Impact of Cultural Practices on Cold Tolerance of Ultradwarf Bermudagrass (*Cynodon dactylon* (L.) *Pers.*× *C. transvaalensis* Burtt-Davy) Putting Greens

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Academic Abstract

Low temperature injury is among the greatest challenges facing golf courses with ultradwarf bermudagrass (UDB) (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) putting greens in Virginia. This research focused on the impact of turf covers, fungicide programming, core aeration, and trinexapac-ethyl (TE) on UDB cold tolerance, winter quality, and cold de-acclimation (CD). Our results indicate that the use of turf covers significantly increased UDB canopy and soil temperatures when air temperatures were below -3.9°C. Air gaps under covers and the use of double turf covers increased soil and canopy temperatures compared to single covers alone in some instances, but results were inconsistent. Late fall and early winter fungicide applications of chlorothalonil and azoxystrobin improved UDB quality throughout winter dormancy and spring green up. The addition of a pigmented phosphonate significantly improved winter and spring UDB quality. The addition of acibenzolar-S-methyl to fungicide programs did not improve winter UDB quality or spring green up. Summer core aeration programs were evaluated for their impact on spring green up, turfgrass quality, surface firmness, and moisture retention. Spring UDB green up was improved incrementally as surface disruption increased. Treatments with 20%, 15%, and 10% surface disruption produced higher color vs treatments with lower surface disruption. Surface firmness and volumetric water content of UDB were impacted by construction method but were not significantly impacted by core aeration programs. Field research revealed that 'fall only' and 'fall and winter' TE applications improved UDB quality but only 'fall and winter' delayed UDB premature CD in early spring when UDB can be susceptible to low temperature injury. Growth chamber studies evaluated the impact of TE on UDB cold tolerance to -9.4°C x time duration. Regression analysis predicted a 50% mortality exposure point for UDB under TE treatments of 9.84 hours at -9.4°C (r^2=0.836) compared to 11.38 hours at -9.4°C (r^2=0.671) for non-treated UDB during cold acclimation. Winter and spring scenarios resulted in delayed CD under TE but no differences in cold tolerance when exposed to -9.4°C. Together, these results increase our understanding of the impact of management practices on UDB winter quality, CD, and low temperature injury.

Impact of Cultural Practices on Cold Tolerance of Ultradwarf Bermudagrass Putting Greens

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General Audience Abstract

Ultradwarf bermudagrass putting greens are commonly found on golf courses in warm climates. These grasses thrive in heat and humidity but are susceptible to injury or death when exposed to cold temperatures. This research is focused on evaluating management practices that may impact bermudagrass' susceptibility to injury from cold temperature exposure. The cultural practices evaluated include turf covers, fungicide programming, core aeration, and the use of plant growth regulators to manipulate the turfgrasses own self defense mechanisms. Our results show that the use of turf covers significantly increased putting green canopy and soil temperatures when air temperatures were below -3.9°C. Air gaps under covers and the use of double turf covers increased soil and canopy temperatures compared to single covers alone in some instances, but results were inconsistent. Late fall and early winter fungicide applications of commonly-used fungicides improved putting green quality throughout winter dormancy and spring green up. The addition of a green-pigmented phosphonate fungicide significantly improved winter and spring putting green quality. The addition of a plant defense activator, acibenzolar-S-methyl to fungicide programs did not improve winter quality or spring green up. Summer core aeration programs were evaluated for their impact on spring green up, turfgrass quality, surface firmness, and moisture retention. Spring green up was improved incrementally as surface disruption increased. Treatments with 20%, 15%, and 10% surface disruption produced higher color vs treatments with lower surface disruption. Surface firmness and soil moisture content of the putting greens were impacted by construction method but were not significantly impacted by core aeration programs. Field research revealed that 'fall only' and 'fall and winter' plant growth regulator applications improved ultradwarf bermudagrass quality but only 'fall and winter' delayed premature green-up in early spring when the turfgrass can be susceptible to low temperature injury. Growth chamber studies revealed that plants treated with the growth regulator, trinexapac-ethyl were more sensitive to low-temperature exposure than non-treated plants. Together, these results increase our understanding of the impact of management practices on UDB winter quality, CD, and low temperature injury.

Dedication

This dissertation is dedicated to my beautiful wife, Dr. Erin Booth for her unwavering support and encouragement and to our boys, Jackson Booth and William 'Ford' Booth.

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Introduction

Low temperature injury is one of the greatest obstacles to successful ultradwarf bermudagrass (UDB, *Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) putting green management on golf courses in colder climates (Anderson et al. 2002; Goatley et al. 2007; Zhang et al 2008). The use of UDB became popular in the 1990's due to its ability to withstand low mowing heights and provide desirable playing conditions (Reasor et al. 2016). The low mowing heights associated with putting greens makes UDB more susceptible to low temperature injury than other hybrid bermudagrasses (Anderson et al. 2002). Based on successful adoption in southern climates, widespread installation of UDB putting greens was observed throughout the southeast and into colder climates in the 2000's and early 2010's (Hartwiger, 2009, O'Brien & Hartwiger, 2011, Richardson et al. 2014).

The basic process of low-temperature injury in turfgrasses is ice formation in and around cells in turfgrass plants (Fry & Huang, 2004). Minimum temperature exposure is the primary factor associated with low-temperature injury, but duration of low-temperature exposure, and length of dormancy following low-temperature exposure also contributes (Chalmers & Schmidt, 1979; Anderson et al. 2003). The ability of turfgrasses to survive low-temperature exposure is impacted by day length, metabolic stage, maturity, and moisture content in the plant (Chalmers & Schmidt, 1979).

