rate of trinexapac-ethyl was used alone and not in conjunction with other PGRs. The turf was also supplied with sufficient nitrogen and moisture throughout the trial. Adjustments in management practices could potentially be utilized to increase the BRD of the experimental genotypes under close management on golf courses. It should be remembered these genotypes are different and would be expected to perform differently than the ultradwarfs many management practices have been adapted. Further research is planned to evaluate the effect these practices have on these non-ultradwarf genotypes to gain a better understanding of their impact on BRD. Even still, some of the genotypes evaluated in this study showed adequate performance above 2.45 m.

While BRD is an important aspect of putting green performance, chasing higher green speeds to the detriment of turfgrass health should be avoided (Moraghan, 2012). Fast greens beyond the ability of many golfers could slow play down considerably as they take longer to make putts on the difficult surface (Moraghan, 2012; Waters, 2023). Previous work has also

shown has shown many golfers struggle in detecting variation in green speeds, so if speeds are kept consistent across the course the specific speed of the green is not as important (Karcher et al., 2001).

Possibly more important than BRD are the visual evaluations of turfgrass performance evaluated in this study. Several genotypes were able to display similar or improved performance compared to 'TifEagle'. As expected, the ratings were significantly impacted by month and were analyzed as such. Genotypes 11x2, 15x9, OKC3920, and MSB1050 consistently were among the highest quality ratings over the summer. Many of the experimental genotypes showed significantly finer leaf texture and improved uniformity when maintained at 3.2 mm compared to 'TifEagle', and OKC0805, OKC0920, 11x2, OKC3920, and MSB1050 showed similar density and mowing stress ratings during the trial. Turfgrass color preference can be a subjective subject with some individuals preferring a darker green while other would prefer the grass have a lighter color. In this study, 11x2, 5x23, 34x20, 63x18, and MSB1050 were consistently similar in color to 'TifEagle' while genotypes such as OKC0920, OKC1609, 12x3, and 'Tahoma 31' had a lighter green color when maintained at this height. Throughout the study, the ultradwarf genotypes had no or very little seedhead production while 63x18 consistently produced seedheads. Other genotypes such as OKC3920, 15x9, OKC0920, OKC0805, and MSB1050 had short periods during the summer when they would produce seedheads.

The genotypes evaluated in this study showed varied response to disease pressures encountered in both years. During the first disease event (the pathogen was unable to be determined), the ultradwarf genotypes showed the most susceptibility to the disease while all others remained above the acceptable threshold, and all showed adequate recovery after a curative application of a fungicide (Mancozeb [ethylene-bis-dithiocarbamate]). However, for the second disease event caused by *Culvularia* and *Exerohilum* ssp., all genotypes displayed significant damage and slow recovery despite the applications of several fungicides during the event

(Mefentrifluconazole[2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1- (1H-1,2,4-triazole-1-yl)propan-2-o], Chlorothalonil [tetrachloroisophthalonitrile], and Acibenzolar-S-methyl [Benzo(1,2,3)thiadiazole-7-carbothioic acid-S-methyl ester]). The causal agent was not able to be identified until after the disease event so fungicides were applied based on visual symptom assessments. However, genotype OKC0805 showed the earliest and most severe symptoms during this time and was unable to recover prior to winter dormancy. Symptoms first appeared on OKC0805 towards the end of August 2022, weather data shows the maximum temperatures were consistently above 30º C during August reaching up to 40 º C and most days above 30º C September as well. Management practices attempting to maintain stress free turf through irrigation and fertilization during these hot periods could have created ideal conditions for the *Exerohilum* spp. to create leaf spot conditions as seen in China on other hybrid bermudagrass cultivars by Zhang et al. (2016). The presence of *Culvularia* ssp. was identified as a not being a primary or secondary fungi by Brecht et al. (2007) and was a saprophyte, feeding on the already senescing tissues.

Fall color retention and spring green up are important for superintendents as they represent the period when the turfgrass is actively photosynthesizing and is more capable to recover from damages occurring during play. Unlike golf courses in the Northern United States, courses in the transition zone and Southern United States do not usually close courses during the entire winter months as the weather does not get as severe to justify closing for the extended period. Because of this, superintendents generally prefer cultivars with late fall color retention and early spring green up. Leading up to winter dormancy, the process of cold acclimation is extremely important for warm season turfgrasses. During cold acclimation, the turfgrass will shift osmotic protectants, protein synthesis, antioxidant production, and fatty acid saturation in preparation for freezing temperatures (Munshaw et al., 2006; Zhang et al., 2006). Cold acclimation is analogous to chilling stress and fall color retention reported in turfgrass when

exposed to low nonfreezing temperatures (Fontanier et al., 2020). The study by Fontanier et al. (2020) observed some chilling-induced changes in lipid composition of the leaves promoted freezing tolerance but negatively affected the chilling tolerance of the plant and better post dormancy regrowth. This idea may explain the reason the present study observed poor fall color retention in some of the genotypes showing improved freeze tolerance over 'TifEagle' such as OKC1609 and OKC0805 (Gopinath et al., 2021). The performance of MSB1050 follows similarly having the best fall color retention with some color persisting through January of the following year but was one of the slower genotypes to begin greening up the next spring. However, this would not explain the performance of 'Tahoma 31' showing similar fall color retention, but slower spring green up observed in the present study. The impact of the cover on the soil and canopy microclimate could have also impacted the observed early spring green up in 'TifEagle' not typically seen in more freeze susceptible genotypes.

Additionally, the second year of fall color retention showed different results in the performance of different genotypes. However, this could be attributed to the disease pressure experienced in the two months leading up to winter dormancy causing the plants to be more susceptible to chilling injuries prior to freezing temperatures. There were also potential discrepancies in the off color resulting from the disease damage or chilling or freezing temperatures with four genotypes removed from evaluations due to them turning off color and remaining well before cool temperatures were present (OKC0805, 5x23, 34x20, and 'Tahoma 31').

One area of significant improvement the experimental genotypes showed over the commercial standards was in their rooting depth. 'TifEagle' has been criticized for its shallow rooting depth when mown at putting green heights (Tucker et al., 2006). In general, it has been observed as mowing height decreases, the plant loses its ability to form deep roots (Turgeon and Kaminski, 2019). In this study, nine genotypes showed significantly deeper root systems when

maintained at the same height as ‘TifEagle’ with OKC0920 averaging almost 22 cm; six genotypes had numerically long roots but were not significantly different, and only one genotype had shorter root systems than ‘TifEagle’. As stated previously, increased rooting depth has been shown to improve turfgrass performance under limited water resources and allows for greater nutrient absorption from the soil solution (Hays et al., 1991; Huang et al., 1997; Thorup-Kristensen et al., 2020).

Ball Roll Distance and Morphological Characteristics

The results of this data follow the general consensus of the turfgrass industry for the understanding of the influences behind BRD. When working on the development of grasses for putting greens, Dr. Burton selected genotypes displaying short, fine leaves and short internodes which made them better adapted for the low mowing heights on putting greens (Hein, 1961). As mutations have occurred over the years, they have been consistently selected for shorter or more dwarfed characteristics as superintendents have lowered the HOC. The model also highlights the importance of average leaf length, even as internode length decreases. Some of the genotypes displayed shorter internodes than the ultradwarf genotypes but did not as long BRDs. Genotypes with longer leaf lengths may still try to reach their desired length even when mown short. This upward growth would then increase the friction imposed on the golf ball as it rolled across the turfgrass surface decreasing its distance. The pairs plot also reveals a strong correlation (r=0.85) between average leaf length and canopy height. Wu et al. (2007) also found significant positive correlation between these two traits in common bermudagrass accession (r=0.53). Additionally, both the current work and that by Wu et al. (2007) found significant positive correlation between internode lengths and leaf lengths and canopy height. These works differ in the relationships between internode length and width; Wu et al. (2007) found a positive correlation between the two whereas this work found a negative relationship. This knowledge could also help turfgrass breeders when selecting genotypes in the field as even though the model indicates the leaf length

plays a more important role in BRD, breeders can visually select shorter growing genotypes before then looking closer at the leaf length. Overall, this model shows evidence supporting the idea that shorter morphological characteristics can improve BRDs on bermudagrass putting greens. Turfgrass breeders can use this data to assist in selecting potential genotypes with the potential for acceptable BRDs before evaluation in a putting green trial to help save time and resources.

Conclusion

This study was conducted on a modified USGA sand based putting in 2021 and 2022 in Stillwater, Oklahoma. Fourteen experimental genotypes from three universities along with two commercial ultradwarf genotypes and one hybrid bermudagrass developed for use on sports fields and golf course fairways. One experimental genotype (ultradwarf mutation from UF) showed similar performance to the top commercial standard, 'TifEagle', in most measurements observed. Several experimental genotypes from OSU and the genotype from MSU demonstrated similar performance in the visual assessment ratings to 'TifEagle' but exhibited lower BRDs. These experimental genotypes did show significant improvement in their root depth abilities under mowing heights of 3.2 mm potentially increasing their performance under limited irrigation.

Despite differences in BRD, this study has identified superior performing experimental bermudagrass genotypes (OKC0920, OKC3920, 11x2, 19x19, and MSB1050) with new genetic backgrounds that can withstand extended periods of low mowing heights used on golf course putting greens (3.2 mm). The information gained from this field study will better inform OSU, MSU, and UF turfgrass breeders to make decisions on whether to pursue the experimental genotypes used in this study for commercial release. Additionally, this study has sparked new projects into gaining more understanding of the impact of different management practices on turfgrass quality and BRD for these new greens-type hybrid bermudagrass genotypes to make

recommendations to golf course superintendents. Additionally, this study has revealed some of the morphological influencers on putting green BRD turfgrass breeders can use when selecting new genotypes for future testing. The model created in this study can be improved through further research including additional genotypes, morphological characteristics, and trial locations, but serves as the first trial to evaluate these relationships.

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Table 1. Experimental genotype/variety name and origin of the 16 *C. dactylon* x *C. transvaalensis* hybrids included in this study.

Entry Number	Entry Name	Origin/Reference
1	OKC0805	Oklahoma State University
2	OKC0920	Oklahoma State University
3	OKC3920	Oklahoma State University
4	OKC1609	Oklahoma State University
5	3x23	Oklahoma State University
6	5x23	Oklahoma State University
7	11x2	Oklahoma State University
8	12x3	Oklahoma State University
9	15x9	Oklahoma State University
10	19x19	Oklahoma State University
11	34x20	Oklahoma State University
12	63x18	Oklahoma State University
13	MSB1050	Mississippi State University
14	FB1901	University of Florida
15	'Tahoma 31'	Wu et al. (2020)
16	'TifEagle'	Hanna and Elsner (1999)
17	'Tifdwarf'	Burton (1966)

Table 2. Test of fixed effects for ball roll distance using Type III analysis for 2022

Source	**Ball Roll Distance**	
	df ‡	F value
Genotype (G)	16,64	52.22***
Month (M)	2,136	402.16***
GxM	32,136	6.62***

‡ Degrees of freedom for numerator, denominator
*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 3. Mean, minimum, and maximum ball roll distances from June to August 2022

Ball Roll Distance (m)†							
Cultivar	June		July		August		
	Mean	Min/Max	Mean	Min/Max	Mean	Min/Max	
OKC0805	2.28r§	2.21/2.40	2.58d-m	2.40/2.72	2.65b-i	2.54/2.76	***
OKC0920	2.37n-r	2.26/2.43	2.60d-j	2.51/2.75	2.59d-k	2.56/2.63	***
OKC3920	2.28r	2.25/2.30	2.47h-r	2.44/2.51	2.39l-r	2.37/2.44	***
OKC1609	2.32pqr	2.25/2.39	2.61d-j	2.54/2.63	2.54e-o	2.49/2.58	***
3x23	2.35o-r	2.27/2.45	2.47h-r	2.34/2.55	2.42j-r	2.30/2.47	**
5x23	2.38m-r	2.25/2.55	2.51f-p	2.38/2.64	2.47h-r	2.38/2.54	**
11x2	2.43j-r	2.33/2.51	2.58d-l	2.49/2.64	2.54e-o	2.50/2.57	***
12x3	2.37n-r	2.32/2.44	2.64c-i	2.59/2.72	2.66b-h	2.57/2.73	***
15x9	2.33pqr	2.23/2.42	2.59d-l	2.47/2.65	2.45i-r	2.32/2.57	***
19x19	2.48g-q	2.39/2.62	2.70b-f	2.63/2.80	2.56e-n	2.53/2.61	***
34x20	2.38n-r	2.32/2.44	2.53e-o	2.42/2.61	2.47h-r	2.38/2.53	***
63x18	2.40k-r	2.34/2.45	2.55e-n	2.45/2.69	2.50g-p	2.43/2.54	***
MSB1050	2.47h-r	2.35/2.54	2.77bcd	2.58/2.91	2.68b-g	2.53/2.76	***
FB1901	2.76bcd	2.69/2.85	3.14a	3.04/3.26	3.23a	3.10/3.33	***
Tahoma 31	2.30qr	2.19/2.38	2.46i-r	2.27/2.62	2.45i-r	2.37/2.56	***
TifEagle	2.71b-e	2.63/2.83	3.11a	3.04/3.19	3.13a	3.08/3.19	***
Tifdwarf	2.60d-j	2.55/2.79	2.83bc	2.73/2.93	2.84b	2.68/2.92	***
	***#		***		***		

†Ball roll distance was measured weekly from June to August in 2022 according to USGA guidelines and monthly averages were calculated

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level across all columns

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

Table 4. Test of fixed effects for visual ratings using Type III analysis for field trial in 2022

Source	Turfgrass Quality		Turfgrass Color		Turfgrass Density		Turfgrass Texture		Turfgrass Uniformity		Turfgrass Mowing Stress	
	df ‡	F value	df	F value	df	F value	df	F value	df	F value	df	F value
Genotype (G)	16,64	15.17***	16,114.9	55.63***	16,66.07	42.05***	16,69.12	39.45***	16,86.49	10.56***	16,88.71	13.36***
Month (M)	4,272	165.05***	4,143.9	142.55***	4,158	8.81***	4,150.8	69.9***	4,154.6	89.21***	4,257.9	5782***
GxM	64,272	13.58***	64,234.7	13.23***	64,221.7	10.34***	64,219.6	3.16***	64,229.1	7.84***	64,263.5	6.21***

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

Table 5. Mean turfgrass quality ratings collected biweekly from June to October 2022

	Turfgrass Quality†					
Cultivar	June	July	August	September	October	
OKC0805	6.7a§	6.5abc	5.4c-o	3.9stu	2v	***
OKC0920	5.6a-l	5g-s	5.2e-q	5.6a-l	3.9stu	***
OKC3920	5.6a-l	6.1a-g	5.8a-j	5.6a-l	3.7tu	***
OKC1609	5.5b-n	4.7j-t	4.3o-u	4.2p-u	3.4u	***
3x23	5.1f-r	5g-s	4.8i-q	4.9h-s	4.5l-u	NS¶
5x23	5.5b-n	5.4c-o	5.2e-q	5.1f-r	3.7tu	***
11x2	6.3a-e	6.6ab	5.7a-k	5.7a-k	4.8i-t	***
12x3	5.4c-o	5.3d-p	5.5b-m	4.4n-u	3.4u	***
15x9	5.8a-j	5.7a-k	5g-s	6a-h	5.6a-k	***
19x19	5.4c-o	5.3d-p	5.2e-q	5.3d-p	4.4l-u	***
34x20	5.2e-q	5.8a-j	5.3d-p	4.9h-s	3.7tu	***
63x18	4.6k-t	4r-u	4.1q-u	4.9h-s	4.8i-t	***
MSB1050	5.6a-l	5.2e-q	5.7a-k	5.8a-j	4.5l-u	***
FB1901	5.4c-o	5.9a-i	6.2a-f	5.9a-i	5.4c-o	**
Tahoma 31	5.6a-l	5.6a-l	5.1f-r	5.6a-l	3.4u	***
TifEagle	6.1a-g	6.1a-g	6.4a-d	6.2a-f	5.2e-q	***
Tifdwarf	3.4u	4.2p-u	4.5l-u	5.8a-j	4.8i-t	***
	***#	***	***	***	***	

