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**INVESTIGATING COLD HARDINESS
AND MANAGEMENT PRACTICES OF WARM-SEASON
PUTTING GREEN SPECIES IN THE TRANSITION ZONE**

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

John Matthew Kauffman
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DEDICATION

I would like to dedicate this dissertation to my paternal grandparents, John R. and Kathryn Kauffman. Their kindness, strength, and perseverance through life's troubles have inspired me throughout the years. I also dedicate this dissertation to my maternal grandfather, Emmett Brock. His love of nature was strong and his knowledge was immeasurable. He taught me that family means more than anything else in life. All of my grandparents valued education and emphasized its importance to their grandchildren. Their positive outlook and influence has been integral in my life. I can't possibly thank them enough.

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Most importantly, I must thank my Lord and Savior, Jesus Christ, for it is through Him that all is possible.

ABSTRACT

Warm-season turf species are becoming increasingly popular for putting green use in the transition zone. Ultradwarf bermudagrass (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy) is the prevalent warm-season putting green species, but seashore paspalum (*Paspalum vaginatum* Swartz) and ‘Diamond’ zoysiagrass [*Zoysia matrella* (L.) Merr.] may also be grown in the transition zone. Warm-season species are susceptible to winter injury and may require different management regimes than cool-season species. Therefore, the objectives of this research were to assess the impacts of various management practices on warm-season putting green species and characterize the physiological basis for differences in freeze tolerance of various warm-season putting green species. Field studies determined sampling procedures for thatch-mat depth and soil organic matter content of warm-season putting greens and assessed the impact of various management practices on different warm-season putting green species/varieties. The relative freeze tolerance of ‘Champion’ and ‘TifEagle’ ultradwarf bermudagrass cultivars, ‘SeaDwarf’ seashore paspalum, and Diamond zoysiagrass were determined, along with the accumulation of proline and polyamines during cold acclimation, in growth chamber studies.

All species/varieties required different sampling numbers for determination of thatch-mat depth and soil organic matter. More sand was incorporated into the turf canopy and surface hardness was increased with brushing and vibratory rolling TifEagle putting greens after sand topdressing application than either treatment alone. Putting green management programs with lower mowing heights and increased mowing

frequencies increased ball roll distance on a MiniVerde putting green without negatively affecting turf quality. Weekly vertical mowing + daily grooming on TifEagle reduced thatch depth and turfgrass quality, while increasing topdressing incorporation over either treatment alone.

Diamond was the most freeze tolerant species/variety, followed by TifEagle, Champion, then SeaDwarf. Cold acclimation increased proline concentration for all species/varieties except SeaDwarf, but had inconsistent effects on polyamines. Spermidine and putrescine concentrations differed with species/variety, but were not correlated to freeze tolerance.

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CHAPTER ONE – LITERATURE REVIEW

Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) is commonly used as putting green turf in the transition zone of the United States, despite its poor adaptation to the region's climate (Salisbury and Ross, 1992). Defined by McCarty (2001) as a 320 km wide belt centered along a line at 37° north latitude (which includes Tennessee), the transition zone's warm and humid climate encourages activity of fungal pathogens, such as *Rhizoctonia* spp. and *Pythium* spp., as well as drought and high temperature stress (Beard, 1997; Murphy et al., 1994). When combined, these conditions are often referred to as summer bentgrass decline (Beard, 1997; Carrow, 1996; Huang and Liu, 2003; Murphy et al., 1994). The increased photo-oxidation and photorespiration that C₃ plants experience during warm periods decreases the efficiency of photosynthesis in creeping bentgrass and increases the rate of cellular respiration (Taiz and Zeiger, 1991). The resulting carbohydrate deficit inhibits the ability of plants to accumulate nonstructural carbohydrates and allocate them to the crown and roots (Hull, 1992; Liu and Huang, 2000; Xu and Huang, 2000). These conditions lead to increased fungicide and water usage, as well as increased labor inputs.

Bermudagrass (*Cynodon dactylon* L.), however, has been used as a putting surface south of the transition zone since the 1930s because of its successful adaptation to the region's climate (Beard and Sifers, 1996; Maples, 2001). This C₄ species has several advantages over C₃ turfgrasses for use in the transition zone, including its resistance to high temperature stress, increased photosynthetic efficiency, reduced water requirement, and decreased susceptibility to fungal pathogens (Beard, 2002). These attributes lead to

decreased fungicide and water usage, and fewer in-season labor inputs. However, warm-season turfgrasses experience dormancy during winter, and are often overseeded with cool-season species or painted for winter play (Goatley et al., 2007).

Common bermudagrass, the only viable warm-season species for southern putting greens until 1956, has coarse texture and poor stand density. The hybrid bermudagrass ‘Tifgreen’ (*C. dactylon* (L.) × *transvaalensis*), released in 1956, soon became the variety of choice for use as putting green turf (Alderson and Sharp, 1994). Developed as a result of crossing selections of common bermudagrass (*C. dactylon*) from a putting green at Charlotte Country Club in Charlotte, NC, and African bermudagrass (*C. transvaalensis*) from East Lake Country Club in Atlanta, GA, Tifgreen exhibited a much higher shoot density than common bermudagrass and could tolerate the 6 mm height of cut that was popular for putting greens during that time (Burton, 1966; Beard and Sifers, 1996). In 1965, a dwarf mutant of Tifgreen, called ‘Tifdwarf,’ was released, beginning a wave of dwarf bermudagrasses for putting green use (Alderson and Sharp, 1994). Dwarf bermudagrasses exhibit slower vertical leaf extension and slower lateral stem development than non-dwarf varieties (Beard and Sifers, 1996; Gray and White, 1999; Hollingsworth et al., 2005). Tifdwarf tolerates cutting heights as low as 4.5 mm, and has a darker color and higher shoot density than Tifgreen (Beard and Sifers, 1996; Moncrief, 1968).

In the 1970s, the demand for faster, higher quality greens rose, as did the need for improved bermudagrass cultivars (McCarty, 2001). As cutting heights were lowered to increase ball roll speed, disease pressure, heat stress, and scalping incidents among traditional cultivars like Tifgreen and Tifdwarf increased (Beard and Sifers, 1996). Amid

the stress, “off-type” bermudagrasses began to appear. These off-type grasses did not phenotypically resemble Tifgreen or Tifdwarf, yet had higher shoot densities, shorter internodes, and a tolerance of lower cutting heights than Tifdwarf (Moncrief, 1975; Foy, 1997). The off-types were genotypically different from Tifdwarf and maintained higher quality than Tifdwarf during stressful periods (Caetano-Anolles et al., 1995; Ho et al., 1997; Zhang et al., 1999).

Selected off-types have been released as ultradwarf cultivars because they exhibit characteristics of both dwarf and non-dwarf plants. Ultradwarf bermudagrasses are divided into two main groups according to their growth habit in relation to Tifdwarf (Beard and Sifers, 1996). One group has slower vertical growth and faster lateral growth than Tifdwarf and includes the cultivars ‘Champion,’ ‘MiniVerde,’ and ‘MS Supreme.’ The second group has a similar lateral growth rate, but slower vertical growth than Tifdwarf. Included in this group are ‘FloraDwarf’ and ‘TifEagle’ (Gray and White, 1999; Hanna and Elsner, 1999).

Zoysiagrass (*Zoysia* spp.), however, requires fewer maintenance inputs than either creeping bentgrass or bermudagrass (Duble, 1996; Beard, 1973). Zoysiagrass is a perennial C₄ turfgrass that spreads by way of stolons and rhizomes. The species is well-adapted to the warm and humid climate of the transition zone, and is more tolerant of shade than bermudagrass (Duble, 1996). Zoysiagrass is also tolerant of moderately saline conditions (Hitchcock and Chase, 1955; Beard, 1973; Anderson, 2000). In addition to having a lower water requirement than bermudagrass, zoysiagrass also requires less nitrogen (N) fertility than bermudagrass (Biran et al., 1981; White et al., 2001). Zoysiagrass also exhibits superior load-bearing capacity, and excellent drought, wear,

heat, and pest tolerance compared to cool-season grasses (Youngner, 1961; Biran et al., 1981; Reinert and Engelke, 2001; White et al., 2001). These traits contribute to reduced maintenance expense compared to cool-season turfgrasses (Duble, 1996).

Zoysiagrass creates a dense, uniform, low-growing turf when maintained on golf course fairways, tees, and bunker faces (Beard, 1973). First introduced to the U.S. around 1900, zoysiagrass did not gain popularity until the 1950s, with the release of the cultivar 'Meyer' (Grau and Radko, 1951; McDonald and Copeland, 1997). Meyer was often established vegetatively, thus the high cost and slow rate of establishment may have lessened its use. However, Meyer remains the most commonly used zoysiagrass cultivar in the transition zone and has been found to be more cold hardy than other zoysiagrass cultivars (Dunn and Diesburg, 2004; Dunn et al., 1999; Patton and Reicher, 2007).

'Diamond' zoysiagrass [*Zoysia matrella* (L.) Merr.] exhibits an even lower water and fertility requirement than 'Meyer', as well as increased shade tolerance relative to other *Zoysia* spp., yet it is much finer textured than other cultivars (Reinert and Engelke, 2001; Engelke et al., 2002). This cultivar tolerates very close mowing, making it a candidate for putting green use (Engelke, et al., 2002). Reduced disease susceptibility, N fertilizer requirement, water use, and growth rate make Diamond less costly to maintain (Youngner, 1961; Biran et al., 1981; Erusha et al., 1999; Reinert and Engelke, 2001; White et al., 2001). Although *Zoysia japonica* usually exhibits excellent cold tolerance (Turgeon, 1991), Diamond, a *Zoysia matrella*, seems to be less tolerant of cold temperatures (Patton and Reicher, 2007), which may be, along with its slow establishment rate, one of the main obstacles preventing its use in the transition zone (Beard, 1973; Busey and Myers, 1979).

Seashore paspalum (*Paspalum vaginatum* Swartz) is another C₄ turfgrass species that is common in coastal areas and may be used on putting greens (Raymer et al., 2007). A marine coastal species, seashore paspalum has excellent drought tolerance and is considered to be the most salt tolerant warm-season turfgrass species (Henry et al., 1979; Marcum and Murdoch, 1994; Huang et al., 1997; Loch et al., 2003). Tolerance of low cutting heights, dark leaf color, high shoot density, and propagation by stolons and rhizomes make seashore paspalum desirable for putting greens (Duncan and Carrow, 2000). Breeding of seashore paspalum began at the University of Georgia in the mid 1990s as a joint project with the USGA to develop paspalum cultivars suitable for golf course use (Raymer et al., 2007). ‘SeaIsle 1’ was released as a fairway variety and ‘SeaIsle 2000’ was released as a putting green variety in 2003 (Raymer et al., 2007). Most recently, ‘SeaDwarf’ was released as a putting green cultivar (Raymer et al., 2007).

Halophytic plants, such as seashore paspalum, that exhibit a tolerance for saline growing conditions, defined as those in which soil electrical conductivity exceeds 4 decismens per meter (dS m⁻¹) (US Salinity Laboratory, 1969). Halophytic plants adjust to saline conditions by two main mechanisms: restriction of salt entry into the plant and minimization of salt concentration in the cytoplasm (Munns, 2002). Salt exclusion is the process by which salt entry into the plant is restricted and is accomplished by selective uptake of ions from the soil solution to exclude Na⁺, loading the xylem with K⁺ to reduce Na⁺ capacity (Jeschke, 1984), and removing Na⁺ from the xylem in stem and petiole tissues before it reaches the leaves (Colmer, 2000; Munns, 2002). Minimization of salt concentrations in the cytoplasm is accomplished by sequestration of ions in vacuoles or by the accumulation of K⁺ ions and organic solutes, such as proline and glycine betaine

(Hasegawa et al., 2000). This ion and solute accumulation adjusts the solute potential in plant tissues to a level more negative than the water potential in solution, thus encouraging water movement into plant cells to maintain turgor and growth (Bohnert and Shen, 1999).

Similar to bermudagrass and zoysiagrass, low temperature tolerance is a limiting factor in the use of seashore paspalum in the transition zone (Duncan and Carrow, 2000). Seashore paspalum cultivars ‘Adalayd’ and ‘Futurf’ were reported to have a cold tolerance range of -4 to -7° C (Ibitayo et al., 1981; Campbell, 1979), suggesting that they may be injured by low winter temperatures in the transition zone. Additionally, Cardona et al. (1997) found ‘HI-1’, ‘Adayalad’, and PI 299042 seashore paspalum to be less cold tolerant than ‘Midiron’ bermudagrass. Cardona et al. (1997) also reported that cold acclimated root tissues of all three paspalum cultivars experienced less electrolyte leakage than non-acclimated root tissues, indicating that seashore paspalum cultivars do experience some acclimation process to cold weather. However, very little research has been conducted on cellular contents of seashore paspalum during freezing stress (Cardona et al., 1997; Raymer et al., 2007).

Cultural Practices for Managing Warm-season Putting Greens

The aggressive growth habit and high shoot density of ultradwarf bermudagrass can lead to problems related to excessive organic matter (OM) accumulation (Hollingsworth et al., 2005). Organic matter in turfgrass systems is often divided into thatch and mat layers (McCarty, 2001). Thatch is a tightly intermingled layer of living and dead tissue that develops between the verdure and the soil surface (Beard, 1973). Mat is a layer below the thatch in which the OM of the thatch is intermixed with the soil

(Carrow et al., 1987). Moderate amounts of surface OM are beneficial for putting greens. Surface OM can cushion turf crowns against foot traffic and incoming golf shots, reduce ammonia volatilization, and slow the leaching of pesticides into groundwater (Horst et al., 1996; McCarty and Miller, 2002; Petrovic, 1990; Snyder and Cisar, 1995; Vermeulen and Hartwiger, 2005). However, OM depths exceeding 1.3 cm can be considered excessive (Christians, 1998; McCarty and Miller, 2002). Excessive accumulation of organic matter near the soil surface can negatively impact the health and playability of ultradwarf bermudagrass putting greens. Large amounts of OM can encumber infiltration of water into the soil profile, slow drainage of excess water away from the putting surface, and plug soil pores, which may decrease gas exchange within the soil profile (Carrow, 2000). Accumulated OM is also associated with reduced tolerance to cold temperatures, increased disease incidence, and may reduce pesticide efficacy (White, 1962; Musser, 1960; Cornman, 1952). Additionally, excessive OM accumulation near the putting surface may result in a soft playing surface, which is not desirable for golf and may lead to mower scalping (Barton et al., 2009; Carrow, 2001; Hurto et al., 1980).

Frequent sand topdressing, vertical mowing, core cultivation, and grooming are often used to alleviate such negative effects of excessive OM accumulation (Beard, 2002; Engle and Alderfer, 1967; McCarty, 2001). Topdressing alone has been shown to reduce thatch layers in ‘Penncross’ creeping bentgrass (*Agrostis stolonifera* L.) (Eggens, 1980), ‘Tifway’ bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Pers. L.) (Carrow et al., 1987), and three different dwarf bermudagrass cultivars (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy) (White and Dickens, 1984). Topdressing with sand has been regarded as an effective method to control thatch by improving conditions for its

decomposition (Ledeboer and Skogley, 1967). Sand topdressing aids in thatch decomposition by diluting the thatch layer, preserving soil porosity, and enhancing water infiltration, which improve conditions for microbial degradation of thatch (Carrow, 2001; Rieke, 1994; Ledeboer and Skogley, 1967). Topdressing alone has been shown to reduce thatch layers in ‘Penncross’ creeping bentgrass (Eggens, 1980), ‘Tifway’ bermudagrass (Carrow et al., 1987), and three different dwarf bermudagrass cultivars (White and Dickens, 1984).

Further research has shown that thatch dilution is as important as improving conditions for thatch decomposition (Rieke, 1994). Specifically, sand topdressing serves to dilute the OM near the putting surface and preserve the majority sand matrix, which helps to preserve soil porosity. Golf course superintendents have reported difficulty incorporating sand topdressing into ultradwarf bermudagrass putting greens and have sought numerous methods to aid incorporation, including dried sand applications and using hover-type mowers to force sand into the canopy by way of air currents (Foy, 1999).

However, ultradwarf bermudagrasses produce thatch aggressively enough that sand topdressing alone can not keep pace with thatch production and mechanical means are required to keep OM levels manageable (Guertal and White, 1998). Secondary cultural practices, such as vertical mowing and grooming, are often used to help maintain surface playability, but may be important pieces of a thatch management program (Beard, 1973; Turgeon, 1991). Vertical mowing cuts stolon tissue and grooves the soil surface using vertically oriented blades (Duble, 1996). On putting greens, vertical mowing blades are often set to cut even with the soil surface or immediately below it, which

severs stolons, encourages lateral growth, increases shoot density, and encourages vigorous juvenile growth (White and Dickens, 1984; McCarty and Canegallo, 2005). Grooming is a process of very light vertical mowing with closely spaced blades that often do not penetrate the turf canopy (McCarty, 2001). Grooming helps to stand up leaf blades vertically, so that the cut is more uniform.

However, as these practices open the turf canopy, they remove organic matter from the thatch layer and open channels in the dense turf canopy for topdressing incorporation. Carrow et al. (1987) reported that vertical mowing at least twice yearly reduced thatch depth in Tifway bermudagrass. White et al. (2004) reported weekly vertical mowing severing stolons, but not penetrating the soil surface, on a Tifdwarf bermudagrass putting green to maintain a similar thatch depth to severe invasive vertical mowing twice yearly, but maintained more acceptable turf quality. Grooming, however, did not consistently affect fall carbohydrate content, shoot density, and thatch accumulation on TifEagle bermudagrass (Salaiz et al., 1995).

Little research regarding the management of putting surface characteristics, such as putting green speed, exists for warm-season putting greens. Putting green speed is a term used to describe the distance that a ball will roll and is an important playing characteristic of golf course putting greens. Ball roll distance is inversely proportional to the coefficient of friction between the ball and the putting surface (USGA, 1993). Many cultural practices have been used to decrease the coefficient of friction on putting greens. Mowing heights, N fertilization, and irrigation levels are often reduced in an attempt to increase ball roll distances (McCarty, 2001). However, these practices can be deleterious to turf health and can result in a thinning of the turf stand that reduces playing surface

quality (Beard, 2002). Previous studies on TifEagle bermudagrass have shown that mowing at 3.2 mm reduced root dry mass and turf color compared to mowing at 4.8 mm (Guertal and Evans, 2006). Similarly, lower N fertilization rates produced fewer total nonstructural carbohydrates in the TifEagle bermudagrass turf than higher N rates (Guertal and Evans, 2006).

Light-weight rollers have also been used to reduce the coefficient of friction on a putting green and increase ball roll speed. Rolling was a popular practice in the early 1900s and was promoted to smooth putting surfaces and increase the slow green speeds that the mower technology of the time could provide (Piper and Oakley, 1921). However, rolling became less popular by the mid-twentieth century because of the increased awareness of compaction in the native soil rootzones in use at the time and the development of improved mower technology (Carrow and Petrovic, 1992). The advent of self-propelled dedicated rolling units and the increased use of high sand content rootzones for putting greens have contributed to bringing rolling back in favor (Nus, 1992). Previous research on the impact of light-weight rolling on putting greens has focused on creeping bentgrass, showing that ball roll distance increased for several hours after rolling (Danneberger et al., 1993; Hamilton et al., 1994). Additional research has shown that rolling may reduce dollar spot (*Sclerotinia homoeocarpa*) infestation, moss occurrence, and black cutworm activity (Nikolai et al., 2001). Further studies have reported that rolling sand-based putting greens up to seven times per week has no deleterious effect on rootzone bulk density, water infiltration, or creeping bentgrass quality (Hamilton et al., 1994; Hartwiger et al., 2001; Nikolai, 2005).

Recent research has explored combinations of mowing height, mowing frequency, and rolling frequency as they relate to putting green speed and turf quality. Decreasing mowing frequency to alternate days instead of daily and increasing rolling frequency to 3 to 6 times per week have been shown to increase creeping bentgrass turf quality while maintaining green speed similar to mowing daily without rolling (Nikolai, 2005; Strunk, 2006; Zarco Perez and Nikolai, 2009). Additionally, no increases in soil bulk density and disease occurrence, or decrease in water infiltration, were observed. Richards et al. (2009) found that mowing creeping bentgrass at 4.0 mm and rolling 3 to 6 times per week increased ball roll distances and green speeds compared to mowing at 3.2 mm without rolling. However, research on the combination of mowing height, mowing frequency, and rolling frequency has not been conducted on ultradwarf bermudagrass cultivars.

Freeze Tolerance Evaluation of Warm-season Species

A relative lack of cold hardiness, or ability to survive low temperature stress (Beard, 2002), is one deterrent to widespread use of ultradwarf bermudagrasses, zoysiagrass, and seashore paspalum for putting greens in the transition zone (Anderson et al., 2003; Forbes and Ferguson, 1947; Fry, 1990; Kopec et al., 2007). Cold hardiness is often reported at LT_{50} , which the temperature at which 50% of the test population is lethally injured. Anderson et al. (2002) evaluated differences in survival of bermudagrass cultivars grown at putting green and fairway heights with a laboratory freezing test and found that cultivars maintained for putting green use were generally less cold tolerant than those maintained at fairway height. Tifdwarf and Tifgreen had LT_{50} values near -6.5°C , MiniVerde and TifEagle had LT_{50} values near -6°C , and Champion, Floradwarf, and MS Supreme were cold tolerant to -5°C . At fairway height 'Midlawn,'

‘Quickstand,’ ‘Yukon,’ and ‘Guymon’ bermudagrasses were cold tolerant to -8.4, -8.0, -7.6, and -7.4° C, respectively. Similarly, Patton and Reicher (2007) evaluated differences in survival of zoysiagrass species and cultivars using a laboratory freezing test. They found Diamond, the only zoysiagrass cultivar potentially suitable for putting green use, to be less tolerant of cold temperatures, being cold tolerant to -8.4° C, than the higher mown Meyer and ‘Zenith,’ which were cold tolerant to -11.5° C.

Cold hardiness has traditionally been evaluated in the field, in which regrowth is evaluated in the spring following a severe winter (Anderson et al., 1988). Although this method is considered a reliable indicator of cold hardiness, a minimum of two years of data collection is required because temperature, moisture, and snow cover differences between years may alter survival rates (Anderson et al., 2002). Additionally, not all winters provide conditions cold enough to be considered good indicators of cold hardiness and may not distinguish hardiness between cultivars. Laboratory methods may need to be used in conjunction with field trials to more accurately estimate cold hardiness of cultivars.

Freeze tolerance of warm-season grasses has been evaluated in the past by measuring electrolyte leakage from plant tissues subjected to freezing temperatures in controlled chambers (Anderson et al., 2003). However, lethal temperature predictions by electrolyte leakage may not be accurate. Electrolyte leakage understated freezing tolerances of centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] (Fry et al., 1993) and seashore paspalum (Cardona et al., 1997), while overstating freezing tolerances of St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze] (Maier et al., 1994).

Anderson et al. (1993) developed a quick and repeatable regrowth method for quantifying the freeze tolerance of bermudagrass that corrects the inconsistency of the electrolyte leakage method and closely matches field test observations. In this method, plants are cold acclimated in a controlled-environment chamber set at 8° C and 2° C day/night cycles with a 10 h photoperiod at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light for four weeks. Controlled-environment chambers provide an acclimation setting for plants that is independent of yearly fluctuations of field trials (Cardona et al., 1997) and have been used successfully to acclimate and evaluate freeze tolerance of other warm-season species such as buffelgrass (*Pennisetum ciliare* L.), seashore paspalum (*Paspalum* spp.), and zoysiagrass (*Zoysia* spp.) (Stair et al., 1998; Cardona et al., 1997; Patton and Reicher, 2007).

Freeze Tolerance Physiology

Winter injury in turfgrass is often caused by direct or indirect low temperature exposure (McCarty, 2001). Direct low temperature exposure occurs when temperatures drop rapidly and ice crystals rupture cell membranes intracellularly (McCarty, 2001). Indirect low temperature exposure is more common than direct low temperature exposure and occurs when cold acclimated grasses are subject to cold temperatures for prolonged periods (Fry, 1990). Both direct and indirect low temperature exposure commonly result in reduced respiration, enzyme activity, carbohydrate translocation, and protein synthesis, as well as tissue desiccation, all of which are due to or confounded by membrane dysfunction (McCarty, 2001; Samala et al., 1998; Taiz and Zeiger, 1991).

Membrane Lipid Desaturation

Plant cell membranes change during cold acclimation and must remain fluid for proper function (Harwood et al., 1994; Steponkus, 1984). When temperatures approach critical values for chilling injury, cell membranes in cold-sensitive plants may transition from a fluid to a gel-like state, which may inhibit H^+ -ATPase activity, transport, energy transduction, and metabolism (Hale and Orcutt, 1987). Membrane fluidity at lower temperatures is maintained by decreasing the saturation of lipids in the phospholipid bilayer (Steponkus, 1984; Taiz and Zeiger, 1991). Lipid desaturation forms double bonds in the fatty acids, decreasing the freezing point of the bilayer and maintaining fluidity. Such lipid desaturation is common in warm-season turfgrasses such as bermudagrass and seashore paspalum, along with other crops (Samala et al., 1998; Cyril et al., 2002; Munshaw et al., 2006; Steponkus, 1984). When cold-sensitive plants undergo acclimation, lipid desaturase activity increases, resulting in greater lipid unsaturation and greater membrane fluidity (Taiz and Zeiger, 1991).

Proline

The non-polar amino acid proline accumulates in plants during dehydrative stress and aids in osmotic adjustment to decrease cellular water potential, quenches singlet oxygen species known to increase during freezing, and helps to stabilize membranes during dehydrative stresses such as freezing (Kavi Kishor et al., 1995; Kendall and McKersie, 1989; Matysik et al., 2002; Rudolph et al. 1986; Santarius, 1992; Saradhi et al., 1995). Freeze tolerance in crops such as alfalfa (*Medicago media* Pers.) (Paquin, 1977), potato (*Solanum* spp.) (van Swaaij et al., 1985), orange (*Citrus sinensis* L.) (Yelenosky and Vu, 1992), and maize (*Zea mays* L.) (Chen and Li, 2002), as well as

bermudagrass and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] (Munshaw et al., 2004; Cai et al., 2004), have been correlated to increases in proline concentration. Research by Akiyama et al. (1994) showed that high proline concentrations in the leaves of three zoysiagrass species increased cold hardiness. Patton et al. (2007) also reported increased proline concentrations in two zoysiagrass species during cold acclimation and reported a correlation between proline concentration and freeze tolerance. Additionally, Levitt (1980) showed that proline levels increase during cold acclimation, decrease during de-acclimation, and are present in greater concentration in cold-hardy plants.

Polyamines

Polyamines, such as spermidine, spermine, and putrescine, occur ubiquitously in plants and are thought to aid in plant defenses to various environmental stresses (Flores, 1990; Kumer et al., 1997; Bouchereau et al., 1999; Smith, 1985). Because polyamines express a polycationic nature at physiological pH, they can bind strongly to the negative charges of nucleic acids, proteins, and phospholipids (Smith, 1985). The interaction between polyamines and membrane phospholipids may stabilize cell membranes during stress conditions (Roberts et al., 1986). Polyamines also protect membranes against lipid peroxidation induced by reactive oxygen species (Tadolini et al., 1984). Cold-tolerant plants have been found to increase endogenous polyamine levels in response to freeze acclimation more than plants of lesser cold tolerance (Guye et al., 1986; Kramer and Wang, 1989; Lee et al., 1997; Nadeau et al., 1987). Increased cold-tolerance in cucumber (*Cucumis sativus*) was closely related to a lower rate of reactive oxygen species generation caused by increased polyamines (Shen et al., 2000). Polyamines have also been associated with low temperature survival in cabbage (*Pringlea antiscorbutica*)

(Hummel et al., 2004). However, no polyamine research in regards to cold-tolerance has been studied in turfgrass.

Practices to Aid Winter Survival and Spring Greenup

Because cold hardiness and winterkill are concerns for managers of warm-season putting greens in the transition zone, practices to enhance winter survival are important. Improving spring greenup may allow play to resume earlier and allow the plant to begin producing photosynthates earlier in the year. Covering the turf is a common method to increase winter survival (Goatley et al., 2005). Winter protective blankets trap heat energy and keep the soil and air around the plant warmer than the ambient temperatures (Goatley et al., 2007; Goatley et al., 2009). Pine straw was used as early as 1929 to protect bermudagrass putting greens in the winter (Beckett, 1929). Enhanced fall color retention, winter survival, and spring regrowth of covered bermudagrass maintained at heights >19 mm have been reported (Sowers and Welterlen, 1988; Schmidt et al., 1989; Shashikumar and Nus, 1993; Goatley et al., 2005). Turf covers have enhanced winter survival of creeping bentgrass and annual bluegrass (*Poa annua* L.) on putting greens (Dionne et al., 1999).

However, golf courses in the transition zone often receive play year-round and require a turf cover that can be removed to accommodate play. Previous research has shown moveable covers to enhance fall color retention, winter survival, and spring regrowth of bermudagrass maintained at heights above 19 mm (Goatley et al., 2005). For putting greens, a study comparing various types of removable turf covers on ‘MS-Express’ bermudagrass (*Cynodon magennissii* Hurc.) found all cover types increased mean daily minimum surface temperatures over uncovered plots, although PAR

transmission, which may increase spring greenup, was highly variable (Goatley et al., 2007).

Bermudagrass growth ceases when temperatures drop below 10° C and turf discolours from green to brown when temperatures fall below 0° C (McCarty, 2001; McCarty and Miller, 2002). Dormant bermudagrass turf can make a playable surface, but is not aesthetically pleasing to golfers. Additionally, temperatures in the transition zone routinely fall below 0° C during the winter and may reach temperatures near -15° C, which is near the LT₅₀ value for ‘Champion’ and ‘TifEagle’ bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burt-Davy), and could cause winter injury (Anderson, 2002). Historically, golf course superintendents have overseeded warm-season putting greens with cool-season species, such as creeping bentgrass (*Agrostis stolonifera* L.), roughstalk bluegrass (*Poa trivialis* L.), or perennial ryegrass (*Lolium perenne* L.), to provide an actively growing and aesthetically pleasing putting surface during winter (McCarty and Miller, 2002; Long, 2006). However, the overseeding process requires aggressive vertical mowing or other surface disruption to open the existing turf canopy and facilitate proper contact between seed and soil (McCarty, 2001). Additionally, the overseeded cool-season species can add to maintenance expenses by requiring fertilizer and pesticide applications during the winter months, as well as chemical aids to transition back to the base bermudagrass turf in the spring (McCarty and Miller, 2002).

Recently, painting putting greens with a green colorant in the winter to provide more acceptable aesthetics has come into favor. The paint used to color putting greens does not impose the competition for resources that overseeding does, nor does the paint last long enough to create shading problems for bermudagrass emerging from dormancy

(McCarty, 2001). Also, using paint to add color to dormant greens is less expensive than overseeding. Painting greens can cost from \$900 to \$3000 per season for an 18-hole golf course depending on the number of paint applications and the price of paint (Carson, 2004). However, overseeding the average 18-hole golf course with 1.2 hectares of putting green area can cost nearly \$6,000 per season in addition to maintenance, fertilizer, and pesticide applications (Carson, 2004; McCarty, 2001; Long, 2006).

Previous studies regarding painting putting greens have focused on different paint brands and comparisons between painting and overseeding. Long (2006) found that applications of two different latex paint brands on Champion bermudagrass putting greens increased soil temperatures at a 7.62 cm depth over the untreated control and encouraged spring green-up over the control, but did not affect ball roll distance during the winter. Liu et al. (2007) found that painting Champion bermudagrass putting greens provided a similar color to overseeding with roughstalk bluegrass, but painting did provide longer ball roll distance than overseeding. Liu et al. (2007) also reported that trinexapac-ethyl and foliar fertilizer applications did not impact winter paint quality, but foliar fertilizer applications did improve overseeded turf over granular applications.

Primary strategies for enhancing cold hardiness of bermudagrass have also included avoiding late season nitrogen (N) applications and increasing potassium (K) applications (Goatley et al., 2005; Reeves et al., 1970; Schmidt and Blaser, 1969). Late season N applications are believed to promote excessive shoot growth and enhance production of young shoot tissue, which creates tissue easily susceptible to low temperature injury and hinders the accumulation of storage carbohydrates and osmotic regulators. However, several studies have suggested that late season N fertility may not

be as detrimental as previously thought. Goatley et al. (1994) reported that late season N improved fall and spring color, while not impacting nonstructural carbohydrates. Schmidt and Chalmers (1993) also reported better fall color and longer color retention with late season N applications, while that no deleterious effects were observed on emergence from dormancy. Reeves et al. (1970) found later N applications to extend green color later into the fall on 'Tifgreen' bermudagrass without negatively affecting freeze tolerance. Additionally, Richardson (2002) reported that late season N fertility had no influence on winter survival of seeded bermudagrass and that late fall N applications enhanced spring green-up of 'Tifway' bermudagrass without sacrificing rhizome cold tolerance.

The role of plant growth regulators (PGRs) in turf management is also becoming increasingly important and varied (Watschke et al., 1992). Plant growth regulators were introduced to suppress seed head development and reduce mowing, but their use has expanded to abiotic stress tolerance and improving turf density and population characteristics (Nabati et al., 1994). Trinexapac-ethyl (TE) is a commonly used PGR in turf management that blocks the formation of 3 β -OH late in the synthesis of gibberellic acid, thus inhibiting plant cell elongation (Adams et al., 1992). Use of TE has been reported to increase levels of total soluble carbohydrates in creeping bentgrass, which may allow photosynthate to be stored for later use or osmotic regulation (Han et al., 1998). Qian and Engelke (1999) found root development and photosynthesis to be enhanced by TE applied to Diamond zoysiagrass grown under shade conditions. Research by Richardson (2002) found summer and fall TE applications to enhance fall

turf color and spring green-up of Tifway bermudagrass without negatively impacting rhizome freezing tolerance.

The extension of green turf color later into the fall and increased pace of spring greenup observed of late season N applications in previous studies, along with the increase in soluble carbohydrates and improvement of rhizome freeze tolerance observed with TE treatments in previous studies, suggest that warm-season putting species and varieties may benefit from those practices (Fagerness et al., 2002; Yelverton et al., 2000).

Objectives

The objectives of the following research were several-fold. This work sought to determine proper sampling procedures for assessing thatch-mat and soil organic matter content in warm-season putting greens. Also, this research sought to examine the effects of management practices important to the maintenance of quality putting surfaces on warm-season putting green species. As well, this research sought to determine the relative freeze tolerance of four warm-season putting green cultivars and investigate the role of proline and polyamines in freeze tolerance of those cultivars. Traditional methods of sand topdressing incorporation were compared to the use of vibratory rollers as a means of incorporating topdressing sand into an ultradwarf bermudagrass turf canopy. Weekly vertical mowing, daily grooming, and the combination of both were also compared to assess their effects on thatch production, topdressing incorporation into putting green turf canopies, and overall turf quality of ultradwarf bermudagrass putting greens. The impact of two mowing heights, three mowing frequencies, and three rolling frequencies on the ball roll distance, percent green turf cover, turf quality, and disease occurrence on MiniVerde bermudagrass was also studied. Finally, the relative freeze

tolerance of TifEagle and Champion bermudagrass, Diamond zoysiagrass, and SeaDwarf paspalum was determined, as well as how stolon and rhizome proline content change with cold acclimation, and how stolon and rhizome polyamine content vary with cold acclimation.