Cold acclimation (CA) is the combination of metabolic and physiological changes to prevent ice formation and low-temperature injury in turfgrasses (Davis & Gilbert, 1970; Chalmers & Schmidt, 1979, Gatschet et al. 1994; Fry & Huang, 2004). While bermudagrasses vary in cold tolerance between varieties and cultivars (Anderson et al. 1993; Gatschet et al. 1994), all bermudagrasses have the capability of going through CA and entering winter dormancy.

Maximum cold tolerance typically occurs during winter dormancy, following CA, and declines in the later stages of dormancy during cold de-acclimation (Davis & Gilbert, 1970; Chalmers & Schmidt, 1979). Cold de-acclimation occurs when sunlight exposure and air temperatures increase. Once de-acclimated, turfgrasses become more sensitive to cold temperature exposure (Fry & Huang, 2004). Research has demonstrated that ultradwarf bermudagrass can withstand temperatures below -9.4°C in the field (DeBoer et al. 2019). However, both laboratory and field research has shown that ultradwarf bermudagrass varieties vary in cold tolerance (Anderson et al. 2002; DeBoer et al. 2019).

Turf covers are used to insulate bermudagrass putting greens during cold weather to buffer the turfgrass plants from low temperature exposure (Goatley et al 2007, 2017; Hartwiger, 2009; Richardson & Booth, 2021). Black, woven, polypropylene covers provide above average temperature moderation coupled with relatively low weight which contributes to ease of installation compared to many other cover sources (Goatley et al. 2017). For these reasons, lightweight, black, polypropylene turf covers have been widely accepted as the preferred option for protecting ultradwarf bermudagrass putting greens during low temperature events (Richardson & Booth, 2021). Until recently, -3.9°C has been the recommended covering threshold for putting greens in adequate growing environments with no history of winter injury (O’Brien & Hartwiger, 2007). The latest threshold is -6.7°C (Richardson & Booth, 2021) based on the field research from Arkansas, USA (DeBoer et al. 2019). In an effort to improve the efficacy of turf covers, golf course superintendents have investigated the use of air gaps and multiple covers to further protect bermudagrass putting greens from low temperature injury (O’Brien, 2017). Further research is warranted to evaluate the impact of these covering methods.

The use of turf covers during cold temperatures can sometimes lead to premature and undesirable UDB growth during a potentially vulnerable period for low temperature damage.

Trinexapac-ethyl (TE) is a Type II, Class A, late-stage gibberellic acid (GA) inhibitor (Rademacher, 2015). Trinexapac-ethyl is commonly applied to actively growing turfgrasses to reduce leaf growth and mowing requirements, leading to smooth, uniform turfgrass surfaces (Rademacher, 2015; Reasor & Brosnan, 2020). Turfgrass color, quality, rooting, shade tolerance, photosynthesis, soluble carbohydrate levels, and drought tolerance improved or increased after applications of TE (Ervin & Koski, 1998; Qian & Engelke, 1999; Steinke & Stier, 2003; Fry & Huang, 2004; Rademacher, 2015). While there is some evidence of TE improving cold tolerance of turfgrasses (Fagerness et al. 2002, Richardson, 2002, Steinke & Stier, 2003) little is understood about the impact of TE on cold tolerance of UDB, particularly during potentially vulnerable periods of suboptimal growth.

Fungal diseases present challenges during the UDB active growing season but are arguably more concerning when UDB is not actively growing preceding, during, and exiting winter dormancy (Inguagiato & Martin 2015). Beneficial, nontarget effects of certain fungicides have been documented including improved rooting in controlled environments, improved visual turfgrass quality, and increased turfgrass density (Dernoeden & McIntosh, 1991; Brosnan et al. 2010). Phosphonate fungicides have been shown to improve stress tolerance of turfgrasses in the absence of disease (Huang & Liu, 2009; McCarty et al. 2013). Several commercially available phosphonate fungicides contain a green pigment. Acibenzolar-S-methyl (ASM) is a plant defense activator and is combined with a fungicide in several commercially available products labeled for use on turfgrasses. While the exact mechanisms are unknown, ASM limits transpiration leading to water conservation and drought tolerance (Shekoofa et al. 2016). Acibenzolar-S-methyl has also been shown to improve heat and drought tolerance in creeping bentgrass when applied as a pre-formulated product with chlorothalonil (Jespersen & Huang 2017). However, the roles of ASM and phosphonate fungicides in UDB cold tolerance have not been explored in on-site research trials.

Ultradwarf bermudagrass is a prolific thatch and organic matter producer (Vines et al. 2017). Thatch is a layer of interwoven organic residue in the upper zone of soil, directly below turfgrass shoots and stems (McCarty et al. 2007; Turgeon & Kaminski, 2019). Excess thatch can exacerbate disease and pest severity in UDB putting greens as well as lead to soft, inconsistent playing conditions (Carrow, 2004; Turgeon & Kaminski, 2019). Thatch accumulates in UDB putting greens when production of organic matter is greater than its rate of decomposition or removal (Carrow, 2004; McCarty et al. 2007; Turgeon & Kaminski, 2019). Ultradwarf bermudagrass putting greens have been grown in Virginia since the early 2010's (Bevard, 2013). Latitude, longitude, elevation, weather patterns, and micro-environments influence growing season duration, minimum and maximum temperature exposure, growth rates, and organic matter decomposition rates that provide challenges with UDB use for putting greens in this area. With more UDB putting greens being grown in temperate regions, adjustments may need to be made to traditional thatch and organic matter management programs tailored from regions with warmer, longer growing seasons.

Advances in management practices including the use of turf covers and wetting agents (Goatley, 2007, DeBoer et al. 2020) and a better understanding of turf cover thresholds (DeBoer et al. 2019) has improved the likelihood of UDB winter survival. Despite these improvements, widespread low temperature injury or "winterkill" still occurred in 2014, 2018, and 2021 in various regions of the country (Richardson et al. 2014, Anonymous, 2018, MacLeod, 2021).