† Turfgrass quality was rated on a 1 to 9 scale where 9 was considered to have exceptionally high quality and 1 was considered to have exceptionally low quality

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

¶ NS not significant at the 0.05 level

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

Table 6. Mean turfgrass genetic color ratings collected biweekly from June to October 2022

	Turfgrass Color†					
Cultivar	June	July	August	September	October	
OKC0805	6.1b-j§	6.7a-d	5.9d-k	5.8d-k	2.1s	***
OKC0920	4r	5.3i-p	5.6f-n	5.4h-o	4.7n-r	***
OKC3920	5.5f-o	5.8d-k	5.7e-m	5.5f-o	4.1r	***
OKC1609	4.5o-r	4.7m-r	5k-q	5k-q	4.2qr	**
3x23	5.9c-k	6c-j	5.7e-m	5.7e-m	5.5g-o	**
5x23	5.8d-l	7ab	6.9abc	6.5b-f	4.6o-r	***
11x2	7.1ab	7.7a-d	6.9abc	6.9abc	5.6f-n	***
12x3	5.1j-q	5.2i-p	5.4h-o	5.6f-n	5.2j-p	NS¶
15x9	5.7d-m	5.5f-o	5.9c-k	6.9abc	6c-j	*
19x19	5.5f-o	5.9c-k	5.5f-o	6c-j	5.8d-l	*
34x20	5.9d-k	6.9abc	6.9abc	6.1b-i	4.5pqr	***
63x18	6.2b-i	6c-j	6.5b-f	6.6b-e	5.9c-k	*
MSB1050	6.7a-d	6.5b-f	6.6b-e	6.9abc	5.1j-p	***
FB1901	6.2b-i	6.6b-e	6c-j	5.9d-k	5.8d-k	***
Tahoma 31	5.2i-p	5.3i-p	5.8d-k	5.7e-m	4.2qr	***
TifEagle	6.4b-g	6.6b-e	6c-j	6c-j	5.7e-m	***
Tifdwarf	5.8d-l	5.5f-o	6.1b-i	5.9c-k	5.6f-m	*
	***#	***	***	***	***	

† Turfgrass color was rated on a 1 to 9 scale where 9 was considered to have dark green color and 1 was considered to have light green

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

¶ NS not significant at the 0.05 level

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

Table 7. Mean turfgrass density ratings collected biweekly from June to October 2022

	Turfgrass Density†					
Cultivar	June	July	August	September	October	
OKC0805	7.7a-d§	6.9a-g	6.9a-g	5.1j-p	6.6b-i	***
OKC0920	7a-g	6.3c-j	6.7a-h	7.1a-e	6.9a-g	**
OKC3920	6.8a-h	6.3c-j	6.9a-g	6.9a-g	6.5b-i	*
OKC1609	6.4b-j	5.7f-m	6e-k	4.8l-p	6.1e-k	***
3x23	5.7f-n	5.2i-p	5k-p	5.7f-m	5.8f-l	***
5x23	6.2d-k	5.1j-p	5.5h-n	5.5h-n	5.5h-n	**
11x2	6.7a-i	6.4b-j	5.6h-n	6e-k	5.9f-l	***
12x3	6.4b-j	5.6g-n	5.6h-n	5k-p	5.2i-p	***
15x9	6.3c-k	5.5h-n	5.4i-o	5.9f-l	5.7f-m	**
19x19	5.7f-n	5.5h-n	5.4i-o	5.3i-o	5.6g-n	NS¶
34x20	6e-l	5.4h-o	5.7f-m	5k-p	5.3i-o	***
63x18	5.2i-p	4.5m-p	4.2op	5.3i-o	5.3i-o	***
MSB1050	7.4a-e	7.1a-f	7.8ab	8a	7.8a	**
FB1901	7a-g	7.7abc	8a	7.8ab	7.8a	**
Tahoma 31	6.2d-k	5.5h-n	5.6g-n	5.7f-m	4.4nop	***
TifEagle	7.4a-e	7.8ab	8a	7.9a	8a	NS
Tifdwarf	3.9p	4.9k-p	6e-k	6.8a-g	6.4c-j	***
	***#	***	***	***	***	

† Turfgrass density was rated on a 1 to 9 scale where 9 was considered to have exceptionally dense canopies and 1 was considered to have exceptionally thin canopies

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

¶ NS not significant at the 0.05 level

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

Table 8. Mean turfgrass texture ratings collected biweekly from June to October 2022

	Turfgrass Texture†					
Cultivar	June	July	August	September	October	
OKC0805	7.3a-d§	6.9a-h	7.5abc	7.7ab	7a-g	***
OKC0920	6.7c-j	6.8b-h	7.5abc	7.8a	7.8a	***
OKC3920	7a-g	7a-g	7.2a-e	7.7ab	7.2a-e	**
OKC1609	7.1a-f	6.7c-j	7.5abc	7.7ab	6.9a-h	***
3x23	6.7d-j	6.7d-j	6.9a-h	6.9a-h	6.7c-j	NS¶
5x23	6.1g-k	6.5d-k	6.9a-h	6.9a-h	6.4d-k	***
11x2	6.5d-k	6.3f-k	6.8b-h	6.9a-h	6.7c-j	***
12x3	6h-k	6.5d-k	6.8b-h	7a-g	6.3e-k	***
15x9	7a-g	7a-g	7a-g	7.1a-f	6.9a-h	NS
19x19	6h-k	6.1g-k	6.6d-j	6.4d-k	6h-k	**
34x20	6.3e-k	6.2g-k	6.8b-i	6.8b-i	6.1g-k	***
63x18	6.2f-k	6h-k	6.4e-k	6.2f-k	6.3e-k	NS
MSB1050	7.1a-f	6.9a-h	7.2a-e	7.6abc	7.6abc	***
FB1901	5.8jk	6h-k	6.7c-j	6.8b-i	6.9a-h	***
Tahoma 31	5.6k	5.8jk	5.7k	5.9ijk	5.8jk	NS
TifEagle	6h-k	6h-k	6.8c-i	6.5d-k	6.6d-j	***
Tifdwarf	5.6k	6h-k	6.5d-k	6.1g-k	6h-k	***
	***#	***	***	***	***	

† Turfgrass texture was rated on a 1 to 9 scale where 9 was considered to have exceptionally fine leaf texture and 1 was considered to have exceptionally course leaf texture

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

¶ NS not significant at the 0.05 level

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

Table 9. Mean turfgrass uniformity ratings collected biweekly from June to October 2022

	Turfgrass Uniformity†					
Cultivar	June	July	August	September	October	
OKC0805	7.1a§	5.9a-g	5.9a-g	3.8klm	4.5f-m	***
OKC0920	5.2b-k	4.5f-m	4.9d-l	5c-k	4.6f-m	*
OKC3920	6.4a-d	6.4abc	6.2a-d	5.2b-k	3.3m	***
OKC1609	6.1a-f	5c-k	5.4b-i	4.9d-l	4.1i-m	***
3x23	6a-g	5.2b-k	5.7a-h	5.4b-i	5c-k	**
5x23	6a-g	4.9d-l	5c-k	5.4b-i	4.4h-m	***
11x2	6.5abc	7.1a	6.9a	6.6ab	5.3b-k	***
12x3	6.6ab	6.2a-d	6.4abc	4.4h-m	3.5lm	***
15x9	6.5abc	6.3a-d	6.3a-d	6.6ab	6.1a-e	NS¶
19x19	6.6ab	6.1a-e	6.3a-d	5.7a-h	5c-k	***
34x20	5.7a-h	5.1b-k	5.1b-k	5.3b-j	4.2i-m	***
63x18	5.3b-k	3.9klm	4.1j-m	4.8e-l	5.3b-i	***
MSB1050	6.4a-d	5.8a-h	6.1a-d	5.3b-j	4.4h-m	***
FB1901	5.6a-i	5.7a-h	6.2a-d	5.7a-h	5.1b-k	**
Tahoma 31	5.8a-h	5.8a-h	6.2a-d	5.4b-i	4.1i-m	***
TifEagle	5.9a-h	5.2b-k	5.9a-g	5.1b-k	5.2b-k	***
Tifdwarf	4.5f-m	4.5g-m	4.7f-l	5.9a-h	5.3b-k	***
	***#	***	***	***	***	

† Turfgrass uniformity was rated on a 1 to 9 scale where 9 was considered to have exceptionally uniformity and 1 was considered to have exceptionally poor uniformity

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

¶ NS not significant at the 0.05 level

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

Table 10. Mean turfgrass mowing stress ratings collected biweekly from June to October 2022

	Turfgrass Mowing Stress†					
Cultivar	June	July	August	September	October	
OKC0805	6.7a-d§	6.5a-f	6.5a-f	5j-n	4.4n	***
OKC0920	6.1a-i	5.7d-l	6.6a-e	6.2a-i	5.7d-l	***
OKC3920	6.5a-f	6.3a-h	5.9b-k	6.3a-h	5.8c-k	**
OKC1609	6.4a-g	5.4g-n	6.7a-d	4.7lmn	5.2i-n	***
3x23	5j-n	5.3h-n	5.3h-n	5.4g-n	5.5f-m	**
5x23	6.3a-h	6a-j	6.4a-g	5.7d-l	5.6e-l	***
11x2	6.8abc	6.7a-d	6.3a-h	6.3a-h	5.7d-l	***
12x3	6.3a-h	5.5f-m	6.3a-h	4.5mn	4.9k-n	***
15x9	6.3a-h	6a-j	6.2a-i	6.3a-h	6.2a-i	NS¶
19x19	6.2a-i	5.8c-k	6.2a-i	5.8c-k	5.6e-l	*
34x20	5.9b-k	6a-j	6.5a-f	5.3h-n	5j-n	***
63x18	5.6e-l	5.3h-n	5.6e-l	5.6e-l	5.9b-k	NS
MSB1050	6a-j	5.5f-m	6.3a-h	6.4a-g	5.2i-n	***
FB1901	6.5a-f	6.3a-h	6.8abc	6.5a-f	6.4a-g	NS
Tahoma 31	6a-j	5.6e-l	6.3a-h	5.6e-l	4.4n	***
TifEagle	6.4a-g	6.8abc	7a	6.9abc	6.5a-f	*
Tifdwarf	5.2i-n	5.9b-k	6.3a-h	6.5a-f	5.9b-k	***
	***#	***	***	***	***	

† Turfgrass uniformity was rated on a 1 to 9 scale where 9 was considered to have exceptionally mowing stress tolerance and 1 was considered to have exceptionally poor mowing stress tolerance

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

¶ NS not significant at the 0.05 level

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

Table 11. Test of fixed effects visual seedhead ratings using Type III analysis for field trial in 2022

Source	**Seedheads**	
	df ‡	F value
Genotype (G)	16,83.06	40.59***
Month (M)	4,129.2	46.45***
GxM	64,222.3	13.29***

‡ Degrees of freedom for numerator, denominator

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

Table 12. Monthly turfgrass seedhead ratings collected from June to October 2022

	Seedheads†					
Cultivar	June	July	August	September	October	
OKC0805	9a§	7.6bcd	8a-d	8.2abc	9a	***
OKC0920	8.6abc	8.4abc	8.2a-d	9a	9a	*
OKC3920	5.8fg	8.6abc	9ab	9a	9a	***
OKC1609	9a	9a	9ab	9a	9a	NS¶
3x23	8a-d	9a	9ab	9a	9a	***
5x23	8.8ab	9a	9ab	9a	9a	NS
11x2	8.6abc	9a	8.6abc	9a	9a	NS
12x3	8.4abc	9a	9ab	9a	9a	NS
15x9	5.6gh	7.4cde	7.2c-f	9a	9a	***
19x19	7.6bcd	9a	9ab	9a	9a	***
34x20	8.2abc	9a	9ab	9a	9a	**
63x18	8.6abc	4.4h	5gh	7def	6.6ef	***
MSB1050	7.2cde	8.8ab	9ab	9a	9a	***
FB1901	9a	9a	9ab	9a	9a	NS
Tahoma 31	9a	9a	9ab	9a	9a	NS
TifEagle	8.8ab	9a	9ab	9a	9a	NS
Tifdwarf	9a	8.2abc	8a-d	9a	9a	***
	***#	***	***	***	***	

† Turfgrass uniformity was rated on a 1 to 9 scale where 9 was considered to have no seedheads and 1 was considered to have exceptionally high number of seedheads
§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level
¶ NS not significant at the 0.05 level
# *, **, *** significant at *P* = 0.05, 0.01, and 0.001, respectively

Table 13. Test of fixed effects visual disease ratings using Type III analysis for field trial in 2021 and 2021

Source	**Summer 2021**		**Summer 2022**	
	df‡	F value	df	F value
Genotype (G)	16,74.12	16.12***	16,60.71	43.90***
Date (D)	1,74.12	324.48***	6,193.6	141.63***
GxD	16,74.12	10.04***	96,315.9	4.08***

‡ Degrees of freedom for numerator, denominator
*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 14a. Visual turfgrass disease ratings collected July 28th and August 5th, 2021

	Turfgrass Disease Ratings†		
Cultivar	28-Jul	5-Aug	
OKC0805	7.8abc§	9a	**
OKC0920	7.6abc	9a	***
OKC3920	6.6cde	9a	***
OKC1609	8.4abc	9a	NS¶
3x23	8.2abc	9a	*
5x23	7.6abc	9a	***
11x2	8.8ab	9a	NS
12x3	8.2abc	9a	*
15x9	8.4abc	9a	NS
19x19	7.6abc	9a	***
34x20	7bcd	9a	***
63x18	7.6abc	9a	***
MSB1050	8.8ab	9a	NS
FB1901	5ef	8.8ab	***
Tahoma 31	7.4bc	9a	***
TifEagle	5.4def	8.8ab	***
Tifdwarf	4.4f	8.2abc	***
	***#	***	

† Turfgrass disease severity was rated on a 1 to 9 scale where 9 indicated no disease presence and 1 indicated complete disease coverage

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

¶ NS not significant at the 0.05 level

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

Table 14b. Visual turfgrass disease ratings collected weekly from September 9th to October 18th, 2022