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**CHAPTER TWO – SAMPLING PROCEDURES FOR
THATCH-MAT DEPTH AND ORGANIC MATTER CONTENT IN
FOUR WARM-SEASON PUTTING GREEN CULTIVARS**

Abstract

Thatch-mat and organic matter (OM) accumulation near the soil surface of putting greens are of concern to turf managers because these factors can affect soil physical properties and turf performance. Surface OM can cushion turf crowns against foot traffic and incoming golf shots. However, excessive thatch and OM can encumber infiltration of water into the soil profile, slow drainage of excess water away from the putting surface, and plug soil pores, which may decrease the infiltration of oxygen into the soil profile. A study was undertaken in Knoxville, TN, and to derive proper sampling procedures to assess thatch-mat depths and OM contents of TifEagle and Champion bermudagrass (*Cynodon dactylon* (L.) $\times$ *C. transvaalensis* Burt-Davy), SeaDwarf seashore paspalum (*Paspalum vaginatum* Swartz), and Diamond zoysiagrass [*Zoysia matrella* (L.) Merr.]. TifEagle and Champion accumulated thatch-mat to a deeper depth than both SeaDwarf and Diamond. However, SeaDwarf had a higher OM content than Diamond and both had higher OM contents than TifEagle and Champion. This study also generated sampling numbers for thatch-mat depth and OM content sampling and found that previous studies often undersampled plots for thatch-mat depth, but previous OM sampling procedures have not traditionally undersampled plots. The sampling numbers generated in this study provide a range of confidence levels and minimum detectable difference levels which may allow future researchers to accurately sample TifEagle, Champion, SeaDwarf, and Diamond putting green plots for thatch-mat depth and OM content.

Introduction

Organic matter (OM) accumulation near the soil surface of putting greens is of concern to turf managers because of its effects on soil physical properties that can influence turf performance. Soil OM is composed primarily of dead and sloughed plant tissues that are most resistant to decay, along with soil microbial biomass (Waddington, 1992; Wolf et al., 1994). In turfgrass systems, OM is often divided into thatch and mat layers (McCarty, 2001). Thatch is a tightly intermingled layer of living and dead tissue that develops between the verdure and the soil surface (Beard, 1980). Thatch includes horizontal stems, nodes, crown tissue, periodically sloughed roots, intact fibrous roots, and vascular strands of stem and leaf sheaths (Engle, 1954; Roberts and Bredakis, 1960; Hurto et al., 1980). The mat layer is the layer below the thatch in which the OM of the thatch is intermixed with soil (Carrow et al., 1987). Thatch and mat accumulate because their constituents have high lignin content relative to other components of OM, such as leaf tissue, and slow microbial degradation of the cellulosic structures comprising this layer (Ledeboer and Skogley, 1967). Meinhold et al. (1973) found that leaf matter in clippings did not add to thatch dry weight, but did influence depth and thickness of the thatch layer, suggesting that leaf matter is degraded more quickly than root or stem tissues, but is still an important constituent of soil OM.

The depth of the organic layer, and the density of that layer in the upper 2.5 cm of the rootzone, can have both positive and negative effects on turf performance (Carrow et al., 1987; Neylan, 1994; Waddington et al., 1974). Moderate amounts of surface OM are beneficial for putting greens (Carrow, 2004). Surface OM can cushion turf crowns against foot traffic and incoming golf shots, reduce ammonia volatilization, and slow the

leaching of pesticides into groundwater (Horst et al., 1996; McCarty and Miller, 2002; Petrovic, 1990; Snyder and Cisar, 1995; Vermeulen and Hartwiger, 2005). However, thatch depths exceeding 13 mm can be considered excessive (Christians, 1998; McCarty and Miller, 2002). Large amounts of OM can encumber infiltration of water into the soil profile, slow drainage of excess water away from the putting surface, and plug soil pores, which may decrease the infiltration of oxygen into the soil profile (Carrow, 2000; Harris, 1978; Murray and Juska, 1977). Accumulated OM is also associated with reduced tolerance to cold temperatures, increased disease and insect incidence, and may reduce pesticide efficacy (Cornman, 1952; Miller, 1965; Musser, 1960; Sprague, 1945; Thompson, 1967; White, 1962). Additionally, excessive OM accumulation near the putting surface may result in soft playing surfaces, which are not desirable for golf and may lead to mower scalping (Barton et al., 2009; Carrow, 2001; Hurto et al., 1980).

Organic matter is often assessed as the depth of the thatch-mat layer, as well as some measure of OM content (Callahan et al., 1998; Waddington, 1992). Thatch-mat depth can be measured by several methods, although no industry-wide standard exists for its measurement (Waddington, 1992). Thatch-mat depth has been measured by ruler both before and after compressing the sample with various weights (Waddington, 1992; Callahan et al., 1997; Volk, 1972; Beard, 1976; Engel and Alderfer, 1967; McWhirter and Ward, 1976). Compressing the sample is often used to correct for differences in thatch density and can also correct for the thatch stretching that can occur when samples are removed from the soil (Waddington, 1992). However, compression can understate the amount of thatch-mat present in samples that have a loose arrangement of thatch (Waddington, 1992). Previous research has found both compressed and uncompressed

thatch thickness to be correlated to thatch dry weights, suggesting that thatch compression may not be necessary in assessing thatch depth (NE-57 Technical Research Committee, 1977).

Organic matter content is often measured using dry combustion, wet chemical oxidation, or weight loss on ignition (Nelson and Sommers, 1982; Combs and Nathan, 1998; Kimble et al., 2001). The dry combustion method is used primarily for assessing soil organic carbon content and involves measuring CO₂ evolved from oxidizing samples at high temperatures, which requires costly equipment (Konen et al., 2002). Wet chemical oxidation (Walkley and Black, 1934), often referred to as the Walkley-Black procedure, has long been the standard for measuring OM concentration in soils, but may overstate OM due to oxidization of materials other than true OM and involves such human and environmental hazards as H₂SO₄ and Cr⁶⁺ (Miyazawa et al., 2000). Weight loss on ignition is the most common method of assessing OM content in turfgrass systems and involves oxidizing samples at moderate to high temperatures, with the weight loss after ignition being proportional to the amount of OM in the sample (Waddington, 1992; Combs and Nathan, 1998). The weight loss on ignition method has produced OM values that correlate well with OM values obtained by the Walkley-Black method, is less costly than other methods, and has been adopted by the American Society for Testing and Materials (ASTM) for use in testing OM content in soils for turfgrass use (ASTM 2000; ASTM 2007).

Although many options exist for assessing thatch-mat and OM in turfgrass systems, preferred methods and sampling procedures do not currently exist. The objective of this study was to determine the optimum sample sizes required to estimate

thatch-mat depth and OM content in ‘TifEagle’ and ‘Champion’ ultradwarf bermudagrass (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy), ‘SeaDwarf’ seashore paspalum (*Paspalum vaginatum* Swartz), and ‘Diamond’ zoysiagrass [*Zoysia matrella* (L.) Merr.] with a pre-determined degree of accuracy and confidence.

Materials and Methods

Experimental area

This study was conducted in April 2010 on a newly constructed putting green comprised of 90% sand and 10% reed sedge peat (v/v) at the East Tennessee Research and Education Center in Knoxville. Sand texture was 15.6% coarse sand (0.5-1.0 mm), 73% medium sand (0.25-0.50 mm), and 11.4% fine sand (0.15-0.25 mm). TifEagle and Champion bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burt-Davy), SeaDwarf seashore paspalum (*Paspalum vaginatum* Swartz), and Diamond zoysiagrass [*Zoysia matrella* (L.) Merr.] were established in 1 × 4 m plots from washed sod in July 2008 in a randomized complete block design with three replications, in which the cultivar was the treatment factor. Beginning in July 2008 and continuing throughout the study, granular nitrogen (N) and potassium (K) were applied to the bermudagrass and seashore paspalum cultivars at 24.4 and 12.2 kg ha⁻¹, respectively, in March and September, then at 48.0 and 36.6 kg ha⁻¹, respectively, each month from April through August. Granular phosphorus (P) was applied at 29.3 kg ha⁻¹ in March and August, then at 9.8 kg ha⁻¹ each month from April through July. For Diamond zoysiagrass, granular nitrogen (N) and potassium (K) were applied to the bermudagrass and seashore paspalum cultivars at 12.2 and 6.1 kg ha⁻¹, respectively, in March and September, then at 24.0 and 18.3 kg ha⁻¹, respectively, each month from April through August. Granular phosphorus (P) was applied at 14.6 kg ha⁻¹

in March and August, then at 4.9 kg ha⁻¹ each moth from April through July. Because each cultivar was fertilized in accordance with recommendations, Diamond received a different fertility regime than TifEagle, Champion, and SeaDwarf (McCarty, 2001; Qian and Engelke, 1999). Plots were maintained as a putting green and were mowed six times per week at 3.2 mm with a triplex greens mower (Jacobsen Greens King IV, Textron Company, Charlotte, NC). Irrigation was applied deeply and infrequently, applying approximately 2.5 cm of water per week, to encourage deep rooting while minimizing drought stress and disease pressure. Plots were topdressed at a rate of 550 g m⁻², using the same sand as used for construction, without reed sedge peat, on 14-day intervals during periods of active growth. Plots were also aerated in May 2009 with a hollow-tine aerator using 1.25 cm diameter tines at a 5.0 × 5.0 cm spacing. After aeration, cores were removed and holes were filled with sand.

Sampling procedure and measurements

A simple random sampling procedure was generated for 25 points in each plot (SAS, Cary, NC). Sampling points represented an x-y coordinate in each plot. One soil core was extracted from each sampling point in a plot using a 10-cm diameter cup cutter, comprising a total sampling area of 4.9% of the plot area. Uncompressed thatch-mat depth was determined by measuring the depth of the thatch-mat layer from the soil surface for each sample using a ruler (Callahan et al., 1998). Each soil core was then used for assessment of OM content. Organic matter content was determined using the weight loss on ignition method (McCarty et al., 2005; Carrow et al., 1987). The verdure was removed from each soil core and the top 2.5 cm of the core was used for analysis. The samples were oven-dried at 105°C for 24 hours, weighed, then ashed at 600°C for 6

hours, in a modification of the American Society for Testing Materials (ASTM) D2974-07a procedure (N. W. Hummel, 2010, personal communication). The ashes were then weighed and the difference between pre- and post-ashing weight was used to find the total organic matter present as a percent of the sample.

Data analysis

The plots were configured in a randomized complete block design with three replications. A one-way analysis of variance was performed using the mixed model procedure in SAS statistical software (SAS, Cary, NC) to determine the effect of cultivar of thatch-mat depth and OM content. Differences among cultivars were deemed significant when the P-value corresponding to the ANOVA F-test was less than 0.05. When cultivation treatment main effects were significant, means were separated using Fisher's protected LSD test ($\alpha = 0.05$) (Kuehl, 2000).

Means, variances, and standard deviations were calculated for TifEagle, Champion, SeaDwarf, and Diamond. The sample sizes needed to detect differences between means at different detectable levels were calculated from the following equation (Cochran and Cox, 1957; Tugel et al., 2008):

$$n = (2 (z_{(1-\alpha/2)} + z_{\text{power}}) \times \sigma^2) / d^2$$

where n = estimated number of samples, $z_{(1-\alpha/2)}$ = z value from 1 – desired alpha level, z_{power} = z value of desired power level, σ^2 = residual sample variance, and d = minimum detectable difference (MDD).

Results and Discussion

Thatch-mat depth and OM content significantly differed among warm-season putting green cultivars (Table 2.1). TifEagle and Champion accumulated thatch-mat

deeper in the soil profile (26.9 and 27.6 mm, respectively) than both SeaDwarf and Diamond (22.4 and 23.3 mm, respectively) (Table 2.2). TifEagle and Champion thatch-mat depth in this study exceeded those reported by Hollingsworth et al. (2005) and White et al. (2004), who found thatch-mat depths for TifEagle and Champion to be between 17 and 20 mm. The larger thatch accumulation in this study may be the result of management practices that differed from the previous studies. Plots were aerated and vertical mowed by Hollingsworth et al. (2005) and White et al. (2004), but plots were only aerated once per year in this study and did not receive any vertical mowing. However, Baldwin et al. (2009) found Champion to accumulate 36 mm of thatch-mat depth when grown in full sun, but only accumulate 28 mm of thatch-mat depth when grown in shade, while aerating twice per year without vertical mowing. Kopec et al. (2004) reported less thatch-mat (ranging between 12 mm and 16 mm) for 'Sea Isle 1' seashore paspalum mowed at 12.2 mm and 15.4 mm than this study found for SeaDwarf mowed at 3.2 mm. Thatch-mat accumulation for Sea Isle 1 was lower than SeaDwarf possibly because SeaDwarf is a more aggressive biomass producer and the lower mowing height for SeaDwarf stimulated more lateral growth than the higher mowing heights for Sea Isle 1, encouraging more thatch production.

SeaDwarf accumulated more OM (7.0% by weight) than all cultivars (Table 2.1). Diamond accumulated more OM than Champion (5.1% versus 4.4%), which accumulated more OM than TifEagle (4.4% versus 4.0%). Rowland et al. (2009) found similar OM values on 8-year-old TifEagle and Champion plots maintained similarly to the plots in this study. Although no studies have been done previously that assessed the OM or thatch-mat accumulation characteristics of seashore paspalum or Diamond zoysiagrass, a

study by Dunn et al. (1981) assessing thatch-mat accumulation of ‘Meyer’ zoysiagrass (*Zoysia japonica* Steud.) reported that Meyer accumulated between 19 mm and 39 mm of thatch-mat depth when mowed at a 19 mm height, which mostly exceeded the thatch-mat depth found for Diamond, likely because of the higher mowing height of the Meyer. The higher OM content for SeaDwarf than TifEagle and Champion may be the result of the aggressive underground biomass production of SeaDwarf and its large and prolific rhizomes (Duncan and Carrow, 2000). Although Diamond zoysiagrass has a slower growth rate than TifEagle or Champion, its tissues have more lignin than the bermudagrass cultivars and decompose more slowly.

Estimating thatch-mat depth sample number

The number of samples required to estimate thatch-mat depth varied with each cultivar being sampled and with the confidence level and MDD desired (Table 2.3). Although increased thatch-mat depth has been linked to decreased water infiltration, slow drainage of excess water, decreased oxygen content in the soil atmosphere, reduced cold tolerance, and increased disease and insect incidences, the impact of specific thatch-mat depths on soil or plant characteristics is not known and may be impacted by many environmental, soil textural, and plant management factors (Horst et al., 1996; Carrow, 2000; Harris, 1978; Murray and Juska, 1977; White, 1962; Sprague, 1945; Musser, 1960; Miller, 1965; Thompson, 1967; Cornman, 1952; Taylor and Blake, 1982). Thus, the confidence level and MDD are variables that are left to the discretion of the investigator and can be altered based on knowledge of the study subject, the confidence level desired, and the expenses incurred during data collection and analysis. To be 95% confident that differences in thatch-mat depth of TifEagle or Champion putting green plots could be

detected within 1 mm, 85 and 95 samples, respectively, would need to be extracted per plot (Table 2.3). However, as the MDD increases to 3 mm, only 11 samples per plot for each cultivar are needed at 95% confidence. Similarly, sample numbers increase or decrease as the desired confidence level increases or decreases and are displayed in Table 2.3 for TifEagle, Champion, SeaDwarf, and Diamond. SeaDwarf requires more samples than all other cultivars for each confidence and MDD level because thatch-mat depth was more variable across the population (Tables 2.2 and 2.3). Previous studies have sampled at varying numbers, ranging from three to ten samples per plot in ‘Tifdwarf’, ‘Tifgreen’, and ‘Dothan’ bermudagrass (Volk, 1972; White and Dickens, 1984), Meyer zoysiagrass (Dunn et al., 1981), TifEagle and Champion ultradwarf bermudagrass, ‘MS Supreme’ and ‘Floradwarf’ bermudagrass (Hollingsworth et al., 2005; Baldwin et al., 2009). Thus, the studies assessing thatch-mat depth on ultradwarf bermudagrass putting green cultivars have been sampling at numbers that can only detect 3 to 5 mm of thatch-mat depth difference at 85 to 95 % confidence. More samples are required from each plot to detect thatch-mat depth differences of less than 3 mm at 85 to 95% confidence.

Estimating organic matter content sample number

Organic matter content was less variable than thatch-mat depth within cultivars (Table 2.2) and required fewer samples per plot to predict OM content for a particular percent of the population mean at a given confidence level (Table 2.4). Twenty-four samples are required to accurately and precisely estimate the OM content of a Diamond zoysiagrass putting green plot within 4.9% of the population mean, which corresponds to a MDD of 0.25% OM content by weight, with 95% confidence (Table 2.4). However, 85 samples per plot are needed to estimate the thatch-mat depth within 4.3% of the

population mean, corresponding to a MDD of 1 mm of thatch-mat depth, with 95% confidence (Table 2.3). Sample numbers increase or decrease as the desired confidence level increases or decreases and are displayed in Table 2.4 for TifEagle, Champion, SeaDwarf, and Diamond. Sampling numbers were similar across cultivars; however, sampling numbers varied within each cultivar according to population mean and variance (Table 2.4). For example, SeaDwarf requires more samples per plot than other cultivars to estimate OM content at each confidence level and each MDD level because its population mean was higher than the other cultivars and its variance was larger (Tables 2.2 and 2.4).

Although the influence of OM content varies with cultivar and various environmental and management factors, such as irrigation or rainfall amount or frequency, OM consistency, nitrogen fertility, aeration program, and soil texture, no studies have correlated particular OM content percentages and plant or soil responses. Although Murphy et al. (1993) suggested that OM exceeding 5% by weight would be detrimental to creeping bentgrass growth and Carrow (2000) suggested that excessive amounts of OM can encumber infiltration of water into the soil profile, slow drainage of excess water away from the putting surface, plug soil pores, and may decrease the infiltration of oxygen into the soil profile, no correlation has been made between particular OM content values and soil physical properties or plant physiological responses. Because OM content samples require a more lengthy and labor intensive data collection and sample analysis procedure than thatch-mat depth samples, an investigator may select a larger MDD to decrease the required sample size. Similar to thatch-mat depth, the confidence level is left to the discretion of the investigator and can be altered

based on knowledge of the study subject and the expense incurred during data collection and analysis.

Previous studies have sampled at varying numbers, ranging from two to five samples per plot in ‘Providence’ and ‘A-1’ creeping bentgrass (Fu et al., 2009; McCarty et al., 2007) and TifEagle, Champion, Tifdwarf, Floradwarf, and ‘MiniVerde’ bermudagrass (White et al., 2004). These sample numbers were able to detect mean OM content differences 0.5 to 1% OM content by weight with 90 to 95% confidence (Table 2.4). Thus, OM sampling procedures commonly used in previous literature were likely sufficient to detect meaningful OM content differences. Further research correlating specific OM content values to specific soil or plant responses can help to refine and guide MDD recommendations.

The size of the cores extracted for OM determination must also be considered when determining sample number. The equation to determine sample number required per plot does not address the size of the individual sample extracted for OM determination. Larger samples will capture a larger proportion of the plot area than smaller samples. Thus, the number of samples required can vary indirectly with the area encompassed by each sample.

Conclusions

Thatch-mat depth and OM content are important characteristics of putting greens and can be indicative of the relative health and performance of putting green soils.

Thatch-mat depth and OM content differed among TifEagle and Champion bermudagrass, SeaDwarf seashore paspalum, and Diamond zoysiagrass. TifEagle and Champion accumulated thatch-mat to a deeper depth than both SeaDwarf and Diamond.

However, SeaDwarf had a higher OM content than Diamond and both had higher OM contents than TifEagle and Champion, suggesting that the ultradwarf bermudagrass cultivars generate less dense and more quickly decomposing underground biomass than SeaDwarf and Diamond. Thus, more aggressive thatch and OM management practices may be needed for SeaDwarf and Diamond.

Sampling procedures for determining thatch-mat depth and OM content are important, as well. This study found that previous studies often undersampled plots for thatch-mat depth, but came close to correctly sampling for OM content. The sampling numbers generated in this study give researchers a range of confidence levels and MDD levels and allow future researchers to accurately sample TifEagle, Champion, SeaDwarf, and Diamond putting green plots for thatch-mat depth and OM content. Future research correlating specific levels of thatch-mat depth and OM content to specific soil and plant responses could aid in determining an appropriate MDD level. Additionally, similar sampling studies could be performed for different cultivars and different evaluations to give additional accuracy and precision to turfgrass scientific measurements.

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Appendix A

Table 2.1. Analysis of variance for effects of block, cultivar, and their interaction on thatch-mat depth and organic matter (OM) content on warm-season putting greens in Knoxville, TN.

Source	df	Thatch-Mat Depth	OM Content
Block (B)	2	0.0125*	0.1102
Cultivar (C) [†]	3	0.0012	0.0001
B × C	6	0.0973	0.1354

* P-value corresponding to ANOVA F-statistic

[†] Cultivars: TifEagle, Champion, SeaDwarf, Diamond

Table 2.2. Sample means, variances, and standard deviations for thatch-mat depth and organic matter (OM) content of four warm-season putting green cultivars in Knoxville, TN.

Cultivar	Thatch-Mat Depth (mm)			OM Content (% by weight)		
	Mean	Variance	Std. Dev.	Mean	Variance	Std. Dev.
TifEagle	26.9 d	5.4	2.8	4.0 a	0.03	0.19
Champion	27.6 c	6.0	3.0	4.4 a	0.09	0.35
SeaDwarf	22.4 a	7.1	2.8	7.0 b	0.26	0.52
Diamond	23.3 b	5.5	2.3	5.1 b	0.09	0.32
LSD _(0.05)	1.9			0.2		

Means sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

Table 2.3. Number of samples required per plot to estimate thatch-mat depth means at specified minimum detectable differences (MDD) and indicated alpha levels for TifEagle, Champion, SeaDwarf, and Diamond.

MDD*	% of mean	Alpha Level						
		0.001	0.005	0.01	0.05	0.1	0.15	0.2
TifEagle								
1 mm	3.7	187	144	127	85	67	57	49
2 mm	7.4	47	34	32	22	17	15	13
3 mm	11.2	21	16	15	11	8	7	6
4 mm	14.9	12	9	8	6	5	4	4
5 mm	18.6	8	6	6	4	3	3	2
Champion								
1 mm	3.6	206	167	141	95	75	63	55
2 mm	7.2	53	41	36	24	19	16	14
3 mm	10.9	23	18	16	11	9	7	7
4 mm	14.5	19	11	9	6	5	4	4
5 mm	18.2	9	7	6	4	3	3	3
SeaDwarf								
1 mm	4.5	242	189	166	112	88	74	64
2 mm	8.9	61	48	42	28	22	19	16
3 mm	13.4	27	21	19	13	10	9	8
4 mm	17.9	16	12	11	7	6	5	4
5 mm	22.4	10	8	7	5	4	3	3
Diamond								
1 mm	4.3	189	148	130	85	69	58	50
2 mm	8.6	48	37	33	22	18	15	13
3 mm	12.9	21	17	15	10	8	7	6
4 mm	17.2	12	10	9	6	5	4	4
5 mm	21.5	8	6	6	4	3	3	2

* MDD = minimum detectable difference of thatch-mat depth (mm)

Table 2.4. Number of samples required per plot to estimate organic matter (OM) content means at specified minimum detectable differences (MDD) and indicated alpha levels for TifEagle, Champion, SeaDwarf, and Diamond.

MDD	% of mean	Alpha Level						
		0.001	0.005	0.01	0.05	0.1	0.15	0.2
TifEagle								
0.05%	1.2	465	363	318	214	169	142	123
0.1%	2.5	117	90	80	54	43	36	31
0.25%	6.2	19	15	13	9	7	6	5
0.5%	12.5	5	4	4	3	2	2	2
1.0%	24.9	2	1	1	1	1	1	1
Champion								
0.05%	1.1	1162	906	795	534	421	354	307
0.1%	2.3	291	227	199	134	106	89	77
0.25%	5.6	47	37	32	22	17	15	13
0.5%	11.3	12	10	8	6	5	4	4
1.0%	22.5	3	3	2	2	2	1	1
SeaDwarf								
0.05%	0.7	3593	2801	2458	1652	1301	1095	949
0.1%	1.4	899	701	615	413	326	274	238
0.25%	3.5	144	113	99	67	53	44	38
0.5%	7.1	36	29	25	17	14	11	10
1.0%	14.2	9	8	7	5	4	3	3
Diamond								
0.05%	1.0	1285	1002	879	591	465	392	339
0.1%	2.0	322	251	220	148	117	98	85
0.25%	4.9	52	41	36	24	19	16	14
0.5%	9.9	13	11	9	6	5	4	4
1.0%	19.8	4	3	3	2	2	1	1

* MDD = minimum detectable difference of organic matter (OM) content (% by weight)

**CHAPTER THREE – VIBRATORY ROLLING ENHANCES TOPDRESSING
INCORPORATION ON ULTRADWARF BERMUDAGRASS PUTTING GREENS**

Abstract

Thatch accumulation and excessive grain in ultradwarf bermudagrass putting greens can negatively impact playability. Frequent sand topdressing is often used to help alleviate these conditions. However, the high shoot density and aggressive growth habit of ultradwarf bermudagrass inhibits the successful incorporation of sand topdressing into the turf canopy. The objective of this study was to compare the use of vibratory rollers to traditional brushing as a means of incorporating sand topdressing into the canopy of ultradwarf bermudagrass putting greens. This study was conducted on a three-year-old ‘TifEagle’ bermudagrass (*Cynodon dactylon* (L.) $\times$ *C. transvaalensis* Burt-Davy) putting green at the East Tennessee Research and Education Center in Knoxville, TN, that had not been topdressed consistently with sand for two years prior to this study. Sand topdressing was applied biweekly at 550 g m⁻² from 28 July to 22 September 2008 and 6 July to 28 September 2009. Standard brushing, vibratory rolling alone, and brushing + vibratory rolling were used as sand incorporation methods, with a non-topdressed control treatment. Vibratory rolling + brushing resulted in less sand removed from the turf surface by mowing after topdressing than either treatment alone and produced higher surface hardness values than rolling alone or the non-topdressed control. However, neither thatch depth nor soil organic matter content were affected by topdressing incorporation method. This study demonstrates that vibratory rolling following brushing can be a successful method of incorporation sand topdressing into TifEagle ultradwarf bermudagrass putting greens.

Introduction

Ultradwarf bermudagrass (*Cynodon dactylon* (L.) $\times$ *C. transvaalensis* Burtt-Davy) is commonly used as a putting surface in the southern United States because of its successful adaptation to the region's climate, high shoot density, and fine leaf texture (Beard and Sifers, 1996; Maples, 2001). This warm-season species has several advantages over cool-season turfgrasses for use in the transition zone, including its resistance to high temperature stress, increased photosynthetic efficiency, reduced water requirement, and decreased susceptibility to fungal pathogens (Beard, 2002). These attributes lead to decreased fungicide and water usage, as well as fewer in-season labor inputs. However, the aggressive growth habit and high shoot density of ultradwarf bermudagrass can lead to problems related to excessive organic matter (OM) accumulation (Hollingsworth et al., 2005).

Moderate amounts of surface OM are beneficial for putting greens. Surface OM can cushion turf crowns against foot traffic and incoming golf shots, reduce ammonia volatilization, and slow the leaching of pesticides into groundwater (Horst et al., 1996; McCarty and Miller, 2002; Petrovic, 1990; Snyder and Cisar, 1995; Vermeulen and Hartwiger, 2005). However, OM depths exceeding 1.3 cm can be considered excessive (Christians, 1998; McCarty and Miller, 2002). Excessive accumulation of organic matter near the soil surface can negatively impact the health and playability of ultradwarf bermudagrass putting greens. Large amounts of OM can encumber infiltration of water into the soil profile, slow drainage of excess water away from the putting surface, and plug soil pores, which may decrease the infiltration of oxygen into the soil profile (Carrow, 2000). Accumulated OM is also associated with reduced tolerance to cold

temperatures, increased disease problems, and may reduce pesticide efficacy (White, 1962; Musser, 1960; Cornman, 1952). Additionally, excessive OM accumulation near the putting surface may result in soft playing surfaces, which are not desirable for golf and may lead to mower scalping (Barton et al., 2009; Carrow, 2001; Hurto et al., 1980).

Frequent sand topdressing, vertical mowing, core cultivation, and grooming are often used to alleviate such negative effects of excessive OM accumulation (Beard, 2002; Engle and Alderfer, 1967; McCarty, 2001). Topdressing alone has been shown to reduce thatch layers in 'Pennecross' creeping bentgrass (*Agrostis stolonifera* L.) (Eggens, 1980), 'Tifway' bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Pers. L.) (Carrow et al., 1987), and three different dwarf bermudagrass cultivars (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy) (White and Dickens, 1984). Topdressing with sand has been regarded as an effective method to control thatch by improving conditions for its decomposition (Ledeboer and Skogley, 1967). However, further research has shown that thatch dilution is as important as improving conditions for thatch decomposition (Rieke, 1994). Specifically, sand topdressing serves to dilute the OM near the putting surface and preserve the majority sand matrix, which helps to preserve soil porosity.

Shoot densities upwards of 2,000 shoots per square decimeter (Beard and Sifers, 1996) and the aggressive growth habit of ultradwarf bermudagrass may inhibit the successful incorporation of sand topdressing into the turf canopy, leaving much of the topdressing material on the surface of the putting where it is removed during mowing. Golf course superintendents have reported difficulty incorporating sand topdressing into ultradwarf bermudagrass putting greens and have sought numerous methods to aid

incorporation, including using dried sand and hover-type mowers to force sand into the canopy by way of air currents (Foy, 1999).

Previous research on ultradwarf bermudagrass thatch management has focused on the effects of nitrogen sources and varying cultivation techniques, including sand topdressing (Hollingsworth et al., 2005; White et al., 2004), but has not addressed the difficulty of incorporating sand topdressing into the turf canopy. To that end, the objective of this study was to compare traditional methods of sand topdressing incorporation with vibratory rollers as a means of incorporating sand topdressing into an ultradwarf bermudagrass putting green.

Materials and Methods

Experimental Area

This study was conducted on a three-year-old ‘TifEagle’ bermudagrass (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy) putting green constructed on Sequatchie silt loam soil (fine-loamy, siliceous, semiactive, thermic Humic Hapludult) at the East Tennessee Research and Education Center in Knoxville, TN. The putting green was mowed six times per week at 4.0 mm with a walk-behind reel mower (Jacobsen PGM 22, Textron Company, Charlotte, NC) and irrigated deeply and infrequently, applying approximately 2.5 cm of water per week, to encourage deep rooting while minimizing drought stress and disease pressure. Granular nitrogen (N) and potassium (K) were applied at 24.4 and 12.2 kg ha⁻¹, respectively, in March and September, then at 48.0 and 36.6 kg ha⁻¹, respectively, each month from April through August in 2008 and 2009. Granular phosphorus (P) was applied at 29.3 kg ha⁻¹ in March and August, then at 9.8 kg ha⁻¹ each month from April through July in 2008 and 2009. Thatch reduction practices,

such as sand topdressing and core aerification, were used sparingly for two years prior to the start of this study.

Treatment Application

A sand meeting United States Golf Association (USGA) putting green construction specifications was applied as topdressing biweekly at 550 g m⁻² from 28 July to 22 September 2008 and 6 July to 28 September 2009 using a Cushman TD 1500 topdressing unit (Cushman TD 1500, Textron, Charlotte, NC). Sand texture was 15.6% coarse sand (0.5-1.0 mm), 73% medium sand (0.25-0.50 mm), and 11.4% fine sand (0.15-0.25 mm). Topdressing material was incorporated into the turf canopy using a brush apparatus pulled behind a utility cart, a vibratory roller (PR 21204 09-584, True-Surface Inc. Moscow Mills, MO), or a brush followed by vibratory rolling. Brushing treatments were made in three directions and vibratory rolling treatments were made in one direction. Additionally, a control treatment that was not topdressed was included. Plots measured 3.0 m × 4.3 m and all plots were lightly irrigated with a two minute irrigation cycle after the topdressing incorporation methods were applied.

Data Collection

Soil organic matter (SOM) accumulation was measured using a weight loss on ignition procedure (Combs and Nathan, 1998). Two cores (5 cm diameter × 5 cm depth) collected from the center of each plot on 31 October 2008 and 6 October 2009, after treatments had ceased. The verdure was removed and the top 2.5 cm of each core was used for analysis, similar to methods used by Carrow et al. (1987) and McCarty et al. (2007). The samples were oven-dried at 105° C for 24 hours, weighed, then ashed at 600° C for 6 hours in a modification of the American Society for Testing Materials

(ASTM) D2974-07a procedure (ASTM, 2007; Carrow et al., 1987; McCarty et al., 2007).

The ashes were then weighed and used to find the total organic matter present as a percent of the entire sample for each plot.

Several options exist for determining organic matter via weight loss on ignition (Kimble et al., 2001). The ASTM F 1647 – 02a procedure is specified for determining the organic matter content of putting green and sports turf root zone mixes and calls for ashing a 5 to 10 g soil sample in a 360° C furnace for 2 hours (ASTM, 2002). This procedure is useful for determining organic matter content of volume ratio mixed rootzones before turf establishment (ASTM, 2002). Although the low ashing temperature and short ashing time of this procedure are designed to prevent the loss of calcium carbonate from calcareous sands, the procedure may understate organic matter content (N.W. Hummel, 2010, personal communication). The ASTM D2974-07a procedure is specified for determining moisture, ash, and organic matter of peat and other organic soils and calls for soil samples of a minimum of 50 g to be ashed at a temperature range from 440° C to 750° C for a time period sufficient to stabilize the mass of the ashed sample (ASTM, 2007). This procedure was used in this study because its larger ashing sample, higher ashing temperature, and longer ashing time provide a more accurate representation of organic matter content than the ASTM F 1647-02a procedure and did not adversely impact the silica sand used as topdressing material in this study (N.W. Hummel, 2010, personal communication).

The dense canopy, high shoot density, and stolon/rhizome structure of ultradwarf bermudagrass can trap topdressing material in the turf canopy, under the leaves, but above the existing soil surface. During the verdure removal process for SOM

determination, this topdressing material that has already been incorporated into the turf canopy, can be lost. Thus, a procedure similar to the SOM determination procedure was conducted to capture the influence of topdressing material that may have been lost during verdure removal. Because this procedure does not remove the verdure and includes the entire above ground biomass, it is referred to as total biomass (TB) content. Total biomass content was determined in a similar manner to SOM, with two 5 cm diameter cores collected from the center of each plot on 31 October 2008 and 6 October 2009. However, the verdure was left intact and the top 2.5 cm of the soil were used during analysis to determine if topdressing sand was lost during verdure removal. Similar to the SOM determination, the TB samples were oven-dried at 105° C for 24 hours, then ashed at 600° C for 6 hours. The ashes were then weighed and used to find the TB present as a percent of the entire sample for each plot.