There is great financial risk to golf courses after lethal low-temperature injury to UDB putting greens (Richardson et al. 2014; DeBoer et al. 2019). This research was conducted to explore opportunities to improve management practices that increase the cold tolerance of UDB.

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Chapter 1: Influence of double turf covers and air gaps under turf covers on canopy and soil temperatures of bermudagrass.

1 Turf Covers for Winter Protection of Bermudagrass

Low temperature injury is among the greatest challenges facing golf courses with ultradwarf bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) putting greens in Virginia and the United States Transition Zone (Anderson et al. 2002; Goatley et al. 2007; Zhang et al 2008). The primary mechanism of low temperature injury in bermudagrass putting greens is ice formation in and around cells in turfgrass plants (Stier et al. 2003; Fry & Huang, 2004). Desiccation or plant dehydration is also a factor in winter injury and can occur regardless of low-temperature exposure (DeBoer et al. 2020).

Turf covers have successfully been used to manipulate turfgrass canopy and soil temperatures as well as retain rootzone and crown hydration during low-temperature events (Roberts, 1986; Shashikumar & Nus, 1993; Dionne et al. 1999; Goatley et al. 2005, 2007, 2017). Temperature and hydration are critical factors involved in low-temperature exposure and desiccation (DeBoer et al. 2019). Turf covers are used for many purposes in turfgrass management and are available in a variety of weights, colors, and materials (Goatley et al, 2007, 2017). Turf covers are used to insulate bermudagrass putting greens during cold weather to buffer the turfgrass plants from low temperature exposure (Goatley et al 2007, 2017; Hartwiger, 2009; Richardson & Booth, 2021). Compared to other covers evaluated, black, woven, polypropylene covers provide above average temperature moderation coupled with relatively low weight which contributes to ease of installation compared to other cover sources (Goatley et al. 2017). For these reasons, lightweight, black, polypropylene turf covers have been widely accepted as a preferred option for protecting ultradwarf bermudagrass putting greens during low temperature events (Richardson & Booth, 2021).

While research has demonstrated that ultradwarf bermudagrass can withstand temperatures below -9.4°C in the field (DeBoer et al. 2019), both laboratory and field research has shown that ultradwarf bermudagrass varieties vary in cold tolerance (Anderson et al. 2002; DeBoer et al. 2019). In laboratory research, 50% of ‘Champion’ ultradwarf bermudagrass suffered lethal injury (LT_{50}) at -4.8°C while ‘MiniVerde’ and ‘TifEagle’ had an LT_{50} of -5.8°C and -6.0° respectively (Anderson et al. 2002). ‘TifEagle’ and ‘MiniVerde’ also suffered less winter injury than ‘Champion’ in field evaluations (DeBoer et al. 2019). Anderson et al. (2002) conceded that ultradwarf bermudagrass cultivars would likely be able to withstand lower ambient temperatures in the field due to cold acclimation and buffering capacity of soil and soil moisture. While ambient air temperature is an important factor, soil and leaf canopy temperatures may be better predictors of low-temperature injury (Dionne et al. 1999). Until recently, -3.9°C has been the recommended covering threshold for putting greens in adequate growing environments with no history of winter injury (O’Brien & Hartwiger, 2007). The latest threshold is -6.7°C (Richardson & Booth, 2021) based on the field research in Arkansas, USA (DeBoer et al. 2019). However, it is still recommended to cover bermudagrass putting greens with a history of low-temperature injury or putting greens in marginal growing environments including shaded or newly established ultradwarf bermudagrass when temperatures are forecast below -3.9°C (Richardson & Booth, 2021).

Cover thresholds and types of covers used on ultradwarf bermudagrass putting greens vary widely by golf course based on site-specific operating budgets, history of low-temperature injury, staffing levels, and objectives (Goatley et al. 2007, 2017; Richardson & Booth, 2021). Golf courses have experienced extensive damage and mortality from low temperature exposure under extreme conditions with and without turfgrass covers (Richardson et al. 2014). During recent winters including 2013-14, 2017-18, and 2020-21 golf courses with bermudagrass putting greens have experienced extensive cold temperature injury across the transition zone and southern United States (Richardson et al. 2014, Anonymous, 2018, MacLeod, 2021). In an effort to improve the efficacy of turf covers, golf course superintendents have investigated the use of air gaps and multiple covers to further protect bermudagrass putting greens from low temperature injury (O'Brien, 2017). Air gaps are created by separating the turfgrass from the turf cover with light-weight objects such as pipe or plastic cones (O'Brien, 2017). There is evidence of air gap (5 cm) under cover decreasing temperature variability and increasing minimum daily soil temperatures of cool-season putting greens in northern environments (Quebec, Canada) compared to a single cover alone (Dionne et al. 1999). However, limited scientific information is available about the impact of air gap and double covering on improved UDB survival from cold injury.

The objectives of this research were to (i) evaluate the impact of air gaps under turf covers on bermudagrass putting green canopy and soil temperatures. (ii) evaluate the use of two turf covers on bermudagrass putting green canopy and soil temperatures. (iii) evaluate different heights of air gap on bermudagrass putting green canopy and soil temperatures.

2 Materials and Methods

2.1 Air Gaps and Double Covers on 'Patriot' bermudagrass (Project 1)

The research was conducted at the Virginia Tech Turfgrass Research Center in Blacksburg, Virginia (37.21, -80.41) on 'Patriot' bermudagrass (*Cynodon dactylon* × *C. transvaalensis)* maintained at 13mm during the winter of 2015/2016 and repeated in 2016/2017. 3m x 3m plots were arranged in a completely randomized design with 3 replications of 5 cover treatments (Table 1).