	Turfgrass Disease Ratings †							
Cultivar	9-Sep	16-Sep	21-Sep	2-Oct	5-Oct	14-Oct	18-Oct	
OKC0805	4.2f-p§	3n-q	3n-q	2qr	1.4r	1.4r	2qr	***
OKC0920	7.4ab	5.2d-i	6b-g	4.8f-l	4.4f-o	4h-p	4.4f-o	***
OKC3920	7.2abc	6b-g	5.4c-i	4.4f-o	4.2g-p	4.8f-k	4.4f-o	***
OKC1609	5.4b-i	4h-p	4h-p	3.4j-q	4.4f-n	2.8o-r	3.2m-q	***
3x23	6.8a-e	5.6b-h	5.6b-h	5.2d-i	5.2d-i	4.8f-j	5e-j	***
5x23	6.2a-f	5e-j	5.6b-h	4.4f-o	4.8f-j	3.2k-q	3.6i-q	***
11x2	7.2abc	6b-g	6.6a-e	5.6b-h	5.4c-i	5.2d-i	5e-j	***
12x3	5.6b-h	4h-p	4.2g-p	3.6i-q	3.6i-q	4h-p	3.4j-q	***
15x9	7.2abc	6.4a-f	6.8a-e	6b-g	5.8b-g	6a-g	6.4a-f	**
19x19	6.2a-f	5.4c-i	6b-g	5e-j	5e-j	4.4f-n	5.2d-i	***
34x20	6a-g	5.2d-i	5.2d-i	4.6f-n	4.6f-m	3.4j-q	3.6i-q	***
63x18	6a-g	5.6b-h	5.2d-i	5e-j	5.4c-i	5.6b-h	5.4c-i	NS¶
MSB1050	8a	6.2a-f	6b-g	5e-j	5.2d-i	4.6f-m	4.8f-j	***
FB1901	6.4a-f	5.4c-i	6.4a-f	5.6b-h	5.6b-h	5.2d-i	5.4c-i	***
Tahoma 31	7.2abc	5.8b-g	6b-g	4.8f-k	4h-p	3.2l-q	2.6pqr	***
TifEagle	7a-d	5.4c-i	6.6a-e	5.8b-g	5.8b-g	5.4c-i	5.4c-i	***
Tifdwarf	6.4a-f	5.8b-g	6.2a-f	5.8b-g	5.6b-h	5.4c-i	5.4b-h	*
	***#	***	***	***	***	***	***	

† Turfgrass disease severity was rated on a 1 to 9 scale where 9 indicated no disease presence and 1 indicated complete disease coverage

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

¶ NS not significant at the 0.05 level

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

Table 15. Test of fixed effects visual fall color retention ratings using Type III analysis for field trial in 2021 and 2021

Source	Fall Color 2021		Fall Color 2022	
	df ‡	F value	df	F value
Genotype (G)	16,66.69	49.27***	12,52.03	16.88***
Date (D)	4,102.9	1767.93***	4,77	412.85***
GxD	64,197.8	8.32***	48,142.2	5.55***

‡ Degrees of freedom for numerator, denominator

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

Table 16a. Fall color retention visual ratings collected weekly from October 25th to November 29th, 2021

	Fall Color Retention †					
Cultivar	25-Oct	1-Nov	8-Nov	15-Nov	29-Nov	***
OKC0805	7.2c-g§	7c-g	5h-l	4.2j-o	1.8rs	***
OKC0920	8a-e	7.2c-g	5.4h-k	5.2h-l	3n-r	***
OKC3920	7.8a-e	7.4a-f	6.2f-i	5.6g-k	2qrs	***
OKC1609	6f-i	4k-p	3.4m-q	2.4o-s	1.4rs	***
3x23	8a-e	7.6a-f	5.6g-j	4j-p	1s	***
5x23	7.4a-f	6f-i	3.4m-q	2.2o-s	1s	***
11x2	9a	8.8ab	6.6e-h	5h-m	2qrs	***
12x3	8a-e	7.2c-g	5h-l	5.2h-l	2.4o-s	***
15x9	8.6abc	8.2a-d	5.8g-j	4.8i-n	2qrs	***
19x19	8.2abc	7.4b-g	6.2f-i	5.4g-k	2.2p-s	***
34x20	7.2c-g	5.6g-j	3.6l-p	2.4o-s	1s	***
63x18	8.2abc	7.6a-f	6.2f-i	4j-p	2qrs	***
MSB1050	9a	8.6abc	8a-e	7.4a-g	5.8g-j	***
FB1901	9a	8.4abc	6.8d-h	6.4e-i	1.8rs	***
Tahoma 31	8.4abc	8.2a-d	6.2f-i	6f-j	2.6o-s	***
TifEagle	9a	8.4abc	6.8d-h	6f-j	2qrs	***
Tifdwarf	8.8ab	8.4abc	7.4b-g	7c-h	2.4o-s	***
	***#	***	***	***	***	

† Turfgrass color retention was rated on a 1 to 9 scale where 9 was considered to be dark green and 1 was considered to be straw brown

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

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

Table 16b. Fall color retention visual ratings collected weekly from October 26th to November 24th, 2022

	Fall Color Retention †					
Cultivar	26-Oct	3-Nov	11-Nov	17-Nov	24-Nov	
OKC0920 ‡	4.2a-h§	3.4c-k	4.6a-e	3d-k	1mn	***
OKC3920	3d-l	2h-n	2i-n	1.4k-n	1mn	***
OKC1609	3.8a-j	2.2g-n	4a-h	2h-n	1mn	***
3x23	3.6b-k	2.2g-n	2.6f-l	1.6j-n	1mn	***
11x2	3d-l	2.4f-n	2.8e-l	1.6j-n	1mn	***
12x3	4.4a-g	4.2a-g	3.6b-j	2.6e-l	1mn	***
15x9	5.2a-d	3.6b-j	4a-h	2.2g-n	1mn	***
19x19	5.8ab	4.6a-e	5a-d	3.6b-j	1mn	***
63x18	4a-i	3d-l	3.2d-k	1.8j-n	1mn	***
MSB1050	4.6a-f	3.4c-k	3.8b-j	2.4f-m	1mn	***
FB1901	5.2a-d	4.2a-g	4.8a-d	4a-h	1.4lmn	***
TifEagle	6.2a	4.6a-e	5.6abc	5.2a-d	2.4f-l	***
Tifdwarf	5.6abc	4.2a-g	5.6abc	4.8a-d	1.2lmn	***
	***#	***	***	***	***	

† Turfgrass color retention was rated on a 1 to 9 scale where 9 was considered to be dark green and 1 was considered to be straw brown

§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level

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

‡ Four genotypes (OKC0805, 5x23, 34x20, and Tahoma 31) were removed from evaluations due to indistinguishable changes in color from disease damage and winter dormancy

Table 17. Test of fixed effects visual spring greenup ratings using Type III analysis for field trial in 2022

Source	**Spring Greenup**	
	df ‡	F value
Genotype (G)	16,63.42	17.21***
Date (D)	4,149.6	1473.98***
GxD	64,215.7	17.30***

‡ Degrees of freedom for numerator, denominator

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

Table 18. Spring greenup visual ratings collected weekly from March 28th to April 25th, 2022

	Spring Green Up †					
Cultivar	28-Mar	5-Apr	11-Apr	18-Apr	25-Apr	
OKC0805	2t-w§	5.2k-o	6g-m	8.2a-d	8.8ab	***
OKC0920	4n-r	6.2g-l	6.4e-l	6.8d-k	8.2abc	***
OKC3920	3.2q-u	5.6j-n	7.2b-j	7.8a-f	9a	***
OKC1609	3.8o-r	6h-m	5.8h-m	5.8h-m	6.2f-l	***
3x23	1.8uvw	3.6p-s	6g-m	7.2c-j	8.4abc	***
5x23	1.4vw	3.6p-s	6.4e-l	7.4a-h	8.4abc	***
11x2	2t-w	4.4m-q	5.6i-n	7c-j	8.2abc	***
12x3	3.2q-u	5.4k-o	5.4j-o	7.4b-i	8a-e	***
15x9	2.2s-w	5.6i-n	6.8c-k	7.6a-g	9a	***
19x19	1.8uvw	3q-u	3.2q-u	6.4f-l	7.6a-g	***
34x20	1w	3.4q-t	6.2f-l	7c-j	8.4abc	***
63x18	2.8r-v	5.2k-o	5.8h-m	7c-j	8a-e	***
MSB1050	1.4vw	2.8r-v	5.6i-n	7.6a-g	8.8ab	***
FB1901	4n-r	5.8h-m	6.4e-l	6.4f-l	6.6e-k	***
Tahoma 31	2t-w	4n-r	5.8h-m	7.4a-h	8.6ab	***
TifEagle	5l-p	6.6e-k	7.4a-i	7.8a-f	8.2abc	***
Tifdwarf	4.4m-q	5.4k-o	4.6l-q	5l-p	5.4k-o	***
	***#	***	***	***	***	

† Turfgrass greenup was rated on a 1 to 9 scale where 9 was considered to be dark green and 1 was considered to be straw brown
§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level
¶ NS not significant at the 0.05 level
# *, **, *** significant at *P* = 0.05, 0.01, and 0.001, respectively

Table 19. Test of fixed effects for root length with repeated measures using Type III analysis for field trial in 2022

Source	Root Length	
	df ‡	F value
Genotype (G)	16,63.95	16.60 ***
Season (S)	2,130.8	23.99***
GxS	32,130.8	1.05 NS¶

‡ Degrees of freedom for numerator, denominator
*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively
¶ NS not significant at the 0.05 level

Table 20. Test of fixed effects for root length using Type III analysis for field trial in 2022

Source	Root Length	
	df ‡	F value
Genotype (G)	16,234	17.24***

‡ Degrees of freedom for numerator, denominator
*, **, *** significant at P = 0.05, 0.01, and 0.001 respectivel

Table 21. Average turfgrass root lengths collected during spring, summer, and fall 2022

Turfgrass Root Length†	
Cultivar	cm
OKC0805	17.09cde§
OKC0920	21.78a
OKC3920	20.39ab
OKC1609	12.14g
3x23	15.62def
5x23	16.7cde
11x2	18.84bc
12x3	14.56efg
15x9	17.63cd
19x19	17.03cde
34x20	16.19c-f
63x18	17.64bcd
MSB1050	16.73cde
FB1901	16.4c-f
Tahoma 31	14.61efg
TifEagle	13.83fg
Tifdwarf	16.3c-f
	***#

† Turfgrass root length was measured to the deepest root observed in three locations three different dates
§ Means accompanied by the same small letter are not significantly different at the P = 0.05 level
# *, **, *** significant at *P* = 0.05, 0.01, and 0.001, respectively

Table 22. Linear regression model for the relationship between leaf length and ball roll distance

Effect	Estimate	SE	95% CI§		p-value
			LL	UL	
Intercept	7.667	1.1345	5.2156	10.1175	<0.001
Leaf Length	-1.124	0.3169	-1.8083	-0.4391	0.0036
Leaf Length^2	0.080	0.0286	0.0187	0.1421	0.0146
Leaf Length^3	-0.002	0.0008	-0.0037	-0.0001	0.0392
Observations	16				
R^2/Adj. R^2	0.93/0.91				
F statistic	55.55***#				

§ CI = confidence interval; LL = lower limit; UL = upper limit

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

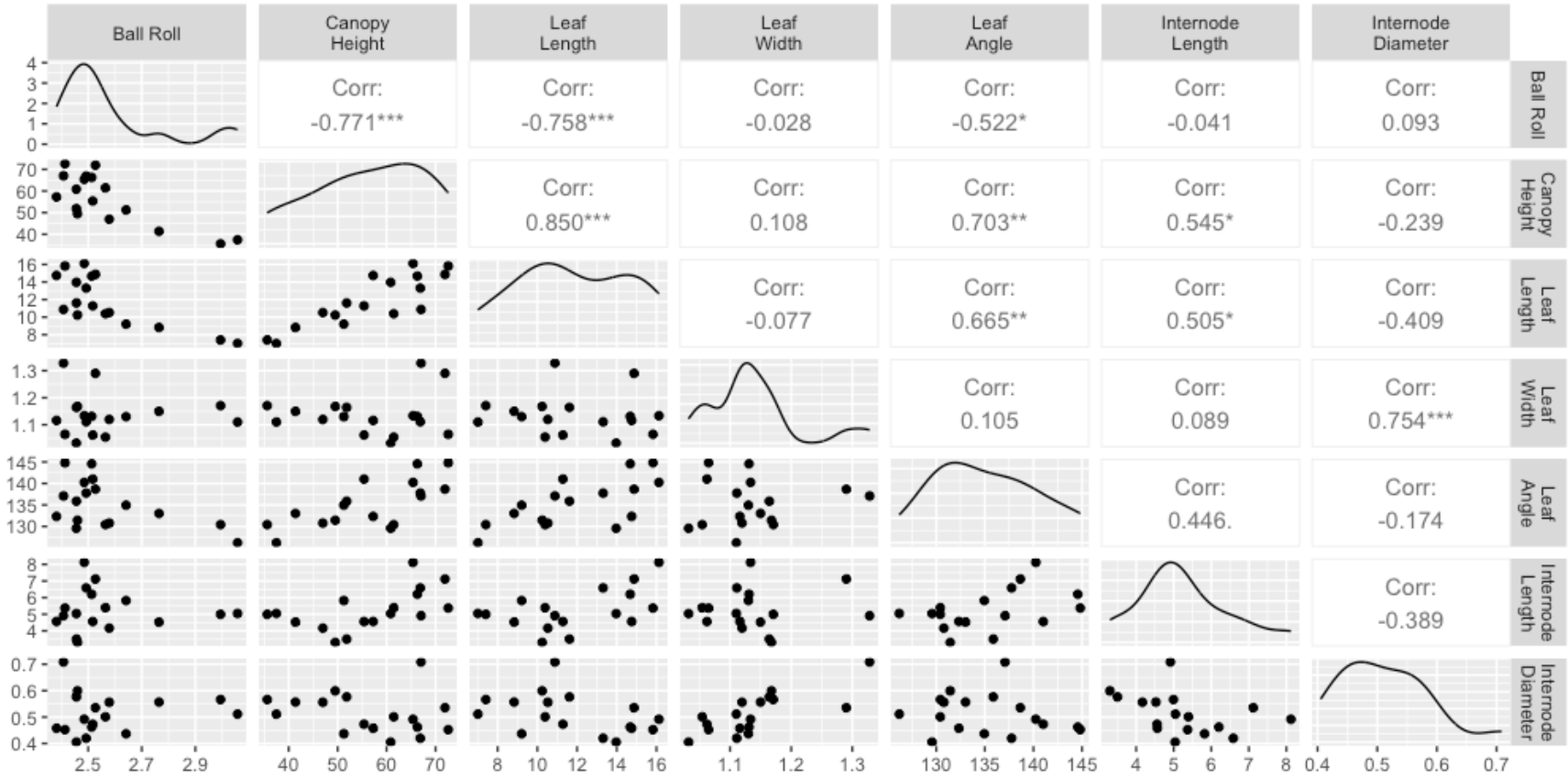

Figure 1. Scatterplot matrix evaluating the relationships between ball roll distance and morphological characteristics. Lower section displays scatter plots between row and column pairs; diagonal section displays density plot of the given trait; upper section displays the Pearson correlation value and significance between the row and column pairs (*, **, *** significant at $P = 0.05$, 0.01, and 0.001, respectively).