Two days following topdressing applications, sand particles and clippings were collected from one mower width on each plot. Similar to Stier and Hollman (2003), collected topdressing material and clippings were dried in an oven at 105° C for 24 hours. The clippings were separated from the sand by adding water and decanting the water and clippings. The remaining sand was oven-dried at 105° C for 24 hours, then weighed to determine the amount of topdressing material that was not incorporated into the turf canopy.

Additionally, the uncompressed depth of the thatch-mat layer was measured. Five samples were extracted from each plot with a soil probe 2.5 cm in diameter to a depth of 10 cm on 31 October 2008 and 6 October 2009. The uncompressed depth of the thatch-mat layer was measured with a ruler (Waddington, 1992; Callahan et al., 1997).

A Clegg Soil Impact Tester (Clegg 95050, Lafayette Instrument, Lafayette, IN) with a 2.25 kg missile was used to determine the surface hardness of each plot on 31 October 2008 and 6 October 2009. The missile of the hammer was raised to 45 cm and dropped on three locations near the center of each plot (Lush, 1985). The impact value was then multiplied by 10 to convert to $g_{\max}$, a peak deceleration value which increases as surface hardness increases (Lush, 1985).

Data Analysis

The plots were configured in a randomized complete block design with three replications. For evaluations made only once each year, a one-way analysis of variance was performed using the mixed model procedure in SAS Statistical Software (SAS Institute, Cary, NC) to determine the effect of the incorporation method. Evaluations made repeatedly during the growing season were analyzed as a repeated measures two-way analysis of variance using the mixed model procedure to determine significance of incorporation treatment, date, and incorporation treatment $\times$ date effects. Incorporation treatment $\times$ date effects were sliced by date to determine the specific dates at which cultivation effects were deemed significant (SAS Institute, Cary, NC). For each evaluation, differences were deemed significant when the P-value corresponding to the ANOVA F-test was less than 0.05. When cultivation treatment main effects were significant, means were separated using Fisher's protected LSD test ($\alpha = 0.05$) (Kuehl, 2000). Because a significant treatment $\times$ year interaction was noted, data for 2008 and 2009 were analyzed and presented separately. Soil organic matter content, TB content, and topdressing removal data were arcsine-square root transformed when necessary to meet normality concerns.

Results and Discussion

Soil Organic Matter Accumulation

Topdressing incorporation methods did not affect SOM accumulation in the upper 2.5 cm of the rootzone with the verdure removed in either 2008 or 2009 (Table 3.1). Each incorporation method returned SOM contents of 8 to 10% by weight in 2008, which exceeded the 4 to 5% OM by weight recommendation by Murphy et al. (1993) for putting greens (Fig. 3.1). Incorporation methods produced SOM content of 5 to 6% by weight in 2009, which were slightly lower than those measured in 2008, perhaps due to the introduction of consistent topdressing applications during this study on a plot that had not been topdressed consistently in the past. McCarty et al. (2007) reported a similar minimal impact of sand topdressing on SOM accumulation in the upper 2.5 cm of the rootzone on creeping bentgrass putting greens. Stier and Hollman (2003) also reported no significant difference in SOM measurements from the presence or lack of sand topdressing.

The lack of difference between incorporation methods, and lack of difference between topdressed and non-topdressed plots, may indicate that the topdressing material did not effectively dilute the organic layer and could not easily penetrate the thick thatch layer immediately under the verdure. Similar reports were made in the early years of Penn A and G series creeping bentgrass (Fraser, 1998). White and Dickens (1984) also found that sand topdressing applications did not penetrate the thatch layer adequately without some type of cultivation to open the canopy and facilitate topdressing incorporation. The similar SOM contents between treatments may also be a result of the verdure removal process. Removing the verdure from the turf sample may have loosened

sand from the sample, facilitating its separation from the sample, thus overstating SOM content.

Total Biomass

Topdressing incorporation methods did impact TB content in the upper 2.5 cm of the rootzone with the verdure included in 2008 and 2009 (Table 3.1). Plots receiving no topdressing contained 22.4% TB by weight in the upper 2.5 cm of the rootzone in 2008, while plots receiving only vibratory rolling contained an average of 19.5% TB by weight (Fig. 3.2). The traditional brushing method resulted in an average of 13.4% TB by weight in the upper 2.5 cm of the rootzone in 2008, while the brushing + vibratory rolling produced only 8.9% TB in the upper 2.5 cm of the rootzone in 2008, with the verdure included. However, all TB levels were lower in 2009. The non-topdressed control returned the highest amount of biomass at 9% by weight (Fig. 3.2). All other treatments had biomass concentrations near 6%. While not significantly different from each other, all topdressing incorporation methods had lower total biomass concentration than the non-topdressed control.

Total biomass levels may have been lower in 2009 than 2008 because of a significant amount of winter injury occurring in early 2009 that delayed emergence from dormancy. The delayed green-up in spring 2009 could have created better conditions for microbial degradation of OM in the soil profile. Additionally, the lack of turf growth during the extended dormant period could have slowed the rate of OM production during the spring and early summer.

The difference between the SOM and TB may indicate that the high shoot density and plant biomass may prevent the sand from penetrating the dense thatch layer and is

diluting the entire organic matter buildup down to the mat layer. A method to open the canopy and allow the sand to reach the soil organic layer, such as vertical mowing, may be beneficial prior to topdressing. Vertical mowing may also serve to decrease OM content, as McWhiter and Ward (1974) found on ‘Tifgreen’ and White and Dickens (1984) found on ‘Tifdwarf’, Tifgreen, and ‘Dothan’.

Two samples were taken from each plot to assess SOM content and TB content. Using two samples to determine SOM content allows 1.0% differences in SOM content to be detected at a 95% confidence level (Chapter 2). Although SOM and TB content are different parameters, they were determined similarly, thus making their sampling procedures related. Statistical differences in SOM content did not exist, but the differences in TB content exceeded the 1% minimum detectable difference value at a 95% confidence level, thus making this a valid test. However, the samples were not taken truly at random, but taken by human randomization, which may be biased. A better sampling scheme would include true non-human randomization of sample locations and one or two additional samples to increase the confidence level of the test.

Topdressing removal

Topdressing incorporation method, observation date, and their interaction (Table 3.1) all significantly impacted the percentage of topdressing material removed by mowing in 2008 and 2009. In 2008, differences among incorporation methods were present on each observation except for the first date, due to a heavy rain that washed away much of the topdressing sand (Fig. 3.3). On each subsequent date, vibratory rolling as the sole means of topdressing incorporation resulted in more than 10% of the applied sand being removed by mowing, which is more than any other method (Fig. 3). Brushing

alone resulted in removal of 4, 6, 6, and 5 %, respectively, of the applied sand by mowing at each observation date after 30 July, which was significantly more sand than was removed by brushing + vibratory rolling on the last three observation dates. Brushing + vibratory rolling resulted in removal of nearly 2% of the applied sand at each collection after 30 July and was not significantly different from the plots receiving no topdressing, which resulted in no sand removed by mowing.

In 2009, differences among incorporation methods were present at all observation dates except 30 September (Fig. 3.3). At all dates other 8 July and 30 September, vibratory rolling alone resulted in more than 10% of the applied topdressing sand removed by mowing, which was more than any other treatment. Brushing alone resulted in significantly more sand being removed by mowing than brushing + vibratory rolling and the control at every observation date except 2 July, 19 August, and 30 September. The amount of sand removed from brushing + vibratory rolling treatments was significantly less than all other treatments on each observation date, except for the three dates when it was not different than brushing alone, but not significantly different than the untreated control on all dates except 22 July and 2 September.

Stier and Hollman (2003) found incorporation of sand topdressing into A and G series creeping bentgrasses similarly difficult, resulting in 1 to 3% of applied topdressing being removed by mowing. Although their findings were statistically significant, they hypothesized that that small proportion of applied sand that was removed by mowing had little bearing on practitioners. However, the amounts of topdressing in this study were larger than those found by Stier and Hollman (2003) and could create problems for turf managers, such as dull mower blades and reduced turf quality from sand abrasion.

Additionally, because the untreated control received no topdressing, no sand could be removed by mowing two days after the topdressing application date, thus very small amounts of sand are removed from brushing + vibratory rolling plots when not significantly different from the control. This indicates that a large majority of the applied sand is being incorporated into the turfgrass canopy with the brushing + vibratory treatments and the amount of sand removed by mowing is negligible. Cultural practices that open the turfgrass canopy and penetrate the thatch layer can also reduce the amount of topdressing sand removed by mowing (Chapter 5).

Surface Hardness

Topdressing incorporation methods significantly impacted surface hardness in both 2008 and 2009 (Table 3.1). Surface hardness values for vibratory rolling were equal to the non-topdressed control in 2008 (Fig. 3.4). However, plots that received topdressing and brushing alone or brushing + vibratory rolling returned $g_{\max}$ values of 81 and 84, respectively, in 2008, indicating that topdressing must be successfully incorporated to increase surface hardness. In 2009, the non-topdressed control returned the lowest $g_{\max}$ value any treatment at 61 (Fig. 2.4). Brushing alone and vibratory rolling alone had higher $g_{\max}$ values than the control, at 71 and 75, respectively, but both values were lower than the brushing + vibratory rolling, which produced the most firm surface and a $g_{\max}$ value of 89.

The method used to incorporate the sand into the turf canopy is also an important component to surface hardness. Brushing the turf helps to reorient leaf blades and open the canopy, allowing the sand to fall in between leaf blades. Brushing has been used to as a cultural practice to vertically orient leaf blades prior to mowing, reduce grain, and

incorporate sand topdressing into the turf canopy (Beard, 2002; McCarty and Canegallo, 2005). Vibratory rolling, when used after brushing, helps to work the sand particles farther into the dense turf canopy by shaking the sand and helping to break the bridging effect that may keep sand particles suspended higher in the canopy. This effect has also been observed on creeping bentgrass putting greens (T.A. Nikolai, 2010, personal communication). Plots receiving only vibratory rolling as the sole means of topdressing incorporation had a firmer surface than the control in 2009, but not in 2008, perhaps indicating that vibratory rolling aids in increased surface hardness or that two seasons were necessary to increase surface hardness. Windows and Bechelet (2007) have also indicated that rolling may increase surface hardness when used in conjunction with topdressing regimes.

Thatch-mat Depth

The depth of the thatch-mat layer in the research plots was not significantly impacted by the different topdressing incorporation methods in 2008 or 2009 (Table 3.1). Thatch depths remained consistent between treatments, ranging from 22 to 26 mm in depth for both years (data not shown). Although brushing alone and brushing + vibratory rolling total biomass, increased surface hardness, and resulted in less topdressing removed by mowing than other treatments, they did not reduce thatch depth. Stier and Hollman (2003) also reported that sand topdressing did not reduce that depth in A and G creeping bentgrass cultivars. Similarly, White and Dickens (1984) reported that topdressing without cultivation did not affect thatch depth in Tifgreen, Dothan, and Tifdwarf bermudagrass. Because this putting green was not core aerified, no channels were present to allow sand to dilute the thatch at lower depths. Sand topdressing only

dilutes thatch from the top and will not dilute thatch deeper in the soil profile without mechanically removing portions of the thatch layer to allow greater sand infiltration. This indicates that proper core aeration and vertical mowing may be an important part of ultradwarf putting green thatch management.

Five samples were taken from each plot to assess thatch-mat depth in this study. This sampling procedure allows 5 mm differences in thatch-mat depth to be detected with 95% confidence (Chapter 2). Because the range of thatch-mat depths was 4 mm wide, true differences in thatch-mat depth between treatments at less than 5 mm were not captured, thus this test may not have been valid as performed. Also, the samples were not taken truly at random, but taken by human randomization, which may introduce bias into the sampling procedure. A better sampling scheme would include true non-human randomization of sample locations and at least 11 samples per plot to detect differences of less than 4 mm, if they exist.

Conclusions

The use of vibratory rolling and brushing helps to overcome the difficulty of incorporating sand into the canopy of a TifEagle ultradwarf bermudagrass putting green. The very high shoot and canopy density of TifEagle suspends topdressing material above the mat layer in the thatch layer, making more invasive procedures like vertical mowing and core aeration necessary to open the canopy and allow sand to penetrate deeper into the mat layer. No topdressing incorporation method decreased the thatch layer, making thatch penetrating cultural practices important. Brushing alone and brushing + vibratory rolling created a more firm putting surface in the beginning of the study, but the continued use of vibratory rolling alone over time also increased surface hardness. Less

sand is removed from the putting green by mowing when incorporated by brushing alone than by vibratory rolling alone. However, the addition of vibratory rolling after brushing further reduced the amount of sand that is removed by mowing. The successful incorporation of topdressing sand is necessary to diluting thatch, maintaining proper drainage, and producing firm playing surfaces. Further research is needed to address the impact that vertical mowing prior to topdressing, using dried topdressing material as opposed to slightly moist material, and varying topdressing frequencies and application rates have on topdressing incorporation into ultradwarf bermudagrass putting greens.

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Appendix B

Table 3.1: Analysis of variance for the effects of topdressing incorporation method, date of observation, and their interaction for evaluations made in 2008 and 2009.

Analysis of Variance					
Effect	2008				
	SOM concentration	TB Concentration	Thatch depth	Surface hardness	Topdressing removal
Incorporation method	% by weight NS	% by weight ***	mm NS	$g_{\max}$ ***	% of applied ***
Date	NA	NA	NA	NA	***
Incorporation method × Date	NA	NA	NA	NA	***
Effect	2009				
	SOM concentration	TB concentration	Thatch depth	Surface hardness	Topdressing removal
Incorporation method	% by weight NS	% by weight ***	mm NS	$g_{\max}$ **	% of applied ***
Date	NA	NA	NA	NA	***
Incorporation method × Date	NA	NA	NA	NA	***

** and *** indicate significance at the 0.01 and 0.001 *P* level, respectively.

NS indicates differences not significant. SOM indicates soil organic matter content. TB indicated total biomass content.

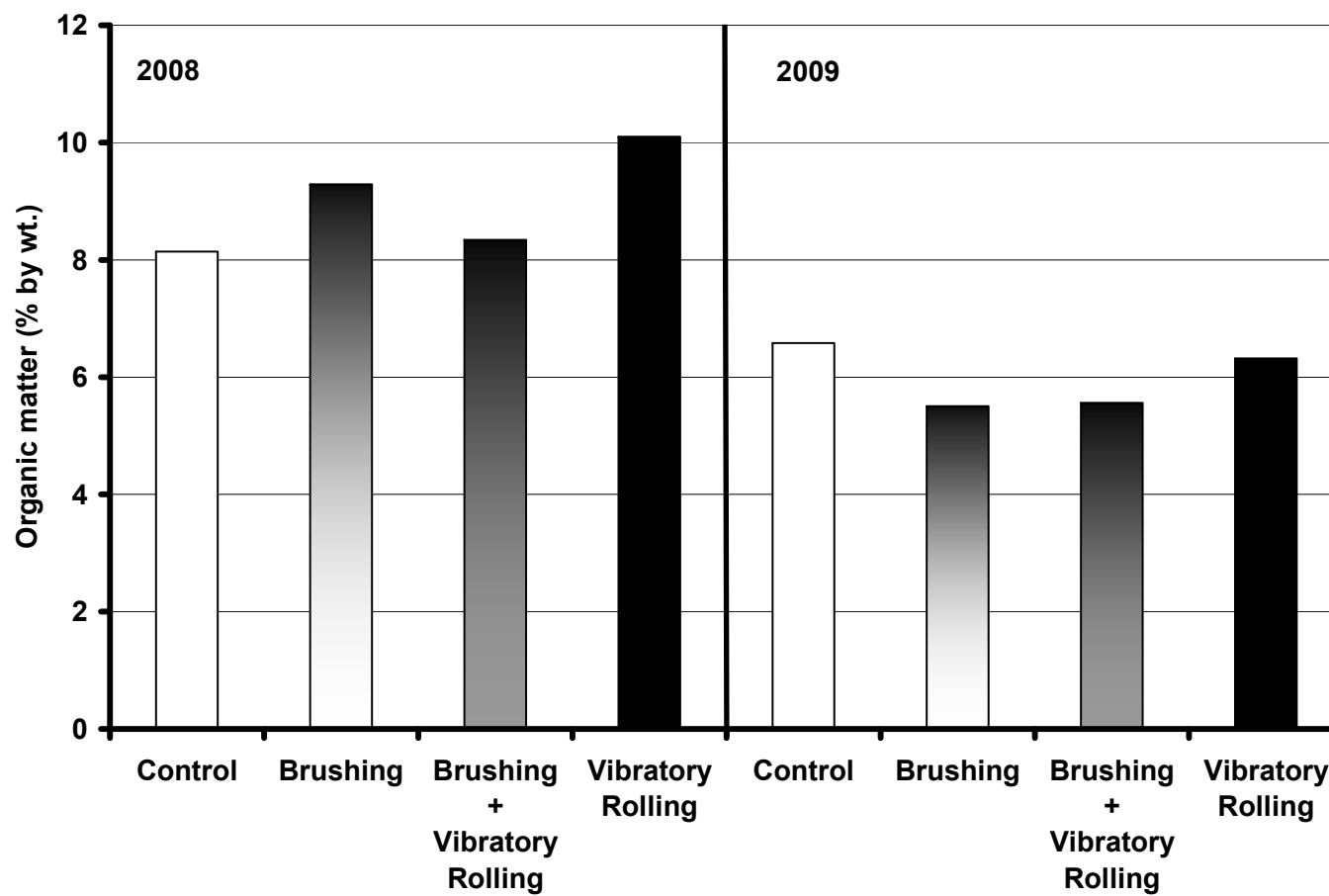


Figure 3.1: Organic matter concentration in upper 2.5 cm of rootzone, with verdure removed, in 2008 and 2009. No significant differences apparent according to Fisher's LSD test ($\alpha = 0.05$)

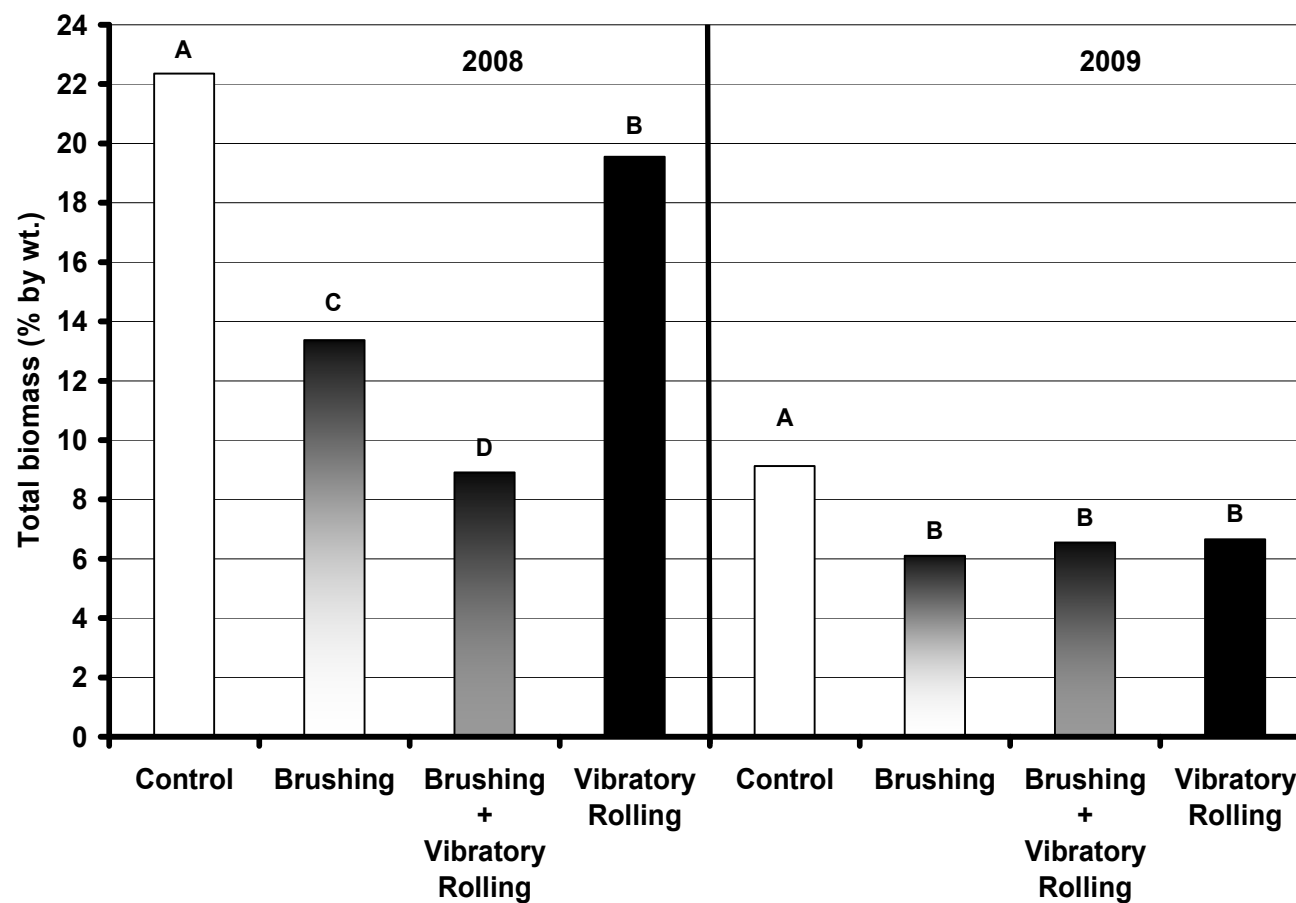


Figure 3.2: Total biomass by weight in upper 2.5 cm of rootzone, with verdure included, in 2008 and 2009. Bars sharing a letter in each year are not significantly different according to Fisher's LSD test ($\alpha = 0.05$)

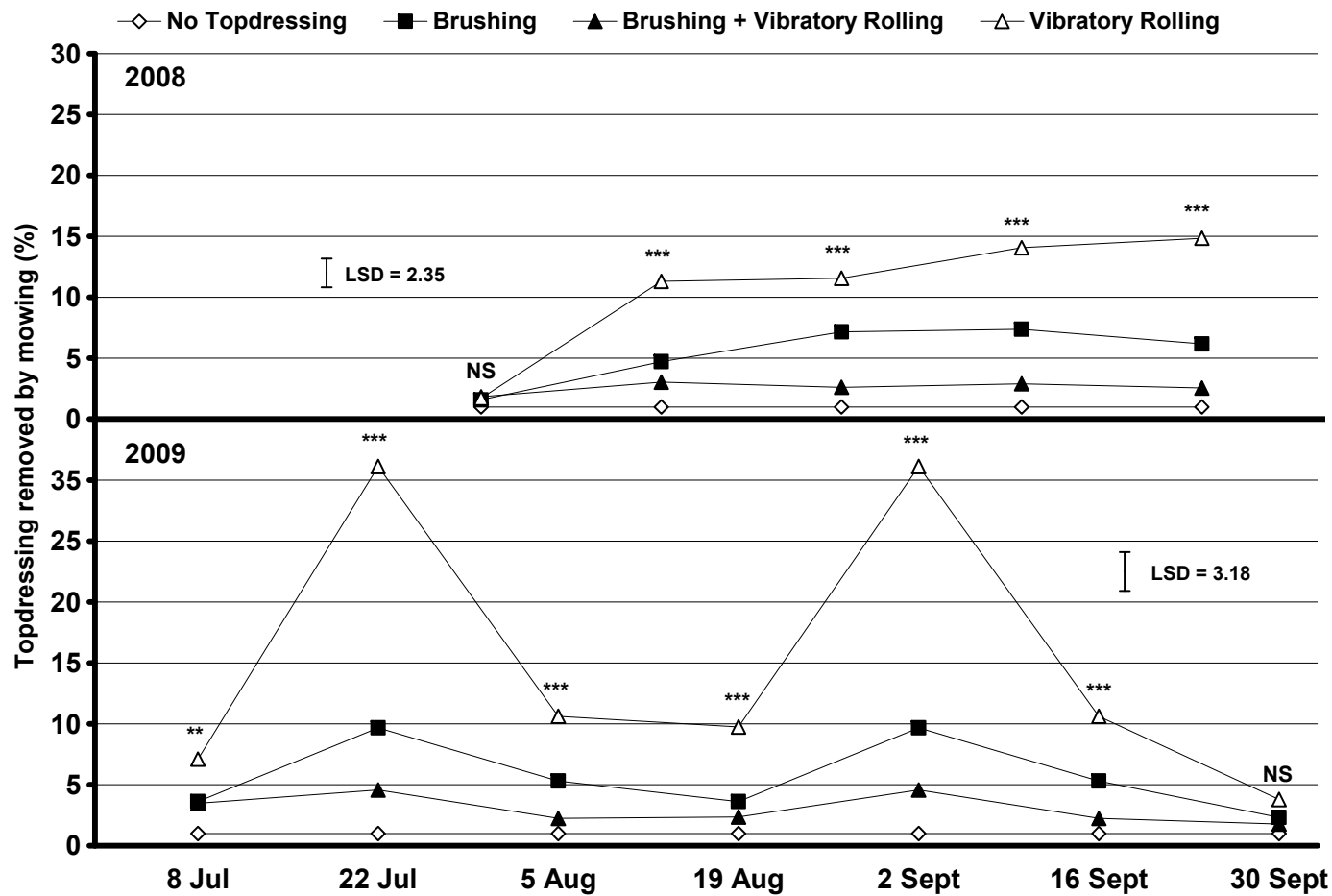


Figure 3.3: Topdressing material removed by mowing as a percentage of total material applied in 2008 and 2009. NS indicates no significant difference. ** and *** indicate significance at the 0.001 and 0.0001 *P* level, respectively.

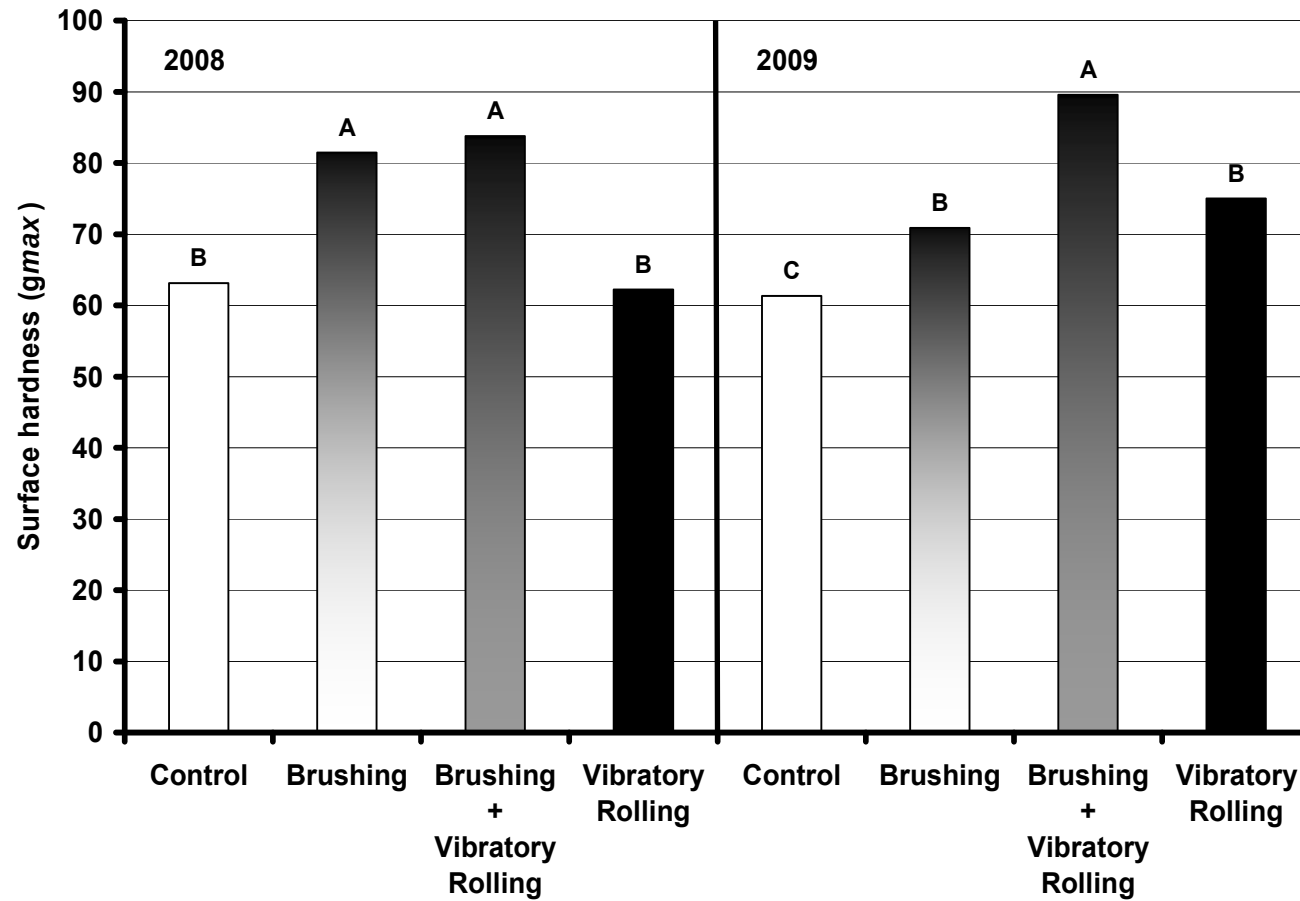


Figure 3.4: Surface hardness as measured by Clegg Soil Impact Tester in 2008 and 2009. Bars sharing a letter in each year are not significantly different according to Fisher's LSD test ($\alpha = 0.05$)

**CHAPTER FOUR – MOWING HEIGHT, MOWING FREQUENCY,
AND LIGHTWEIGHT ROLLING EFFECTS ON ULTRADWARF
BERMUDAGRASS PUTTING GREENS IN THE TRANSITION
ZONE**

Abstract

Prior research examining the impact of rolling in conjunction with varied mowing heights and frequencies on creeping bentgrass putting greens indicates that increasing lightweight rolling frequency, increasing mowing heights, and decreasing mowing frequencies can improve putting green quality without sacrificing putting green speed. However, minimal research is available regarding the impacts of mowing and rolling on ultradwarf bermudagrass putting greens in the transition zone. A two-year study was conducted on a 'MiniVerde' bermudagrass putting green in Knoxville, TN, to assess the impact of 10 different putting green management programs, that varied in mowing height, mowing frequency, and lightweight rolling frequency, on ball roll distance, percent green turf cover, turf quality, and disease occurrence. The management programs consisted of two mowing heights, 3.2 and 4.0 mm, mowed either daily or three times per week. For each daily mowing treatment, plots were rolled three times per week, six times per week, or not at all. For each mowing treatment applied three times per week, plots were rolled six times per week or on days when not mowed, thus alternating the mowing and rolling days. Programs mowed at 3.2 mm from increased ball roll distance by an average of 15 cm over those mowed at 4.0 mm. Programs with daily mowing and lightweight rolling increased ball roll distance by an average of 10 cm over those not rolled. However, no management program affected turf quality, cover, dollar spot occurrence, or spring dead spot occurrence. Programs with daily rolling did produce better turf color ratings than those not rolled daily. Thus, lower mowing heights and the use of lightweight rolling can increase green speed of ultradwarf bermudagrass putting greens without sacrificing quality.

Introduction

Ultradwarf bermudagrasses (*Cynodon dactylon* (L.) $\times$ *C. transvaalensis* Burt-Davy) are quickly replacing dwarf cultivars as putting surfaces in the southern United States and are increasingly being grown in the transition zone (McCarty and Miller, 2002). Little research regarding the management of putting surface characteristics, such as putting green speed, of these cultivars exists. Putting green speed is a term used to describe the distance that a golf ball will roll when struck and is an important play characteristic of golf course putting greens (Hartwiger, 1996; McCarty and Miller, 2002). Ball roll distance is inversely proportional to the coefficient of friction between the ball and the putting surface (USGA, 1993; Weber, 1997). Many cultural practices have been used to decrease the coefficient of friction on putting greens. Mowing heights, nitrogen (N) fertilization, and irrigation levels are often reduced and plant growth regulators, such as trinexapac-ethyl, have been used in an attempt to increase ball roll distances (McCarty, 2001; McCullough et al., 2006; McCullough et al., 2007). However, some of these practices can be deleterious to turf health and can result in a thinning of the turf stand, thus reducing playing surface quality (Beard, 2002). Previous studies on 'TifEagle' bermudagrass have shown that a putting green mowed at 3.2 mm has a lower root dry mass than at 4.8 mm and a lower color rating when mowed at 3.2 mm than 4.8 mm (Guertal and Evans, 2006). Similarly, N fertilization rates below $3.4 \text{ g m}^{-2} \text{ wk}^{-1}$ produced fewer total nonstructural carbohydrates in the TifEagle bermudagrass turf than higher N rates (Guertal and Evans, 2006).

Lightweight rollers are also used to reduce the coefficient of friction on a putting green and increase ball roll speed. Rolling was a popular practice in the early 1900s and

was promoted to smooth putting surfaces and increase green speeds beyond what mower technology could provide (Travis, 1901; Piper and Oakley, 1921). However, rolling became less popular by the mid-twentieth century because of the increased awareness of compaction on native soil (Carrow and Petrovic, 1992). The advent of self-propelled dedicated rolling units and the increased use of high-sand rootzones for putting greens have lead to rolling becoming a more common management practice (Nus, 1992).

Previous research on the impact of light-weight rolling on putting greens has focused on creeping bentgrass (*Agrostis stolonifera* L.). Several studies have reported that creeping bentgrass green speed increases for several hours after rolling (Danneberger et al., 1993; Hamilton et al., 1994; Nikolai, 2004; Nikolai, 2005). Additional research has shown that rolling may reduce dollar spot (*Sclerotinia homoeocarpa*) infestation, moss occurrence, and black cutworm activity (Nikolai et al., 2001). Further studies have reported that rolling sand-based putting greens up to seven times per week has no deleterious effect on rootzone bulk density, water infiltration, or creeping bentgrass quality (Hamilton et al., 1994; Hartwiger et al., 2001; Nikolai, 2005).

Recent research has explored combinations of mowing height, mowing frequency, and rolling frequency as they relate to putting green speed and creeping bentgrass turf quality. Decreasing mowing frequency and increasing rolling frequency to 3 to 6 times per week can increase creeping bentgrass turf quality while maintaining putting green speed similar to mowing daily without rolling (Nikolai, 2005; Strunk, 2006; Zarco Perez and Nikolai, 2009). Additionally, no increases in soil bulk density and disease occurrence, or decrease in water infiltration, were observed. Richards et al. (2009) found

that creeping bentgrass putting greens mowed at 4.0 mm and rolled 3 to 6 times per week exhibited longer ball roll distance values than those mowed at 3.2 mm without rolling.