Table 2: List of cover treatment abbreviations and descriptions for Project 1 (2015-2017) evaluating the impact of air gaps and double covers on canopy temperature of ‘Patriot’ hybrid bermudagrass during low temperature exposure in Blacksburg, VA, USA.

Treatment Abbreviation	Treatment Description
UC	Uncovered Control
SC	Single Cover
SC+AG	Single Cover with Cones (45mm Air Gap)
DC	Double Cover
DC+AG	Double Cover with Cones (45mm Air Gap)

‘Xton Black’ woven, polypropylene covers (Xton Inc., Florence, AL) were used and treatments were applied in December, secured to the ground with 25cm x 8mm diameter stakes, and remained in place for the entire study. In the treatments with an air gap (SC+AG, DC+AG), plastic cones were placed between the cover(s) and the turfgrass to create an air gap of 45mm. The cones were arranged in a 0.6 m x 0.6m pattern for a total of 16 cones per plot. Canopy temperature was collected using Onset® HOBO™ temperature sensor data loggers (Onset Computer Corp, Bourne, MA) placed directly in the center of each plot. Data collected in the uncovered plots was used as the ambient air temperature. Temperature data were collected every 30 minutes from December 21, 2015 until March 8, 2016 and again from December 14, 2016 until March 28, 2017.

2.2 Different air gap heights on ‘Patriot’ bermudagrass (Project 2)

The research was conducted at the Virginia Tech Turfgrass Research Center in Blacksburg, Virginia (37.21, -80.41) on ‘Patriot’ bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) maintained at 13mm during the winter of 2017/2018. 3m x 3m plots were arranged in a completely randomized design with 3 replications of 4 cover treatments (Table 2).

Table 2: List of cover treatment abbreviations and descriptions for Project 2 (2017-2018) evaluating the impact of various air gap heights under turf cover on canopy temperatures of ‘Patriot’ hybrid bermudagrass during low temperature exposure in Blacksburg, VA, USA.

Treatment Abbreviation	Treatment Description
UC2	Uncovered Control
SC2	Single Cover
SC+45AG	Single Cover with 45mm Air Gap
SC+89AG	Single Cover with 89mm Air Gap

‘Xton Black’ woven, polypropylene covers (Xton Inc., Florence, AL) were used and treatments were applied in December, secured to the ground with 25cm x 8mm diameter stakes, and remained in place for the entire study. In treatment SC+45AG, plastic cones were placed between the cover and the turfgrass to create an air gap of 45mm and plastic drainpipe was placed under covers in treatment SC+89AG to create an 89mm air gap. The cones were arranged in a 0.6 m x 0.6m pattern for a total of 16 cones per plot (SC+45AG). Four sections of 89mm diameter x 5 m drainpipe was installed 0.6 m apart under the cover in treatment SC+89AG. Canopy temperature was collected as previously described every 30 minutes from December 4, 2017 until March 1, 2018.

2.3 Air Gaps and Double Covers on ‘TifEagle’ ultradwarf bermudagrass (Project 3)

This project was conducted at the Virginia Tech Research Short Course at Independence Golf Club in Midlothian, VA (37.55 N, -77.69 E) on a ‘TifEagle’ ultradwarf bermudagrass putting green maintained at 4mm during the winter of 2018/2019. This putting green was constructed with an 85/15 blend of sand to peat rootzone mix to USGA specifications (United States Golf Association, 2018) in 2000 and planted to ‘TifEagle’ in 2017 through a no-till conversion. Plots were 1m x 1m and arranged in a completely randomized design with 3 replications of 5 cover treatments (Table 3). The treatments were applied when forecast temperatures were expected to drop below -3.9°C. Air gaps were installed for treatments SC+AG3 and DC+AG3 with 90mm corrugated pipe, cut in half. Two sections of 0.5 m length x 90 mm width x 45mm height corrugated pipe were installed 0.25 m apart to create the air gaps in treatments SC+AG3 and DC+AG3.

Table 3: List of cover treatment abbreviations and descriptions for Project 3 (2018-2019) evaluating the impact of air gap under turf cover and double covers on soil temperatures of 'TifEagle' ultradwarf bermudagrass during low temperature exposure in Midlothian, VA, USA.

Treatment Abbreviation	Treatment Description
AT3	Ambient Air Temperature
SC3	Single Cover
SC+AG3	Single Cover with 45mm Air Gap
DC3	Double Cover
DC+AG3	Double Cover with 45mm Air Gap

Black, woven, polypropylene covers (S and S Turf Covers, Covington, GA) were cut to size and stapled to a 1m x 1m wooden frame (7.5 x 3.75 cm x 1 m wooden sections) to secure the cover. Treatments were applied from December 10 – 14, 2018, January 18, 2019 – February 2, 2019 and again from March 4 - 7, 2019. Soil temperatures were collected beneath each treatment using Onset® HOBO™ temperature sensor data loggers (Onset Computer Corp, Bourne, MA). Sensors were inserted vertically 25mm into the putting green in the center of each plot to record soil temperature. Ambient air temperature and light levels were recorded using Onset® HOBO™

pendant MX data loggers. Temperature data were collected every 30 minutes. There was a total of eleven unique nights under -3.9°C during this study.