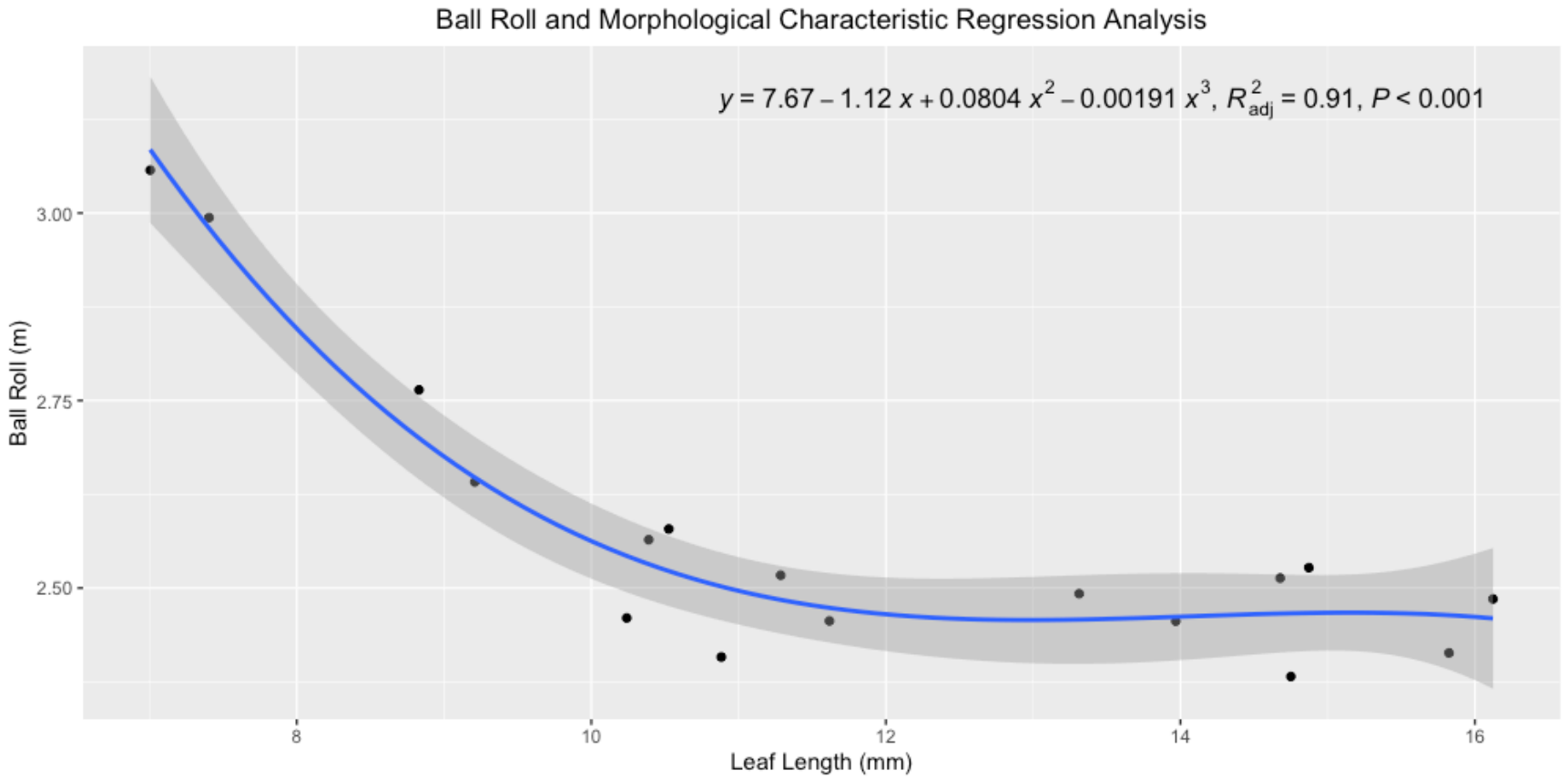

Figure 2. Linear regression model between ball roll distance and leaf length. Upper right corner displays the equation, adjusted R^2, and significance level. The blue line indicates the fitted line, with the dark gray area surrounding indicating the confidence interval.

CHAPTER IV

PHONE-APP AND DRONE-BASED IMAGERY AS NEW METHODS IN EVALUATING PERCENT GREEN COVER IN ADVANCED TURFGRASS PHENOTYPING

Abstract

The use of visual rating methods in turfgrass systems can introduce bias between different raters, raters' fatigue in large trials, as well as difficulty separating similar performing treatments. However, as remote sensing technologies advance and become more accessible, they can be a quick and effective method to evaluate large scale turfgrass trials for determining canopy coverage and other phenotypic traits. Studies have been conducted to evaluate the performance of remote sensing methods in short term periods, but not from establishment to full coverage. In this trial, 85 plots of greens-type hybrid bermudagrass (*Cynodon dactylon x C. transvaalensis*) cultivars were evaluated during the establishment period as well as fall, spring, and summer once fully covered. The percent green cover was measured by taking images using a lightbox and calculating the number of green pixels using TurfAnalyzer software. Five alternative methods to determine green coverage were tested by using the Canopeo application (RGB-imagery), and drone-based multispectral and RGB imageries to calculate the normalized difference vegetation index (NDVI), green normalized difference vegetation index (GNDVI), normalized difference red edge index (NDRE), and the green leaf index (GLI). A linear model using a logit transformation of the PGC was fit using a random sample of the data set for each collection method to determine

its predictive ability to the digital image analysis method. The results produced by four alternative methods were significantly associated with digital image analysis with an adjusted R^2 ranging from 0.71 to 0.81 and root mean square error ranging from 1.82 to 2.22. The NDVI model showed the best performance followed by NDRE, GLI, Canopeo, and GNDVI. Additionally, there was a significant difference between the models for the establishment period and after complete establishment for all the remote sensing methods. This study shows the potential in using these remote sensing methods for evaluating turf coverage, but they may not be best suited for directly estimating percent canopy coverage.

Introduction

Many turfgrass evaluations utilize subjective rating methods such as the National Turfgrass Evaluation Program (NTEP) of utilizing a 1 to 9 scale (Morris and Shearman, 1998). While these methods can be useful in evaluating turfgrass characteristics, variability between rating dates, raters, and rater's fatigue can increase experimental errors. It would be ideal to evaluate performance using objective methods to better understand turfgrass performance (Horst et al., 1984). One of the current methods to quantify this objective measurement is the use of digital image analysis (DIA) to determine the percent green cover (PGC) (Richardson et al., 2001). While this method can produce accurate and reproducible data for turfgrass canopy cover, the time and labor this method takes can limit the ability to collect comprehensive data particularly on large selection nurseries.

Recent advancements in remote sensing technologies can provide an opportunity for turfgrass research to use these methods to objectively assess turfgrass performance in selection nurseries and advanced trials. Previous research has shown various ground-based vegetation indices can be used to predict turfgrass quality and nitrogen content (Bell et al., 2004; Bremer et al., 2011; Fitz–Rodríguez and Choi, 2002). Unmanned aerial vehicle (UAV) systems and methods

for analysis have advanced quickly in recent years allowing for researchers to obtain high spatial resolution images of the entire field and the ability to collect data frequently to better understand environmental relationships such as drought stress (Roberson et al., 2021; Xiang and Tian, 2011).

Recent studies using UAVs on various turfgrass studies have demonstrated a promising future for these methods. Zhang et al. (2019) used multispectral and RGB imagery to evaluate performance of various remote sensing methods during temporary drought stress of a replicated turfgrass field trial with two species hybrid bermudagrass (*Cynodon* spp.) and zoysiagrass (*Zoysia* spp.). They found UAV-based NDVI and visible atmospherically resistant index were able to estimate ground percent green cover ($R^2 = 0.86$ and $R^2 = 0.87$, respectively). Wang et al. (2022) evaluated thirty approaches ranging in complexity for high-throughput estimation of turfgrass PGC. The levels used included vegetation indices as well as supervised and unsupervised machine learning classification. Their results found that using a Hue-Saturation-Value color space-based method for green pixel identification had a coefficient of determination of 0.86-0.96 when compared to the ground PGC. Overall, they found that RGB images from UAV systems could be used to accurately determine the PGC within an established plot, but multispectral methods could offer a solution when trying to determine between turfgrass and soil background during establishment (Wang et al., 2022). Similar to work done to evaluate the nitrogen status and crop biomass of different agricultural crops (Hunt et al., 2005), Caturegli et al. (2016) demonstrated the ability of remote sensing to assess the nitrogen status of three turfgrass species [hybrid bermudagrass (*C. dactylon* x *C. transvaalensis*), zoysiagrass (*Zoysia matrella*), and seashore paspalum (*Paspalum vaginatunt*)] and its ability to evaluate the spatial variability over large areas such as golf courses and sod farms.

In addition to UAV based methods to assist in decreasing the time and labor needed to collect subjective canopy coverage measurements, a potentially cheaper method would be to use a phone-based method of PGC measurements. One such application is FieldScout GreenIndex+

Turf (Spectrum Technologies, Aurora, IL, USA) which has been used to evaluate stress responses of warm-season turfgrasses (Xiang et al., 2017). One drawback to this application is the cost. However, a relatively recent application developed at Oklahoma State University (OSU) by Patrignani and Ochsner (2015), which is free for use on both Android (Google, Mountain View, CA, USA) or iOS (Apple, Cupertino, CA, USA) operating systems has been well documented for use in evaluating crop canopy coverage (Govindasamy et al., 2022; Shepherd et al., 2018), yield (Chung et al., 2017; Jáuregui et al., 2019), herbicide injury (Abreu, 2019), as well as disease severity (Yellareddygari and Gudmestad, 2017). Chhetri and Fontanier (2021) found Canopeo was able to determine the green coverage of bermudagrass as it exited winter dormancy; however, to our knowledge no work has been done to evaluate this method on turfgrass from establishment to fall color retention and spring green up.

As these methods begin to be used in turfgrass settings, more information is needed to evaluate the performance of these UAV and phone-based methods and better understand the relationship between these and current ground-based methods across the span of a replicated turfgrass field trial. Therefore, the overall goal of this study was to further assess the use of visual (RGB) and multispectral images collected with a UAV platform and phone in a replicated turfgrass field trial from establishment to full coverage throughout the year. The objective of this study was to examine the relationship between the remote sensing measurements and the ground measurements from 0-100% PGC during two different growth periods (establishment and fully established) to see if there is any interaction between the growth period and relationship to PGC.

Materials and Methods

Study Site

This study was conducted at The Botanic Gardens at Oklahoma State University (OSU) in Stillwater, Oklahoma (36° 7' 18.2964" N 97° 6' 11.6964" W), and the study site was part of a

putting green type bermudagrass replicated test funded by the United States Golf Association (USGA). The plots were sprigged at a 1:10 ratio onto a modified USGA rootzone mixture in May of 2021. A total of 17 bermudagrass entries were arranged as a randomized complete block design with 5 replications; the plot size was 4.57 by 1.83 meters. Twelve advanced lines from OSU's turfgrass breeding program along with one experimental line from Mississippi State University and University of Florida turfgrass breeding programs, respectively, and three commercial cultivars ('Tifdwarf', 'TifEagle', and 'Tahoma 31') were used in the trial. Mowing was initiated one month post planting three times a week at 1.27 cm using a reel mower; the mowing height was lowered to 0.318 cm over the next three months transitioning to mowing five times a week. Manual weeding was performed as needed to maintain weed free plots, along with irrigation to prevent drought stress to promote growth and establishment of the plots.

Data Collection

Two drones, DJI Matrice 200 V2 and a DJI Mavic 2 Pro (DJI Technology Inc., Shenzhen, China), were used to collect multispectral and RGB images, respectively. For the establishment period, data was collected weekly following planting for 22 weeks until October 18, 2021. After this, data was collected for 6 weeks to begin the fully established period. The following year (2022), data were collected weekly for 7 weeks during spring green up, then biweekly beginning June 2022. Data were then collected weekly again beginning October 2022 for 4 weeks, for a total of 49 data collections. The UAV system can fly either manually or autonomously using Global Positioning Systems (GPS) and waypoint navigation system. The UAV system consisted of the drone, controller, and ground station that uses software to plan flight missions, control, and telemetry (Torres-Sánchez et al., 2013). For this study, the DJI Pilot app was used to plan and fly the multispectral flights and the Pix4DMapper (Pix4DMapper, SA, Lausanne, Switzerland) application was used for the RGB flights.

A Micasense RedEdge MX camera (Micasense, Seattle, WA, United States) with 5 narrow spectral bands (Blue: 475 ± 32 nm; Green: 560 ± 27 nm; Red: 668 ± 16 nm; Red edge: 717 ± 12 nm; NIR: 842 ± 57 nm) was used for the multispectral images. The camera used for the RGB images was a Hasselblad L1D-20c camera (Hasselblad, Gothenburg, Sweden) with a 28 mm 20 mega-pixel 1" CMOS sensor that captures images in true color (Red, Green, and Blue). The flight altitude for both drones was 30 m, resulting in image resolution of 0.7 cm per pixel for the RGB camera and 2 cm per pixel for the multispectral camera. The flights were flown at 3.2 ms^{-1} and 1.5 ms^{-1} for the RGB and multispectral, respectively. The software used maintained the desired speed and altitude during the flights and captured images at 80% front and side overlaps. Four ground control points were placed at the four corners of the field for geo-referencing.

Image Processing

The image processing for all flights followed protocol described by Zhang et al. (2019). All images were geotagged using on-board GPS in the Matrice and Mavic drones during flight. These geotagged images were then used to create an orthomosaic with the information from each individual band using Pix4Dmapper (Pix4 SA, Lausanne, Switzerland). Standard templates within Pix4Dmapper, "Ag RGB" and "Ag Multispectral", were used to stitch the RGB and Multispectral images respectively. The georeferenced orthomosaic images were then used for further analysis in ArcGIS (Esri, Redlands, CA, United States) using a TIFF format. A shape file was created with the individual plot information in ArcMap Version 10.7.1 (Esri, Redlands, CA, United States) for data analysis. The zonal statistics feature was then used to extract data from each polygon (plot). Three vegetation indices, Normalized Difference Vegetation Index (NDVI), Green NDVI (GNDVI), and Normalized Difference Red Edge Index (NDRE), were calculated using the multispectral images (Table 1). Additionally, one index, Green Leaf Index (GLI), was calculated using bands captured by the RGB images.

Ground Measurements

In addition to the UAV based methods, a ground-based method for estimation of percent green cover (PGC) was collected using the phone-based application Canopeo (Oklahoma State University Department of Plant and Soil Science, Stillwater, OK, USA) (Patrignani and Ochsner, 2015). Images of each plot were taken in the middle of the plot approximately 3-4 feet above the canopy.

Digital image analysis PGC was used as the ground truth for this study was estimated from digital images collected using a Canon G9X camera (Canon Inc., Ōta, Tokyo, Japan) (Shutter: 1/160, F-stop: 2.2, ISO: 200) mounted to a lightbox (0.91 x 0.6 x 0.6m) with four lights. Each image was cropped to exclude the sides of the box using FastStone Photo Resizer (FastStone Soft), and then analyzed using TurfAnalyzer (Green Research Services, LLC, Fayetteville, AR, USA) for PGC (0-100%) using a hue range of 70 – 170, saturation range of 10 – 100, and brightness range of 0 – 100 using the TurfAnalyzer recommended values.

Data Analysis

Multiple linear regression analysis between remote sensing measurements and PGC measurements were conducted in R (Version 4.2.2) (R Core Team, 2022) using the packages "tidyverse"(Wickham H et al., 2019), "readxl" (Hadley Wickham and Bryan, 2022), "ggplot2" (H Wickham, 2016), "lubridate" (Grolemund and Wickham, 2011), "GGally" (Schloerke et al., 2021), "effects" (Fox, 2019), and "performance" (Lüdecke et al., 2021). For all models, a logit transformation () was done on the PGC variable, where = PGC/100, before conducting the multiple linear regression analysis to linearize the sigmoid curve of the relationships.