Research on mowing height, mowing frequency, and rolling frequency programs has not been conducted on ultradwarf bermudagrass cultivars. To that end, the objective of this research was to assess the impact of putting surface management programs that differed in mowing heights, mowing frequencies, and rolling frequencies on the ball roll distance, percent green turf cover, turf quality, and disease occurrence on a 'MiniVerde' ultradwarf bermudagrass (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy) putting green.

Materials and Methods

Experimental Area

This study was conducted on a three-year-old MiniVerde bermudagrass (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy) putting green constructed on Sequatchie silt loam soil (fine-loamy, siliceous, semiactive, thermic Humic Hapludult) at the East Tennessee Research and Education Center in Knoxville, TN. The area was maintained as a typical golf course putting green and irrigated as needed to encourage deep rooting while minimizing drought stress and disease pressure. Irrigation was applied at a 2.5 cm depth weekly. Nutrients were applied granularly. Nitrogen (N) and potassium (K) were applied at 24.4 and 12.2 kg ha⁻¹, respectively, in March and September, then at 48.0 and 36.6 kg ha⁻¹, respectively, each month from April through August. Phosphorus (P) was applied at 29.3 kg ha⁻¹ in March and August, then at 9.8 kg ha⁻¹ each month from April through July. The entire experimental area was core aerified on 15 May and 25 July in 2008 and 12 May and 20 July in 2009 with 2.5 cm diameter hollow tines on a 5 cm × 5

cm spacing. The resulting holes were backfilled with a sand meeting specifications for United States Golf Association (USGA) putting green construction. Sand texture was 15.6% coarse sand (0.5-1.0 mm), 73% medium sand (0.25-0.50 mm), and 11.4% fine sand (0.15-0.25 mm) and was incorporated into the turf canopy using a brush apparatus pulled behind a utility cart. Biweekly applications of the same sand for topdressing were made at a rate of 550 g m⁻² from 1 May to 20 September in both 2008 and 2009.

Treatment Application

Ten different greens management programs, differing in their mowing heights, mowing frequencies, and rolling frequencies, were replicated three times for this study and are summarized in Table 4.1. Briefly, plots were mowed at 3.2 or 4.0 mm either six times per week or three times per week. For each program in which plots were mowed six times per week, rolling was applied either three times per week, six times per week, or not at all. For each program in which plots were mowed three times per week, plots were rolled either six times per week or on days when not mowed, thus alternating mowing and rolling days. The program with a 3.2 mm mowing height and six times per week mowing frequency is often used by golf superintendents and was considered the control treatment. Treatments were applied to 1.2 × 5.4 m plots from 7 July to 3 October in 2008 and from 20 July to 2 October in 2009 using a Toro Flex 21 walk-behind greens mower (Toro Flex 21, Toro Company, Bloomington, MN). After plots were mowed, rolling treatments were applied as a single pass over indicated plots using a commercially available greens roller (RS48-11C Golf Roll 'n' Spike, Tru-Turf Rollers, Ernest Junction, Queensland, Australia).

Data Collection

Ball roll distance was evaluated using a Pelzmeter (Nikolai, 2005). The Pelzmeter was released in 2002 as an alternative to the Stimpmeter for measuring putting green speed (Pelz, 2002). The Stimpmeter is a v-shaped aluminum ramp that is 91 cm long and has a precisely milled ball-release notch 76 cm from one end (USGA, 1979). To measure ball roll distance, a golf ball is placed in the notch, then the operator slowly raises the notched end until the ball starts to roll down the ramp. Once the ball starts to roll, the Stimpmeter must be held steady until the ball reaches the putting surface to ensure measurement accuracy (Richards et al., 2009). The distance travelled by the ball from the end of the ramp is then measured. Three golf ball rolls in one direction, then three rolls in the opposite direction are averaged for a composite ball roll distance value (USGA, 1979).

The Pelzmeter, designed to reduce variability common among Stimpmeter operators, employs a bubble-level and tapered ramp that ensures that golf balls are released from a consistent height and release onto the putting surface horizontally. Golf balls are released from the top of the Pelzmeter apparatus and the ball roll distance is measured from the end of the Pelzmeter ramp to the point at which the golf ball stops rolling. The Pelzmeter has been used to assess ball roll distances of putting greens that were mowed with different bedknife thicknesses, as well as a study assessing the impact of various mowing and rolling treatments on creeping bentgrass putting green speed (Carson, 2007; Richards et al., 2009).

To evaluate ball roll distance, three golf balls were released from the ramp of the Pelzmeter from opposite ends of each plot and a ball roll distance was measured from the end of the ramp to the average resting place of the three balls. The resulting ball roll

distances were averaged to obtain a single ball roll distance measurement for the entire plot (Pelzmetr User Manual, 2004). Two ball roll distance measurements from each plot were collected biweekly from 7 July to 3 October in 2008 and 21 July to 2 October in 2009, one on a day in which all mowing treatments were applied, referred to as “day of mowing” and one on a day in which plots mowed three times per week were not mowed, referred to as “day after mowing.”

Turfgrass quality was also rated on 1 to 9 scale, with 1 representing dead turf and 9 representing green, dense, and uniform turf. Turf color and percent green turfgrass cover were determined weekly using digital image analysis during 2008 and 2009 according to the methods of Richardson et al. (2001) and Karcher and Richardson (2003). Images were captured using a digital with a Canon G5 camera (Canon Inc., Japan) mounted on a 0.28 m² light box illuminated with four TCP 40w compact fluorescent lamps (Lighthouse Supply Co., Bristol, VA). Digital images, with a total picture size of 307,200 pixels, were analyzed for turfgrass color and percent green turf cover using SigmaScan Pro software (v. 5.0, SPSS, Inc., Chicago, IL). A customized macro (Karcher, 2007; Karcher and Richardson, 2003; Richardson et al., 2001) defined green turf as pixels with a hue range of 45 to 120° and a saturation between 0 and 100 percent, and compared the relative color of each image, as well as determined the percent of pixels with green turf. Turf color was rated on a 1-9 scale, with 1 representing dead turf and 9 representing ideal dark green turf. Percent green turf cover was rated as a percent of green pixels in the digital image.

Additionally, dollar spot (*Sclerotinia homoeocarpa*) ratings were taken in 2008 and 2009 when symptoms occurred by counting the number of individual spots per plot.

Spring dead spot (*Ophiosphaerella korrae* spp.) ratings were also taken in 2009 when symptoms occurred and were rated by visual assessment of the percentage of plot area affected by spring dead spot.

Data Analysis

Plots were arranged in a randomized complete block design with three replications. For evaluations made only once during the study, a one-way analysis of variance was performed using the mixed model procedure in SAS Statistical Software (SAS Institute, Cary, NC) to determine the effect of putting green management programs. Evaluations made repeatedly during the growing season were analyzed as a repeated measures two-way analysis of variance using the mixed model analysis of variance procedure in SAS statistical analysis software (SAS Institute, Cary, NC) to determine significance of mowing and rolling program, date, and program $\times$ date effects. Program $\times$ date effects were sliced by date to determine the specific dates at which the mowing and rolling program effects were deemed significant. For each evaluation, differences were deemed significant when the P-value corresponding to the ANOVA F-test was less than 0.05. When cultivation treatment main effects were significant, means were separated using Fisher's protected LSD test ($\alpha = 0.05$) (Kuehl, 2000). Percent turf cover and percent plot coverage of spring dead spot data were arcsine square root transformed when necessary to normalize data. Because a significant year $\times$ treatment was noted, 2008 and 2009 data were analyzed and presented separately.

Results and Discussion

Ball roll distance

On days in which all plots were mowed, 3.2 MD R3x, 3.2 MD RD, and 3.2 M3x RD had the longest ball roll distances for most evaluation dates in 2008 and 2009 (Tables 4.4 and 4.5). 3.2 MD and 3.2 Alt produced similar ball roll distances similar to or less than those produced by 3.2 MD R3x, 3.2 MD RD, and 3.2 M3x RD at most evaluation dates, but higher than those produced by 4.0 M3x RD, 4.0 MD RD, and 4.0 MD R3x. the 4.0 MD program and 4.0 Alt generally produced the lowest ball roll distance values across both 2008 and 2009. Programs mowed at 3.2 mm increased ball roll distance an average of 24 cm over those mowed at 4.0 mm in 2008 and an average of 13 cm in 2009. These distances are greater than or near the 15 cm difference in ball roll distance that an average golfer can detect (Karcher et al., 2001) and are similar to the difference reported on bentgrass by Zarco Perez and Nikolai (2009). Ball roll distances increased approximately 15 cm for the 3.2 MD RD and 4.0 MD RD over 3.2 MD and 4.0 MD programs, respectively. However, ball roll distances for 3.2 MD RD generally did not differ from 3.2 MD R3x and 3.2 M3x RD, and those produced by 4.0 MD RD generally did not differ from 3.2 MD RD, which indicates that daily mowing and daily rolling may not increase ball roll speed over less intensive procedures.

When data were averaged across the entire season for each year, ball roll distance was generally higher for programs mowed at 3.2 mm than programs mowed at 4.0 mm (Figs. 4.1 and 4.2). Additionally, programs that included rolling produced higher ball roll distance values than those that did not include rolling. The 3.2 M3x RD, 3.2 MD RD, and 3.2 MD R3x programs increased ball roll distance 10 cm over the control, indicating

that the addition of rolling to daily mowed programs will increase putting green speed. However, 3.2 Alt produced a ball roll distance that did not differ from the control, indicating that rolling without daily mowing may not increase putting green speed. All programs mowed at 4.0 mm produced ball roll distances that were 10 to 40 cm shorter than the 3.2 MD control in 2008 and 2009 (Figs. 4.1 and 4.2). However, programs with a rolling component did impact ball roll distance. The 4.0 MD R3x program produced ball roll distances 15 cm below the control in 2008, but did not affect ball roll in 2009. However, 4.0 MD RD, which includes rolling three times per week more than 4.0 MD R3x, produced ball roll measurements not different from the 3.2 mm control in both years. These departures in ball roll distance from the control suggest that increased rolling frequency may increase ball roll distance at higher mowing heights. Richards et al. (2009) and Nikolai et al. (2001) found increased rolling frequency to increase ball roll distance on creeping bentgrass, while Kopec et al. (2007) found rolling to increase ball roll distance on 'Sea Isle 2000' seashore paspalum (*Paspalum vaginatum* Swartz).

On the day after mowing, all 3.2 mm mowing height programs produced longer ball roll distances than any the 4.0 mm mowing height programs at most evaluation dates in 2008 and 2009 (Tables 4.6 and 4.7). Programs mowed at 3.2 mm increased ball roll distance an average of 23 cm over those mowed at 4.0 mm in 2008, but increased ball roll distance only by an average of 14 cm in 2009. The lower ball roll distance in 2009 may be a result of increased rainfall that year, which could have produced more succulent growth that could have increased the turf resistance to rolling. Within each mowing height, plots that were mowed on the day of evaluation significantly increased ball roll distance over plots that were not mowed, indicating the lack of residual effect that

mowing had on ball roll distance (Tables 4.4 and 4.5). Although Hartwiger (2001), Hamilton et al. (1994), and Langolis (1985) found lightweight rolling to have a residual impact on ball roll distance of creeping bentgrass at least 48 hours after rolling, this study did not find a similar effect, perhaps because of the increased rate of growth of ultradwarf bermudagrass over creeping bentgrass during the summer months. However, programs alternating mowing and rolling did not increase ball roll distance over mowing three times per week and rolling daily for either mowing height, indicating that mowing and rolling on one particular day does not affect ball roll speed the next day. Across mowing heights, 3.2 M3x RD produced a ball roll distance similar to 4.0 MD R3x, suggesting that that increased rolling frequency and decreased mowing frequency at 3.2 mm may delivers a similar putting green speed result to mowing at an increased mowing height and mowing daily, but rolling less frequently.

When ball roll distance data from the day after mowing are averaged across the entire season each in 2008 and 2009, all programs mowed at 4.0 mm decreased ball roll distance from the control plot, except for 4.0 MD RD (Fig. 4.1 and 4.2). The 3.2 MD RD program increased ball roll distance over the control by 17 and 12 cm in 2008 and 2009, respectively. The 3.2 M3x RD program produced ball roll distances 13 cm shorter than the control in 2008, but was not different from the control in 2009. Conversely, the 3.2 Alt program decreased ball roll distance from the control by 18% each year.

These results indicate that mowing height, daily mowing, and the presence of rolling are important factors affecting putting green speed on ultradwarf bermudagrass. Mowing at 3.2 mm increased ball roll distance more than 13 cm over mowing at 4.0 mm, similar to work done on creeping bentgrass (Richards et al., 2009). However, daily

mowing on ultradwarf bermudagrass for both heights increased ball roll distance over plots that were not mowed daily, contrasting with findings by Richards et al. (2009), who reported ball roll speed to increase on creeping bentgrass by mowing and rolling on alternate days. This discrepancy may be due to the increased growth of bermudagrass during to summer months and the decreased growth on creeping bentgrass in the summer. The frequency of rolling also had little effect on ball roll distance on ultradwarf bermudagrass, as programs rolling six times per week provided no increase in ball roll distance over those rolling three times per week when mowed at 3.2 mm. Conversely, Richards et al. (2009) and Hartwiger et al. (2001) found additional rolling to yield additional ball roll distance on creeping bentgrass. Ultradwarf bermudagrass may not gain additional ball roll distance from additional rolling because its prostrate growth habit does not promote vertical leaf growth (Duble, 1996). Lightweight rolling reduces the coefficient of friction by orienting vertical leaf blades in a prostrate manner (Weber, 1997; Hamilton et al., 1994). The degree of this reduction is lessened if leaf blades are already oriented in a prostrate manner, as in ultradwarf bermudagrass.

Turfgrass quality and percent cover

No putting green management program impacted turfgrass quality or percent green cover in 2008 or 2009 (Table 4.3). Turf quality ratings for all treatments averaged between 8 and 9 and all plots had more than 95% green turf cover throughout both seasons (data not shown). The lack of impact of mowing and rolling programs did not impact turf quality or green turf cover on MiniVerde ultradwarf bermudagrass because they were applied during the summer months, in which weather conditions favor warm-season grass growth and environmental stresses for the turf are relatively low (Duble,

1996, Beard and Sifers, 1996). However, altering mowing and rolling regimes during stressful periods may impact turf quality and cover. Richards (2009) found quality to decrease on creeping bentgrass that was mowed daily at 3.2 mm and rolled either three or six times per week, while quality increased for all 4.0 mm mowing heights, presumably due to the stresses placed on creeping bentgrass during the summer months. Increasing mowing heights can turf quality and decrease the need for fungicide inputs on creeping bentgrass putting greens (Stier, 2006; Bruneau et al., 2001). Increasing mowing heights can also be linked to higher stolon, rhizome, and root dry weights and total non-structural carbohydrate content on TifEagle ultradwarf bermudagrass putting greens (Guertal and Evans, 2006). Similarly, Hartwiger et al. (2001) found quality to decrease when creeping bentgrass plots were rolled four or more times per week in the summer. However, Hartwiger et al. (2001) considered rolling a plot to be a single pass in one direction, then returning to the starting point over the same plot, thus applying two passes of rolling per plot in each rolling event. Therefore, Harwtiger et al. (2001) found eight single pass rolls per plot to be excessive on creeping bentgrass during the summer months. Future research could assess potential differences in turf quality or green cover of ultradwarf bermudagrass putting greens between mowing and rolling regimes if studied under particular stress conditions, such as water stress, nutrient stress, traffic stress, or cool temperature stress, such as the spring or fall.

Turfgrass Color

Mowing and rolling programs significantly impacted turfgrass color in both 2008 and 2009 (Table 4.3). In both years, all plots exceeded the minimally acceptable color rating of 6. In 2008, 3.2 MD RD and 4.0 MD RD produced a turf color rating of 8, which

was higher than all other treatments, but not significantly different from the 7.7 rating of 3.2 MD R3x, 3.2 M3x RD, 4.0 MD R3x, and 3.2 M3x RD (Fig. 3.3). At both mowing heights, the daily mowed plots and those alternating mowing and rolling had the lowest color ratings, between 6.7 and 7.1, which were significantly lower than the daily mowed and rolled plot, but similar to the treatments mowed daily and rolled and daily rolled and mowed three times per week. In 2009, all 3.2 mm mowing height treatments, except for daily mowing and rolling, as well as the 4.0 mm treatments mowed daily and rolled either three or six times per week, produced color ratings near 8. 3.2 MD, 4.0 MD, and 4.0 M3x RD returned the lowest color ratings, ranging between 6.5 and 7 (Fig. 3.4). These results differ from those of Kopec et al. (2007), who found that turf color improved with increased mowing height on ‘Sea Isle 2000’ seashore paspalum. Mowing is known to elicit plant wounding responses, such as the reduction of ascorbate peroxidase, catalase, glutathione reductase, and superoxide dismutase, that reduce the ability to detoxify reactive oxygen species (Dat et al., 2000; Foyer and Halliwell, 1976; Mittler, 2002). As well, some tissue abrasion and wounding can occur during lightweight rolling (Howieson, 2005). However, ultradwarf bermudagrass may be better equipped to handle reactive oxygen species during summer stress than creeping bentgrass or seashore paspalum, which may explain how turf color improved during more frequent mowing and rolling. These results showed that color may be positively impacted by additional rolling, although turf quality does not appear to be affected by mowing or rolling strategies.

Disease Occurrence

No differences in dollar spot occurrence or spring dead spot occurrence were apparent in 2008 or 2009 (Table 4.3). Early morning mowing that is common on golf

courses may allow guttation water to more easily escape from mowing wounds. Guttation exudates are rich in nutrients and sugars, which can harbor turf pathogens (Williams and Powell, 1995). Because the movement and proliferation of dollar spot, and other turf pathogens, is hypothesized to be exacerbated by movement of infected plant material from equipment or humans, the potential exists for dollar spot activity to increase during mowing and rolling activities. However, previous research has shown that dollar spot activity on creeping bentgrass has been reduced by rolling (Nikolai et al., 2001). Nikolai et al. (2001) hypothesized that rolling after early morning mowing may help to remove fungal inoculum left behind in excess clippings or may reduce disease severity by disperse concentrated guttation water. In this study, no increase or decrease in dollar spot occurrence was noted across treatments, indicating that dollar spot response to rolling on MiniVerde may be different than its response on creeping bentgrass.

Conclusions

Lightweight rolling has been previously studied in conjunction with varied mowing heights and mowing frequencies on creeping bentgrass putting greens. Increasing lightweight rolling frequency, increasing mowing heights, and decreasing mowing frequencies have been shown to increase putting green quality without sacrificing putting green speed. However, this research shows that ultradwarf bermudagrass putting greens maintained in the summer in the transition zone do not behave in a similar manner. Given these conditions, putting green management programs with lower mowing heights and more frequent mowing have a larger impact on putting green speed than lightweight rolling frequency. All programs mowed at 3.2 mm increased putting green speed an average of 20 cm over those mowing at 4.0 mm. Also,

all programs mowed daily had higher putting green speeds than those mowed less frequently at each mowing height. Daily mowing at 3.2 mm and rolled six times per week, along with daily mowing at 3.2 mm and rolling 3 times per week increased green speed over those daily mowing at 3.2 mm without rolling by 10 cm, indicating that rolling frequency may not have as much impact on putting green speed as the mere presence of rolling. No mowing and rolling program influenced turfgrass quality, cover, dollar spot occurrence, or spring dead spot occurrence. However, programs with daily mowing and more frequent rolling did improve turfgrass color for each mowing height. Because ultradwarf bermudagrass is not as susceptible to decline during stressful summer periods as creeping bentgrass, mowing at lower heights and increasing rolling frequency may increase putting green speed without sacrificing quality. Future research can be used to examine how mowing height, mowing frequency, and rolling frequency impacts turf health and putting green speed during periods stressful to ultradwarf bermudagrass, such as cooler periods in the spring and fall.

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Appendix C

Table 4.1. Summary of mowing and rolling programs applied from 7 July to 3 October in 2008 and from 20 July to 2 October in 2009.

Program ID	Mowing height	Mowing frequency	Rolling frequency
	Mm	days wk ⁻¹	days wk ⁻¹
3.2 MD (control)	3.2	6	0
3.2 Alt	3.2	3	3
3.2 MD R3x	3.2	6	3
3.2 MD RD	3.2	6	6
3.2 M3x RD	3.2	3	6
4.0 MD	4.0	6	0
4.0 Alt	4.0	3	3
4.0 MD R3x	4.0	6	3
4.0 MD RD	4.0	6	6
4.0 M3x RD	4.0	3	6

Table 4.2: Analysis of variance for the effects of putting greens management program, date of observation, and their interaction on ball roll distance (BRD) and its change from the control in 2008 and 2009.

Analysis of Variance					
2008					
Effect	df	BRD Day of Mowing cm	BRD Day After Mowing cm	Change in BRD Day of Mowing cm	Change in BRD Day After Mowing cm
Program	9	***	***	***	***
Date	6	***	***	NA	NA
Program × Date	54	***	**	NA	NA
2009					
Effect	df	BRD Day of Mowing cm	BRD Day After Mowing cm	Change in BRD Day of Mowing cm	Change in BRD Day After Mowing cm
Program	9	***	***	***	***
Date	5	***	***	NA	NA
Program × Date	45	**	**	NA	NA

** and *** indicate significance at the 0.05 and 0.001 *P* level, respectively.
NA indicates that effects were not determined for that evaluation.

Table 4.3: Analysis of variance for the effects of putting greens management program, date of observation, and their interaction for evaluations made in 2008 and 2009.

Analysis of Variance						
2008						
Effect	df	Turfgrass Quality	Turfgrass Cover	Turfgrass Color	Dollar Spot	Spring Dead Spot
		1-9	% green turf	1-9	number of spots	% area affected
Program	9	NS	NS	*	NS	NS
Date	6	NS	NS	*	NS	NS
Program × Date	54	NS	NS	NS	NS	NS
2009						
Effect	df	Turfgrass Quality	Turfgrass Cover	Turfgrass Color	Dollar Spot	Spring Dead Spot
		1-9	% green turf	1-9	number of spots	% area affected
Program	9	NS	NS	*	NS	NS
Date	5	NS	NS	*	NS	NS
Program × Date	45	NS	NS	NS	NS	NS

*, **, and *** indicate significance at the 0.05, 0.01, and 0.001 *P* level, respectively.
NS indicates differences not significant.

Table 4.4. Effect of mowing and rolling treatments on ball roll distance as measured on the day that all mowing treatments were applied in 2008.

Treatment	Ball roll distance (cm)						
	11 July	25 July	8 Aug	22 Aug	5 Sept	19 Sept	3 Oct
3.2 MD (control)	209 bcd	204 ab	231 bc	220 abc	227 cd	220 abc	227 cd
3.2 Alt	206 bcd	199 bcd	225 cd	216 bc	220 cd	216 bc	220 cde
3.2 MD R3x	218 ab	209 ab	244 ab	220 abc	242 ab	220 abc	242 ab
3.2 MD RD	223 a	213 a	254 a	231 a	233 bc	231 a	233 bc
3.2 M3x RD	202 abc	209 ab	242 ab	222 ab	248 a	222 ab	248 a
4.0 MD	186 e	187 d	205 e	185 f	193 g	185 f	193 g
4.0 Alt	197 de	188 d	202 e	184 f	194 g	184 f	194 g
4.0 MD R3x	197 de	203 abc	215 de	193 ef	211 ef	193 ef	211 ef
4.0 MD RD	203 cd	206 ab	225 cd	209 cd	220 de	209 cd	220 de
4.0 M3x RD	200 cd	191 cd	206 e	199 de	202 fg	199 de	202 fg
Significance	***	***	***	***	***	***	***

Within each date, means sharing a letter are not different according to Fisher's protected LSD test ($\alpha = 0.05$). *** indicates significance at the 0.001 *P* level.

Table 4.5. Effect of mowing and rolling treatments on ball roll distance as measured on the day that all mowing treatments were applied in 2009.

Treatment	Ball roll distance (cm)					
	24 July	7 Aug	21 Aug	4 Sept	18 Sept	2 Oct
3.2 MD (control)	228 ab	220 bcd	215 ab	216 cde	220 bcd	220 bcd
3.2 Alt	229 ab	214 cde	205 bc	211 def	214 cde	214 cde
3.2 MD R3x	238 a	232 ab	226 a	232 ab	232 ab	230 ab
3.2 MD RD	227 abc	239 a	223 a	237 a	239 a	237 a
3.2 M3x RD	231 ab	238 a	227 a	226 abc	238 a	236 a
4.0 MD	213 d	210 cde	197 c	206 ef	210 cde	210 cde
4.0 Alt	214 cd	204 e	192 c	201 f	204 e	204 e
4.0 MD R3x	217 bcd	224 bc	215 ab	222 bcd	224 bc	224 bc
4.0 MD RD	221 bcd	223 bcd	214 ab	219 bcde	223 bcd	223 bcd
4.0 M3x RD	223 bcd	209 de	202 bc	220 bcde	209 de	209 de
Significance	***	***	***	***	***	***

Within each date, means sharing a letter are not different according to Fisher's protected LSD test ($\alpha = 0.05$). *** indicates significance at the 0.001 *P* level.

Table 4.6. Effect of mowing and rolling treatments on ball roll distance as measured on the day when treatments mowed three times per week were not applied in 2008.

Treatment	Ball roll distance (cm)						
	8 July	22 July	5 Aug	19 Aug	2 Sept	16 Sept	30 Sept
3.2 MD (control)	228 ab	217 b	212 ab	217 ab	200 b	231 b	255 b
3.2 Alt	194 ef	199 cd	195 cd	189 de	188 bc	216 cd	239 cd
3.2 MD R3x	220 bc	219 b	213 ab	220 a	202 b	237 ab	259 b
3.2 MD RD	236 a	236 a	221 a	231 a	223 a	248 a	284 a
3.2 M3x RD	200 de	209 bc	199 bc	194 cd	193 bc	228 bc	251 bc
4.0 MD	206 de	195 cd	183 de	191 de	162 d	200 ef	218 ef
4.0 Alt	186 f	187 d	176 e	180 e	167 d	195 f	210 f
4.0 MD R3x	205 de	201 cd	194 cd	190 de	171 d	202 ef	234 d
4.0 MD RD	214 cd	220 b	204 bc	206 bc	186 c	226 bc	255 b
4.0 M3x RD	186 f	191 d	182 de	181 de	162 d	209 de	226 de
Significance	***	***	***	***	***	***	***

Within each vote, means sharing a letter are not different according to Fisher's protected LSD test ($\alpha = 0.05$). *** indicate significance at the 0.001 *P* level.

Table 4.7. Effect of mowing and rolling treatments on ball roll distance as measured on the day when treatments mowed three times per week were not applied in 2009.

Treatment	Ball roll distance (cm)					
	21 July	4 Aug	18 Aug	1 Sept	15 Sept	29 Sept
3.2 MD (control)	231 ab	231 ab	241 ab	242 a	220 bcd	225 b
3.2 Alt	212 cd	212 cd	230 bcd	222 b	205 e	208 cd
3.2 MD R3x	229 ab	229 ab	237 abc	250 a	228 b	227 b
3.2 MD RD	238 a	238 a	249 a	244 a	246 a	242 a
3.2 M3x RD	222 bc	221 bc	228 bcd	242 a	226 b	226 b
4.0 MD	209 cde	208 cde	227 bcd	217 b	211 cde	215 bcd
4.0 Alt	197 e	197 e	224 cd	200 c	197 e	193 e
4.0 MD R3x	221 bc	221 bc	232 bcd	226 b	220 bcd	220 bc
4.0 MD RD	229 ab	229 ab	235 abcd	246 a	226 bc	223 b
4.0 M3x RD	203 de	203 de	222 d	212 bc	210 de	203 de
Significance	***	***	**	***	***	***

Within each date, means sharing a letter are not different according to Fisher's protected LSD test ($\alpha = 0.05$). **, *** indicate significance at the 0.01 and 0.001 *P* level, respectively.

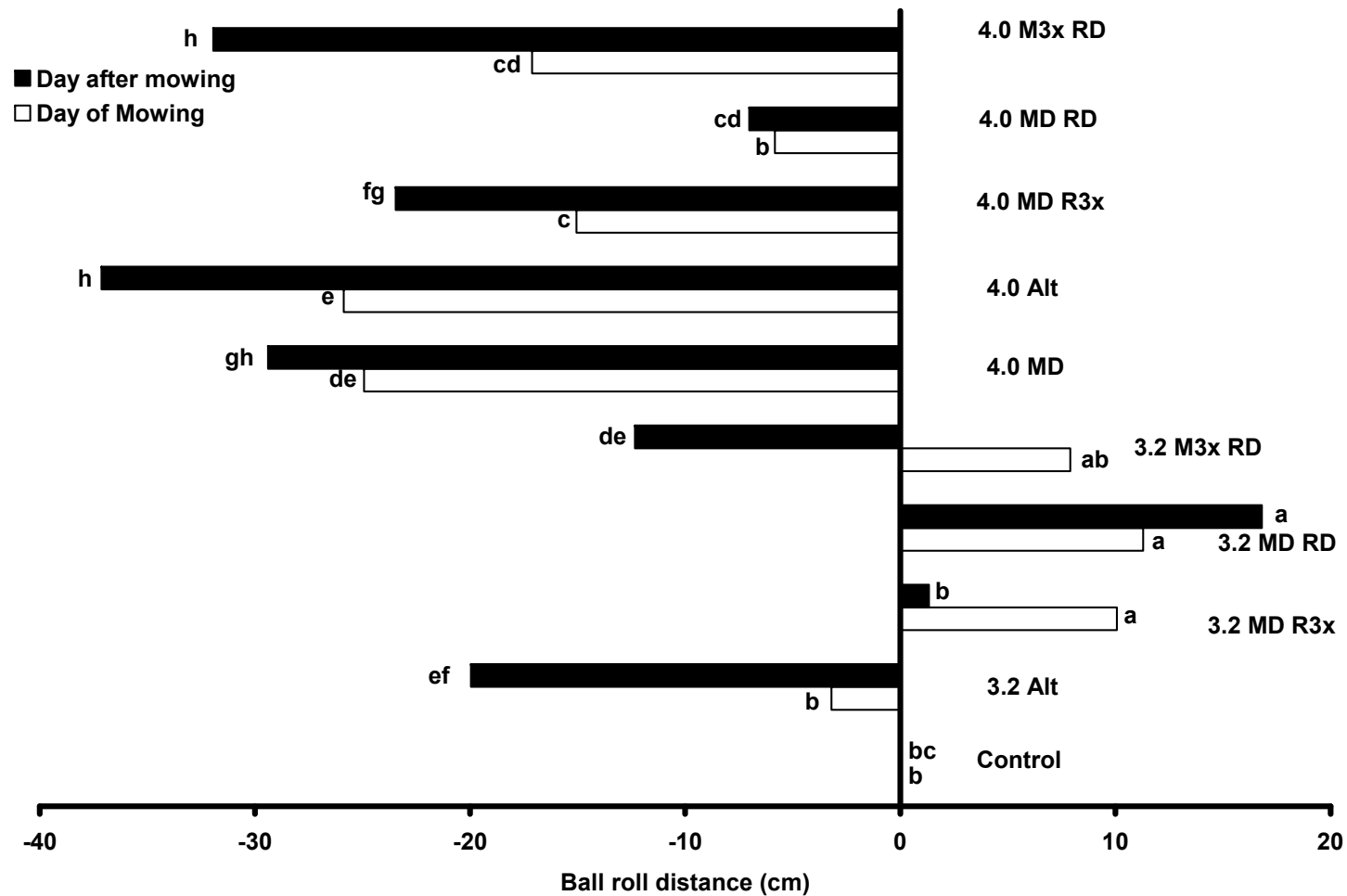


Figure 4.1. Change in ball roll distance from control on MiniVerde bermudagrass as measured on the day all mowing treatments were applied and the day after treatments mowed three times per week were applied in 2008. Within days, bars sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

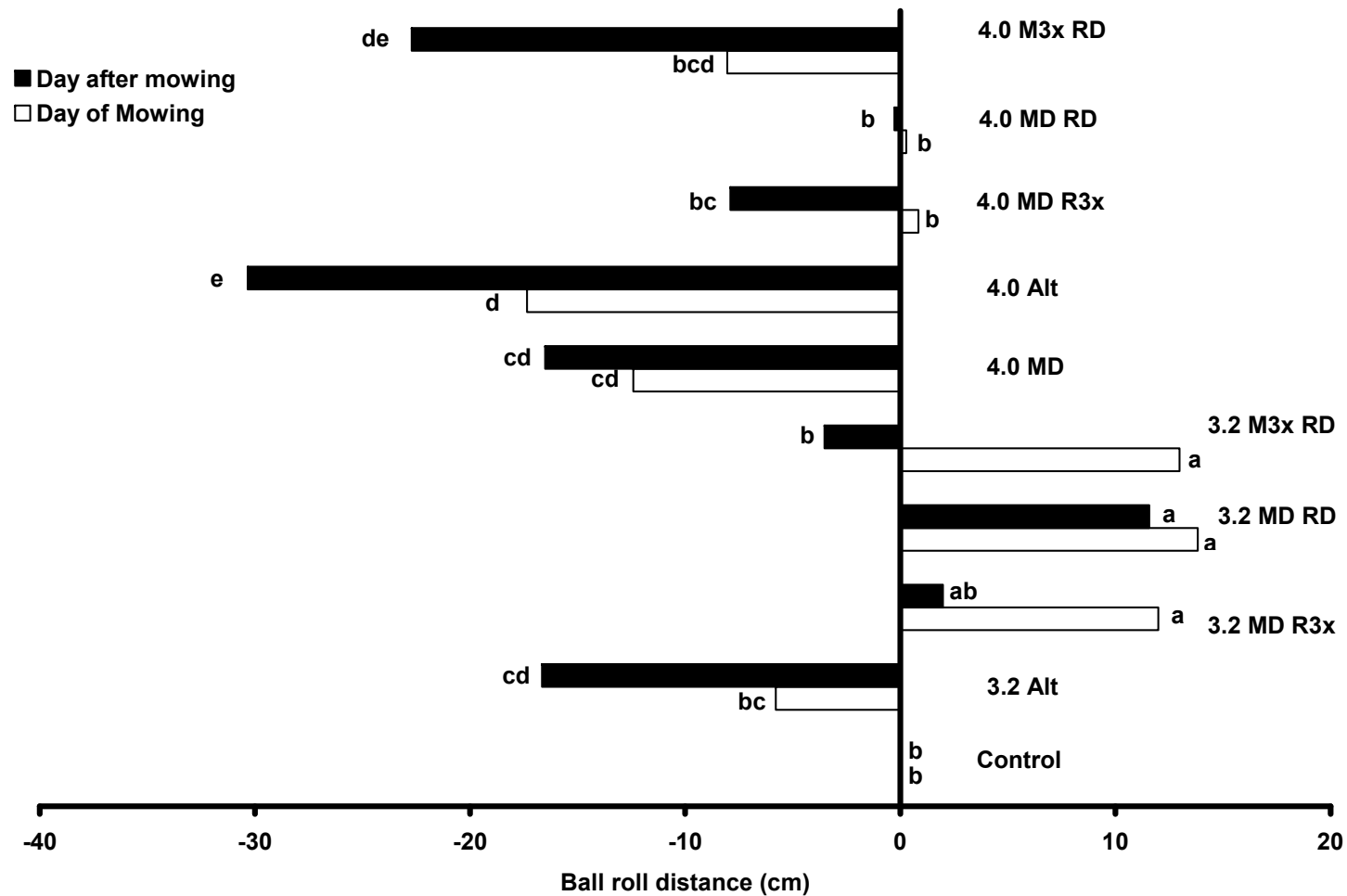


Figure 4.2. Change in ball roll distance from control on MiniVerde bermudagrass as measured on the day all mowing treatments were applied and the day after treatments mowed three times per week were applied in 2009. Within days, bars sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