2.4 Different air gap heights on ‘Champion’ ultradwarf bermudagrass

This project was conducted at the Virginia Tech Research Short Course at Independence Golf Club in Midlothian, VA (37.55 N, -77.69 E) on a ‘Champion’ ultradwarf bermudagrass putting green maintained at 4mm during the winter of 2019/2020. This putting green was constructed with an 85/15 blend of sand to peat rootzone mix to USGA specifications in 2000 and planted to ‘Champion’ in 2017 through a no-till conversion. Plots were 3m x 3m and arranged in a completely randomized design with 4 replications of 5 cover treatments with 3m x 3m buffers were between each plot (Table 4). Air gaps were installed with 19mm diameter PVC pipe (SC+19AG), 50mm drainage pipe (SC+50AG), and 100mm drainage pipe (SC+100AG) respectively. A single cover was secured with 25cm x 8mm diameter stakes across the entire green and around each plot to create the distinct cover treatments when forecast temperatures were below -3.9°C.

Table 4: List of cover treatment abbreviations and descriptions for Project 4 (2020) evaluating the impact of various air gap heights under turf cover on soil temperatures of ‘Champion’ ultradwarf bermudagrass putting greens during low temperature exposure in Midlothian, VA, USA.

Treatment Abbreviation	Treatment Description
AT4	Ambient Air Temperature
SC4	Single Cover
SC+19AG	Single Cover with 19mm Air Gap
SC+50AG	Single Cover with 50mm Air Gap
SC+100AG	Single Cover with 100mm Air Gap

A black, woven, polypropylene cover (S and S Turf Covers, Covington, GA) was used in these treatments. Treatments were applied from January 6-9, 2020, January 18-23, 2020, and February 13-March 2, 2020. Soil temperatures were collected beneath each treatment using Onset® HOBO™ temperature sensor data loggers (Onset Computer Corp, Bourne, MA). Sensors were inserted vertically 25mm into the putting green in the center of each plot to record soil temperature. Ambient air temperature and light levels were recorded using Onset® HOBO™ pendant MX data loggers. Temperature data were collected every 30 minutes. There was a total of eleven (11) unique nights under -3.9°C during this study.

2.5 Data Collection and Analysis

Canopy temperatures were collected under covers in Projects 1 and 2. Uncovered control plots were used as the reference air temperature in these projects. Soil temperatures were collected in Projects 3 and 4. Air temperature data were collected in Projects 3 and 4 to be used as the control as we did not have the ability to leave any plots uncovered at this research facility. While air temperatures may be the most effective predictors of covering thresholds, we feel as though soil temperatures are critical to understanding low-temperature injury as the crowns, stolons, and rhizomes of bermudagrass putting greens are found in the soil.

Temperature data were compiled for all times when the air temperature was less than -3.9°C. Temperature data were also compiled by nighttime temperatures when temperatures dropped below -3.9°C for the hours when no light levels were measured (nighttime). Analysis of variance (ANOVA) (JMP Pro 16, SAS Institute, Cary, NC) was used to compare treatments when air temperatures were under -3.9°C and for minimum nighttime temperatures. Dunnett’s method was used to compare each treatment to the uncovered control or air temperature, showing the buffering capacity of each treatment. Treatment means were separated using Fisher's protected LSD when appropriate ($\alpha = 0.05$).

3 Results

3.1 Double Covers and Air Gap

When air temperatures were below -3.9°C in Project 1, there was a significant year by treatment interaction (Table 5), so years were separated and data subjected to ANOVA. There was no significant year by treatment interaction for minimum nighttime temperature, so years were combined for ANOVA. Double cover (DC) treatments had a significant impact on canopy temperature for each year ($p \leq 0.0001$). Double cover treatments provided significantly higher canopy temperatures compared to SC or UC plots when air temperatures were below -3.9°C and for minimum nighttime temperature. Dunnett’s method revealed a 4.15°C (Year 1) and 3.04° (Year 2) difference between canopy temperatures and air temperatures compared to 2.56°C (Year 1) and 1.85° (Year 2) when air temperatures were below -3.9°C. Dunnett’s method also showed improvement between double covers (2.86°C) and air temperatures compared to single covers (1.64°C) for minimum nighttime temperature data.

The results of Project 1 led to investigating the impact of these treatments on USGA specified, sand-based ultradwarf putting greens (Project 3). There was no significant covering event x treatment interaction for either temperatures below -3.9° or minimum nighttime temperature, so all data were combined for ANOVA (Table 6). Double cover (DC3) and SC3 treatments had a significant impact on canopy temperatures compared to air temperatures ($p \leq 0.0001$) but DC3 did not have significantly higher canopy temperatures compared to SC3 for either dataset.

Table 5: Treatment differences for Project 1: Effects of Air Gaps (AG) of 45 mm and Double Covers (DC) on covered ‘Patriot’ bermudagrass when nighttime temperatures were less than -3.9^{o}C and for overall minimum nighttime temperature cover treatment response. Means denoted by a different letter indicate significant differences between treatments in a given column ($p < 0.05$). Dunnett’s method = absolute difference between treatments – the least significant difference (LSD). UC = uncovered control.

	All data when temperatures <-3.9°C						**Minimum nighttime temperature**		
	Year 1			**Year 2**			**Years Combined**		
Treatment	**Canopy °C**	**Dunnett's**	**p-Value**	**Canopy °C**	**Dunnett's**	**p-Value**	**ANOVA**	**Dunnett's**	**p-Value**
DC+AG	-2.64 a*	4.73	<.0001	-2.59 a	3.84	<.0001	-2.59 a	3.43	<.0001
DC	-3.21 b	4.15	<.0001	-3.39 b	3.04	<.0001	-3.15 b	2.86	<.0001
SC+AG	-6.55 d	0.82	<.0001	-5.16 d	1.27	<.0001	-5.31 d	0.70	<.0001
SC	-4.80 c	2.56	<.0001	-4.56 c	1.87	<.0001	-4.38 c	1.64	<.0001
UC	-7.57 e	N/A		-6.61 e	N/A		-6.56 e	N/A	
LSD	0.17	0.21		0.14	0.18		0.44	0.55	

[*] Means followed by the same letter within columns are not significantly different according to Fisher’s protected LSD (α=0.05).