Results

Distribution of Ground Truth and Remote Sensing Based Measurements

Ground percent green cover, UAV-based NDVI, NDRE, GNDVI, GLI, and phone-based Canopeo during the establishment period ranged from 0 to 99.999%, 0.06 to 0.905, 0.011 to 0.597, 0.242 to 0.8, 0.012 to 0.325, and 0.19 to 99.99%, respectively. The same parameters for the full establishment period ranged from 0 to 99.961%, 0.253 to 0.909, 0.126 to 0.563, 0.439 to 0.791, -0.045 to 0.334, and 0.43 to 100%, respectively. Parameters during the establishment period were affected by the rate of establishment from sprigs filling in the plot area as well as the soil background resulting in a different ratio of reflected light for the VIs. Similarly, after the plots were fully established, the parameters were affected by the different fall color retention and spring green up for the different genotypes and impact of reflected light different between dormant and actively growing turfgrass.

Correlation between Ground and Remote Sensing Measurements

Percent green cover was positively correlated with the multispectral UAV-based VIs NDVI (0.867, $p<0,001$), NDRE (0.874, $p<0.001$), and GNDVI (0.714, $p<0.001$) (Table 2). Additionally, the RGB UAV-based GLI was positively correlated with PGC (0.89, $p<0.001$) as was the phone-based Canopeo (0.933, $p<0.001$).

Model Performance

To evaluate the relationship between PGC and the various VIs, a multiple linear regression analysis was conducted to evaluate the relationships and determine if there is an interaction between the VIs and the establishment period (Tables 3 – 7; Figures 7 – 11). Each model was tested against a reduced version without the interactions between the VIs and establishment period using ANOVA methods to determine if the two models had similar slopes.

The analysis revealed all of the models had significant interaction between the given VI and the establishment period when the data was collected ($p<0.001$ for all models).

After the best model for each VI was chosen, the performance of each fitted model was evaluated to determine how well each model is able to predict the logit(PGC) value given the VI value and the establishment period. Two common methods of evaluating a model's performance were used: adjusted R^2 and Root Mean Square Error (RMSE) (Table 8). For the models evaluated, the NDVI model had an adjusted R^2 of 0.81 and RMSE of 1.82; the NDRE model had 0.8 and 1.83, respectively; the GNDVI model had 0.71 and 2.22, respectively; the GLI model had 0.80 and 1.84, respectively; and the logit(Canopeo) model had 0.77 and 1.97, respectively.

Discussion

Each of these VIs have a similar goal to evaluate the "greenness" of the plot to determine plant health. When the plant is healthy, it will absorb most of the red and blue wavelengths, while reflecting a little green and significantly more NIR. An unhealthy plant on the other hand will reflect similar amounts of blue, green, red, and NIR. The VIs used in this study use different combinations of wavelengths to determine the "greenness" of the plot and subsequently the overall health. Unlike the DIA methods, these indices take into account the soil and dormant turfgrass reflectance which can decrease the index value. This can be seen by the significantly different models between the establishment period when there was soil background and after the plots fully established when there might be dormant turfgrass background.

While the studies evaluating the performance of different VIs for use in turfgrass systems have shown positive results, they have only looked at short periods of stress or only during establishment and not comparing the two (Wang et al., 2022; Zhang et al., 2019). The present work highlights the importance of understanding how site-specific factors can influence the indices values and how they relate to PGC. In order to use the methods demonstrated in this

study, ground truthing should also be done to determine the most effective model to estimate the PGC. Additionally, the models can be complex when evaluating across the growing season as shown by the logit transformation and cubic interaction models used here. A simpler method used in other studies is to apply a threshold to the index above which is considered a "green" pixel and then the percent of these can be determined similar to DIA methods. This method however loses the information provided by these indices particularly on the higher end of the index. The scatterplots of NDRE and GLI (Figures 3 and 4) show a wider range of index values for similar PGC values. This indicates the index is picking up variation in the plots where the DIA is not; this can be particularly useful when attempting to separate high performing treatments. However, applying a threshold would not pick up on these differences. Similarly, the NDVI and GNDVI scatterplots (Figures 1 and 2) show variation on the lower end of the PGC which is not seen in the DIA methods. This information could be useful when evaluating establishment rates of different treatments.

Additionally, the Canopeo application was designed to be a simple and rapid to analyze fractional green canopy cover of various crops (Patrignani and Ochsner, 2015). The original paper by Patrignani and Ochsner (2015) as well as others have shown good relationships between Canopeo and other digital image analysis software (Chhetri and Fontanier, 2021; Govindasamy et al., 2022). The present work showed similar correlation (r=0.93) to the work by Chhetri and Fontanier (2021) when no turf colorant was used (r=0.91).

While several methods, including the HSV method by Wang et al. (2022), are still being evaluated to determine the most effective and simple way to estimate PGC using remote sensing methods, the actual VIs themselves should not be viewed as just a way to reach the PGC value. These VIs can help provide valuable information about the turfgrass plot as a whole and can be particularly useful when current DIA methods have difficulty separating plots.

Conclusion

This study shows there is a significant relationship between logit(PGC) and the VIs/phone application used. The more complex relationship between the PGC and VIs when evaluating the entire range of possible values throughout the season makes relating and predicting PGC based on the VI value more difficult as several factors can influence the VI value including soil background, canopy density, dormant turfgrass background, and overall turfgrass health. However, when comparing similar data sets over a select period of time NDVI can be useful to separate out lower values of PGC while NDRE and GLI could be used to separate higher values of PGC when evaluating turfgrass health and performance. Additionally, the use of the Canopeo application could allow for quick estimation of turfgrass coverage following proper calibration while in the field. The use of the VIs by themselves could help to provide more information about a plot than only PGC as well. More evaluation under varying soil conditions and stressors is needed to further understand the place UAV-based remote sensing has in turfgrass research.

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Table 1. Equations for vegetation indices used in this study

Indices	**Equation**	**Reference**
NDVI: normalized difference vegetation index	$(R_{NIR}-R_{Red})/(R_{NIR}+R_{Red})$	Lee et al. (2011)
GNDVI: green normalized difference vegetation index	$(R_{NIR}-R_{Green})/(R_{NIR}+R_{Green})$	Gitelson et al. (1996)
NDRE: normalized difference red edge	$(R_{NIR}-R_{Red\ edge})/(R_{NIR}+R_{Red\ edge})$	Mutanga and Skidmore (2004)
GLI: green leaf index	(2g-b-r)/(2g+b+r)	Louhaichi et al. (2001)

Table 2. Pearson correlation coefficients between ground measurements (PGC and Canopeo) and UAV-based measurements (NDVI, GNDVI, NDRE, GLI) for combined establishment periods

	Canopeo	NDVI	GNDVI	NDRE	GLI
PGC	0.93†	0.87	0.71	0.87	0.89
Canopeo		0.87	0.71	0.88	0.88
NDVI			0.94	0.96	0.82
GNDVI				0.87	0.65
NDRE					0.87

† All coefficients significant at $p < 0.001$

Table 3. Multiple linear regression model output for NDVI

Variable	Coefficient	Standard error	p-value
Intercept	-10.76	0.75	***
NDVI	39.69	4.95	***
EP†	8.02	2.42	***
NDVI*EP	-76.30	13.2	***
$NDVI^2$	-59.86	9.82	***
$NDVI^2$*EP	150.54	23.03	***
$NDVI^3$	41.67	5.99	***
$NDVI^3$*EP	-86.48	12.90	***

†EP, Establishment period: 0 = Establishment, 1 = Fully Established
*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 4. Multiple linear regression model output for GNDVI

Variable	Coefficient	Standard error	p-value
Intercept	-14.89	4.36	***
GNDVI	37.11	25.72	
EP†	333.56	24.97	***
GNDVI*EP	-1751.99	121.93	***
$GNDVI^2$	-25.11	47.16	
$GNDVI^2$*EP	2961.78	196.88	***
$GNDVI^3$	15.84	28.47	
$GNDVI^3$*EP	-16.32.34	105.18	***

†EP, Establishment period: 0 = Establishment, 1 = Fully Established
*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 5. Multiple linear regression model output for NDRE

Variable	Coefficient	Standard error	p-value
Intercept	-8.88	0.45	***
NDRE	65.21	5.75	***
EP†	3.74	1.39	**
NDRE*EP	-105.94	14.63	***
$NDRE^2$	-143.33	19.87	***
$NDRE^2$*EP	428.81	47.21	***
$NDRE^3$	141.35	20.53	***
$NDRE^3$*EP	-472.49	47.92	***

†EP, Establishment period: 0 = Establishment, 1 = Fully Established

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

Table 6. Multiple linear regression model output for GLI

Variable	Coefficient	Standard error	p-value
Intercept	-7.83	0.35	***
GLI	117.29	9.38	***
EP†	3.39	0.37	***
GLI*EP	-115.17	10.03	***
GLI^2	-433.25	65.90	***
GLI^2*EP	934.38	72.12	***
GLI^3	774.49	136.24	***
GLI^3*EP	-2173.95	151.74	***

†EP, Establishment period: 0 = Establishment, 1 = Fully Established
*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 7. Multiple linear regression model output for logit(Canopeo)

Variable	Coefficient	Standard error	p-value
Intercept	0.36	0.08	***
logit(Canopeo)	1.46	0.03	***
EP†	-1.63	0.10	***
logit(Canopeo)*EP	-0.31	0.04	***
$logit(Canopeo)^2$	-0.09	0.01	***
$logit(Canopeo)^2$*EP	0.03	0.01	***

†EP, Establishment period: 0 = Establishment, 1 = Fully Established
*, **, *** significant at P = 0.05, 0.01, and 0.001 respectively

Table 8. Regression model performance between ground measurements and remote sensing methods

Model	Adjusted R^2	p-value	RMSE†
logit(PGC) ~ NDVI model	0.81	***	1.82
logit(PGC) ~ GNDVI model	0.71	***	2.22
logit(PGC) ~ NDRE model	0.8	***	1.83
logit(PGC) ~ GLI model	0.8	***	1.84
logit(PGC) ~ logit(Canopeo) model	0.77	***	1.99

*, **, *** indicate model significance at P = 0.05, 0.01, and 0.001 respectively
†RMSE is the standard deviation of the error

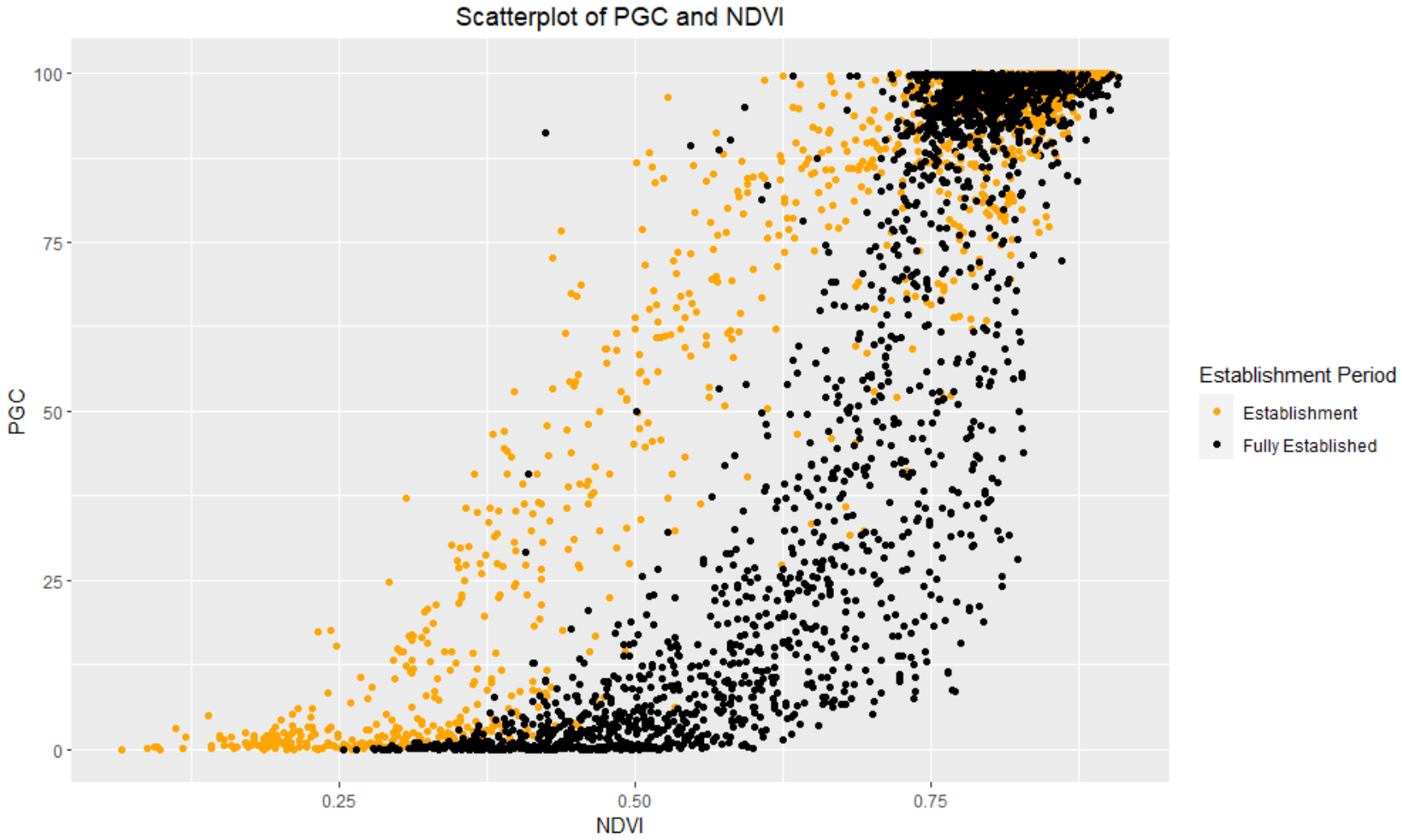


Figure 1. Scatterplot of NDVI and PGC during establishment and once fully established