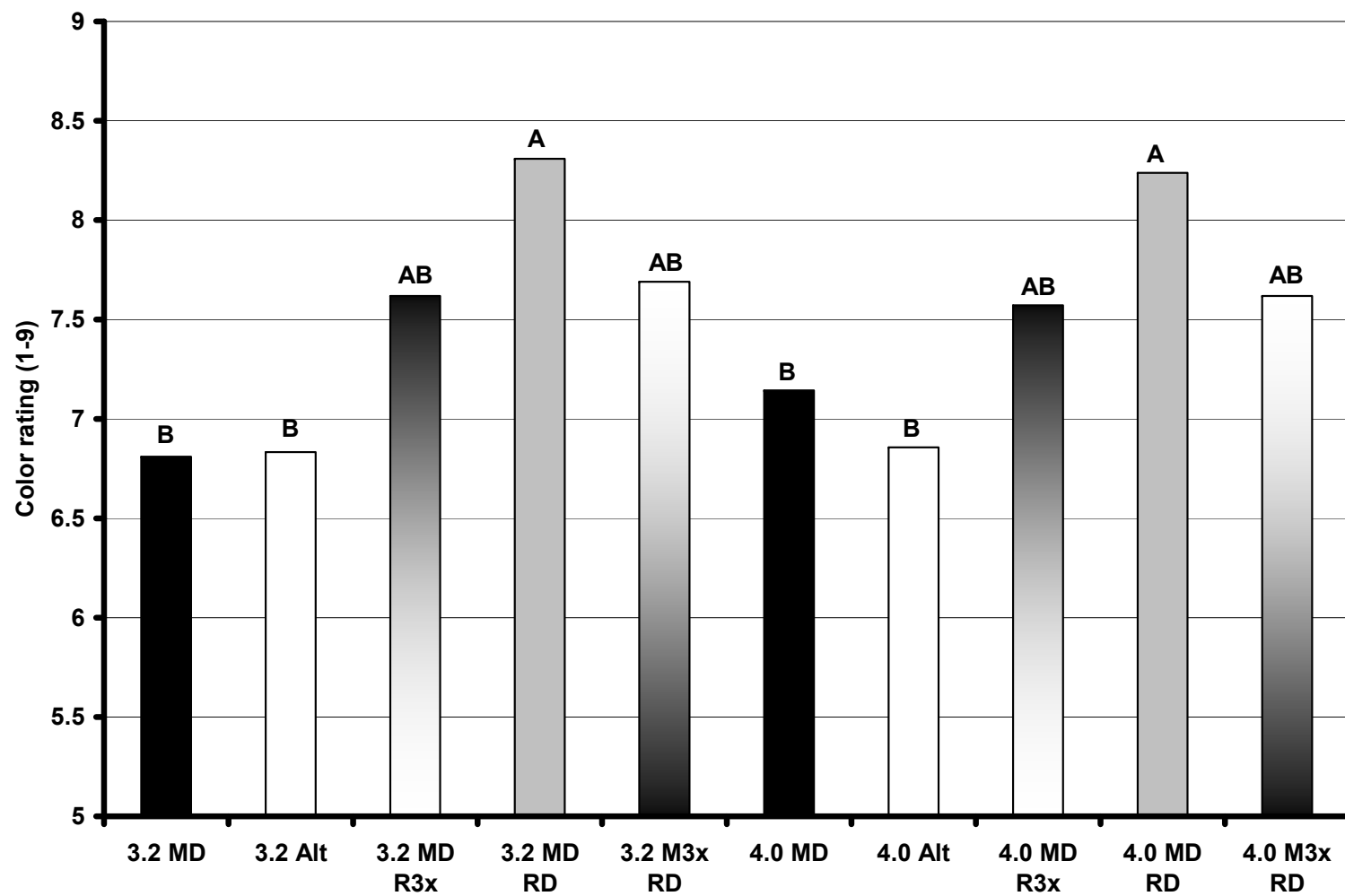


Figure 4.3. Turfgrass color rating as influenced by mowing and rolling treatments in 2008. Bars sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

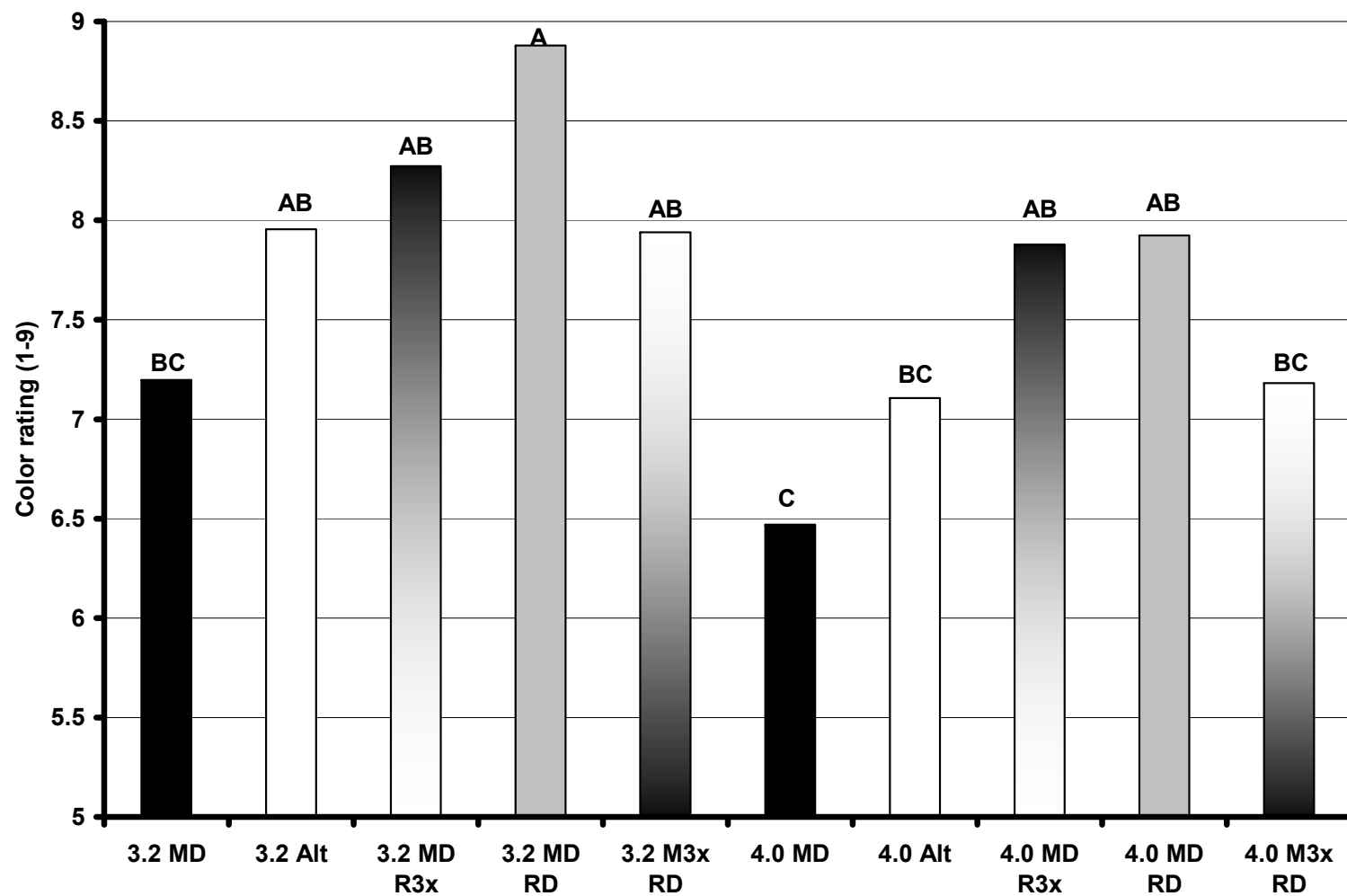


Figure 4.4. Turfgrass color rating as influenced by mowing and rolling treatments in 2009. Bars sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

**CHAPTER FIVE – VERTICAL MOWING AND GROOMING ARE
IMPORTANT THATCH CONTROL MEASURES FOR
ULTRADWARF BERMUDAGRASS PUTTING GREENS**

Abstract

The dense and aggressive growth habit of ultradwarf bermudagrass often results in excessive thatch accumulation, which can adversely affect turfgrass health and functionality. Light vertical mowing and grooming are often used as components of thatch management programs, but have not been studied in combination. The objective of this study was to determine the effect of light vertical mowing at weekly intervals, grooming at daily intervals, and their combination on soil physical properties and turfgrass quality of an ultradwarf bermudagrass putting green. This study was conducted on a one-year-old 'TifEagle' bermudagrass (*Cynodon dactylon* (L.) $\times$ *C. transvaalensis* Burt-Davy) putting green at the East Tennessee Research and Education Center in Knoxville. All plots were mowed daily at 3.9 mm, topdressed bi-weekly throughout the growing season, and irrigated to prevent stress. The study was conducted in 2008 and 2009 with plots receiving treatments of daily grooming, weekly light vertical mowing, a combination of both, or an untreated control. Vertical mowing + grooming reduced thatch depth, total biomass concentration, and turfgrass quality over all treatments, but increased topdressing incorporation. Grooming alone reduced thatch depth similar to the combination, but yielded the highest turf quality. The untreated control incorporated the least amount of sand topdressing into the turfgrass canopy and had the highest levels of thatch and total biomass. Although daily grooming and weekly vertical mowing may be too aggressive for sustained use at the parameters in this study, alteration of the frequency or invasiveness of both practices may result in a practice that can help control thatch accumulation and produce a quality putting surface.

Introduction

Ultradwarf bermudagrasses (*Cynodon dactylon* (L.) $\times$ *C. transvaalensis* Burt-Davy) are quickly replacing dwarf cultivars as putting surfaces in the southern U.S. and are increasingly being grown in the transition zone (McCarty and Miller, 2002), which is an area defined by McCarty (2001) as a 320 km wide belt centered along a line at 37 degrees north latitude that includes Tennessee. Ultradwarf cultivars, such as ‘TifEagle’ and ‘Champion’, arose as mutations of ‘Tifdwarf’, which was the first true dwarf bermudagrass in common use (Burton, 1966; Beard and Sifers, 1996; Moncrief, 1968). The ultradwarf bermudagrasses have higher shoot densities and shorter internodes than Tifdwarf and tolerate lower mowing heights (Foy, 1997; Moncrief, 1975), which makes them desirable for putting green use. In the transition zone, ultradwarf bermudagrasses are increasingly being used in place of creeping bentgrass for putting greens because they require less water, are susceptible to fewer fungal pathogens in the summer season, and are better adapted to the warm and humid growing conditions of the region (Beard and Sifers, 1996).

Ultradwarf bermudagrass cultivars are often divided into two groups, based on their growth characteristics relative to Tifdwarf. One group has slower vertical growth and faster lateral growth than Tifdwarf and includes the cultivars Champion, ‘MiniVerde’, and ‘MS Supreme’. The second group has a similar lateral growth rate, but slower vertical growth than Tifdwarf. Included in this group are ‘FloraDwarf’ and TifEagle (Gray and White, 1999; Hanna and Elsner, 1999). Both groups, however, accumulate organic matter (OM) at much faster rates than Tifdwarf and require more intensive thatch management programs (Hollingsworth et al. 2005; McCarty, 2001).

Organic matter in turfgrass systems is often divided into thatch and mat layers (McCarty, 2001). Thatch is a tightly intermingled layer of living and dead tissue that develops between the verdure and the soil surface (Beard, 1973). The mat layer is the layer below the thatch in which partially decomposed thatch is intermixed with soil (Carrow et al., 1987). Some level of thatch is beneficial to turf systems. Thatch can harbor beneficial microbes and protect turfgrass crowns from traffic damage (McCarty, 2001). However, excessive thatch can be detrimental by reducing drought tolerance, cold tolerance, and water infiltration. Additionally, excessive thatch can result in soft playing surfaces, which can lead to mower scalping (Barton et al., 2009; Hurto et al., 1980;). Although the mat layer helps to hold approach shots on a putting green, provide traffic tolerance, and hasten ball mark recovery, excessive organic matter accumulation in this layer can encumber diffusion of oxygen into the rootzone, soften the playing surface, and promote shallow rooting (McCarty, 2001; Meinhold et al., 1973).

Frequent sand topdressing and core cultivation are often used to alleviate these conditions (Beard, 2002; Carrow et al., 1987; McCarty, 2001). Topdressing with sand dilutes the thatch layer, preserves soil porosity, enhances water infiltration, and helps to improve conditions for microbial degradation of thatch (Carrow, 2001; Ledebøer and Skogley, 1967; Rieke, 1994). Topdressing alone has been shown to reduce thatch layers in ‘Pennncross’ creeping bentgrass (*Agrostis stolonifera* L.) (Eggens, 1980), ‘Tifway’ bermudagrass (Carrow, 1987), and three different dwarf bermudagrass cultivars (White and Dickens, 1984). However, ultradwarf bermudagrasses produce thatch aggressively enough that sand topdressing alone cannot keep pace with thatch production and mechanical means are required to keep OM levels manageable (Guertal and White, 1998)

Secondary cultural practices, such as vertical mowing and grooming, are often used to help maintain surface playability (Beard, 1973; Turgeon, 1991). Vertical mowing penetrates the turf canopy, cuts stolon tissue, and lightly disrupts the soil surface using vertically oriented blades (Duble, 1996). On putting greens, vertical mowing blades are often set to cut even with the soil surface or immediately below it, which severs stolons, encourages lateral growth, increases shoot density, and encourages vigorous juvenile growth (McCarty and Canegallo, 2005; White and Dickens, 1984). Grooming is a process of very light vertical mowing that often does not penetrate the turf canopy and does not contact the soil (McCarty, 2001). Grooming is intended to vertically orient leaf blades, so that the cut is more uniform, but is not intended as a tool to remove large amounts of thatch. However, as these practices open the turf canopy, they remove OM from the canopy and thatch layer, which opens channels in the dense turf canopy for topdressing incorporation. Carrow et al. (1987) reported that vertical mowing at least twice yearly reduced thatch depth in Tifway bermudagrass. White et al. (2004) reported that weekly light vertical mowing on a Tifdwarf bermudagrass putting green maintained a similar thatch depth to severe invasive vertical mowing twice yearly, but maintained more acceptable turf quality. Grooming, however, did not consistently affect fall carbohydrate content, shoot density, and thatch accumulation on TifEagle bermudagrass (Salaiz et al., 1995).

Previous research on ultradwarf bermudagrass thatch management has focused on the effects of nitrogen sources and varying cultivation techniques, including sand topdressing, aggressive vertical mowing, and infrequent applications of light vertical mowing (Hollingsworth et al., 2005; White et al., 2004), but has not addressed the effects

of light and frequent vertical mowing along with regular grooming on thatch production, OM status, or surface quality of ultradwarf bermudagrass putting greens. Thus, the objective of this study was to compare weekly vertical mowing, daily grooming, and the combination of both on thatch production, topdressing incorporation into putting green turf canopies, and overall turf quality of ultradwarf bermudagrass putting greens.

Materials and Methods

Experimental Area

This study was conducted on a two-year-old ‘TifEagle’ bermudagrass (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy) putting green constructed on a Sequatchie silt loam soil (fine-loamy, siliceous, semiactive, thermic Humic Hapludult) at the East Tennessee Research and Education Center in Knoxville, TN. The putting green was mowed six times per week at 3.9 mm with a walk-behind reel mower (Jacobsen PGM 22, Textron Company, Charlotte, NC) and irrigated deeply and infrequently, approximately to a 2.5 cm depth weekly, to encourage deep rooting while minimizing drought stress and disease pressure. Granular nitrogen (N) and potassium (K) were applied at 24.4 and 12.2 kg ha⁻¹, respectively, in March and September, then at 48.0 and 36.6 kg ha⁻¹, respectively, each month from April through August in 2008 and 2009. Granular phosphorus (P) was applied at 29.3 kg ha⁻¹ in March and August, then at 9.8 kg ha⁻¹ each month from April through July in 2008 and 2009. Sand topdressing was applied at 14-day intervals from 4 August to 15 September 2008 and 29 July to 23 September 2009 at a rate of 550 g m⁻². Sand texture was 15.6% coarse sand (0.5-1.0 mm), 73% medium sand (0.25-0.50 mm), and 11.4% fine sand (0.15-0.25 mm).

Treatments

Weekly vertical mowing, daily grooming, vertical mowing + grooming, and an untreated control treatment were applied to 3 × 3 m plots. Vertical mowing was performed once weekly from 1 August to 3 October in 2008 and 24 July to 2 October in 2009 using vertical mowing units mounted on a triplex greens mower (Jacobsen Greens King IV, Textron Co., Charlotte, NC). Vertical mowing blades were spaced 1.3 cm apart and set to a depth that scratched the soil. Grooming treatments were made daily during the same time period using a walk-behind greens mower with a grooming attachment (Jacobsen PGM 22, Textron Co., Charlotte, NC). The grooming blades were 0.5 cm apart and set even with the height of cut at 3.9 mm. Combined vertical mowing and grooming treatments were also applied from 1 August to 3 October in 2008 and 24 July to 2 October in 2009.

Data Collection

Soil organic matter (SOM) accumulation was measured using a weight loss on ignition procedure (Combs and Nathan, 1998). Two cores (5 cm diameter × 5 cm depth) collected from the center of each plot prior to the initiation of treatments and after cessation of treatments in 2008 and 2009. The verdure was removed and the top 2.5 cm of each core was used for analysis, similar to methods used by Carrow et al. (1987) and McCarty et al. (2007). The samples were oven-dried at 105° C for 24 hours, weighed, then ashed at 600° C for 6 hours in a modification of the American Society for Testing Materials (ASTM) D2974-07a procedure (Carrow et al., 1987; McCarty et al., 2007). The ashes were then weighed and used to find the total organic matter present as a percent of the entire sample for each plot.

Several options exist for determining organic matter via weight loss on ignition (Kimble et al., 2001). The ASTM F 1647 – 02a procedure is specified for determining the organic matter content of putting green and sports turf root zone mixes and calls for ashing a 5 to 10 g soil sample in a 360° C furnace for 2 hours (ASTM, 2002). This procedure is useful for determining organic matter content of volume ratio mixed rootzones before used for plant growth (ASTM, 2002). Although the low ashing temperature and short ashing time of this procedure are designed to prevent the loss of calcium carbonate from calcareous sands, the procedure may understate organic matter content (N.W. Hummel, 2010, personal communication). The ASTM D2974-07a procedure is specified for determining moisture, ash, and organic matter of peat and other organic soils and calls for soil samples of a minimum of 50 g to be ashed at a temperature range from 440° C to 750° C for a time period sufficient to stabilize the mass of the ashed sample (ASTM, 2007). This procedure was used in this study because its larger ashing sample, higher ashing temperature, and longer ashing time provide a more accurate representation of organic matter content than the ASTM F 1647-02a procedure and did not adversely impact the silica sand used as topdressing material in this study (N.W. Hummel, 2010, personal communication).

The dense canopy, high shoot density, and stolon/rhizome structure of ultradwarf bermudagrass can trap topdressing material in the turf canopy, under the leaves, but above the existing soil surface. During the verdure removal process for SOM determination, this topdressing material that has already been incorporated into the turf canopy, can be lost. Thus, a procedure similar to the SOM determination procedure was conducted to capture the influence of topdressing material that may have been lost during

verdure removal. Because this procedure does not remove the verdure and includes the entire above ground biomass, it is referred to as total biomass (TB) content. Total biomass content was determined in a similar manner to SOM, with two 5 cm diameter cores collected from the center of each plot prior to the initiation of treatments and after cessation of treatments in 2008 and 2009. However, the verdure was left intact and the top 2.5 cm of the soil were used during analysis to determine if topdressing sand was lost during verdure removal. Similar to the SOM determination, the TB samples were oven-dried at 105°C for 24 hours, then ashed at 600 °C for 6 hours. The ashes were then weighed and used to find the organic matter present as a percent of the entire sample for each plot.

Two days following topdressing applications, sand particles and clippings were collected from one mower width on each plot. Similar to Stier and Hollman (2003), collected topdressing material and clippings were dried in an oven at 105° C for 24 hours. The clippings were separated from the sand by adding water and decanting the water and clippings. The remaining sand was oven-dried at 105° C for 24 hours, then weighed to determine the amount of topdressing material that was not incorporated into the turf canopy.

Additionally, the uncompressed depth of the thatch-mat layer was measured prior to the initiation of treatments and after their cessation. Five samples were extracted from each plot with a soil probe 2.5 cm in diameter to a depth of 10 cm on 29 July and 3 October, 2008, and 16 July and 2 October, 2009. The uncompressed depth of the thatch-mat layer was measured with a ruler (Callahan et al., 1997; Waddington, 1992).

A Clegg Soil Impact Tester (Clegg 95050, Lafayette Instrument, Lafayette, IN) with a 2.25 kg missile was used to determine the surface hardness of each plot before treatments began and after cessation of treatments in 2008 and 2009. The missile of the hammer was raised to 45 cm and dropped on three locations near the center of each plot. The impact value was then multiplied by 10 to convert to $g_{\max}$, a peak deceleration value which increases as surface hardness increases (Lush, 1985).

Turfgrass color and percent green turfgrass cover were determined weekly using digital image analysis weekly from 1 August to 3 October in 2008 and 24 July to 2 October in 2009 using the methods of Richardson et al. (2001). Images were captured using a digital with a Canon G5 camera (Canon Inc., Japan) mounted on a 0.28 m² light box illuminated with four TCP 40w compact fluorescent lamps (Lighthouse Supply Co., Bristol, VA). Digital images, with a total picture size of 307,200 pixels, were analyzed for turfgrass color and percent green turf cover using SigmaScan Pro software (v. 5.0, SPSS, Inc., Chicago, IL). A customized macro (Karcher, 2007; Karcher and Richardson, 2003; Richardson et al., 2001) defined green turf as pixels with a hue range of 45 to 120° and a saturation between 0 and 100 percent, and compared the relative color of each image, as well as determined the percent of pixels with green turf. Percent green turf cover was rated as a percent of green pixels in the digital image.

Additionally, turfgrass quality was assessed on a visual basis weekly from 1 August to 3 October in 2008 and 24 July to 2 October in 2009. Turfgrass quality was rated on 1 to 9 scale, with 1 representing dead turf and 9 representing green, dense, and uniform turf.

Data Analysis

Treatments were arranged in a randomized complete block design with six replications. For observation evaluated singularly in time, such as initial and final SOM contents, a one-way analysis of variance was performed using the mixed model procedure in SAS Statistical Software (SAS Institute, Cary, NC) to determine the effect of putting green management programs. Evaluations made repeatedly during the growing season were analyzed as a repeated measures two-way analysis of variance using the mixed model analysis of variance procedure in SAS statistical analysis software (SAS Institute, Cary, NC) to determine significance of secondary cultivation treatment, date, and secondary cultivation treatment $\times$ date effects. Secondary cultivation treatment $\times$ date effects were sliced by date to determine the specific dates at which cultivation effects were deemed significant. For each evaluation, differences were deemed significant when the P-value corresponding to the ANOVA F-test was less than 0.05. When secondary cultivation treatment main effects were significant, means were separated using Fisher's protected LSD test ($\alpha = 0.05$) (Kuehl, 2000). Because a significant year $\times$ treatment interaction was noted, data are shown separately for 2008 and 2009.

Results and Discussion

Soil Organic Matter Accumulation

The average SOM content for all plots before treatments began in 2008 was 8.45% by weight. The secondary cultural practices of weekly vertical mowing, daily grooming, and their combination did not significantly affect SOM in the upper 2.5 cm of the rootzone during the 2008 season (Table 5.1). Over the 2008 season, SOM content

decreased between 10 and 34% for all treatments, resulting in an average SOM content of 6.5% by weight (Table 5.2). Although these changes were not significantly different from each other, meaning that no treatment decreased SOM content more than any other treatment over the 2008 season, SOM content decreased for all treatments. This decrease may have been partly the result of secondary cultivation treatments, but, because SOM content also decreased in untreated control plots, the decrease is likely due to the sand topdressing that was applied.

Soil organic matter content also did not change between treatments over the 2009 season (Table 5.1). Average SOM content before treatments began in 2009 was 4.9% by weight, which was nominally less than the final SOM content from 2008 (Table 5.2). The extended cool weather in the spring of 2009 delayed emergence from dormancy, which could have allowed an extended period of microbial breakdown of SOM without accumulation from actively growing turf. Additionally, the extended dormancy left the turf canopy and soil surface open to weathering, which could have lead to physical removal of organic matter by weather elements. Over the 2009 season, SOM content decreased between 0.9 and 16.4% for all treatments, resulting in an average SOM content of 4.6% by weight (Table 4.2). The lack of difference in SOM between secondary cultivation treatments across the 2009 season indicate that the treatments were aggressive enough in removing OM to hold SOM steady, but not aggressive enough to decrease SOM.

The reduction in SOM content in the upper 2.5 cm of the rootzone for all treatments, with no treatment providing an additional reduction from any other treatment, suggests that sand topdressing applications were responsible for more SOM reduction

than secondary cultivation treatments. Also, the lack of difference in SOM content between secondary cultivation treatments and the untreated control indicates that weekly vertical mowing, daily grooming, and their combination are not effective practices to reduce SOM content. This ineffectiveness may be because vertical mowing and grooming do not penetrate the organic matter layer and do not remove organic matter already in the soil. The similar SOM contents between treatments may also be a result of the verdure removal process. Removing the verdure from the turf sample may loosen sand from the sample and facilitate its separation from the sample, thus overstating SOM concentration.

After two years of treatment applications, each cultivation method returned SOM contents meeting the recommendation by Murphy et al. (1993) of 4 to 5% OM by weight for putting greens. Because treatment differences were not apparent in the SOM change in either year, secondary cultivation practices have shown to have little effect on SOM concentration in the upper 2.5 cm of putting green rootzones. Primary cultural practices, such as hollow-tine cultivation, may be important in reducing SOM, as reported by McWhirter and Ward (1976).

Previous research has also found the effects of secondary cultural practices on SOM content to be inconsistent. Although McCarty et al. (2007) found vertical mowing and grooming to have no affect on SOM concentration of creeping bentgrass putting greens, Carrow et al. (1987) found that vertical mowing with blades set at the soil surface to effectively decrease SOM in bermudagrass cut at 1.9 cm. Additionally, McWhirter and Ward (1976) found aggressive vertical mowing, to a depth penetrating the organic layer underneath the soil line, every two or four weeks to be as effective as core aerifying

three times yearly at decreasing SOM. White and Dickens (1984) found biweekly light vertical mowing, to a depth scratching the soil surface, of a bermudagrass green to be no more effective than twice yearly severe vertical mowing, and that the twice yearly treatment was less deleterious to the turf surface than was the biweekly treatment.

Total Biomass

Although secondary cultivation practices did not significantly impact SOM over the course of a season, they did influence TB in the upper 2.5 cm of the putting green in 2008 and 2009 (Table 5.1). Before treatments began in 2008, TB contents averaged 15.2% by weight. Over the course of the season, vertical mowing and vertical mowing + grooming reduced TB content by 1.0 and 4.3% by weight, respectively (Table 5.3). However, TB content rose for the grooming treatment and the untreated control by 5.0 and 5.5% by weight, respectively (Table 5.3). Before treatments began in 2009, TB content averaged 10.7% by weight. Over the course of the season, the untreated control reduced TB content over all other treatments, decreasing TB content 7.5% by weight, while all other treatments decreased TB content by 5.1% or less (Table 5.3). Although the untreated control reduced TB content more than all other treatments over the 2009 season, it ended the season with a TB content not different than the other treatments. The significant reduction in TB content was due to the higher initial TB content in the 2009 season, which could have resulted from the lack of secondary cultivation in 2008. Because no secondary cultivation treatments were applied to the control plots, they retained more dormant turf cover during the winter and early spring, which could have overstated the initial TB content measurement in 2009. Because the final TB content

measurement did not include excess dormant shoot or leaf material, its TB content may be more accurate.

Soil organic matter values ranged lower than TB values because of the inclusion of verdant plant material in TB samples. The differences in TB between treatments after the 2008 season indicate that the secondary cultivation practices affect only the canopy and thatch layer, but not the mat layer or soil. The leveling of TB concentration in 2009 suggests that, while secondary cultivation practices do decrease TB concentration, their effect plateaus once it reaches a maximum level.

Previous studies involving vertical mowing treatments have used a more aggressive or invasive vertical mowing technique, which involves setting blades to at least 6 mm below the soil line and is used to remove SOM and soil from the profile (Guertal and White, 1998; Hollingsworth et al., 2005; McCarty et al., 2007; White et al., 2004). However, the vertical mowing blades were set even with the soil surface in order to cut stolons and remove thatch, but not remove soil, similar to studies by Johnson (1979) and Callahan et al. (1998), in which vertical mowing blades were set at or just below the soil surface. Light vertical mowing and its combination with daily grooming appear to thin the canopy, and reduce organic matter concentration, which may allow for improved incorporation of sand topdressing.

Two samples were taken from each plot to assess SOM content and TB content. Using two samples to determine SOM content allows 1.0% differences in SOM content to be detected at a 95% confidence level (Chapter 2). Although SOM and TB content are different parameters, they have a similar determination procedure, thus making their sampling procedures related. Differences in SOM content did not exist, but the

differences in TB content exceeded the 1.0% minimum detectable difference value at a 95% confidence level, thus making this a valid test. However, the samples were not taken truly at random, but taken by human randomization, which may be biased. A better sampling scheme would include true non-human randomization of sample locations and additional samples to increase the confidence level of the test and reduce the minimum detectable difference.

Thatch-mat Depth

Neither vertical mowing, grooming, nor vertical mowing + grooming significantly impacted uncompressed thatch-mat depth in 2008 or 2009 (Table 5.1). Five samples were taken from each plot to assess thatch-mat depth. This sampling procedure allows 5 mm differences in thatch-mat depth to be detected with 95% confidence (Chapter 2). Because the range of thatch-mat depths was 4 mm wide, true differences in thatch-mat depth between treatments at less than 5 mm were not captured, thus this test may not have been valid as performed. Also, the samples were not taken truly at random, but taken by human randomization, which may introduce bias into the sampling procedure. A better sampling scheme would include true non-human randomization of sample locations and at least 11 samples per plot to detect differences of less than 4 mm, if they exist.

Prior to initiating treatments in 2008, thatch-mat depths averaged 13.1 mm (Table 5.4). After treatments ended that same year, each cultivation treatment had a shallower thatch-mat depth than the untreated control, though not statistically significant, even as sand topdressing was consistently applied. White et al. (2004) found sand topdressing alone to be an inadequate method to control thatch on bermudagrass putting greens, as

bermudagrass accumulates thatch more quickly than topdressing can be applied to dilute the thatch. Although thatch-mat depth was unchanged across the season for daily grooming, the 1.9 mm (14%) decrease in thatch-mat depth for weekly vertical mowing was not statistically different, indicating that grooming did not impact thatch-mat depth more or less than vertical mowing (Table 5.4). Similarly, McCarty et al. (2007) found creeping bentgrass plots subjected to topdressing alone had a significantly deeper thatch-mat layer than plots topdressed and either groomed or vertically mowed. Additionally, McCarty et al. (2007) found vertical mowing alone to be no more effective at reducing thatch-mat depth than grooming twice weekly. However, thatch-mat depth decreased 2.8 mm (21.2%) for the combination of weekly vertical mowing and daily grooming in this study, indicating that no treatment other than the combination of vertical mowing and grooming reduced thatch depth (Table 5.4).

Before treatments began, thatch depths averaged 20.5 mm, which was greater than the ending values in 2008 (Table 5.4). This increase may be due to a lack of cultivation practices that penetrate the thatch layer, such as hollow tine cultivation, and is indicative of the importance of such practices in a putting green management program. Hollow tine cultivation was not applied so that its effects would not confound the effects of the specific secondary cultivation treatments in this study. Although the change in thatch depth over the 2009 season was not impacted by secondary cultivation treatments thatch depths tended to increase for all treatments except weekly vertical mowing (Table 5.4). White et al. (2004) saw a similar increase in thatch depth during the second year of a study of nitrogen and vertical mowing programs on bermudagrass putting greens. Both light, frequent and heavy, infrequent vertical mowing increased shoot density for

TifEagle ultradwarf bermudagrass, which contributed to an increase in thatch depth (White et al., 2004). Although vertical mowing resulted in a nominal decrease in thatch depth in this study, grooming and vertical mowing + grooming may have increased shoot density to the point of increasing thatch depth in 2009.

Weekly vertical mowing removes stolons and organic matter near the soil surface, along with opening the turfgrass canopy to allow better topdressing incorporation. Daily grooming is less aggressive and does not remove stolons, but does remove some organic matter and thins the canopy, which may also enhance topdressing incorporation. The combination of both practices, however, removes organic matter and thins the turf canopy, which reduces the excess growth that can lead to increased thatch accumulation (Hollingsworth et al., 2005; Salaiz et al., 1995; White et al., 2004). Similar to this study, Carrow et al. (1987) reported vertical mowing to reduce thatch depth over an untreated control. White and Dickens (1984) also found vertical mowing to reduce thatch depth on Tifgreen and Tifdwarf grown at putting green height. However, Carrow et al. (1987) and White and Dickens (1984) used vertical mowing techniques that penetrated the soil between 1.2 and 2.5 cm, while this study used a less aggressive vertical mowing technique that only scratched the soil surface. This technique removes less SOM, but is less invasive and is used at a greater frequency by practitioners than more aggressive techniques. Although secondary cultivation practices do not reduce thatch depth in the absence of more aggressive techniques, such as hollow-tine cultivation, this study shows that secondary cultivation practices can prevent the excessive accumulation of thatch between applications of more aggressive techniques. Cultivation techniques that penetrate the entire depth of the thatch layer, remove the OM, and allow material with

less OM, such as sand, to be incorporated into the area of removed OM, are critical in the management of putting green OM. Secondary cultivation practices can be used between applications of more aggressive OM control techniques to prevent the excessive accumulation of OM.