Table 6: Treatment differences for Project 3: Effects of Air Gaps (AG) of 45 mm and Double Covers (DC) on covered 'Tifeagle' bermudagrass putting greens when nighttime temperatures were less than -3.9^{o}C and for overall minimum nighttime temperature cover treatment response. Means denoted by a different letter indicate significant differences between treatments in a given column ($p < 0.05$). Dunnett's method = absolute difference between treatments – the least significant difference (LSD). AT = air temperature.

	All temperatures <-3.9°C			**Minimum nighttime temperature**		
Treatment	**Temp °C**	**Dunnett's**	**p-Value**	**ANOVA**	**Dunnett's**	**p-Value**
DC+AG3	2.68 c*	8.99	<.0001	2.02 a	8.28	<.0001
DC3	3.10 ab	9.40	<.0001	2.24 a	8.51	<.0001
SC+AG3	3.25 a	9.55	<.0001	2.50 a	8.76	<.0001
SC3	2.96 b	9.27	<.0001	2.20 a	8.47	<.0001
AT3	-6.63 d	N/A		-7.56 b	N/A	
LSD	0.19	0.40		0.74	1.61	

*Means followed by the same letter within columns are not significantly different according to Fisher's protected LSD (α=0.05).

3.2 Air Gaps

Air Gap treatments (DC+AG and SC+AG) had a significant impact on canopy temperatures of 'Patriot' bermudagrass compared to air temperatures (p≤0.0001) in Project 1 for all temperatures below -3.9°C and minimum nighttime temperatures (Table 5). Air gaps under double covers (DC+AG) had a significantly higher impact on canopy temperatures than DC but SC+AG had a significantly lower impact on canopy temperatures than SC. Similar results were found for minimum nighttime temperatures in Project 1.

Air Gap treatments (DC+AG3 and SC+AG3) had a significant impact on soil temperatures of 'TifEagle' ultradwarf bermudagrass compared to air temperatures (p≤0.0001) in Project 3 for all temperatures below -3.9°C and minimum nighttime temperatures (Table 6). Air gaps under double covers (DC+AG) had a significantly lower impact on soil temperatures than DC but

SC+AG3 had a significantly higher impact on canopy temperatures than SC. No differences were found between cover treatments for minimum nighttime temperatures in Project 3.

All cover treatments positively impacted cumulative and minimum nighttime canopy temperatures relative to the uncovered control (Table 7). Air gap height did not influence the minimum nighttime canopy temperature of single covering, as both SC+45AG and SC+89AG compared similarly to plots receiving single covering without an air gap. However, the cumulative canopy temperature was higher in plots with an air gap than in plots receiving only a single cover directly on the canopy surface. Height of the air gap did not significantly impact cumulative canopy temperature.

All cover treatments, regardless of air gap height, had significantly higher canopy temperatures than the ambient air temperature control on ‘Champion’ ultradwarf bermudagrass (Table 8). The cumulative canopy temperature was highest in plots receiving a single cover with an air gap of 100 mm. However, the cumulative canopy temperature was higher in plots with a 19 mm air gap than in plots with a 50 mm air gap. Further, the plots covered with a 50 mm air gap had a similar cumulative canopy temperature than plots with a single covering alone without an air gap. The minimum nighttime soil temperature was greater in single cover plots with a 100 mm air gap than in plots with a single cover alone without an air gap. The minimum nighttime soil temperature of plots with 19 mm and 50 mm air gaps were similar to both the single cover without an air gap and with a 100 mm air gap.

Table 7: Treatment differences for Project 2: Effects of Air Gap (AG) heights of 45 or 89 mm and Single Cover (SC) on covered ‘Patriot’ bermudagrass when nighttime temperatures were less than -3.9^{o}C and for overall minimum nighttime temperature cover treatment response. Means denoted by a different letter indicate significant differences between treatments in a given column ($p < 0.05$). Dunnett’s method = absolute difference between treatments – the least significant difference (LSD). UC = uncovered control.

	Air temperature <-3.9°C			Minimum nighttime canopy temperature °C		
Treatment	Canopy °C	Dunnett's	p-Value	Min°C	Dunnett’s	p-Value
SC+89AG	-4.93 A	3.56	<.0001	-6.90 a	3.25	<.0001
SC+45AG	-5.05 A	3.44	<.0001	-7.08 a	3.08	<.0001
SC2	-5.63 B	2.86	<.0001	-7.64 a	2.51	<.0001
UC2	-8.71 C	N/A		-11.48 b		
LSD	0.18	0.22		1.11		

[*]Means followed by the same letter within columns are not significantly different according to Fisher’s protected LSD (α=0.05).

Table 8: Treatment differences for Project 4: Effects of Air Gap (AG) heights of 19, 50, or 100 mm and Single Cover (SC) on covered ‘Champion’ ultradwarf bermudagrass when nighttime temperatures were less than -3.9^{o}C and for overall minimum nighttime temperature cover treatment response. Means denoted by a different letter indicate significant differences between treatments in a given column ($p < 0.05$). Dunnett’s method = absolute difference between treatments – the least significant difference (LSD). AT = air temperature.

	Air temperature <-3.9°C			Minimum nighttime canopy temperature °C		
Treatment	Canopy °C	Dunnett's	p-Value	Min°C	Dunnett’s	p-Value
SC+100AG	4.17 a*	8.85	<.0001	2.86 a	8.41	<.0001
SC+50AG	3.73 c	8.41	<.0001	2.43 ab	7.97	<.0001
SC+19AG	3.93 b	8.62	<.0001	2.52 ab	8.06	<.0001
SC4	3.62 c	8.31	<.0001	2.10 b	7.64	<.0001
AT4	-5.00 d	N/A		-6.40 c	N/A	
LSD	0.17	0.40		0.46	1.09	

[*]Means followed by the same letter within columns are not significantly different according to Fisher’s protected LSD (α=0.05).