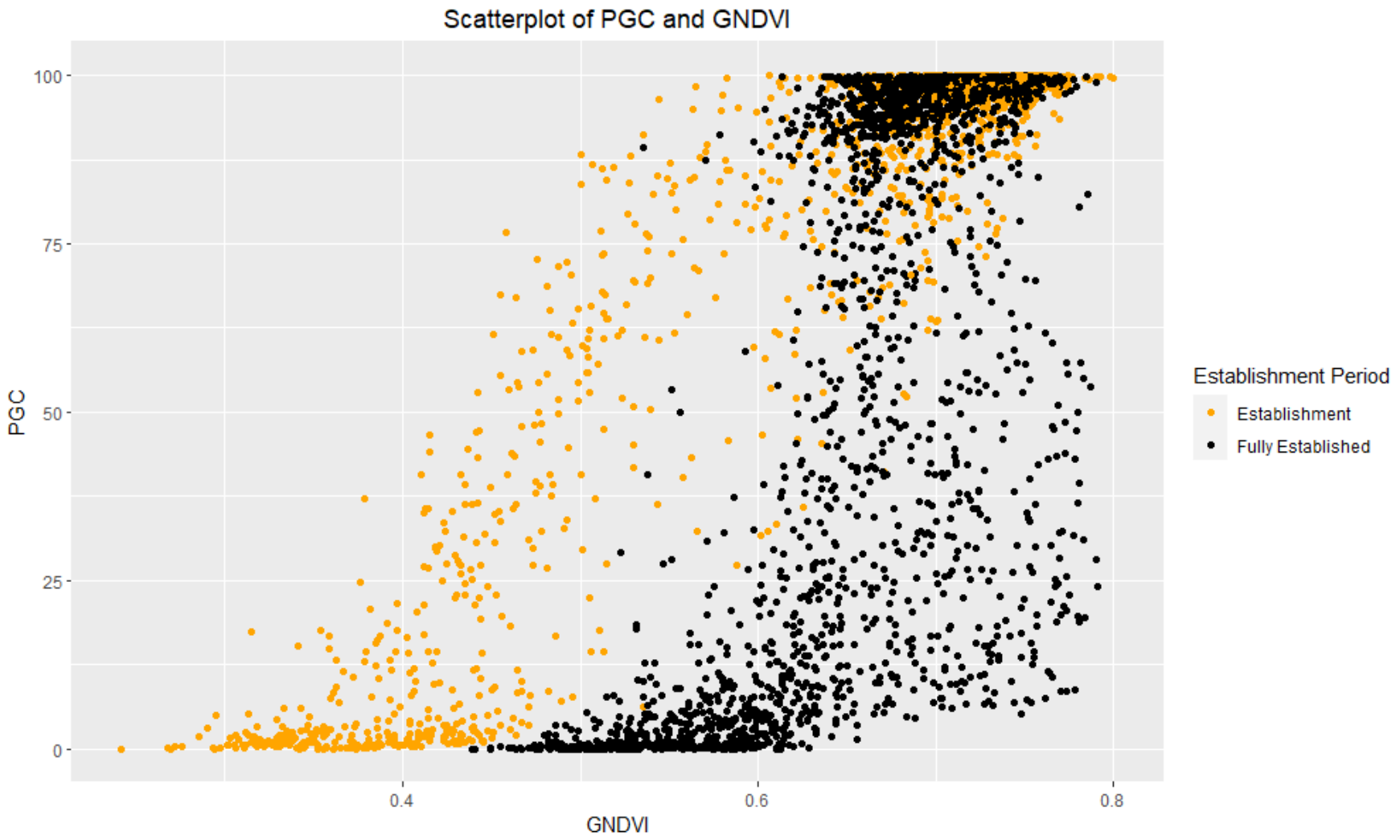


Figure 2. Scatterplot of GNDVI and PGC during establishment and once fully established

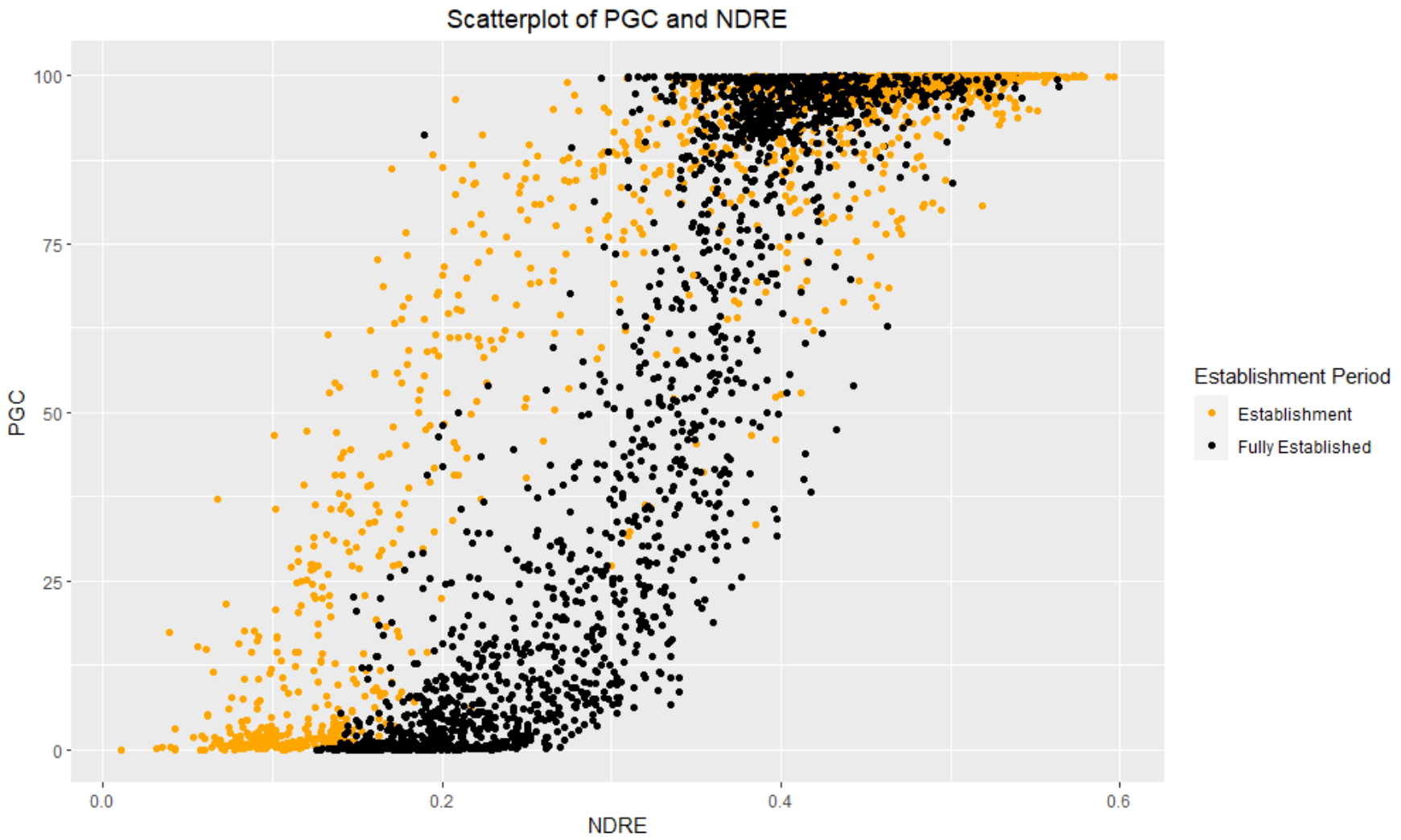


Figure 3. Scatterplot of NDRE and PGC during establishment and once fully established

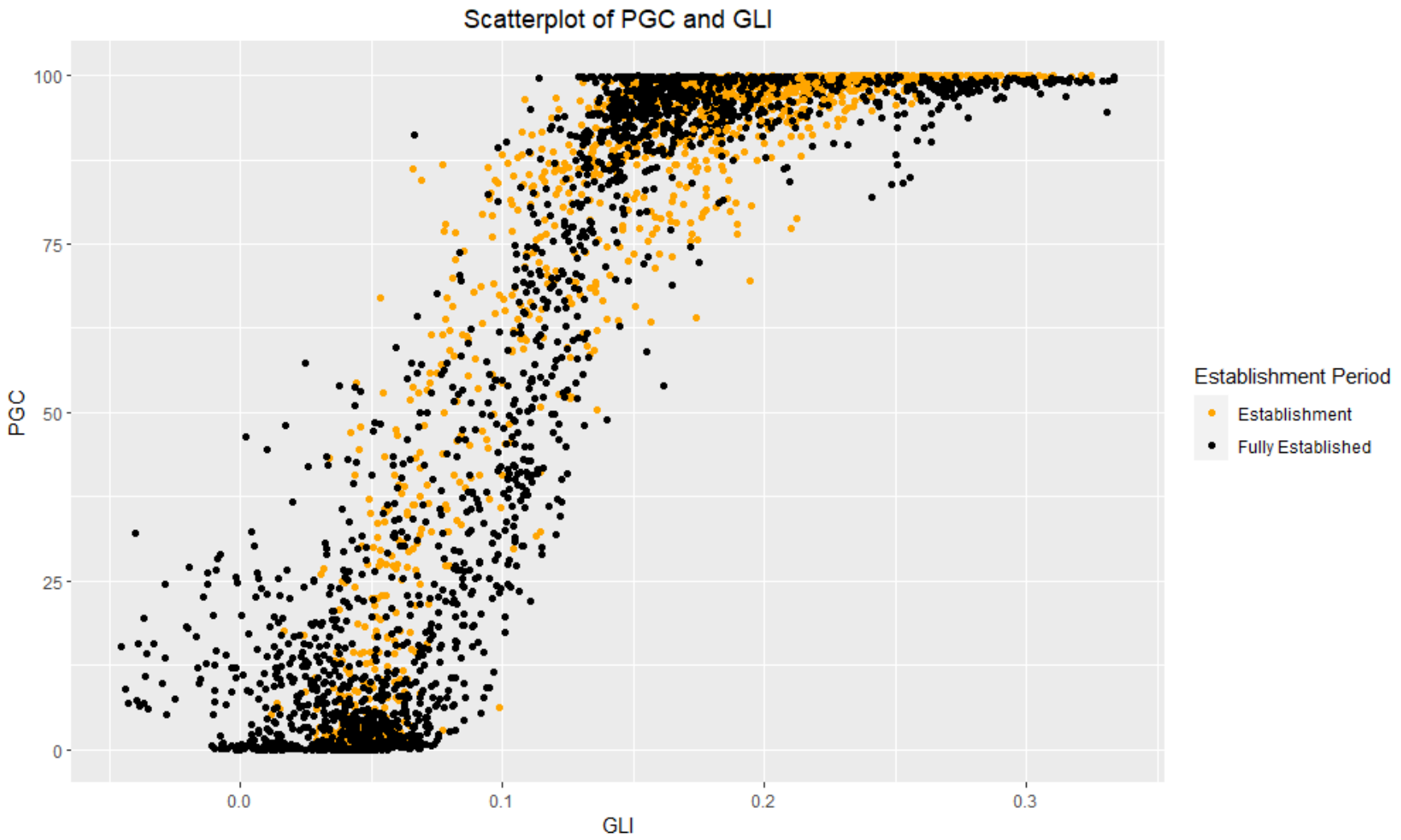


Figure 4. Scatterplot of GLI and PGC during establishment and once fully established

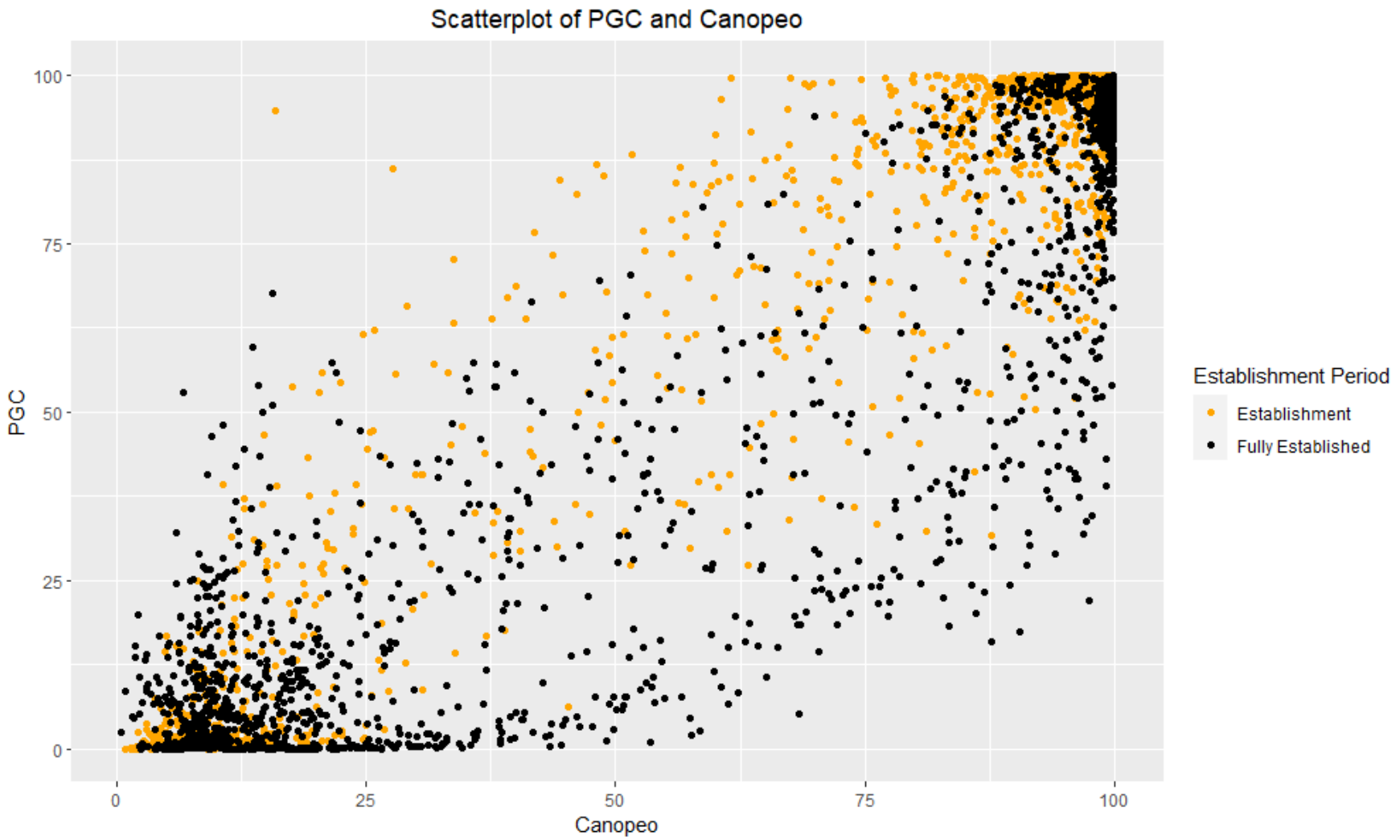


Figure 5. Scatterplot of Canopeo and PGC during establishment and once fully established