Surface Hardness

Before treatments began in 2008, each plot exhibited similar surface hardness values ($g_{\max}$), ranging from 84 to 88 (Table 5.5). Over the 2008 season, secondary cultivation treatments significantly impacted surface hardness (Table 5.1). Although daily grooming produced a more firm surface than the untreated control, both treatments had a similar impact on surface hardness, increasing $g_{\max}$ values 11.5 and 3.6%, respectively (Table 5.5). Weekly vertical mowing increased $g_{\max}$ 35% over the 2008 season to 114.2, which is more firm than daily grooming and the untreated control. However, vertical mowing + grooming created the most firm surface and increased $g_{\max}$ 72% to 144.7 (Table 5.5).

Before treatments began in 2009, both treatments containing vertical mowing had similar surface hardness values at 106.8 and 118.0, respectively, for vertical mowing alone and vertical mowing + grooming (Table 5.5). Grooming alone yielded a $g_{\max}$ value of 103.1, which was not different than vertical mowing alone, but was significantly larger than the 80.7 in the untreated control. After treatments ended in 2009, surface hardness for all treatments increased. Vertical mowing + grooming and weekly vertical mowing increased $g_{\max}$ 22.5 and 17.2%, respectively, over the 2009 season. Daily grooming and the untreated control had similar effects on surface hardness, as they increased surface hardness 8.4 and 14.5%, respectively, over the 2009 season. At the end of 2009, the

control remained the softest surface, followed by grooming, vertical mowing, then vertical mowing + grooming (Table 5.5). Although soil moisture content can affect surface hardness values (Rogers III, 1988), it was not measured in this study. All surface hardness measurements were taken in the afternoon during day on which rain did not fall and irrigation was not applied.

The effect of secondary cultivation on surface hardness has been inconsistent in previous studies. Although Kauffman (2007) found aggressive vertical mowing, to a depth of 2.5 cm, to increase surface hardness in sand-based creeping bentgrass putting greens, McCarty et al. (2007) found both light and aggressive vertical mowing to have no effect on surface hardness. McCarty et al. (2007) also found that plots topdressed with sand to have little effect on surface hardness. The significant impact of grooming and vertical mowing on surface hardness in this study could be a result of the lack of soil disturbance that the previous studies had, the thinning of the turf canopy by cultivation, and allowing more sand topdressing to enter the canopy. Thinning the turf canopy by vertical mowing or grooming provides less plant material to cushion the putting green surface. Allowing more sand topdressing to enter the canopy changes the texture of the soil surface to include more sand, which resists compaction and forms a more firm surface. Untreated control plots increased in surface hardness because of the incorporation of some amount of sand topdressing, but retained more turf cover that cushioned the turf surface. Because the age of this putting green is quite young, further studies assessing the impact of vertical mowing practices on older putting greens is needed.

Topdressing removal

Secondary cultivation treatment, date of collection, and their interaction were all significant factors in the percentage of topdressing material removed by mowing in 2008 and 2009 (Table 5.1). In 2008, the amount of topdressing sand removed by mowing varied due to secondary cultivation treatment at each observation date (Fig. 5.1). More topdressing sand was removed from the untreated control plots on each observation date than from any of the secondary cultivation treatments, indicating that some sort of secondary cultural practice may enhance topdressing incorporation. On 6 August, daily grooming and weekly vertical mowing resulted in removal of similar amounts of topdressing material (0.23 and 0.33%, respectively) (Fig. 5.1). Vertical mowing + grooming removed 0.14% of the applied topdressing, which was significantly less than the daily grooming alone, but similar to weekly vertical mowing. No differences between treatments were present on 20 August; however, on 3 September and 17 September, daily grooming resulted in more topdressing material removed by mowing than weekly vertical mowing. Also, vertical mowing + grooming resulted in less material removed by mowing than either weekly vertical mowing or daily grooming (Fig. 5.1). Differences between treatments increased going into the fall season in 2008, presumably because the cooler temperatures and shorter day length of the fall season slowed turf growth and ability to recover from the injury of cultivation.

The amount of topdressing sand removed by mowing varied due to secondary cultivation treatment only at the last three collection dates in 2009 (Fig. 5.2). On 28 August, 11 September, and 25 September, the untreated control and vertical mowing treatments resulted in similar amounts of topdressing material removed by mowing, averaging 16.5 and 11%, respectively (Fig. 5.2). Grooming alone and vertical mowing +

grooming both resulted in less topdressing material removed by mowing than the untreated control, averaging 6 and 2.8%, respectively. However, grooming and vertical mowing treatments did not differ significantly (Fig. 5.2).

Vertical mowing alone resulted in less topdressing material removed by mowing than the control in 2008 and returned consistently lower values than grooming alone in 2008, but resulted in more topdressing material removed by mowing in 2009 than grooming alone. The larger amounts of topdressing material removed by mowing in 2009 than 2008 could be the result of the age of the putting green and accumulated topdressing applications. Regular topdressing applications began a year after TifEagle sprigs were planted, thus 2008 topdressing applications were made to plots that had not been topdressed the year before. This lack of topdressing left many open spaces in the turf canopy and thatch layer to accept topdressing sand in 2008. In 2009, however, many of these open spaces in the turf canopy and thatch layer had been filled by 2008 topdressing applications, thus less space was available to accept topdressing material.

Data describing the effects of management practices on the removal of topdressing sand by mowing are limited. Stier and Hollman (2003) studied cultivation and topdressing effects on thatch management in creeping bentgrass and annual bluegrass (*Poa annua* L.) and found that vertical mowing decreased the amount of topdressing material removed by mowing. The combination of brushing and vibratory rolling has also been found to decrease the amount of topdressing sand removed by mowing on TifEagle bermudagrass (Chapter 3). The difficulty of incorporating sand topdressing into dense creeping bentgrass and ultradwarf bermudagrass cultivars has been noted, but little research has been performed on the subject (Chapter 3; Foy, 1999; McCarty and

Canegallo, 2005). Vertical mowing and grooming can thin the turf canopy by removing leaf and stolon material, which may facilitate increased topdressing incorporation.

Turfgrass cover

Percent green turf cover varied due to secondary cultivation treatment on the last three rating dates in 2008 (Table 5.1; Fig. 5.3). On the last three rating dates, vertical mowing + grooming yielded 78.5, 75.7, and 73.4% green turf cover, respectively (Fig. 5.3). All other treatments did not differ from each other and had green turf cover percentages greater than 90, 84, and 82%, respectively, at the same three dates. Although not statistically significant, the untreated control tended have less green turf cover during the last three rating dates than either the vertical mowing or grooming by themselves due to scalping of the softer surface during mowing.

In 2009, percent green cover varied due to secondary cultivation treatment on six dates (Table 5.1; Fig. 5.4). On those dates, the untreated control, vertical mowing alone, and grooming alone were not statistically different from each other and averaged 95% green turf cover in late July and early August, while their amount of green cover decreased similarly going into the fall, as temperatures declined and turf entered dormancy (Fig. 5.4). Vertical mowing + grooming consistently had lower percent green turf cover than all other treatments, averaging 85% in late July and early August. On the last two observation dates in 2009, vertical mowing + grooming exhibited at least 10% less green turf cover than all other treatments.

Although the vertical mowing technique in this study is considered a light vertical mowing, it may still be too aggressive for weekly application. Mazur and Wagner (1987) found a similar reduction in turfgrass cover when vertical mowing in two

directions at a 2 mm depth, as did White and Dickens (1984). Vertical mowing also reduced turfgrass cover in a Tifway bermudagrass fairway (Johnson, 1986). White et al. (2004) found a reduction in turf cover and quality for Tifdwarf, Champion, Floradwarf, MiniVerde, and TifEagle in late summer after vertical mowing to be due increased bermudagrass decline. However, those symptoms were not present in this study. In this study, the defoliation caused by vertical mowing appeared to be the cause of the loss in turf cover. A less aggressive vertical mowing depth or less frequent applications may reduce the loss of turf cover.

Turfgrass quality

Turfgrass quality varied due to secondary cultivation treatment, observation date, and their interaction in 2008 and 2009 (Table 5.1). In 2008, turfgrass quality differed between treatments on all dates after 1 August (Fig. 5.5). The untreated control maintained significantly higher turfgrass quality (rating 9.0) throughout the study until the last two rating dates, when quality dropped to 8.3, which was similar to the daily grooming treatment (Fig. 5.5). The decrease in turfgrass quality of the untreated control was the result of scalping that occurred from the softer turf surface. Plots receiving the daily grooming treatment returned higher turfgrass quality ratings (rating 8.0) than either those receiving the weekly vertical mowing treatment or vertical mowing + grooming. The combination of weekly vertical mowing and daily grooming had lower turfgrass quality ratings than all other treatments after 8 August because of the large amount of defoliation caused by the treatments. Turfgrass quality ratings for the vertical mowing + grooming began at 9 on 1 August, went to 7.3 on 8 August, then dropped steadily until 3 October, when a final quality rating of 2.7 was reached (Fig. 5.5). Weekly vertical

mowing treatments produced higher quality values than the combination at all dates after 8 August, but still produced lower quality ratings than daily grooming.

Turfgrass quality as affected by secondary cultivation treatment in 2009, however, was not consistent across observation dates (Fig. 5.6). No significant differences between treatments were seen on 24 July, 14 and 28 August, and 4, 18, and 25 September. On dates where significant differences were apparent, the relationship between treatments was inconsistent. Vertical mowing + grooming returned turfgrass quality ratings at least two units lower than vertical mowing alone, but not different than all other treatments in late July and early August. In late August, grooming alone had the lowest quality rating, while all others did not differ from one another. On 11 September, vertical mowing + grooming had the highest turfgrass quality rating of all treatments (8.5) (Fig. 5.6). However, on 2 October, as dormancy began, a relationship between treatments similar to 2008 developed and vertical mowing + grooming had the lowest quality rating (2.8), while vertical mowing alone had a quality rating of 6.2, and grooming alone and the untreated control had ratings of 8.0 and 8.3, respectively. The inconsistent turfgrass quality ratings could have been the result of a cool and wet summer in 2009 in which damage from secondary cultivation practices was not subjected to heat or drought stress and could recover from damage caused by secondary cultivation. The drop in turfgrass quality of vertical mowing + grooming on 2 October could have been exacerbated by the decreasing temperatures in the fall and the biweekly defoliation during the season, which could have decreased the amount of carbohydrates produced and stored by the bermudagrass plant and allowed the plant to enter an early dormancy (Taiz and Zeiger, 1991).

Vertical mowing and grooming have generally decreased shoot density and quality immediately after treatment. Salaiz et al. (2005) found that turf quality was lower immediately after vertical mowing and increased over time, along with finding that increased vertical mowing frequency decreased turf quality over less frequent treatments. White et al. (2004) found frequent light vertical mowing to produce higher turf quality values than infrequent aggressive vertical mowing. Using grooming as a secondary cultural practice, however, is often less injurious than vertical mowing. Dunnivant (2008) found differing grooming spacings and frequency to have inconsistent effects on shoot density and little sustained impact on overall TifEagle performance.

Conclusions

Secondary cultural practices, such as vertical mowing and grooming, are important factors in ultradwarf putting green management. Daily grooming of TifEagle bermudagrass thinned the turf canopy, enhanced surface hardness, and allowed for increased topdressing incorporation while maintaining acceptable turf cover for much of the season. Weekly vertical mowing to a depth even with the soil line increased turf surface hardness and topdressing incorporation, but reduced thatch depth and TB content. However, vertical mowing at weekly intervals decreased turf quality and cover to unacceptable levels in one year. Vertical mowing + grooming provided the firmest surface, resulted in the largest amount of sand topdressing incorporation, reduced thatch depth, and reduced TB content more than any other treatment in the first year. However, this treatment was much too injurious to the turf to be used in a golf course setting, as it reduced green turf cover and turfgrass quality to unacceptable levels. All treatments aided in keeping SOM within the 4 to 5 % by weight recommendation and decreased TB

content after two years. Many options exist for modifying a secondary cultural practice system, including altering the depths and frequencies of cultivations. The depth and frequency of vertical mowing in this study were too aggressive for golf course use. Either factor could be adjusted to reduce invasiveness and maintain its positive attributes. Because the untreated control returned low topdressing incorporation values, a soft surface, and a deep thatch layer, secondary cultural practices should not be thought of as only surface treatments, but as pieces of a broader thatch management program.

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Appendix D

Table 5.1: Analysis of variance for the effects of cultivation, date of cultivation, and their interaction for evaluations made in 2008 and 2009.

Analysis of Variance							
2008							
Effect	SOM concentration	TB concentration	Thatch depth	Surface hardness	Topdressing removal	Turf cover	Turf quality
	Change % by weight	Change % by weight	Change mm	Change $g_{\max}$	% of applied	% green turf	rating value
Cultivation	NS	***	NS	***	***	*	***
Date	NA	NA	NA	NA	***	***	***
Cultivation $\times$ Date	NA	NA	NA	NA	***	*	***
2009							
Effect	SOM concentration	TB concentration	Thatch depth	Surface hardness	Topdressing removal	Turf cover	Turf quality
	Change % by weight	Change % by weight	Change mm	Change $g_{\max}$	% of applied	% green turf	rating value
Cultivation	***	***	NS	***	**	***	**
Date	NA	NA	NA	NA	***	***	*
Cultivation $\times$ Date	NA	NA	NA	NA	*	*	***

*, **, and *** indicate significance at the 0.05, 0.01, and 0.001 *P* level, respectively.

NS indicates differences not significant.

NA indicates that effects were not determined for that evaluation.

SOM indicates soil organic matter

TB indicates total biomass

Table 5.2: Effect of secondary cultivation on soil organic matter concentration in the upper 2.5 cm of the rootzone in 2008 and 2009.

Treatment	Organic Matter							
	2008				2009			
	Initial	Final	Change	Reduction or increase	Initial	Final	Change	Reduction or increase
	——— % by weight ———			%	——— % by weight ———			%
Vertical Mowing	9.9	6.5	-3.4	-34	4.7 bc	4.6 ab	-0.1	-2.1
Grooming	9.8	7.3	-2.5	-26	4.9 b	4.6 ab	-0.3	-6.1
Vertical Mowing + Grooming	6.5	5.6	-0.9	-13	4.2 c	4.1 b	-0.1	-2.4
Control	7.6	6.8	-0.8	-11	6.1 a	5.1 a	-1.0	-16.4
LSD _(0.05)	NS	NS	NS		0.5	0.5	NS	

Means sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).
NS indicates differences not significant.

Table 5.3: Effect of secondary cultivation on total biomass content to a 2.5 cm depth in 2008 and 2009.

Treatment	Total Biomass							
	2008				2009			
	Initial	Final	Change	Reduction or increase	Initial	Final	Change	Reduction or increase
	—— % by weight ——			%	—— % by weight ——			%
Vertical Mowing	15.6	14.6 b	-1.0 b	-6.4	9.8 bc	5.8	-4.0 a	-40.8
Grooming	15.2	20.2 a	5.0 a	32.9	10.8 b	5.7	-5.1 a	-47.2
Vertical Mowing + Grooming	13.6	9.3 c	-4.3 b	-31.6	8.8 c	5.1	-3.7 a	-42.0
Control	16.6	22.1 a	5.5 a	-33.1	13.6 a	6.1	-7.5 b	-55.1
LSD _(0.05)	NS	3.7	5.4		1.1	NS	1.7	

Means sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).
NS indicates differences not significant.

Table 5.4: Effect of secondary cultivation on uncompressed thatch-mat depth in 2008 and 2009.

Treatment	Thatch-Mat Depth							
	2008				2009			
	Initial	Final	Change	Reduction or increase	Initial	Final	Change	Reduction or increase
	mm	mm		%	mm	mm		%
Vertical Mowing	12.9	14.8	1.9	14.7	20.8	20.2	- 0.6	-2.9
Grooming	13.1	13.1	0	0	20.1	23.1	3.0	14.9
Vertical Mowing + Grooming	13.2	10.4	-2.8	-21.2	16.6	17.1	0.5	3.0
Control	13.1	20.9	7.8	59.5	24.6	27.3	2.7	10.9
LSD _(0.05)	NS	NS	NS		NS	NS	NS	

Means sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).
NS indicates differences not significant.

Table 5.5: Effect of secondary cultivation on surface hardness in 2008 and 2009.

Treatment	Surface Hardness							
	2008				2009			
	Initial	Final	Change	Reduction or increase	Initial	Final	Change	Reduction or increase
	$g_{\max}$	$g_{\max}$		%	$g_{\max}$	$g_{\max}$		%
Vertical Mowing	84.8	114.2 b	29.4 b	34.7	106.8 ab	125.1 b	18.3 ab	17.1
Grooming	88.3	99.9 c	11.6 c	13.1	103.1 b	111.7 c	8.6 b	8.3
Vertical Mowing + Grooming	84.4	144.7 a	60.3 a	71.4	118.0 a	144.6 a	26.6 a	22.5
Control	84.8	88.3 d	3.5 c	4.1	80.7 c	92.4 d	11.7 b	14.5
LSD _(0.05)	NS	6.8	10.2		13.3	13.3	11.5	

Means sharing a letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

NS indicates differences not significant.

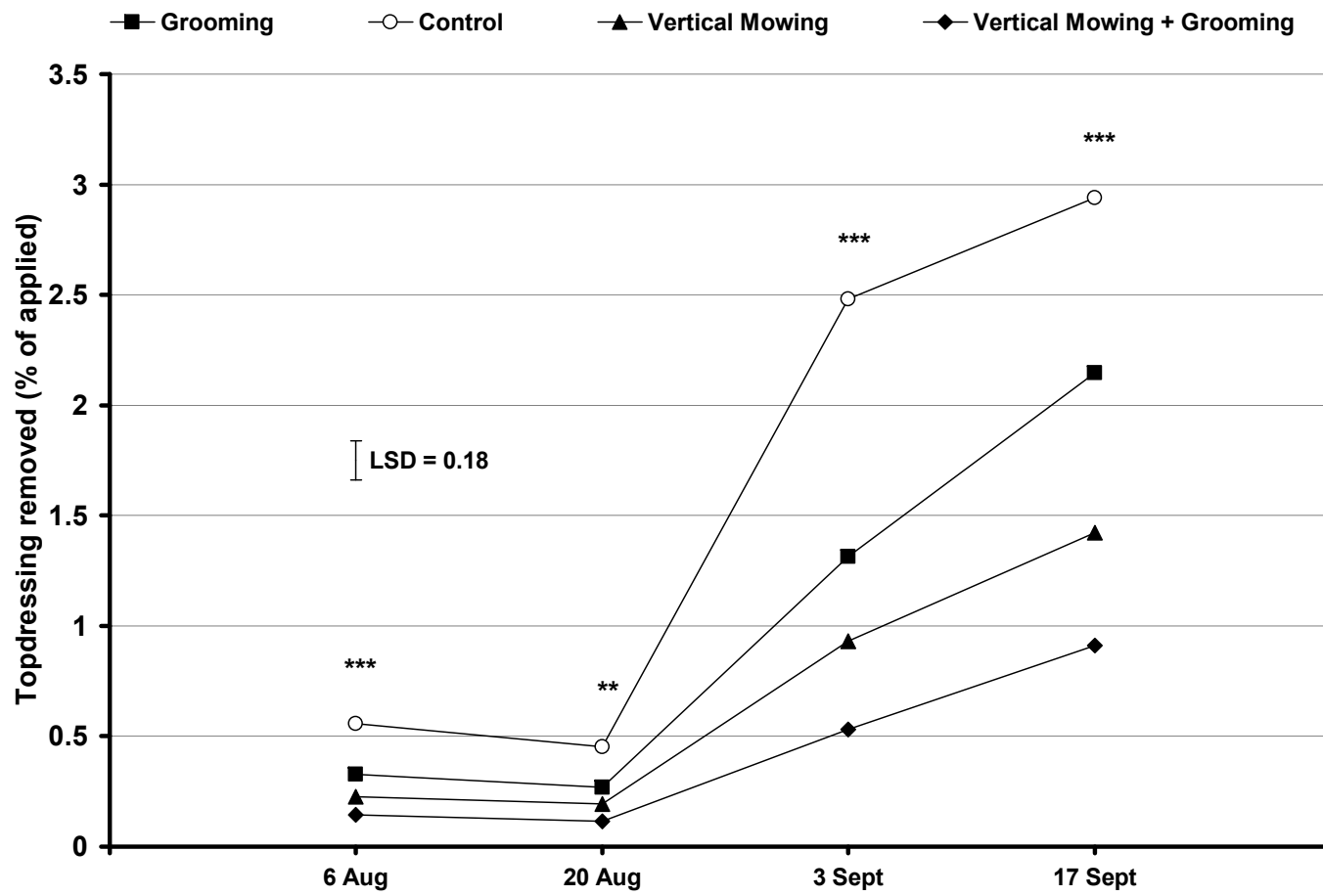


Figure 5.1: Effect of secondary cultivation on amount of topdressing material removed by mowing as a percentage of applied sand in 2008. ** and *** indicate significance at the 0.01 and 0.001 *P* level, respectively.

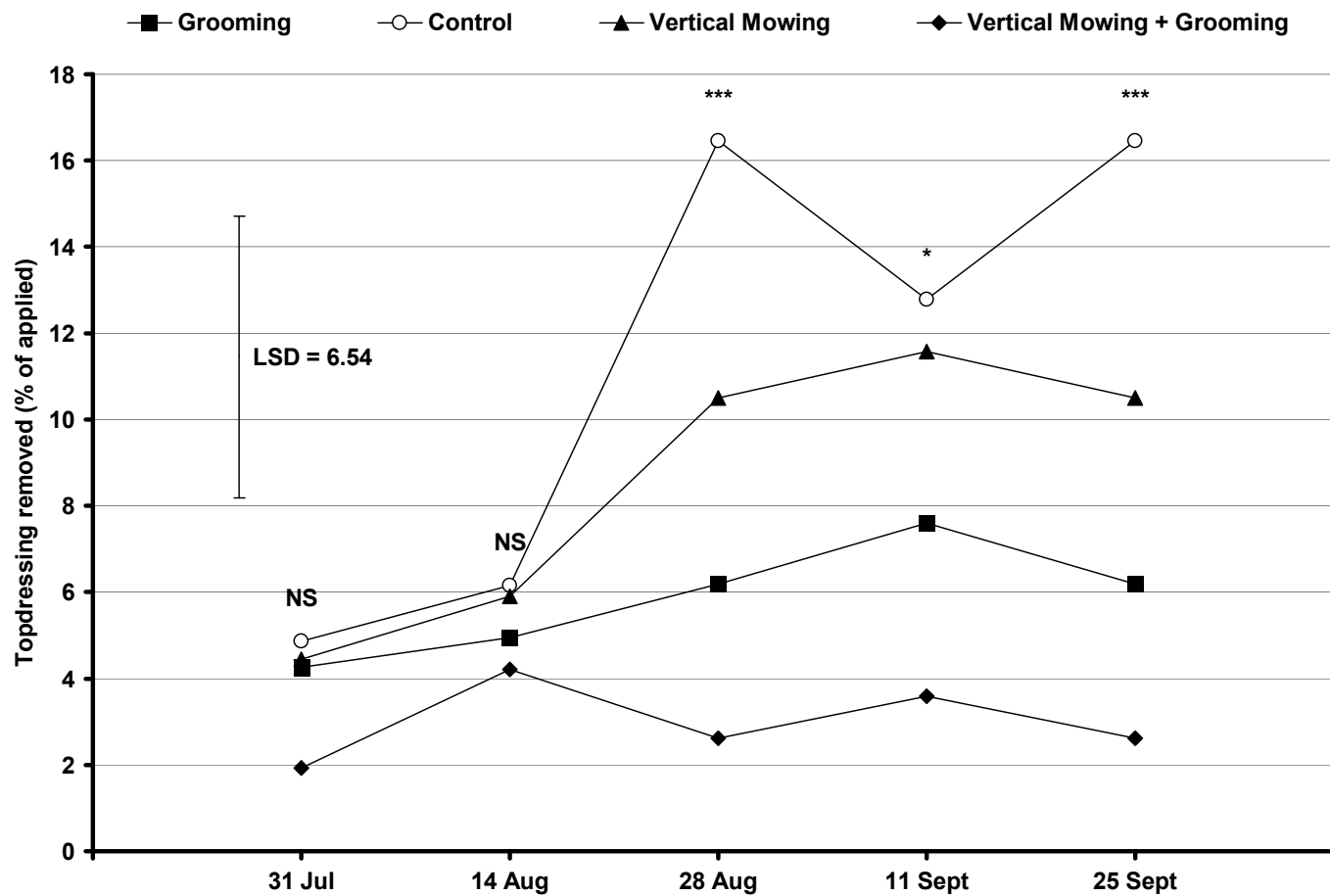


Figure 5.2: Effect of secondary cultivation on amount of topdressing material removed by mowing as a percentage of applied sand in 2009. * and *** indicate significance at the 0.05 and 0.001 *P* level, respectively. NS indicates no significant difference.

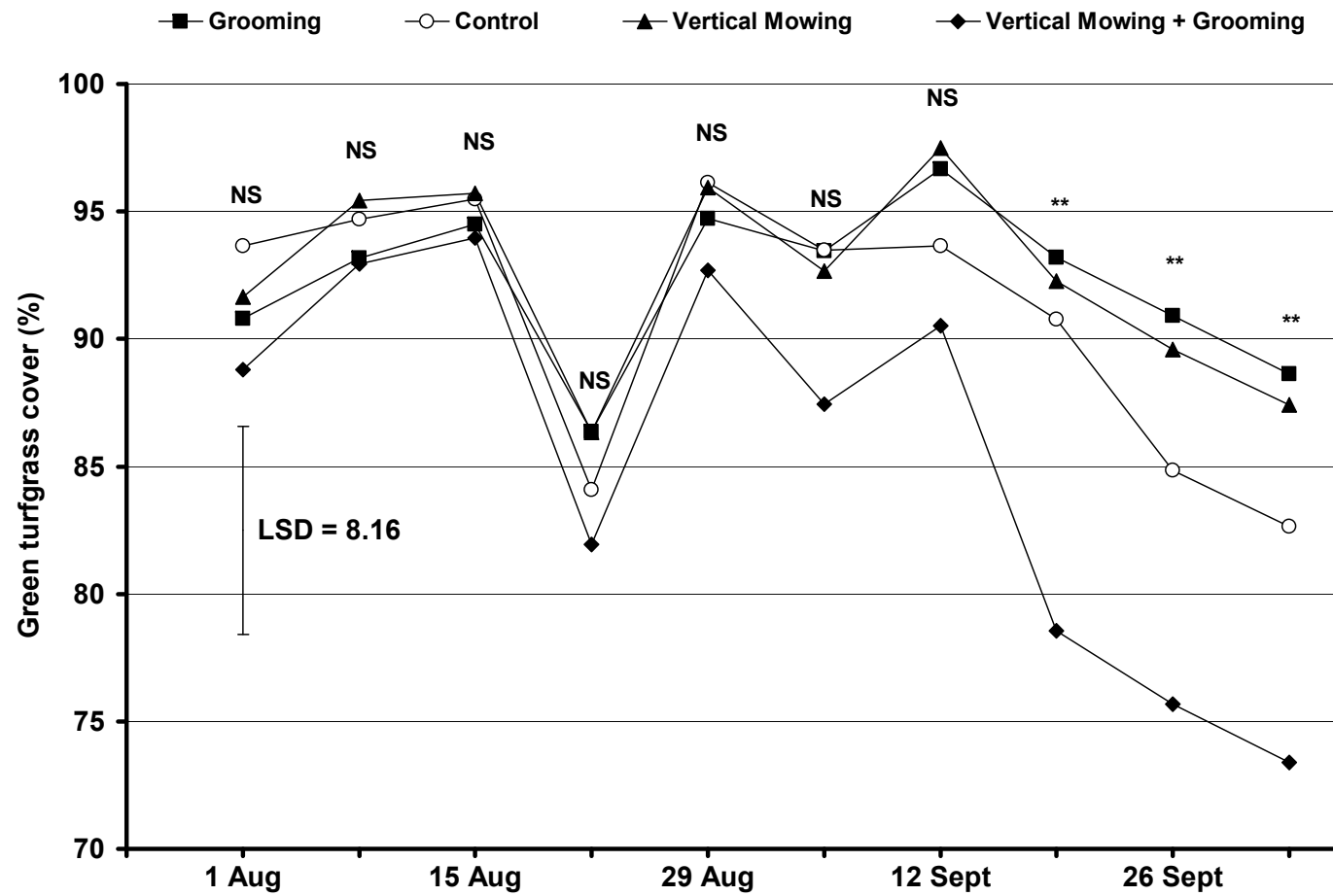


Figure 5.3: Effect of secondary cultivation on percent green turfgrass cover in 2008. ** indicates significance at the 0.01 *P* level. NS indicates no significant difference.

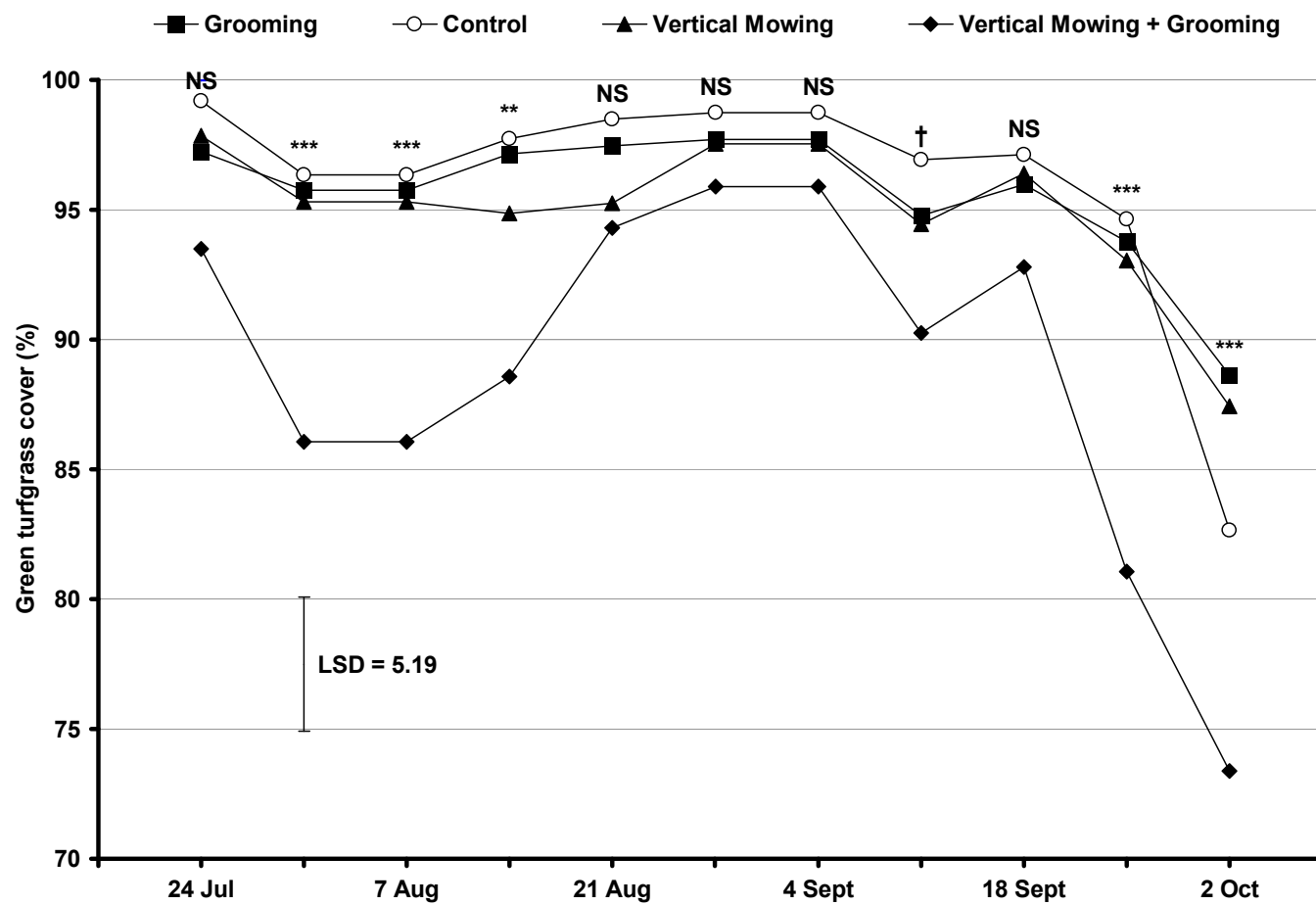


Figure 5.4: Effect of secondary cultivation on percent green turfgrass cover in 2009. †, **, *** indicates significance at the 0.1, 0.01, and 0.001 *P* level, respectively. NS indicates no significant difference.

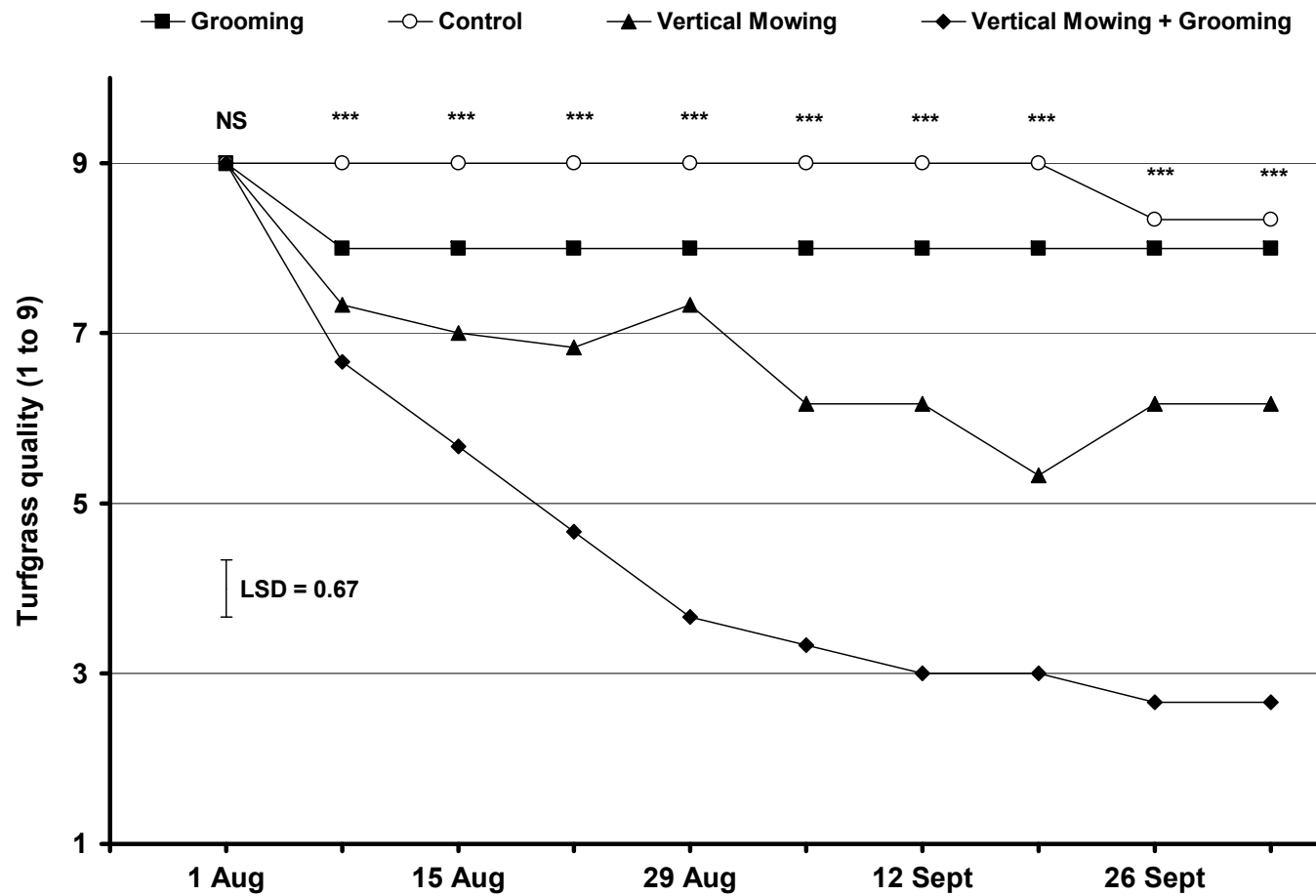


Figure 5.5: Effect of secondary cultivation on turfgrass quality in 2008. Turf quality is rated on a 1-9 scale, with 1 being dead turf, 6 being minimally acceptable, and 9 being ideal turf.