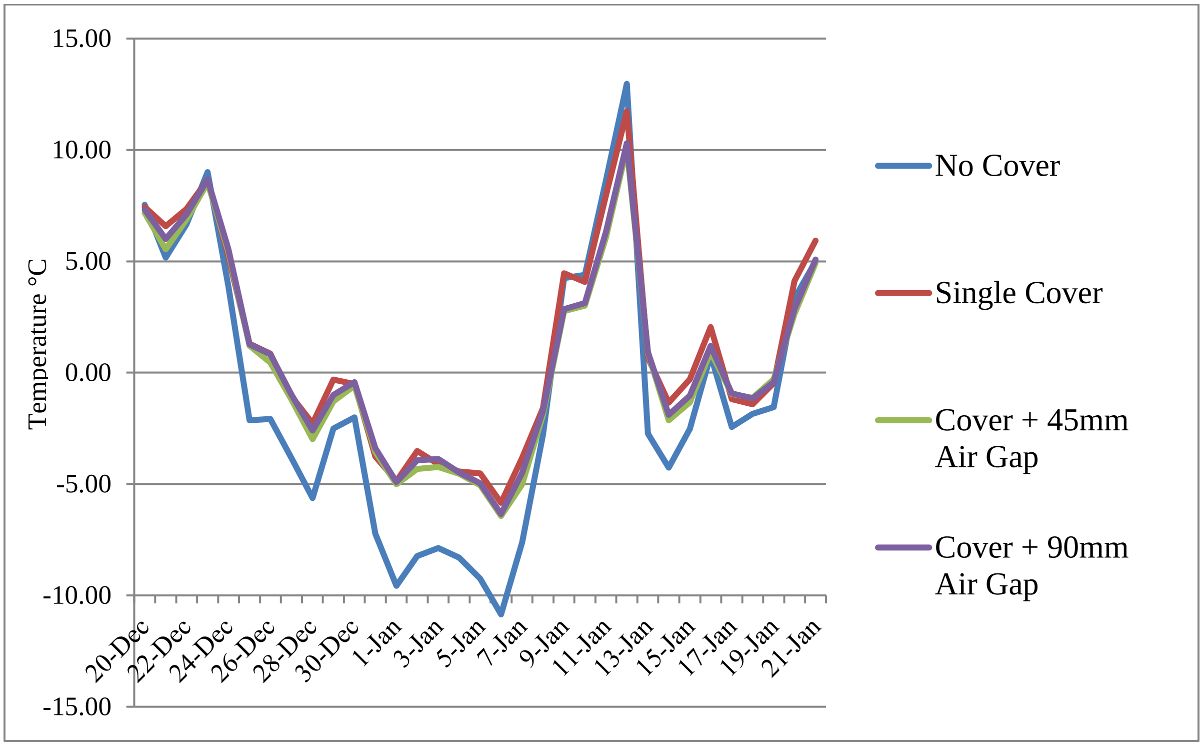

Figure 13: Continuous buffering capacity of turf covers alone and with air gaps on canopy temperatures of ‘Patriot’ hybrid bermudagrass from December 20, 2017 through January 21, 2018 in Blacksburg VA.

4 Discussion

There was statistical and biologically significant increase of canopy temperatures under double covers on ‘Patriot’ bermudagrass (Table 5). However, similar results were not observed with double covers on ‘TifEagle’ ultradwarf bermudagrass (Table 6). This could be due to the differences in treatment applications as Project 1 was installed for months at a time while Project 3 was installed for days at a time on a functioning golf course. Further research needs to explore the impact of double covers on both canopy and soil temperatures as well as winter survival on sand-based, ultradwarf bermudagrass putting greens.

While there is evidence of air gaps providing statistically higher soil and canopy temperatures compared to covers alone, biologically there is little apparent benefit. Air gaps performed quite similarly to covers without air gaps (Figure 1). The impact of air gap height showed little numerical improvement and there were mixed results on both ‘Patriot’ hybrid bermudagrass (Project 1) and the ‘TifEagle’ ultradwarf bermudagrass putting green study (Project 3). Interestingly, the single cover with air gap treatment maintained higher soil temperatures than the single cover alone and was statistically equivalent to the temperature buffering capacity of the double covers. However, the double cover with air gap treatment underperformed compared to the double cover (Project 3, Table 6). The inconsistency of the impact of air gaps on canopy and soil temperatures in Projects 1 and 3, led us to investigate the impact of air gap heights on soil and canopy temperatures. Perhaps the amount of air between the cover and the turfgrass may have impacted the buffering capacity of the air gap. In both studies all of the cover treatments successfully buffered the canopy and soil temperatures compared to the air temperatures (Tables 7 and 8). There is evidence for higher air gap heights being more beneficial on ultradwarf bermudagrass (Project 4, Table 8) but again, what we felt were minimal biological differences between air gap and single cover alone.

5 Implications for Turfgrass Managers

This study confirmed the benefit of turf covers for winter protection of bermudagrass and ultradwarf bermudagrass putting greens (Goatley et al. 2007). While the use of double covers was beneficial in studies on ‘Patriot’ bermudagrass where covers remained in place for the duration of the winter, these results were not confirmed on ultradwarf bermudagrass managed on an in-play golf course. Further investigation into double covers on ultradwarf bermudagrass putting greens is warranted as numerous anecdotal benefits have been realized in the field. This research does not support the use of air gaps under turf covers as a practical way to significantly buffer soil or canopy temperatures.