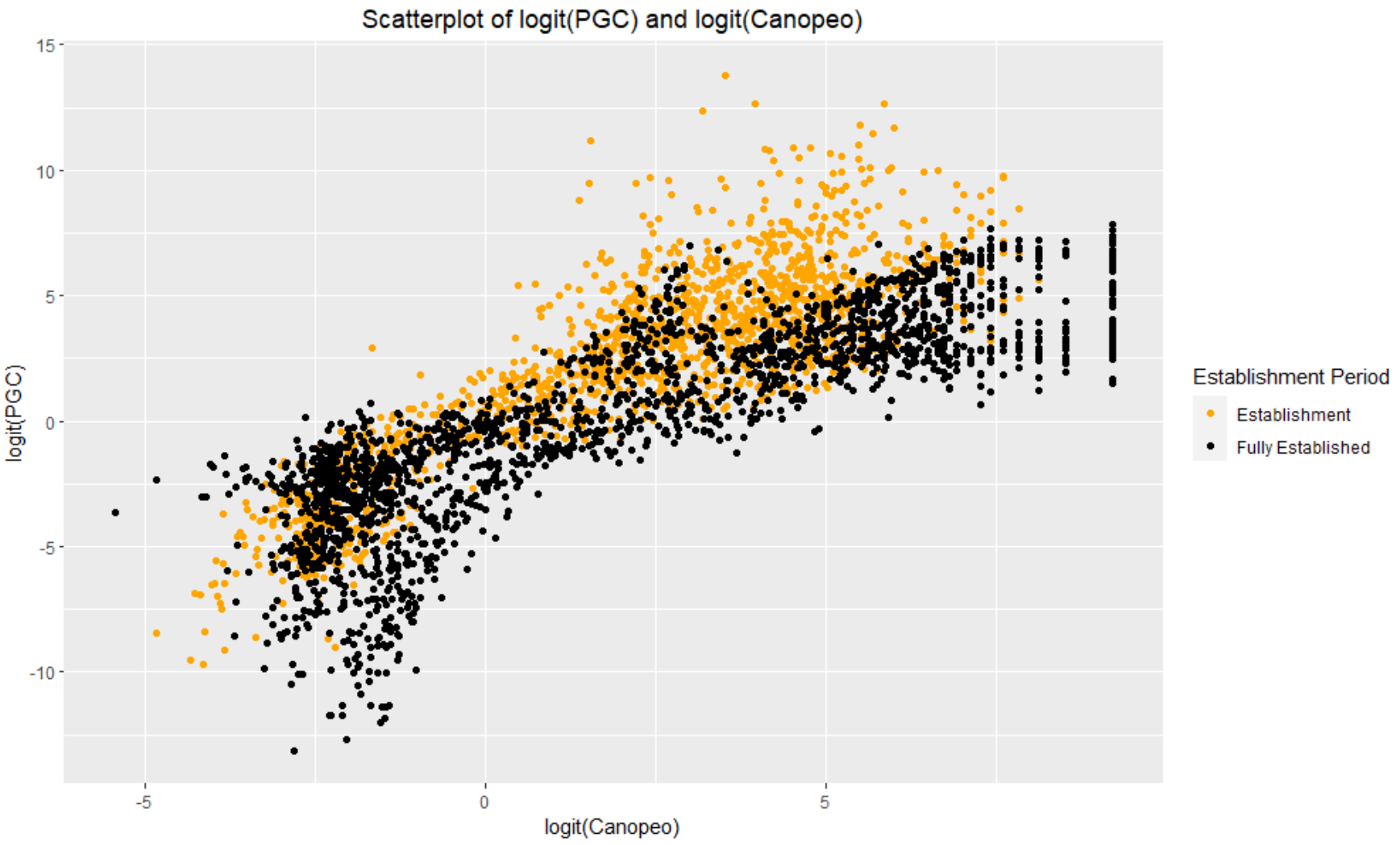


Figure 6. Scatterplot of logit(Canopeo) and logit(PGC) during establishment and once fully established

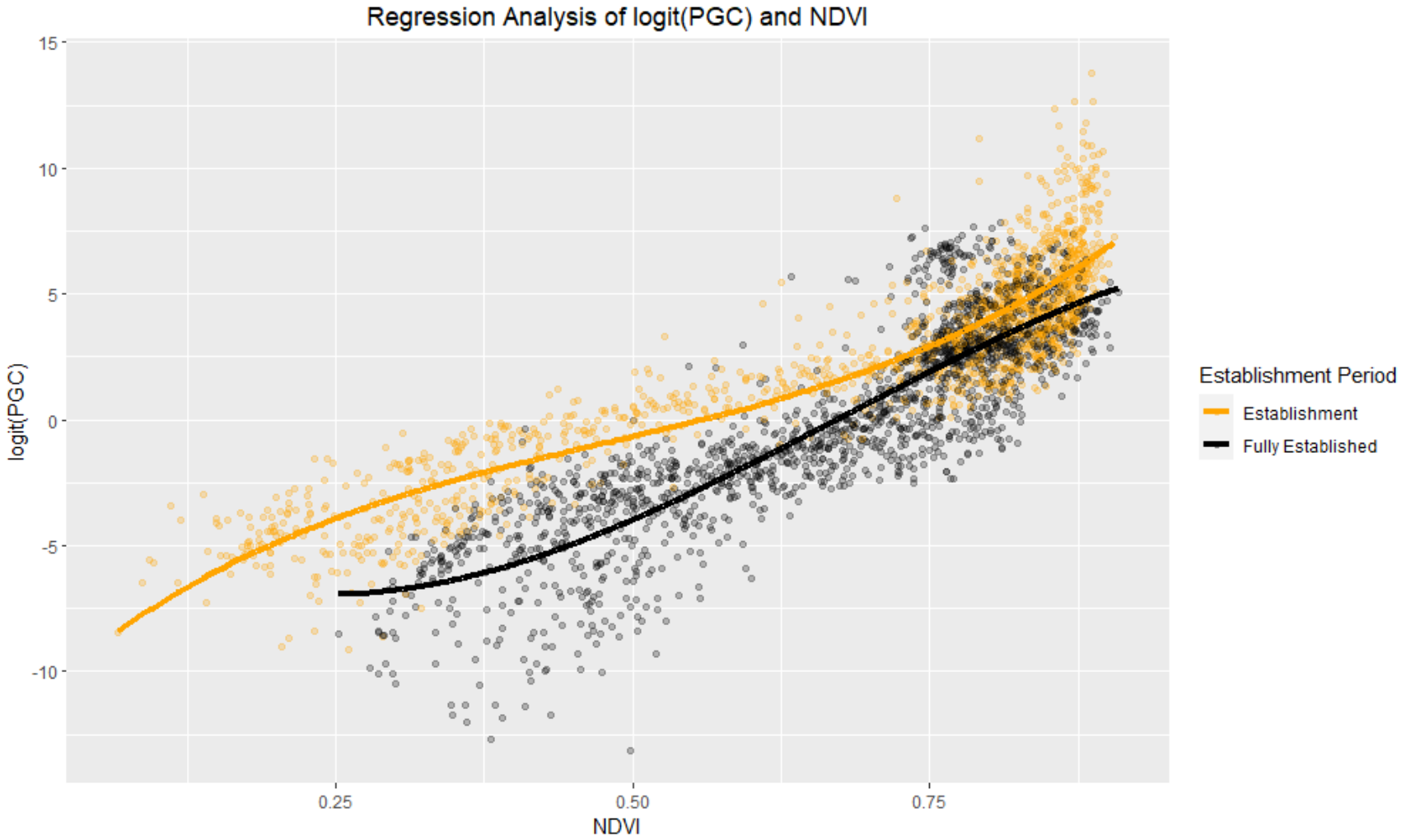


Figure 7. Fitted multiple linear regression model predicting logit(PGC) using UAV-based NDVI for establishment and once fully established

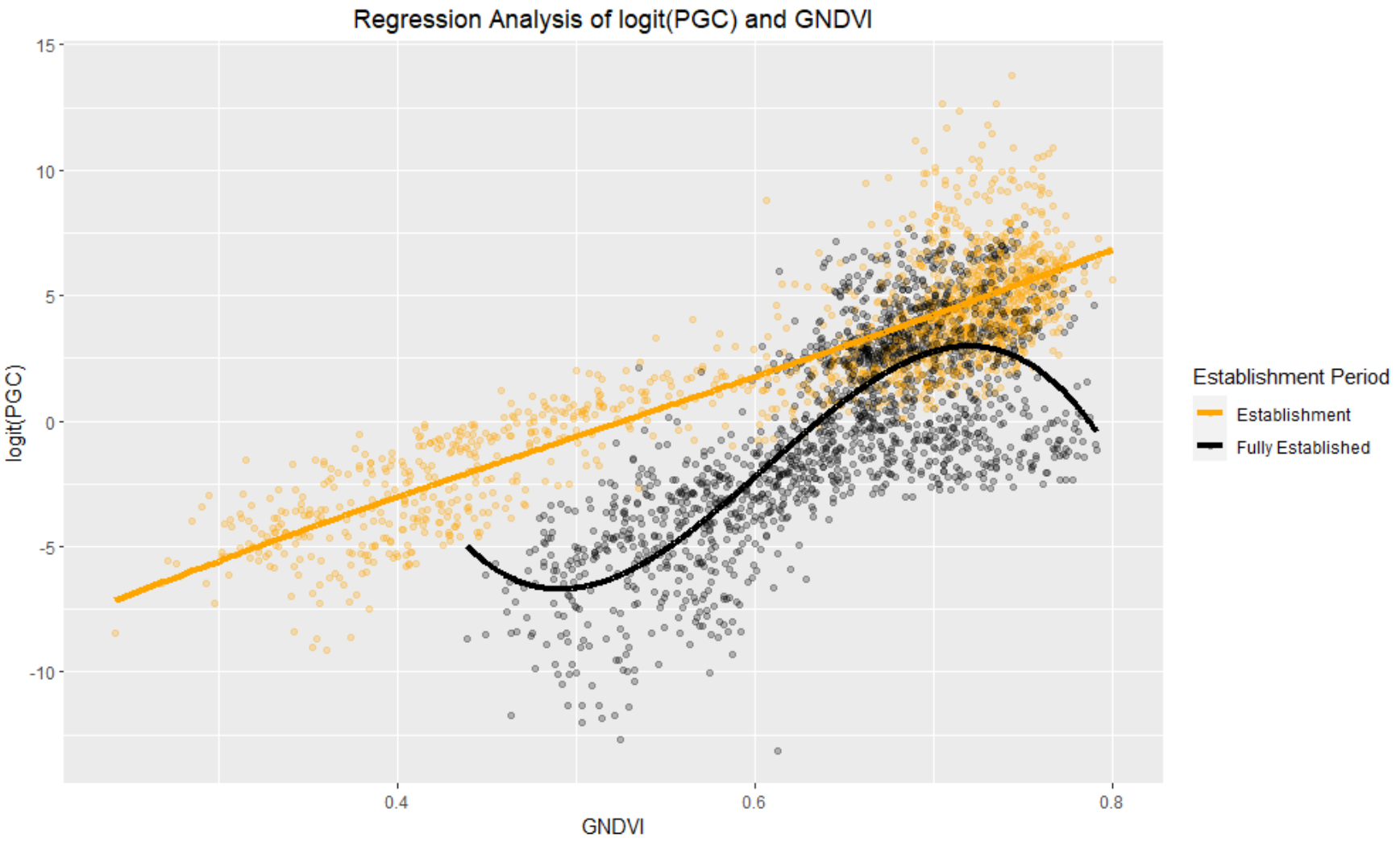


Figure 8. Fitted multiple linear regression model predicting logit(PGC) using UAV-based GNDVI for establishment and once fully established

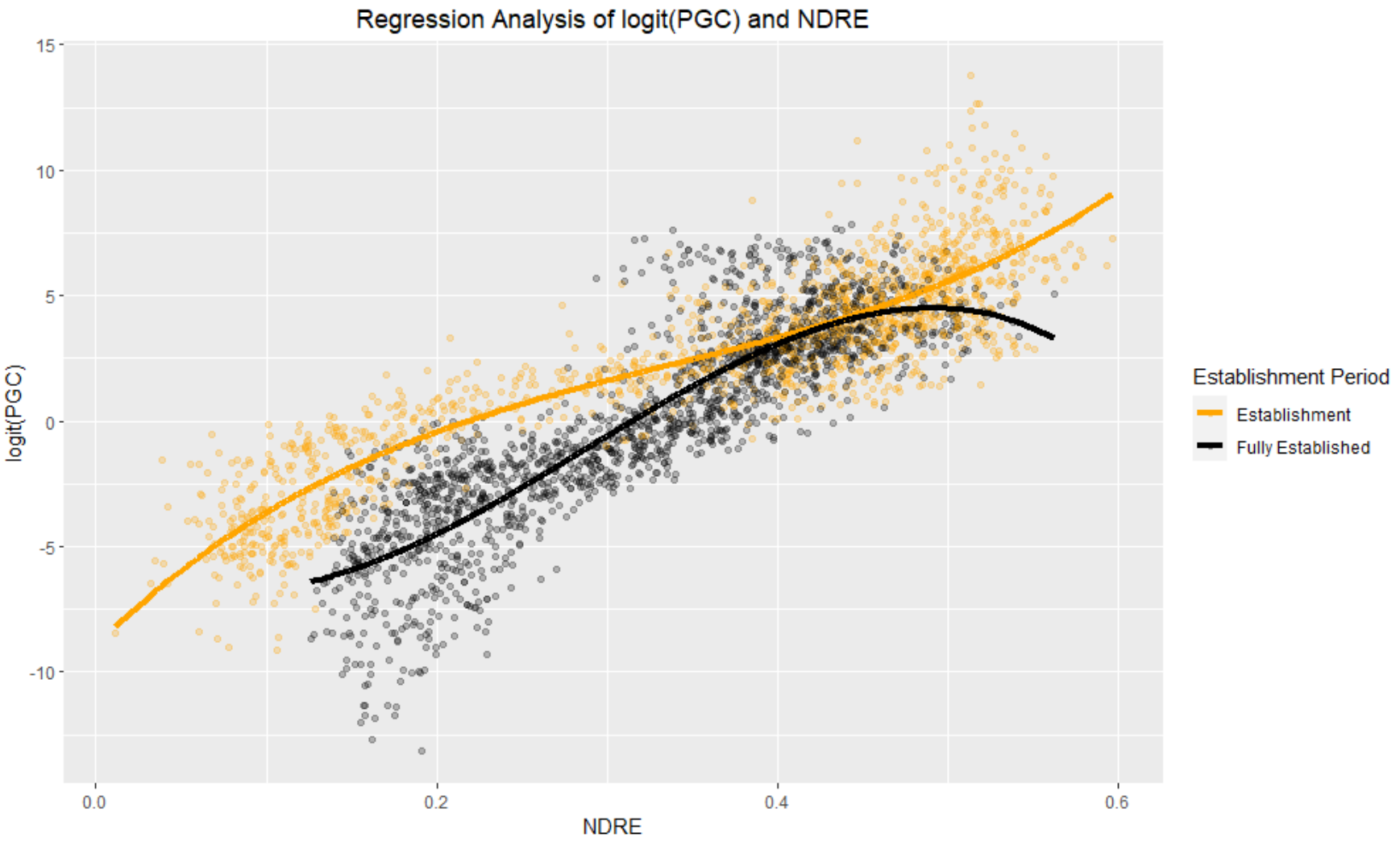


Figure 9. Fitted multiple linear regression model predicting logit(PGC) using UAV-based NDRE for establishment and once fully established