*** indicates significance at the 0.001 *P* level.

NS indicates no significant difference.

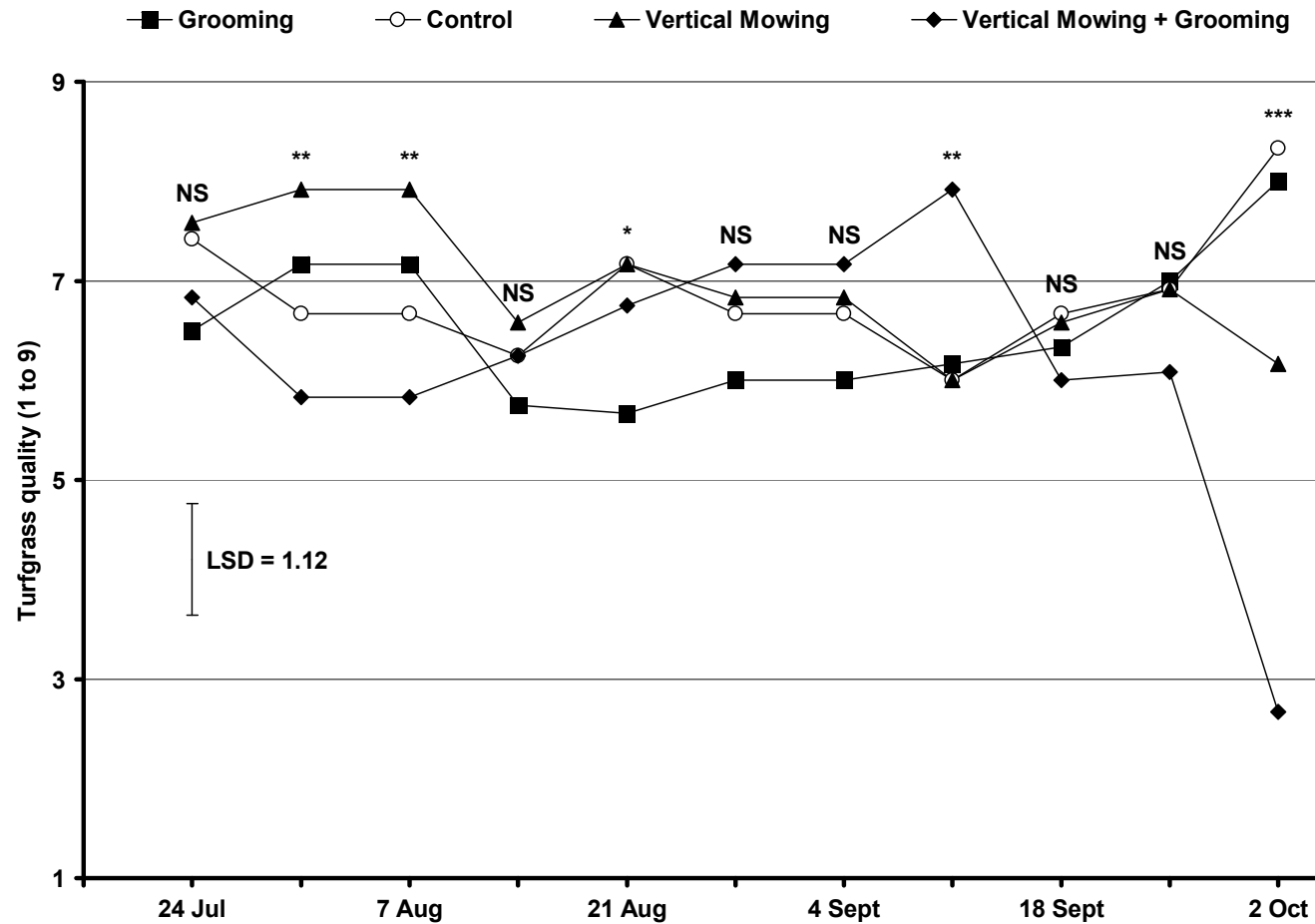


Figure 5.6: Effect of secondary cultivation on turfgrass quality in 2009. Turf quality is rated on a 1-9 scale, with 1 being dead turf, 6 being minimally acceptable, and 9 being ideal turf.

*** indicates significance at the 0.001 *P* level.

NS indicates no significant difference.

**CHAPTER SIX – DETERMINING FREEZE TOLERANCE OF FOUR
WARM-SEASON PUTTING GREEN VARIETIES AND THE
IMPACT OF COLD ACCLIMATION ON PROLINE AND
POLYAMINE ACCUMULATION**

Abstract

A relative lack of cold hardiness and early onset of dormancy are deterrents to widespread use of warm-season turfgrass species for putting greens in the transition zone. Few studies have been conducted to determine the freeze tolerance (LT_{50}) of warm-season putting green species. While proline and polyamines have been linked to cold-hardiness in other plants, their roles in ultradwarf bermudagrass (*Cynodon dactylon* (L.) $\times$ *C. transvaalensis* Burt-Davy), seashore paspalum (*Paspalum vaginatum* Sw.), or ‘Diamond’ zoysiagrass [*Zoysia matrella* (L.) Merr.] freeze tolerance are not well understood. As such, this study sought to determine the relative freeze tolerance (LT_{50}) of ‘Champion’ and ‘TifEagle’ bermudagrass, ‘SeaDwarf’ seashore paspalum, and ‘Diamond’ zoysiagrass, assess the impact of cold acclimation on proline and polyamine concentrations in the stolon and rhizome tissues of these species/varieties, and examine any correlations between cellular contents and freeze tolerance. Cold acclimation was induced with 4 weeks of 8/2° C day/night cycles and a 10 hour photoperiod of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in a controlled environment chamber. The same chamber was used to subject plants to a range of cold temperatures to determine freeze tolerance (LT_{50}). Diamond had the lowest LT_{50} (-10.9° C) of the selected species. Champion and TifEagle did not differ in their freeze tolerance (-9.0 and -10.0° C, respectively), while SeaDwarf was the least freeze tolerant, with an LT_{50} of -7.9° C. Diamond also reached higher amounts of recovery from freezing at particular temperatures than Champion, TifEagle, and SeaDwarf. Cold acclimation increased proline concentration in the rhizomes and stolons of Champion, TifEagle, and Diamond, but not SeaDwarf. However, cold acclimation had

inconsistent effects on polyamines. Spermidine concentration for SeaDwarf increased during cold acclimation, but not for Champion, TifEagle, or Diamond. Putrescine concentrations were highest in the ultradwarf bermudagrass varieties, but lower for SeaDwarf and Diamond. Cold acclimation did increase putrescine concentration in all species/varieties, but neither species/variety nor acclimation status influenced spermine concentrations. Proline concentrations were correlated to freeze tolerance for Champion, TifEagle, and Diamond, but not for SeaDwarf. However, polyamine concentrations were inconsistent between species/varieties and were not linked to freeze tolerance for the grasses in this study, similar to other monocot crops in which polyamines have been studied.

Introduction

Warm-season turf species, such as ultradwarf bermudagrass (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy), seashore paspalum (*Paspalum vaginatum* Sw.), and ‘Diamond’ zoysiagrass [*Zoysia matrella* (L.) Merr.], are gaining popularity for golf course putting green use in the southern United States because of their high shoot density, fine leaf texture, and successful adaptation to the high temperatures and humidity characteristic of summer weather in the region (Beard and Sifers, 1996; Maples, 2001). Warm-season species hold several distinct advantages over cool-season species for putting green use in the transition zone portion of the United States, including resistance to high temperature stress, increased photosynthetic efficiency, and reduced water requirement (Duble, 1996). Summer temperatures in the transition zone, which is a 320 km wide belt centered near 37° N latitude, often exceed the optimal range for cool-season species, but winter temperatures often dip low enough to cause freeze damage and periodic winterkill to warm-season species (Anderson et al., 1997; Anderson et al., 2003; McCarty, 2001; McCarty and Miller, 2002).

Winter injury in turfgrass is often caused by direct or indirect low temperature exposure (McCarty, 2001). Direct low temperature exposure occurs when temperatures drop rapidly and ice crystals rupture cell membranes intracellularly. Indirect low temperature exposure is more common than direct low temperature exposure and occurs when cold acclimated grasses are subject to cold temperatures for prolonged periods (Fry, 1990). Both low temperature conditions commonly result in reduced respiration, enzyme activity, carbohydrate translocation, and protein synthesis, as well as tissue desiccation,

all of which are due to or confounded by membrane dysfunction (McCarty, 2001; Samala et al., 1988; Taiz and Zeiger, 1991).

Plant cell membranes must remain fluid for proper function (Steponkus, 1984; Harwood et al., 1994.). When temperatures approach critical values for chilling injury, cell membranes in cold-sensitive plants may transition from a fluid to a gel-like state, which may inhibit H^+ -ATPase activity, transport, energy transduction, and metabolism (Hale and Orcutt, 1987). Membrane fluidity at lower temperatures is maintained by decreasing the saturation of lipids in the membrane phospholipid bilayer (Steponkus, 1984; Taiz and Zeiger, 1991). Lipid desaturation forms double bonds in the fatty acids, decreasing the freezing point of the bilayer to maintain fluidity. Such lipid desaturation is common in warm-season turfgrasses such as bermudagrass and seashore paspalum, along with other crops (Cyril et al., 2002; Munshaw et al., 2006; Samala et al., 1988; Steponkus, 1984). When cold-sensitive plants undergo acclimation, lipid desaturase activity increases, resulting in greater lipid unsaturation and greater membrane fluidity (Taiz and Zeiger, 1991).

Membrane fluidity in cold weather may also be influenced by polyamines, such as putrescine, spermidine and spermine, which occur ubiquitously in plants and are thought to aid in plant defenses to various environmental stresses (Bouchereau et al., 1999; Flores, 1990; Kumer et al., 1997; Smith, 1985). Because polyamines express a polycationic nature at physiological pH, they can bind strongly to the negative charges of nucleic acids, proteins, and phospholipids (Smith, 1985). The interaction between polyamines and membrane phospholipids may stabilize cell membranes during stress conditions (Roberts et al., 1986). Polyamines also protect membranes against lipid

peroxidation induced by reactive oxygen species (Tadolini et al., 1984). Cold-tolerant plants have been found to increase endogenous polyamine levels in response to freeze acclimation more than plants of lesser cold tolerance (Guye et al., 1986; Kramer and Wang, 1989; Lee et al., 1997; Nadeau et al., 1987). Increased cold-tolerance in cucumber (*Cucumis sativus* L.) was closely related to a lower rate of reactive oxygen species generation caused by increased polyamines (Shen et al., 2000). Polyamines have also been associated with low temperature survival in cabbage (*Pringlea antiscorbutica* R. Br.) (Hummel et al., 2004). However, no polyamine research in regards to cold-tolerance has been studied in turfgrass.

Proline accumulates in plants during dehydrative stress and aids in osmotic adjustment to decrease cellular water potential, quenches singlet oxygen species known to increase during freezing, and helps to stabilize membranes during dehydrative stresses such as freezing (Kavi Kishor et al., 1995; Kendall and McKersie, 1989; Matysik et al., 2002; Santarius, 1992; Saradhi et al., 1995; Rudolph et al., 1986). Freeze tolerance in crops such as alfalfa (*Medicago media* Pers.) (Paquin, 1977), potato (*Solanum* spp.) (van Swaaij et al., 1985), orange (*Citrus sinensis* L.) (Yelenosky and Vu, 1992), and maize (*Zea mays* L.) (Chen and Li, 2002), as well as bermudagrass and centipedegrass [*Eremuchloa ophiuroides* (Munro) Hack] (Cai et al., 2004; Munshaw et al., 2004), have been correlated to increases in proline concentration. Research by Akiyama et al. (1994) showed that high proline concentrations in the leaves of three zoysiagrass species increased cold hardiness. Additionally, Levitt (1980) showed that proline levels increase during cold acclimation, decrease during de-acclimation, and are present in greater concentration in cold-hardy plants.

Cold hardiness of warm-season turfgrasses, such as bermudagrass and zoysiagrass, has traditionally been evaluated in the field, in which regrowth is evaluated in the spring following a severe winter (Anderson et al., 1988). Although this method is considered a reliable indicator of cold hardiness, a minimum of two years of data collection is required because temperature, moisture, and snow cover differences between years may alter survival rates (Anderson et al., 2002). Additionally, not all winters exhibit conditions extreme enough to be considered sufficient indicators of cold hardiness and may not distinguish hardiness between cultivars. Anderson et al. (2003) developed a quick and repeatable regrowth method for quantifying the LT_{50} value of bermudagrass that closely matches field test observations. Defined as the temperature at which 50% of the test population does not survive, LT_{50} is a common measure of lethal temperatures. In this method, plants are cold acclimated in a controlled-environment chamber set at 8° C and 2° C day/night cycles with a 10 h photoperiod at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light for four weeks. Controlled-environment chambers provide an acclimation setting for plants that is independent of yearly fluctuations of field trials (Cardona et al., 1997) and have been used successfully to acclimate and evaluate freeze tolerance of other warm-season species such as buffelgrass (*Pennisetum ciliare* L.), seashore paspalum (*Paspalum* spp.), and zoysiagrass (*Zoysia* spp.) (Cardona et al., 1997; Patton and Reicher, 2007; Stair et al., 1998).

Anderson et al. (2002) evaluated differences in survival of bermudagrass cultivars grown at putting green and fairway heights using this laboratory freezing test and found that cultivars maintained for putting green use were generally less cold tolerant than those maintained at fairway height. ‘Tifdwarf’ and ‘Tifgreen’ had LT_{50} values of -6.5° C,

‘MiniVerde’ and ‘TifEagle’ had LT₅₀ values of -6° C, and ‘Champion’, ‘Floradwarf’, and ‘MS Supreme’ had LT₅₀ values of -5° C. At fairway height ‘Midlawn’, ‘Quickstand’, ‘Yukon’, and ‘Guymon’ bermudagrasses had LT₅₀ values of -8.4, -8.0, -7.6, and -7.4° C, respectively. Similarly, Patton and Reicher (2007) evaluated differences in survival of zoysiagrass species and cultivars using a laboratory freezing test. They found Diamond, the only zoysiagrass cultivar potentially suitable for putting green use, to be less tolerant of cold temperatures, with an LT₅₀ of -8.4° C, than the higher mown ‘Meyer’ and ‘Zenith,’ with LT₅₀ values of -11.5° C.

The role of proline and polyamines in the freeze tolerance of warm-season putting green species and varieties is not well known. Understanding how proline and polyamine concentrations vary between species and varieties, along with understanding how cold acclimation impacts proline and polyamine status in warm-season putting green turfs, can lead to valuable information for turf breeders and managers in the transition zone. Thus, the objectives of this study were to examine the freezing tolerance of two commonly used ultradwarf bermudagrass cultivars, TifEagle and Champion, SeaDwarf seashore paspalum, and Diamond zoysiagrass, quantify proline concentrations and polyamine concentrations between the species/varieties, examine any correlation between freeze tolerance and proline or polyamine accumulation, and determine the influence of cold acclimation on proline and polyamine concentrations of these warm-season putting green turfs.

Materials and Methods

Freeze Tolerance Champion and TifEagle bermudagrass, Diamond zoysiagrass, and SeaDwarf seashore paspalum, as well as ‘Tifway’ bermudagrass and ‘Southern

Dominant' creeping bentgrass, were established from sod the greenhouse for four weeks at 27/20° C under natural photoperiod (approximately 10 hour photoperiod). Plugs measuring 5 cm in diameter were removed from the sod and placed in conetainers (Ray Leach Cone-tainer Nursery, Canby, OR) filled with a potting mix (BM-2, Berger Peat Moss Ltd., Saint-Modeste, PQ, Canada). After two weeks of additional growth in the greenhouse, the conetainers were placed in a controlled-environment chamber (Thermotron 2800, Thermotron Co., Holland, MI) in a completely randomized fashion for cold acclimation. Cold acclimation was performed using the controlled-environment chamber set at 8/2° C day/night cycles and a 10 hour photoperiod of 300 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetically active radiation, similar to the methods of Anderson et al. (1988) and Patton and Reicher (2007). Plants were removed from the chamber every three days for watering and were re-randomized before replacement into the chamber. After four weeks of cold acclimation, some samples were removed for tissue analysis, while others remained in the chamber for cold stress simulation.

Cold stress simulation was performed using the same controlled-environment chamber from cold acclimation. After cold acclimation, the chamber was held at -3° C for 15 h to simulate a cold night in the transition zone, then programmed to cool 1° C h⁻¹ and set t cover a range from complete survival to complete death (-6° C to -20° C), similar to Anderson et al. (1993). Six conetainers of each species/variety were removed from the freeze chamber at each temperature interval, thawed in a 5° C refrigerator for 12 h, then transferred to a greenhouse and evaluated for regrowth for four weeks, in a procedure similar to that used by Patton and Reicher (2007). The plants remaining in the chamber were re-randomized before replacement into the chamber each time samples

were removed. The temperature resulting in no regrowth from 50% of the plants (LT₅₀) was determined using a nonlinear regression equation from Ingram and Buchanan (1984), also used by Anderson et al. (2002), Cardona et al. (1997), Stair et al. (1998), Vainola et al. (1999), and Patton and Reicher (2007) to predict survivability of bermudagrass, seashore paspalum, and zoysiagrass, respectively. Similar to Patton and Reicher (2007), Anderson et al (2002), and Cardona et al. (1997), freeze tolerance was determined using the following equation:

$$\text{Survival} = a + (b-a)/(1 + e^{c(T_m-T)})$$

where a is the base line of survival, b is the maximum survival, c is a function of the slope of the line at the inflection point, T_m is the temperature at the inflection point, which is also the LT₅₀, and T is the treatment temperature. This experiment was repeated four times.

Tissue Preparation for Proline and Polyamine Analysis

Because of their similar anatomy, freeze tolerance, and winter carbohydrate concentrations, stolons and rhizomes from each species/variety were used for proline and polyamine analysis (Rogers et al., 1975, 1977). Samples were taken before and after cold acclimation, washed of soil, placed in liquid N to halt respiration, then stored at -80°C. Samples for proline analysis were lyophilized (Freezone 6, Labconco, Kansas City, MO), then ground with a mortar and pestle in liquid N and stored at -80°C until analyzed. Samples for polyamine determination were not lyophilized, but were analyzed on a fresh weight basis.

Proline Determination

A modification of the Bates et al. (1973) procedure was used to extract and quantify proline, similar to Patton and Reicher (2007). Fifty mg of lyophilized, ground stolon and rhizome tissue from each variety was combined with 1 mL of 3% sulfosalicylic acid, then vortexed three times at 15 s each to suspend tissues. After vortexing, 100 μ L aliquots were placed into test tubes and raised to 1 mL with double-deionized water. One mL acid ninhydrin and 1 mL glacial acetic acid were then added. The test tubes were again vortexed for 15 s and incubated for 1 h at 100°C. After terminating the reaction by placing the test tubes in an ice bath, 4 mL of toluene will be added to each tube and the tubes were vortexed for 20 s and 5 minutes were allowed for phase separation. Three mL of the upper chromophore containing toluene were pipetted into 13 mm test tubes and their absorbance read at 250 nm in a spectrophotometer (BioMate 5, Thermo Electron Corporation, Waltham, MA) using toluene as a blank. Proline concentrations were determined using L-proline as a standard.

Polyamine Determination

A modification of the Hennion and Martin-Tanguy (1999) procedure was used to determine polyamine content. Twenty mg of rhizome and stolon tissues were ground in liquid N₂, then mixed with 0.5 mL of 1 M perchloric acid. After extraction for 1 h, samples were centrifuged at 18,000 *g* for 30 min and the supernatant, containing free amines, was used for analysis. The amine fraction was analyzed by HPLC using LKB 2152 plus and LKB 2150 chromatography system through a 4.6 x 250 mm, 5 μ m reverse-phase C₁₈ column. Twenty μ L samples of the amine fractions were applied to the column and eluted with a methanol:water solvent gradient, changing from 60% to 95% over 23

min at a flow rate of 0.8 mL min⁻¹. Elution was completed after 7 min. For detection of dansyl amines, an excitation wavelength of 365 nm was used, with an emission wavelength of 510 nm. Results were standardized with equimolar (0.1 nmol) mixtures of dansylated amines.

Data analysis

Freeze tolerance data were fit to sigmoidal curves using PROC NLIN (Gauss-Newton method) (Fig. 6.1) and LT₅₀ values were analyzed using PROC ANOVA (SAS Institute, Cary, NC). Means were separated using Fisher's protected least significant difference when *F* tests were significant at $\alpha \leq 0.05$. Regrowth data were analyzed with the mixed models procedure in SAS statistical analysis software (SAS Institute, Cary, NC) as a completely randomized design with six samples per each of four replications. Orthogonal polynomial contrasts were used to determine the relationship of temperatures within weeks and weeks within temperatures. Differences among proline and polyamine concentrations in species/varieties and acclimation status were analyzed using the mixed model procedure of SAS statistical analysis software (SAS Institute, Cary, NC) as a completely randomized design with three samples per each of three replications, since the cost of processing limited the number of samples that could be processed. Means were separated using Fisher's protected least significant difference when *F* tests were significant at $\alpha \leq 0.05$. Correlation coefficients between LT₅₀ and cell content in cold acclimated tissue and non-acclimated tissue were determined using the correlation procedure in SAS (Kuehl, 2000).

Results and Discussion

Freeze Tolerance

Freeze tolerance (LT_{50}) of warm-season putting green species/variety ranged from -7.8°C (SeaDwarf) to -10.9°C (Diamond) (Fig. 6.2). Champion had a LT_{50} value of -9°C , which was not different from SeaDwarf, but higher than Diamond. Additionally, TifEagle reported a LT_{50} value of -10°C , which is not different than Champion or Diamond, but is lower than SeaDwarf. The LT_{50} of the experimental control ‘Tifway’ bermudagrass was -12.3°C , which was similar to previous reports (Anderson et al., 1993). Additionally, ‘Southern Dominant’ creeping bentgrass had a LT_{50} outside the range of temperatures used for this experiment ($<-20^{\circ}\text{C}$), which was consistent with the reported LT_{50} of other creeping bentgrass cultivars (Gusta et al., 1980).

Diamond yielded a lower LT_{50} value in this study than what had been previously reported (Patton and Reicher, 2007; Zhang et al., 2006). Studies investigating the impacts of late season N and trinexapac-ethyl applications, as well as painting and covering warm-season putting greens during the winter, found Diamond to enter dormancy later and emerge from dormancy earlier than ultradwarf bermudagrass cultivars, as well as lose less turf to winter injury (unpublished data). The lack of freeze tolerance for SeaDwarf also corresponds to field observations made during the same studies (unpublished data). Additionally, this study found Champion and TifEagle to have similar freeze tolerances to earlier reports (Anderson et al., 2002).

The difference in LT_{50} value for Diamond between what was seen here and the higher LT_{50} value reported by Patton and Reicher (2007) may be due to a difference in cold chamber methods or greenhouse growth methods prior to freezing. This study used

a cold chamber that was specifically designed for this use and had an air circulation system designed to keep temperatures as consistent as possible during operation. Patton and Reicher (2007) used a modified chest freezer that may not have kept temperatures as consistent inside the cooling unit. Additionally, this study transferred full plugs of washed sod to containers for freezing treatments. Patton and Reicher (2007) planted one phytomere, or shoot containing one growing point and at least one leaf, into each container, then allowed the plants to grow and fill the container. Using a single phytomere per container could have produced a turf plug that was not as mature as those harvested from sod or grown in the field, which may not have been an accurate representation of field conditions.

Low temperatures near the reported LT_{50} values for these warm season putting green species/varieties can be reached on multiple occasions during the winter in the transition zone, making low temperature stress an important factor in cultivar selection for putting greens. However, freeze tolerance is not necessarily indicative of absolute cold hardiness because environmental factors such as soil moisture, duration of cold temperature, and sunlight, which are not captured in controlled chamber simulation (Anderson et al., 2002).

Cold temperature recovery

Regrowth from simulated cold temperature stress in the cold chamber for each species/variety varied by week and temperature (Tables 6.4 to 6.7). Champion, TifEagle, SeaDwarf, and Diamond exhibited linear and cubic trends in each week after cold temperatures were applied (Tables 6.4 to 6.7); however, quadratic trends were inconsistent. Across weeks, Champion, TifEagle, SeaDwarf, and Diamond exhibited

linear trends in regrowth percentage at each temperature with statistically significant differences, but quadratic trends were inconsistent (Tables 6.4 to 6.7).

Regrowth percentages differed each week and each temperature for Champion, TifEagle, SeaDwarf, and Diamond, except for -15° C and -20° C, which did not differ across weeks for any species/variety (Tables 6.4 to 6.7). No species/variety reached 50% regrowth in the first week after cold simulation was applied. Champion reached 50% regrowth after two weeks at -3° C, and three weeks for -9° C (Table 6.4), while TifEagle reached 50% regrowth after two weeks at -7° C, three weeks at -8° C, and four weeks at -9° C (Table 6.5). Thus, TifEagle reached higher regrowth percentages earlier at lower temperatures than Champion. Regrowth did not reach 50% for temperatures $\leq -10^{\circ}\text{C}$. Four weeks after cold simulation, the maximum regrowth percentage for Champion was 79%, but was 96% for TifEagle, suggesting that TifEagle may be more aggressive than Champion during recovery from sub-lethal cold temperatures.

SeaDwarf did not reach 50% regrowth during the first two weeks after cold simulation, but Diamond reached 50% regrowth during the second week after cold simulation (Tables 6.6; 6.7). SeaDwarf reached 50% regrowth after three weeks at -8° C, but did not reach 50% regrowth for any temperature under -8° C (Table 6.6). Diamond reached 50% regrowth after two weeks at -7° C, three weeks at -9° C, and four weeks at -10° C (Table 6.7), indicating that Diamond will recover to 50% coverage more quickly and from lower temperatures than all other species/varieties. Similar to Champion, SeaDwarf reached a maximum regrowth of 76% after four weeks, while Diamond reached a maximum regrowth of 92% after four weeks, similar to TifEagle (Table 6.5).

Minimal data exists on expected recovery times and percentages for cold chamber studies. Patton and Reicher (2007) correlated cold chamber values to winter survival in the field of several *Zoysia* species and cultivars, while Munshaw et al. (2006) assessed spring greenup of four bermudagrass cultivars subjected to freezing, nitrogen applications, seaweed extract applications, and iron applications in the field. However, neither study reported the expected recovery times and percentages for their cold chamber work. This data from this study can be used by turf managers to select putting green species and varieties that are best suited for the manager's climate, as well as serving as a guide for turf managers to know how much injury to expect at different cold temperatures and how long recovery can be expected.

Proline

Proline concentrations varied according to species/variety and acclimation status (Table 6.1). Non-acclimated (NCA) plants had proline concentrations ranging from 6.6 to 66.6 $\mu\text{mol g}^{-1}$ dry wt., while cold-acclimated plants had proline concentrations ranging from 40.6 to 145.4 $\mu\text{mol g}^{-1}$ dry wt. (Table 6.8). However, proline concentration may vary naturally between species and cultivars. Proline concentrations increased after cold acclimation for all species/varieties except SeaDwarf, which had lower proline concentrations after acclimation than before. Although Diamond had the lowest proline concentration among the three species/varieties that increased proline concentrations (77.9 $\mu\text{mol g}^{-1}$ dry wt.), it had the largest increase in proline concentration during cold acclimation (1080%) and had the lowest LT_{50} value in this study (Table 6.8; Fig. 6.2). TifEagle had a higher proline concentration after cold acclimation than Diamond (95.2 $\mu\text{mol g}^{-1}$ dry wt.), yet had a lower increase in proline concentration after acclimation than

Diamond (319.4%) and a similar LT₅₀ value (Table 6.8; Fig. 6.2). Champion produced more proline than both TifEagle and Diamond after acclimation (145.4 $\mu\text{mol g}^{-1}$ dry wt.), yet had an increase in proline concentration after acclimation similar to TifEagle (294.0%) and an LT₅₀ value less than Diamond, but similar to TifEagle. This data suggests that the relative proline concentration between species/varieties is not as important for freeze tolerance as the magnitude of the increase in proline concentration.

Many plants, such as bermudagrass, centipedegrass, zoysiagrass, barley, potato, and maize, are known to accumulate proline during the cold acclimation process (Paquin, 1977; Dorffling et al, 1997; Chen and Li, 2002; Munshaw et al., 2006; Patton et al., 2007). Similar to the findings of this study, Munshaw et al. (2006) reported proline to be linked to freeze tolerance of 'Midiron', 'Riviera', 'Tifway', and 'Princess-77' bermudagrass. Also, Patton et al. (2007) found proline to be correlated to freeze tolerance of different *Zoysia* species, including *Zoysia matrella* (L.) cv. Diamond.

In this study, proline concentration decreased by 39% after cold acclimation for SeaDwarf, while all other species/varieties increased proline concentration after acclimation. Previous research has not always found a link between proline concentration and cold tolerance. Hoffman et al. (2010) did not find a consistent link between proline and freeze tolerance of perennial ryegrass (*Lolium perenne* L.). Similarly, Dionne et al., (2001) reported inconsistent relationships between proline concentration and freeze tolerance in *Poa annua* (L.). Additionally, Wanner and Junttila (1999) and Koster and Lynch (1992) reported inconsistent relationships between proline and freeze tolerance in *Arabidopsis* and 'Puma' rye (*Secale cereale* L.), suggesting that proline accumulation occurs primarily from low temperature exposure and that plants not

accumulating proline are not acclimating to cold conditions. Perhaps SeaDwarf does accumulate proline during cold weather because such weather is uncommon in its native areas, the sub-tropical and tropical coastal regions of North and South America (Duncan and Carrow, 2000).

Across all species/varieties, stolon and rhizome concentrations of proline in the CA plants were not correlated with their LT₅₀ (Table 6.2). This lack of correlation is not consistent with reports of proline concentration and freeze tolerance on *Zoysia* spp. and other crops (Patton et al., 2007; Akiyama et al., 1994; Paquin, 1977; Dorffling et al., 1997; Chen and Li, 2002; Munshaw et al., 2006). However, when SeaDwarf is removed from the analysis, proline concentration is positively correlated with LT₅₀ (Table 6.3), indicating that seashore paspalum may accumulate proline differently in response to cold acclimation than many other plants. This could explain the relative lack of cold tolerance of SeaDwarf.

Although the physiological basis of previous correlations between proline concentration and freeze tolerance is not entirely understood, proline accumulation was found to stabilize chloroplast membranes of spinach under freezing conditions (Santarius, 1992). Patton et al. (2007) found proline concentrations to be less beneficial for freeze tolerance at lower temperatures than higher temperatures in *Zoysia* spp., but still found a correlation between proline concentration and freeze tolerance. Further research may be needed to elucidate the specific cold tolerance mechanism of seashore paspalum.

Spermine

Spermine concentrations were log transformed to correct for non-normality of the data distribution. Differences between species/variety, acclimation status, and their

interaction were not significant for spermine concentration (Table 6.1). Back-transformed spermine concentrations ranged from 0 to 1.3 nmol g⁻¹ fresh wt. across species/varieties and acclimation status, indicating no significant difference in spermine concentration between species/varieties and no change in spermine concentration during cold acclimation (data not shown). Spermine concentrations were also not correlated to freeze tolerance, indicating that rhizome and stolon spermine concentration is not an important factor in freeze tolerance of Champion, TifEage, SeaDwarf, or Diamond (Table 6.2).

The specific role of spermine in plant freeze tolerance is not known (Flores, 1990; Roberts et al., 1986). Songstad et al. (1989) and Shen et al. (2000) reported a similar lack of spermine concentration in maize (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) tissues during chilling, concluding that spermine was not important in their freeze tolerance. Lee et al. (1997) also found spermine concentrations to be unchanged by cold acclimation of rice (*Oryza sativa* L.) seedlings. Similarly, spermine does not appear to be important in cold tolerance of Champion and TifEagle ultradwarf bermudagrass, SeaDwarf seashore paspalum, or Diamond zoysiagrass.

Spermidine

Spermidine concentrations were square root transformed transformed to correct for non-normality of the data distribution. Differences in spermidine concentration were influenced by the interaction of species/variety and acclimation status (Table 6.1) and back-transformed spermidine concentrations ranged from 0.4 to 13.2 nmol g⁻¹ fresh wt. (Fig. 6.4). Before cold acclimation, Diamond had the highest spermidine concentration (9.1 nmol g⁻¹ fresh wt.) of all species/varieties. Champion, TifEagle, and SeaDwarf had

spermidine concentrations that were lower than those of Diamond, but were not different from each other (2.1, 0.4, and 3.6 nmol g⁻¹ fresh wt., respectively). After cold acclimation, Diamond and SeaDwarf had similar spermidine concentrations (9.3 and 13.2 nmol g⁻¹ fresh wt., respectively). Champion and TifEagle also had spermidine concentrations that were similar to each other (0.4 and 0.3 nmol g⁻¹ fresh wt., respectively), yet were lower than Diamond and SeaDwarf. However, the change in spermidine concentration for Champion, TifEagle, and Diamond after cold acclimation was similar, as Champion decreased spermidine concentration by 81%, TifEagle by 25%, and Diamond increased spermidine concentration by 2.2% (Table 6.8). To the contrary, SeaDwarf increased spermidine concentration by 266% after cold acclimation (Table 6.8).