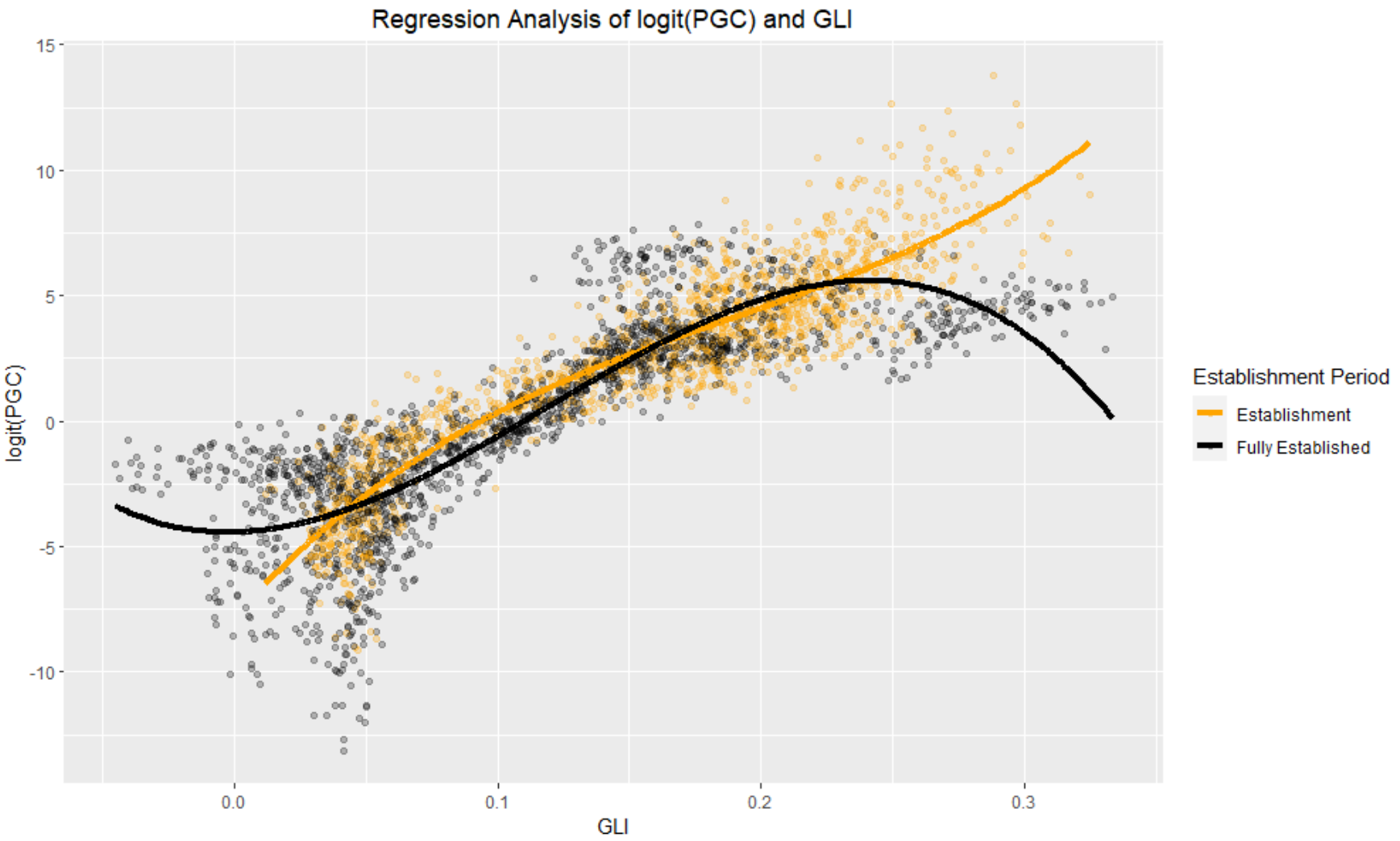


Figure 10. Fitted multiple linear regression model predicting logit(PGC) using UAV-based GLI for establishment and once fully established

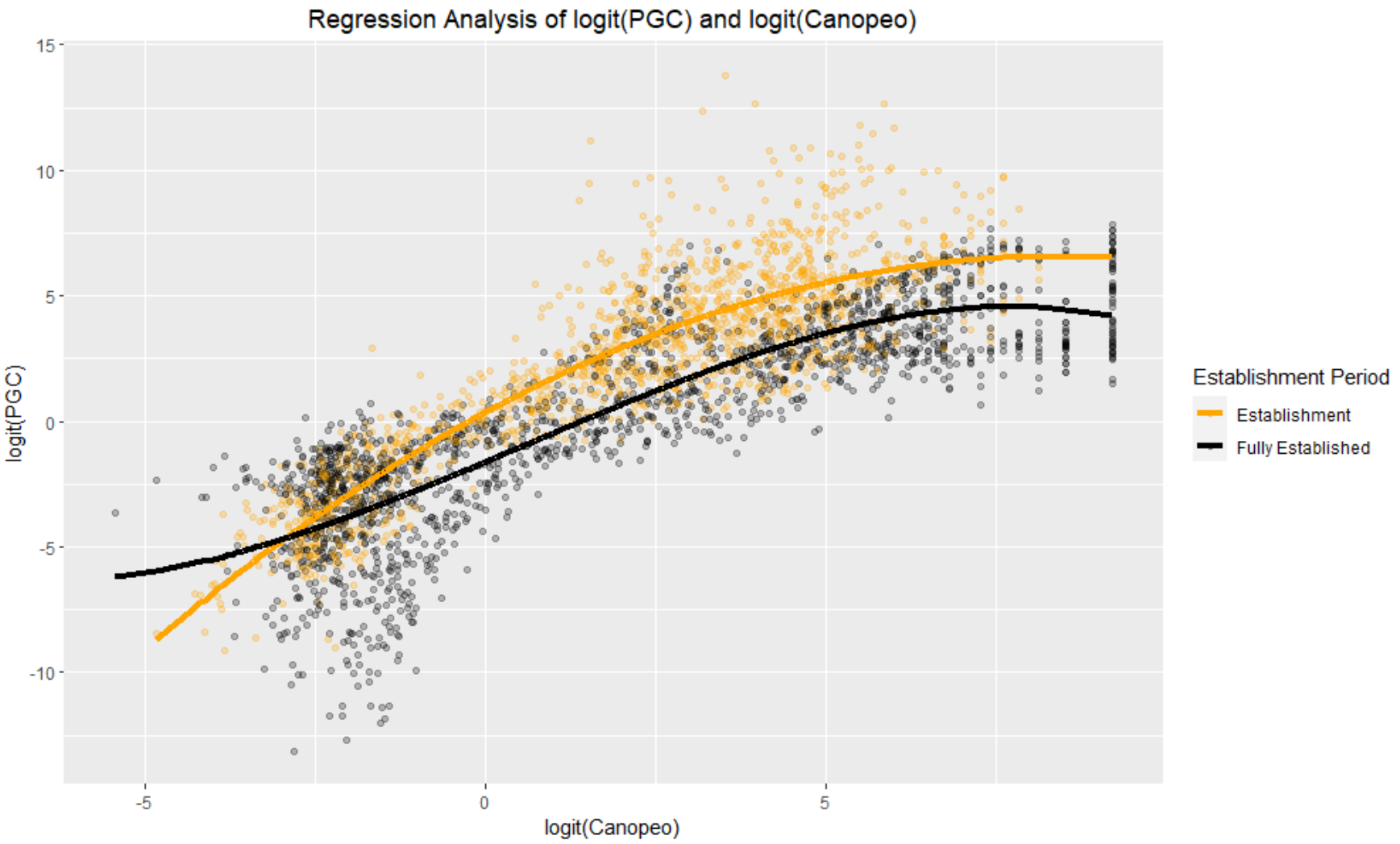


Figure 11. Fitted multiple linear regression model predicting logit(PGC) using ground-based logit(Canopeo) for establishment and once fully established

CHAPTER V

CONCLUSIONS

Chapter 2 and 3

Ultradwarf bermudagrass cultivars are commonly used in the southern United States and up into the lower parts of the transition zone as putting green surfaces on golf courses due to their dwarfed characteristics. However, the ultradwarfs currently available have come as the result of random or induced mutations going back to the original cultivar developed in 1956 by Dr. Glenn Burton, 'Tifgreen'. These mutations have resulted in limited genetic diversity among the genotypes, which could cause issues as superintendents try to find a genotype suited for their location. This is particularly true as superintendents of courses farther north in the transition zone look to use bermudagrass for their putting surfaces.

Oklahoma State University has been actively engaged in bermudagrass breeding since the mid-1980s. One of the goals of the turfgrass breeding program has been to develop genotypes with improved cold hardiness. This goal has also been implemented in the development of new interspecific hybrids for use on putting green surfaces. Some of these grasses have been shown to have increased freeze tolerance compared to the ultradwarf standards, but the genetic diversity between these new genotypes and the ultradwarfs using both DNA and morphological information has not been established. Additionally, the performance of these new genotypes under putting green management has also not been extensively studied. The understanding

provided by the evaluation of the genetic diversity and morphological traits, as well as the performance under putting green management will help turfgrass breeders select genotypes genetically distinct from the ultradwarfs but also have adequate performance desired by superintendents and golfers. Additionally, using the data collected on the morphological characteristics as well as the ball roll performance of these genotypes under field settings, the relationships between these can be established and further understood to assist breeders in selecting genotypes which will have desired ball roll distance performance.

The data in these studies showed the new genotypes were significantly distinct compared to the ultradwarfs, while the ultradwarfs showed high similarity to each other. When evaluating the morphological diversity among all genotypes, there was also a significant difference among the genotypes, but some of the new genotypes grouped closely to the ultradwarfs, indicating new genetic material was introduced but similar morphological traits were obtained. Under the field setting, the new genotypes did not have ball roll distances similar to the common ultradwarf 'TifEagle' but did show similar or improved visual and rooting characteristics. These results show these new genotypes could be used for putting green surfaces in the southern United States and transition zone, but more research should be done to understand the impact of different management practices on ball roll distance. There is also a significant relationship between the leaf blade length of the genotype and the ball roll distance when mown at 3.2 mm. There also exists significant relationships between leaf blade length and other morphological characteristics like canopy height, which could be used to assist breeders in selecting high performing genotypes early in the breeding process.

Chapter 4

Common methods of turfgrass evaluation can suffer from raters' bias and fatigue or take several hours to collect accurate subjective data. The implementation of remote sensing

technologies into turfgrass evaluations could provide an efficient method to collect critical data throughout the growing season. Five alternative methods were compared to digital image analysis results using a lightbox: NDVI, GNDVI, NDRE, GLI, and the Canopeo phone application.

An advance putting green selection nursery was used to evaluate these indices using RGB and multispectral drone cameras. Based on the sigmoid curved relationship present comparing the percent green cover and vegetation index, a logit transformation was performed on the percent green cover, and then a multiple linear regression was fitted taking into account the interaction between the vegetation index and establishment period. The regression model showed that there was a significant relationship between each of the vegetation indices and percent green cover, with their performance ranging. The adjusted R^2 values for the models ranged from 0.71 to 0.81, and the RMSE ranged from 1.82 to 2.22. Overall, the NDVI model performed the best followed by NDRE, GLI, Canopeo, and GNDVI. Additionally, for each model there was a significant difference in the relationship during the establishment period and after the plots were fully established. This was likely due to the influence of the soil or dormant turf had on the average index value. Because of this difference, care should be taken when attempting to compare two index values from different establishment periods to compare the percent green cover. However, when looking at the scatterplots of the original data, NDVI and GNDVI show an extended range of index values for lower percent green cover values while NDRE and GLI showed similar trends for higher percent green cover values. This relationship could allow for more separation of plots with similar percent green cover values on the lower and higher end of the scale. This could be especially beneficial in an advanced selection trial when all genotypes tend to perform very well overall. These or other vegetation indices could provide a new subjective method to evaluate overall turfgrass health and performance.

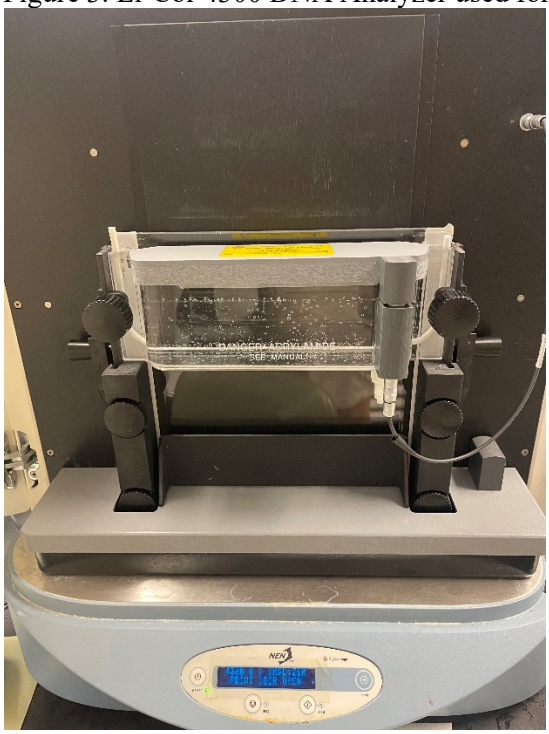
APPENDICES

Chapter 2

Figure 1. Greenhouse morphology trial setup

Figure 2. Collecting canopy height measurements for the field morphology trial

Figure 3. Li-Cor 4300 DNA Analyzer used for SSR marker analysis



Chpater 3

Figure 4. Progagation materials preprared for sprigging putting green trial

Figure 5. Sprigs applied to treatment plot using a 1:10 rate

Figure 6. Completed plots after sprigging and rolling

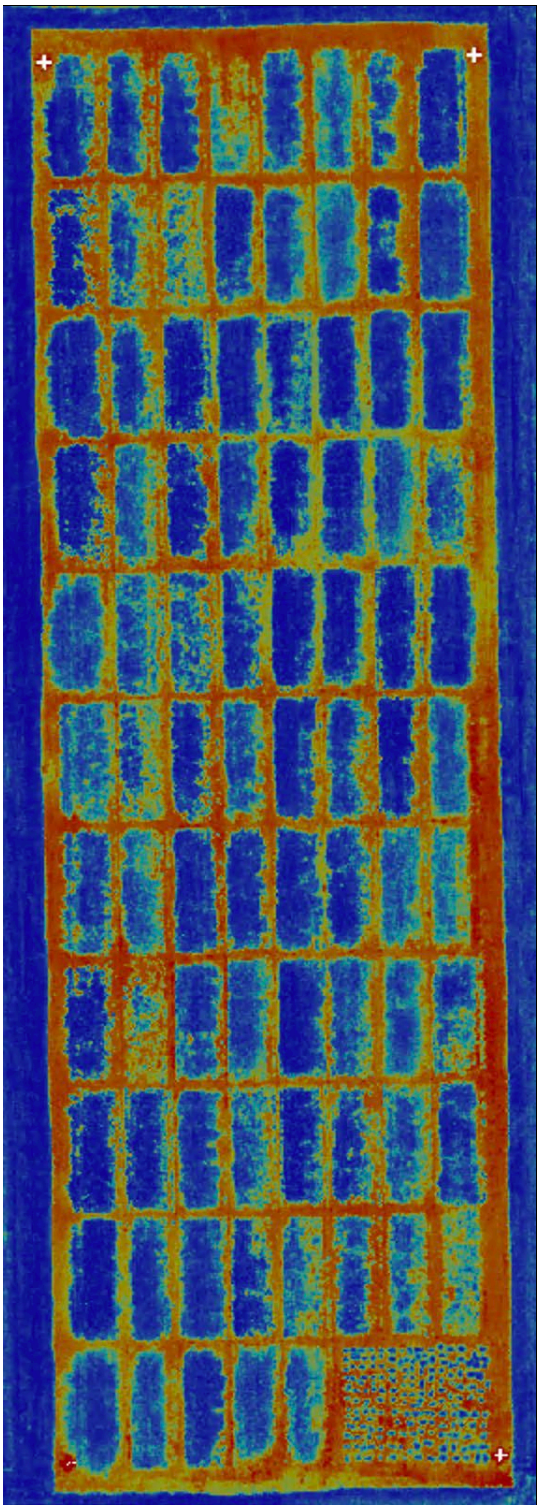
Figure 7. Collecting ball roll measurements using a USGA Stimpmeter

Figure 8. Turfgrass covers utilized to cover putting green when temperatures were predicted to fall below -4 degrees Celsius

Figure 9. DJI Mavic 2 Pro and Matrice V200 used for drone data collection

Figure 10. Orthomosaic image from Mavic 2 Pro

Figure 11. NDVI color map created from Micasense Rededge MX sensor



VITA

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