Spermidine concentration in cold acclimated tissues was not correlated to freeze tolerance with SeaDwarf included or removed (Tables 6.2; 6.3). The most freeze tolerant and least freeze tolerant species/varieties, Diamond and SeaDwarf, had similar spermidine concentrations that were several times higher than those of Champion, which was not different from SeaDwarf in terms of freeze tolerance, and TifEagle, which was not different from Diamond in terms of freeze tolerance.

The influence of spermidine on freeze tolerance is inconsistent in previous work. Shen et al. (2000) found spermidine to increase in leaves of cucumber (*Cucumis sativus* L.) during chilling and correlated spermidine to cucumber freeze tolerance. Kasukabe et al. (2004) found freezing tolerance to increase in *Arabidopsis thaliana* mutants overexpressing spermidine synthase. However, Songstad et al. (1990) and Lee et al. (1997) found spermidine to have little impact on freezing tolerance of

maize and rice. Perhaps spermidine is more important in freezing tolerance of dicot plants than monocots, which could explain the lack of correlation of spermidine and LT_{50} in the grasses studied here, as well as the discrepancy in the work of Shen et al. (2000) and Kasukabe et al. (2004) versus that of Songstad et al (1990) and Lee et al. (1997).

Putrescine

Putrescine concentrations did not require transformation, since the data were normally distributed. Putrescine concentrations differed between species/variety and acclimation status separately, but did not differ for their interaction (Table 6.1). Differences in putrescine concentrations ranged from 36.0 nmol g⁻¹ fresh wt. to 146.2 nmol g⁻¹ fresh wt (Table 6.8). Before cold acclimation, Diamond and SeaDwarf had similar Putrescine concentrations (40.9 and 36.0 nmol g⁻¹ fresh wt., respectively). Champion and TifEagle also did not differ in non-acclimated Putrescine concentrations (87.9 and 98.0 nmol g⁻¹ fresh wt., respectively). Also, putrescine contents for Diamond and Champion did not differ significantly. After cold acclimation, Diamond and SeaDwarf increased Putrescine contents by similar percentages (167.3 and 191.9%, respectively). Champion and TifEagle increased putrescine contents by 66.3 and 68.7 %, respectively, which was not less than SeaDwarf, but not significantly different from Diamond.

Putrescine concentrations in cold acclimated tissues were not correlated to freeze tolerance when analyzed both with and without SeaDwarf (Tables 6.2; 6.3). The most freeze tolerant and least freeze tolerant species/varieties, Diamond and SeaDwarf, had similar increases in putrescine concentrations, which were nominally larger than those of TifEagle, which was freeze tolerant to a lower temperature than SeaDwarf, and

Champion, which had a freeze tolerance similar to SeaDwarf. An increase in putrescine concentration has led to increased cold tolerance in rice, wheat (*Triticum aestivum* L.), alfalfa (*Medicago sativa* L.), maize, cucumber, and *Arabidopsis* (Lee et al, 1995; Lee et al., 1997; Songstad et al., 1990; Nadeau et al., 1987; Kasukabe et al., 2004).

Additionally, root growth of *Pringlea antiscorbutica* increased in plants with higher putrescine concentrations (Hummel et al., 2004), indicating that putrescine can aid in biological functions during low temperature stress. Thus, the increased cold tolerance from an increase in putrescine concentration in this study matches previous studies.

However, putrescine content in SeaDwarf increased as much as after cold acclimation as putrescine in content in Diamond, which was more freeze tolerant than SeaDwarf. Verma and Mishra (2005) reported that increased putrescine levels increased glutathione and carotenoid levels in salt-stressed *Brassica juncea*. As well, increased putrescine levels reduced H₂O₂ levels, reduced lipid peroxidation, and decreased electrolyte leakage in salt-stressed *Brassica juncea*. Because SeaDwarf seashore paspalum is a salt-tolerant species (Duncan and Carrow, 2000), it may have a genetic tendency to accumulate putrescine for salt stress conditions, possibly indicating that increased putrescine levels in SeaDwarf are not involved in cold tolerance.

Conclusions

Freeze tolerance is an important factor when selecting species or varieties for warm-season putting green use in the transition zone. Winter temperatures often dip below the freezing point and can cause injury to dormant turf. SeaDwarf seashore paspalum yielded an LT₅₀ value of -7.9° C, the highest of all species/varieties tested. Champion and TifEagle bermudagrass did not significantly differ in their freeze

tolerance, yielding LT_{50} values of -9.0 and -10.0°C , respectively. Diamond zoysiagrass had the lowest LT_{50} of all the selected species/varieties at -10.9°C , which differed from previous research that found Diamond to be much less freeze tolerant. Proline concentrations in stolon and rhizomes differed by species/variety and acclimation status. Cold acclimation increased cellular proline concentrations increased for all species/varieties except for SeaDwarf, in which proline concentrations decreased after cold acclimation. Proline concentrations increased with freeze tolerance for Champion, TifEagle, and Diamond, but not SeaDwarf. Among the polyamines, only spermidine and putrescine concentrations differed with cold acclimation and species/variety. Cold acclimation increased spermidine concentrations in SeaDwarf, but not Diamond, Champion, or TifEagle. Putrescine concentrations were highest in Champion and TifEagle, but the same for SeaDwarf and Diamond. Additionally, cold acclimation increased putrescine accumulation. SeaDwarf may have behaved differently to cold acclimation than Champion, TifEagle, or Diamond because cold temperatures are not often seen in the sub-tropical and tropical coastal regions from which seashore paspalum originates.

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Appendix E

Table 6.1. Analysis of variance for effect of species/variety, acclimation status, and their interaction on LT₅₀, spermine concentration, spermidine concentration, putrescine concentration, and proline concentration.

Effect	LT ₅₀	Analysis of Variance			
		Spermine	Spermidine	Putrescine	Proline
	°C	nmol g ⁻¹ fresh wt.	nmol g ⁻¹ fresh wt.	nmol g ⁻¹ fresh wt.	μmol g ⁻¹ dry wt.
S*	**	NS	***	***	***
A [†]	NA	NS	NS	***	***
S × A	NA	NS	**	NS	***

Means sharing a letter are not different according to Fisher's protected LSD test ($\alpha = 0.05$). **, *** indicate significance at the 0.01 and 0.001 *P* level, respectively. NS indicates difference not significant. NA indicates effect not applicable.

* indicates Species/variety

† acclimation status

Table 6.2. Correlation coefficients between LT₅₀ and spermine, spermidine, putrescine, and proline in cold acclimated tissues of Champion, TifEagle, SeaDwarf, and Diamond.

	Spermine	Spermidine	Putrescine	Proline
LT ₅₀	0.23 ^{NS}	0.24 ^{NS}	-0.10 ^{NS}	-0.20 ^{NS}
Spermine		0.45 [*]	-0.21 ^{NS}	-0.25 ^{NS}
Spermidine			-0.19 ^{NS}	-0.59 ^{***}
Putrescine				0.54 ^{**}
Proline				

*, **, *** indicate significance at the 0.05, 0.01, and 0.001 *P* level, respectively.
NS indicates difference not significant.

Table 6.3. Correlation coefficients between LT₅₀ and spermine, spermidine, putrescine, and proline in cold acclimated tissues of Champion, TifEagle, and Diamond.

	Spermidine	Putrescine	Proline
LT ₅₀	-0.06 ^{NS}	0.19 ^{NS}	0.46 [*]
Spermidine		-0.20 ^{NS}	-0.46 ^{**}
Putrescine			0.15 ^{NS}
Proline			

^{*}, ^{**} indicate significance at the 0.05 and 0.01 *P* level, respectively.
NS indicates difference not significant.

Table 6.4. Percent regrowth and orthogonal polynomial contrasts of Champion ultradwarf bermudagrass each week after cold acclimation and low temperature simulation.

	-3°C	-6°C	-7°C	-8°C	-9°C	-10°C	-11°C	-12°C	-13°C	-14°C	-15°C	-20°C	LSD	Linear	Quadratic
Wk 1	23.3	22.1	20.4	18.1	10.2	9.8	7.5	6.9	6.7	5.4	2.5	0	3.8	***	**
Wk 2	53.8	51.0	45.2	28.9	18.1	16.5	12.7	10.2	8.1	4.4	3.8	0	8.4	***	NS
Wk 3	76.5	66.9	61.0	52.9	50.4	37.3	34.2	25.0	14.4	17.1	10.4	0	13.4	***	***
Wk 4	79.6	72.9	72.3	56.5	51.7	37.9	34.4	27.1	18.9	13.3	11.0	0	11.8	***	***
LSD	12.3	11.2	15.1	14.5	11.4	11.5	8.5	14.5	7.7	6.5	NS	NS	—	—	—
Linear	***	***	***	***	***	***	***	***	*	**	NS	NS	—	—	—
Quadratic	**	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	—	—	—

*, **, *** indicate significance at the 0.05, 0.01, and 0.001 *P* level, respectively.
NS indicates difference not significant.

Table 6.5. Percent regrowth and orthogonal polynomial contrasts of TifEagle ultradwarf bermudagrass each week after cold acclimation and low temperature simulation.

	-3°C	-6°C	-7°C	-8°C	-9°C	-10°C	-11°C	-12°C	-13°C	-14°C	-15°C	-20°C	LSD	Linear	Quadratic
Wk 1	36.0	27.5	22.1	21.7	21.3	19.8	18.8	12.5	8.9	7.7	3.5	0	1.2	***	***
Wk 2	56.3	56.3	55.2	45.2	41.5	30.4	23.1	22.3	13.3	12.7	5.6	0	12.9	***	NS
Wk 3	90.6	88.5	68.5	59.2	48.9	39.4	30.8	30.4	29.2	13.9	6.3	0	11.9	***	NS
Wk 4	96.9	94.8	83.3	70.6	54.4	46.9	39.6	25.8	20.0	13.9	6.5	0	8.9	***	NS
LSD	10.6	12.3	11.2	13.2	16.8	13.3	11.5	12.5	10.8	10.9	NS	NS	——	——	——
Linear	***	***	***	***	***	***	***	NS	***	***	NS	NS	——	——	——
Quadratic	***	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	——	——	——

*, **, *** indicate significance at the 0.05, 0.01, and 0.001 *P* level, respectively.
NS indicates difference not significant.

Table 6.6. Percent regrowth and orthogonal polynomial contrasts of SeaDwarf seashore paspalum each week after cold acclimation and low temperature simulation.

	-3°C	-6°C	-7°C	-8°C	-9°C	-10°C	-11°C	-12°C	-13°C	-14°C	-15°C	-20°C	LSD	Linear	Quadratic
Wk 1	10.8	19.4	12.1	11.5	13.5	8.9	7.5	7.7	6.5	4.8	1.8	0	7.6	***	*
Wk 2	38.8	45.2	35.8	16.5	12.1	14.6	13.1	11.5	10.6	5.0	2.7	0	10.6	***	*
Wk 3	72.9	61.0	69.6	50.0	35.4	29.8	17.7	16.3	15.0	15.0	6.0	0	13.9	***	NS
Wk 4	76.9	74.8	67.7	53.8	37.5	32.1	31.5	23.5	25.0	16.1	6.9	0	11.2	***	NS
LSD	14.4	14.7	11.2	15.0	15.9	16.6	9.4	9.7	8.2	5.7	NS	NS	——	——	——
Linear	***	***	***	***	***	***	***	***	*	NS	NS	NS	——	——	——
Quadratic	*	NS	***	NS	NS	NS	NS	NS	*	*	NS	NS	——	——	——

*, **, *** indicate significance at the 0.05, 0.01, and 0.001 *P* level, respectively.
NS indicates difference not significant.

Table 6.7. Percent regrowth and orthogonal polynomial contrasts of Diamond zoysiagrass each week after cold acclimation and low temperature simulation.

	-3°C	-6°C	-7°C	-8°C	-9°C	-10°C	-11°C	-12°C	-13°C	-14°C	-15°C	-20°C	LSD	Linear	Quadratic
Wk 1	37.7	41.7	36.5	31.9	35.0	27.5	14.2	9.6	7.3	6.3	0	0	11.78	***	***
Wk 2	62.1	67.9	62.1	28.8	46.3	46.3	34.6	16.5	10.0	12.5	7.1	0	12.5	***	***
Wk 3	86.9	82.5	68.8	70.8	56.25	39.6	45.4	22.5	19.2	18.8	6.3	0	12.2	***	NS
Wk 4	92.7	89.6	82.3	76.0	61.3	50.0	45.5	25.6	19.2	18.8	8.5	0	11.16	***	*
LSD	11.6	11.5	14.6	12.5	12.3	14.3	15.0	11.7	10.7	10.2	NS	NS	——	——	——
Linear	***	***	***	***	***	**	***	*	NS	NS	NS	NS	——	——	——
Quadratic	*	*	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	——	——	——

*, **, *** indicate significance at the 0.05, 0.01, and 0.001 *P* level, respectively.
NS indicates difference not significant.

Table 6.8. Cellular concentrations and percent change of proline, spermidine, and putrescine in Champion, TifEagle, SeaDwarf, and Diamond.

Species/variety	Proline (Pro)			Spermidine (Spd)			Putrescine (Put)		
	NCA [†]	CA [‡]	Change	NCA	CA	Change	NCA	CA	Change
	$\mu\text{mol g}^{-1}$ dry wt.	$\mu\text{mol g}^{-1}$ dry wt.	%	Nmol g ⁻¹ fresh wt.	nmol g ⁻¹ fresh wt.	%	nmol g ⁻¹ fresh wt.	nmol g ⁻¹ fresh wt.	%
Champion	36.9 b	145.4 a	294.0 b	2.1 b	0.4 b	-81.0 b	87.9 ab	146.2 ab	66.3 b
TifEagle	22.7 c	95.2 b	319.4 b	0.4 b	0.3 b	-25.0 b	98.0 a	165.3 a	68.7 b
SeaDwarf	66.6 a	40.6 d	-39.0 c	3.6 b	13.2 a	266.7 a	36.0 c	105.1 b	191.9 a
Diamond	6.6 d	77.9 c	1080.3 a	9.1 a	9.3 a	2.2 b	40.9 bc	127.5 ab	167.3 ab
LSD _(0.05)	9.1	16.6	316.7	5.1	6.1	117.3	47.7	47.0	110.4

Means sharing a letter are not significantly different, according to Fisher's LSD ($\alpha = 0.05$)

[†] NCA indicates non-cold-acclimated

[‡] CA indicates cold-acclimated

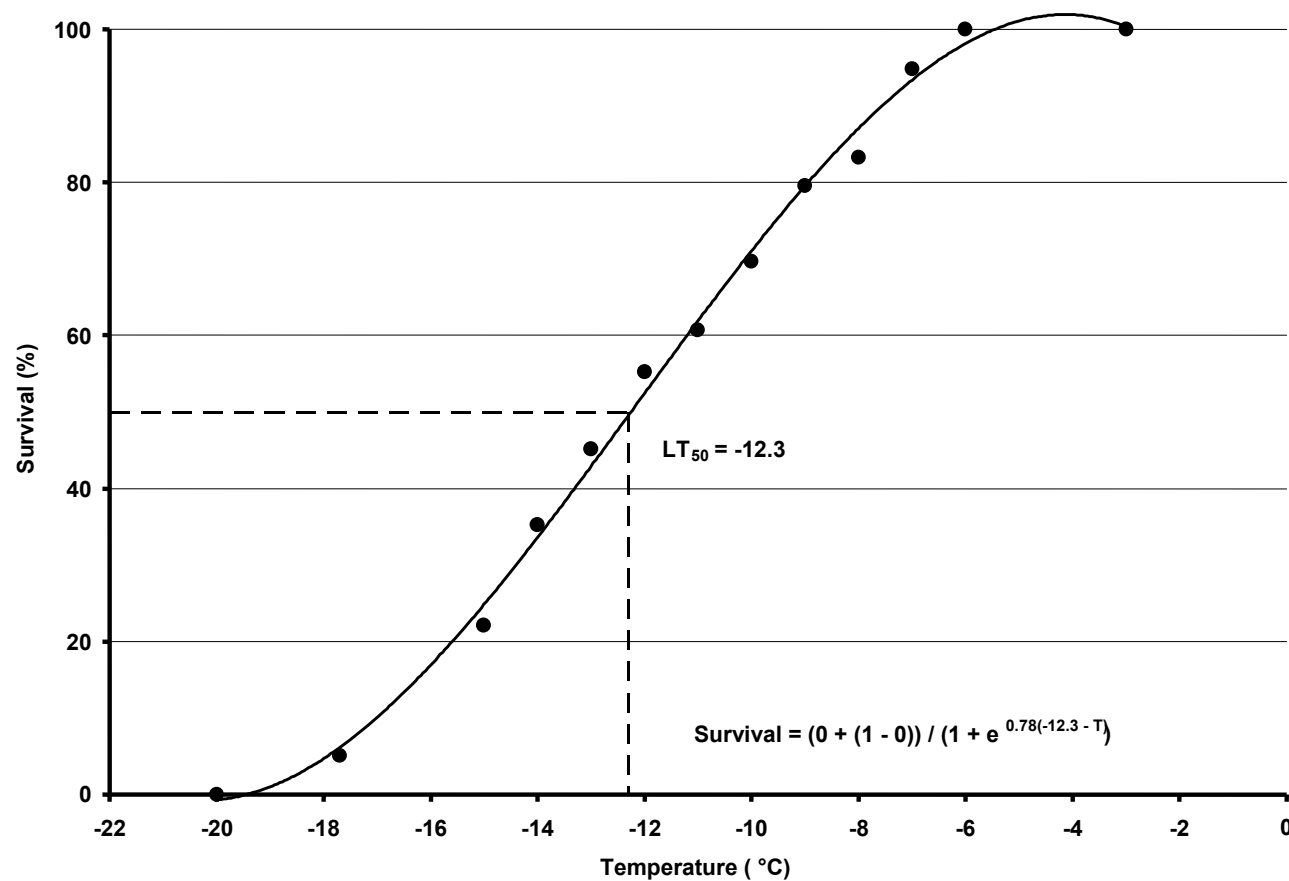


Figure 6.1. Sigmoidal curve fit to regrowth data from experimental control 'Tifway' bermudagrass. Freeze tolerance (LT₅₀, temperature resulting in no regrowth from 50% of test population) was determined by non-linear regression using the following equation: $\text{Survival} = a + (b - a) / (1 + \exp [c(T_m - T)])$, where a is the base line of survival, b is the maximum survival, c is a function of the slope of the line at the inflection point, T_m is the temperature at the inflection point (LT₅₀), and T is the treatment temperature.

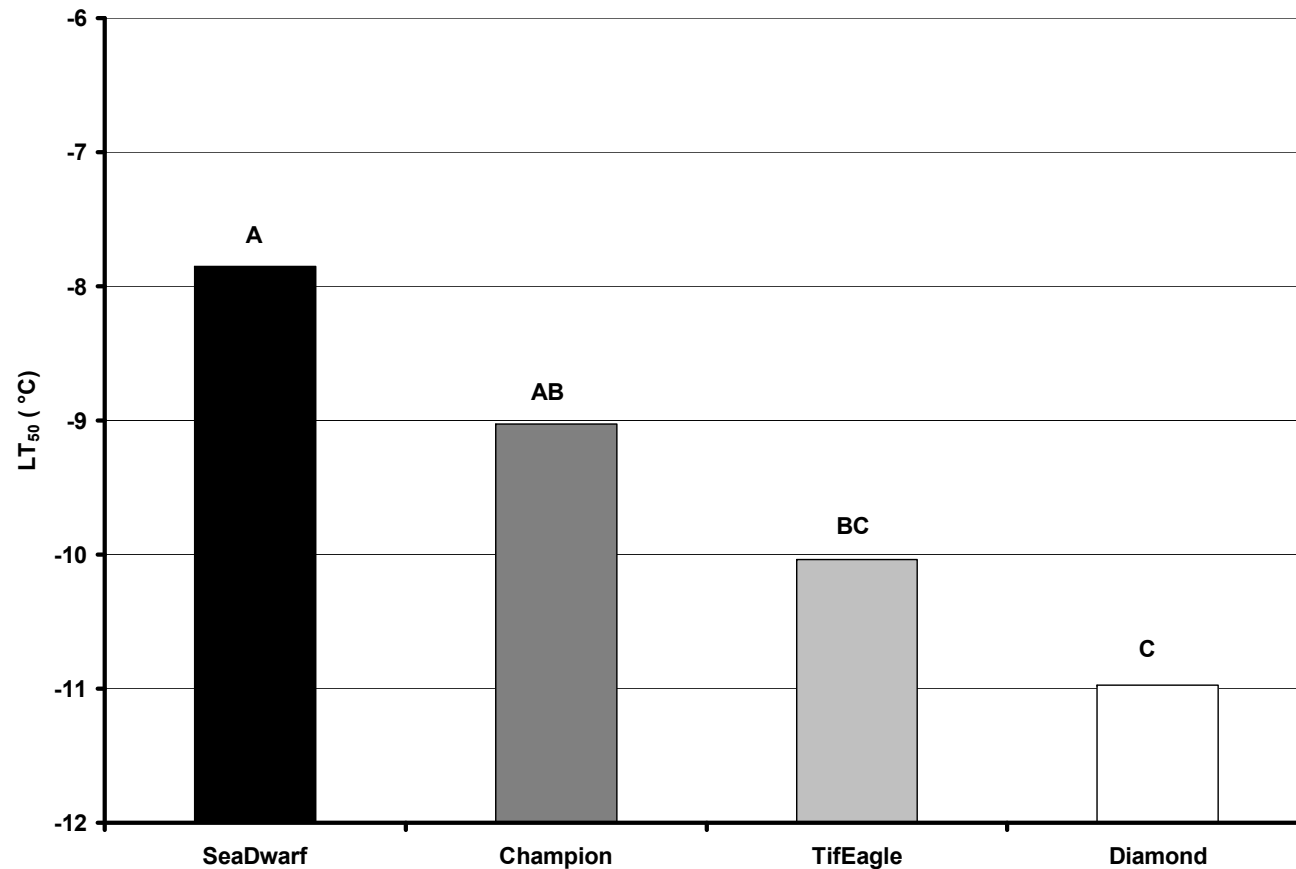


Figure 6.2. Turf species/variety freeze tolerance (LT₅₀, temperature resulting in no regrowth from 50% of the plants) as determined by nonlinear regression. Plants were grown in a greenhouse for at least 10 wks, cold acclimated in a growth chamber at 8/2° C day/night cycles and a 10-h photoperiod of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 4 wks and then placed in a cold stress simulator and subjected to temperatures ranging from -3 to -20° C. Bars sharing a letter are not significantly different, according to Fisher's LSD test ($\alpha = 0.05$).

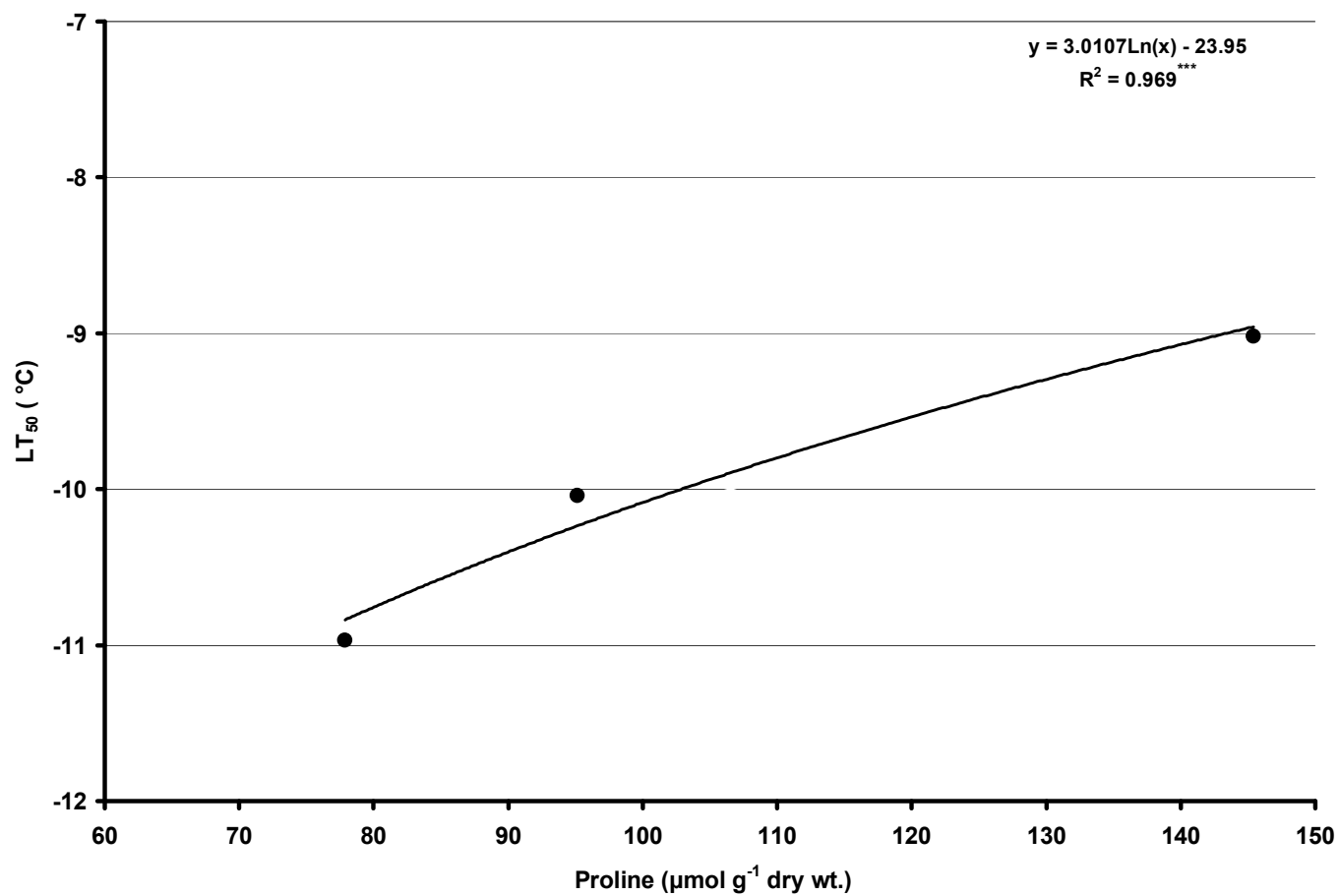


Figure 6.3. Proline concentrations in cold-acclimated rhizomes and stolons of Champion, TifEagle, and Diamond versus freeze tolerance (LT₅₀). *** indicates significance at the 0.001 *P* level.

CHAPTER SEVEN – SUMMARY AND CONCLUSIONS

Warm-season turf species are becoming increasingly popular for putting green use in the transition zone. Ultradwarf bermudagrass (*Cynodon dactylon* (L.) × *C. transvaalensis* Burt-Davy) is the prevalent warm-season putting green species, but seashore paspalum (*Paspalum vaginatum* Swartz) and ‘Diamond’ zoysiagrass [*Zoysia matrella* (L.) Merr.], are other options for putting green turf in the transition zone. Warm-season species hold several advantages over cool-season species for transition zone use, such as heat tolerance, drought tolerance, increased photosynthetic efficiency, decreased water requirements, and higher turf quality during the summer golf season. However, warm-season species require different management regimes than cool-season species and are susceptible to winter injury. This dissertation presents novel work investigating sampling procedures for thatch-mat depth and soil organic matter (SOM) content, which are assessed often in turfgrass research. Additionally, this dissertation includes studies of management practices that address aspects of warm-season putting green maintenance that are of particular importance in the transition zone, as well as a determination of the relative freeze tolerance of four warm-season species/varieties and the role of proline and polyamines in that freeze tolerance.

Sample sizes needed to measure thatch-mat depth and SOM content to a particular minimum detectable difference (MDD) and confidence (alpha) level were determined for ‘Champion’ and ‘TifEagle’ ultradwarf bermudagrass, ‘SeaDwarf’ seashore paspalum, and Diamond zoysiagrass. Measuring thatch-mat depth to a particular MDD and confidence level requires more samples than measuring SOM content because thatch-mat depth was more variable than SOM content. Although this research indicated that previous work in turfgrass may have not sampled intensively enough to determine thatch-

mat depth and SOM content, these results provide a guide for future researchers to determine proper sample sizes for thatch-mat and SOM content determination.

The use of vibratory rolling and brushing helped to overcome the difficulty of incorporating sand into the canopy of a TifEagle ultradwarf bermudagrass putting green in this study. Brushing alone and vibratory rolling + brushing created a more firm putting surface in the beginning of the study, but the continued use of vibratory rolling alone over time also increased surface hardness. Less topdressing sand was removed by mowing when incorporated by brushing alone than by vibratory rolling alone, but vibratory rolling + brushing further reduced the amount of sand removed by mowing to a negligible amount. Using brushing and vibratory rolling to successfully incorporate topdressing material into ultradwarf bermudagrass turf canopies will allow turf managers to make efficient use of their topdressing sand and improve putting green smoothness, firmness, and playability.

This research showed that mowing and rolling programs with a 3.2 mm mowing height influenced putting green speed more than those programs with a 4.0 mm mowing height on a 'MiniVerde' bermudagrass putting green in the summer in the transition zone. All programs mowed at 3.2 mm increased putting green speed an average of 20 cm over those mowing at 4.0 mm. Daily mowing at 3.2 mm and rolled six times per week, along with daily mowing at 3.2 mm and rolling 3 times per week increased green speed over those daily mowing at 3.2 mm without rolling by 10 cm, indicating that rolling frequency may not have as much impact on putting green speed as the mere presence of rolling. No mowing and rolling program influenced turfgrass quality, cover, dollar spot occurrence, or spring dead spot occurrence. However, programs with daily mowing and more

frequent rolling did improve turfgrass color for each mowing height. Because ultradwarf bermudagrass is not as susceptible to decline during stressful summer periods as creeping bentgrass, mowing at lower heights and increasing rolling frequency may increase putting green speed without sacrificing quality.

Secondary cultural practices, such as vertical mowing and grooming, are also important factors in ultradwarf putting green management. Daily grooming of TifEagle bermudagrass thinned the turf canopy, enhanced surface hardness, and allowed for increased topdressing incorporation while maintaining acceptable turf cover for much of the season. Weekly vertical mowing at the soil line increased soil hardness and topdressing incorporation, but reduced thatch depth and total biomass. However, vertical mowing at weekly intervals decreased turf quality and cover to unacceptable levels in one year. Vertical mowing + grooming provided the firmest surface, best topdressing incorporation, a much reduced thatch depth, and the lowest total biomass content in the first year. However, this treatment decreased quality levels below the threshold acceptable for use in a golf course setting. All secondary cultural practices aided in keeping SOM under 5% SOM by weight range and decreased total biomass after two years. Because the untreated control returned low topdressing incorporation values, a soft surface, and a deep thatch layer, secondary cultural practices may be thought of not only as treatments to produce a desirable putting surface, but also as pieces of a broader thatch management program.

Freeze tolerance is another important factor when selecting species or varieties for warm-season putting green use in the transition zone. SeaDwarf seashore paspalum had an LT₅₀ (freeze tolerance) value of -7.9° C, which was highest value, and thus, least

freeze tolerant, in this study. Champion and TifEagle did not significantly differ in their freeze tolerance and had LT_{50} values of -9.0° and -10.0° C, respectively, indicating that the two ultradwarf bermudagrass cultivars might perform equally under winter conditions. Diamond zoysiagrass had the lowest LT_{50} of all the selected species/varieties at -10.9° C, which differed from previous research that found Diamond to be much less freeze tolerant. However, further field studies (Appendix Q, R) have corroborated this finding, showing Diamond to have less winter injury than Champion, TifEagle, or SeaDwarf. Additionally, this research showed that Diamond may recover from cold injury in less time than all other species/varieties. TifEagle recovers more quickly from cold injury than Champion, while SeaDwarf recovers from cold injury the slowest.

Cold acclimation increased as cellular proline concentrations increased for all species/varieties except for SeaDwarf, in which proline concentrations decreased after cold acclimation. When all four species/varieties were analyzed together, proline concentrations did not correlate with freeze tolerance. However, when SeaDwarf was removed, the change in proline concentration after acclimation for Champion, TifEagle, and Diamond positively correlated with freeze tolerance. This suggests that Champion, TifEagle, and Diamond behave similarly to other crops and turfgrass species during cold acclimation, but SeaDwarf does not.

Among the polyamines, only spermidine and putrescine concentrations differed with cold acclimation and species/variety. Cold acclimation increased spermidine concentrations in SeaDwarf, but not Diamond, Champion, or TifEagle. Putrescine concentrations were highest in Champion and TifEagle, but the same for SeaDwarf and Diamond. Additionally, cold acclimation increased putrescine accumulation. However,

no polyamine concentrations were correlated to freeze tolerance. Although polyamine concentrations have been linked to freeze tolerance in dicots, other plants, they have not been linked to freeze tolerance in monocots, indicating that monocot physiology may be responsible for polyamines having no impact on freeze tolerance of Champion, TifEagle, SeaDwarf, or Diamond. Thus, polyamines do not appear to be indicators of freeze tolerance in ultradwarf bermudagrass, seashore paspalum, or Diamond zoysiagrass; however, proline may be used as a breeding screen for cold tolerance in ultradwarf bermudagrasses and Diamond zoysiagrass.

The results of this research will help managers of warm-season putting greens in the transition zone to make more informed choices about the species and cultivars they choose to grow, as well as help them to make better decisions regarding the management of their warm-season putting greens. Additionally, the sampling methods outlined here will help turf researchers to improve thatch-mat depth and SOM content determinations. Also, the growth chamber studies add to the scientific body of knowledge about cold stress physiology of warm-season putting green species and may be useful information for breeders seeking to breed more cold tolerant cultivars.

VITA

John Kauffman was born in Niles, Michigan, to John D. and Patricia Kauffman, and was raised in Stevensville, Michigan, along with his brothers, Shawn and Patrick. John's love for sports and the outdoors grew in that small town and he played football and lettered in wrestling at Lakeshore High School, where he graduated with highest honors in 2000. During his high school years, John seized an opportunity to work with the grounds crew at Berrien Hills Country Club in Benton Harbor, Michigan. Under the mentorship of golf course superintendent Greg Ellis, John discovered his passion for turfgrass management. John earned an Associate Degree in Business Administration from Lake Michigan College in 2003 and a Bachelor of Science degree in Turf and Landscape Horticulture from the University of Arkansas in 2005. During his undergraduate years, John completed an internship at the prestigious Southern Hills Country Club in Tulsa, Oklahoma and earned numerous academic awards, including the Chester Mendenhall award, which is given by the Golf Course Superintendents Association of America and is the highest award given to an undergraduate turfgrass student. After marrying the former Jennifer Hansen in 2005, John stayed in Arkansas to pursue his Master of Science degree in Horticulture under the direction of Dr. Douglas Karcher at the University of Arkansas, studying soil physical properties of sand-based putting greens. While in Arkansas, John was named a Dr. James Watson Fellow, the Golf Course Superintendents Association of America's highest graduate student award. After graduating in 2007, John moved on to the University of Tennessee to pursue a Ph.D. degree in Turfgrass Physiology under the direction of Dr. John Sorochoan.