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Chapter 2: Evaluation of fall and winter trinexapac-ethyl applications on cold tolerance of ultradwarf bermudagrass putting greens

1 Introduction

Cold tolerance is one of the limiting factors reducing expansion of ultradwarf bermudagrasses (*Cynodon dactylon* (L.) *Pers.*× *C. transvaalensis* Burtt-Davy) (UDB) into the U.S. transitional climate zone and further north (Anderson et al. 2002; Goatley et al. 2007; Zhang et al 2008). Compared to creeping bentgrass (*Agrostis stolonifera* L.), UDB offers improved heat and traffic tolerance in the summer months and allows optimal golf play during the peak season (Hartwiger, 2009). These advantages have made UDB the dominant putting green turf in the Southern U.S., and improved management practices have fostered their use well into the transition zone since the early 2010's (Richardson et al. 2014). While converting to UDB on putting greens has improved the playability of many golf courses in warmer months, the risk of low-temperature injury and winter mortality remain high in northern climates (Goatley et al. 2007).

The primary methods employed to reduce winter mortality of UDB is turf covering and moisture management during cold stress, but integration of additional strategies is needed in the north (Goatley et al. 2007; DeBoer et al. 2019). Research has sought to better understand factors associated with cold acclimation and turfgrass response to cold stress in other types of turf and some of these approaches may have applicability to UDB.

Cold acclimation (CA) is the combination of metabolic and physiological changes to prevent ice formation and low-temperature injury in turfgrasses (Davis & Gilbert, 1970; Chalmers & Schmidt, 1979, Gatschet et al. 1994; Fry & Huang, 2004). While bermudagrasses vary in cold tolerance between varieties and cultivars (Anderson et al. 1993; Gatschet et al. 1994; Anderson et al. 2002), all bermudagrasses have the capability of going through CA and entering winter dormancy. Accumulated cryoprotectants including soluble proteins, amino acids, and carbohydrates serve as ‘antifreeze’ material to reduce the freezing point of water in the turfgrass cells (Gatschet et al. 1994; Fry & Huang, 2004). During CA, changes in plant hormone levels slow turfgrass growth, alter cell membrane properties, reduce cell water content, and induces accumulation of these cryoprotectants (Davis & Gilbert, 1970; Gatschet et al. 1994; Fry & Huang, 2004; Zhang et al. 2006;). Plant growth regulators that alter plant hormone levels, increase stress responses, and/or reduce carbohydrate expenditure could improve UDB tolerance to cold stress by inducing or maintaining CA or by bolstering plant stress defenses.

Trinexapac-ethyl (TE) is a Type II, Class A, late-stage gibberellic acid (GA) inhibitor (Rademacher, 2015). Turfgrass color, quality, rooting, shade tolerance, photosynthesis, and drought tolerance improved after applications of TE (Ervin & Koski, 1998; Qian & Engelke, 1999; Fry & Huang, 2004; Rademacher, 2015,). Total soluble carbohydrates increase in turfgrasses following TE applications, perhaps by reduced carbohydrate consumption associated with leaf growth (Qian & Engelke, 1999, Steinke & Stier, 2003). While there is some evidence TE improves cold tolerance of turfgrasses (Fagerness et al. 2002, Richardson, 2002, Steinke and Stier, 2003), little is understood about the impact of TE on cold tolerance of UDB.

Gibberellins antagonize abscisic acid (ABA), a stress defense hormone. While not evaluated in turfgrasses, ABA increases as GA decreases in other plant species (Shu et al. 2018). Likewise, GA is negatively associated with cold tolerance while ABA increases during cold acclimation and is positively associated with enhanced cold tolerance (Fry & Huang, 2004; Zhang et al. 2008) and lower lethal temperatures in bermudagrasses (Zhang et al. 2008). Decreased carbohydrate loss and increased ABA may both promote freezing tolerance by increasing dehydration tolerance (Fry & Huang, 2004). The reduction of GA through TE applications before, during, and after CA may lead to increased levels of ABA and stored carbohydrates, resulting in improved cold tolerance in turfgrasses under TE treatments (Zhang et al. 2006). We hypothesized that TE could play a role in UDB CA and/or winter stress tolerance. Since neither of these factors have been previously investigated in peer-reviewed literature, studies were designed to 1) evaluate the impact of fall and winter TE applications on UDB color and quality when the turfgrass is not actively growing and 2) evaluate the impact of fall and winter TE applications on cold tolerance of UDB during cold acclimation, winter dormancy, and cold de-acclimation.

2 Materials and Methods

2.1 Field Evaluation

Studies were conducted in Midlothian, Virginia on UDB putting greens built to USGA specifications at the Virginia Tech Research Short Course at Independence Golf Club (United States Golf Association, 2018). Four total greens were used each year (2 ‘TifEagle’ and 2 ‘G12’) during the winters of 2018/19 and 2019/20 for a total of eight site years. The putting greens were maintained at 3.25 mm height of cut with a reel mower (2500A Triplex Mower. Deere and Co. Moline, IL) in the growing season and the height of cut was raised to 4mm in the fall of the year. Irrigation was applied to prevent visual moisture stress and fungicides and wetting agents were applied as needed to maintain acceptable turfgrass quality. The greens received TE applications of 0.026 kg a.i. ha^{-1} every 14 days during the growing season. Vertical mowing followed by sand topdressing occurred every 2 weeks during the growing season from May - September. Plots were 1.2m x 1.8m and arranged in a completely randomized design with 4 replications of 7 treatments at each location. ‘Fall Only’ TE treatment applications (2-4) began on October 4, 2018 (year 1) and October 15, 2019 (year 2) after traditional TE applications ended during the

UDB active growing season. ‘Fall Only’ applications (2-4) ended on November 27, 2018 (year 1) and November 26, 2019 (year 2). ‘Fall only’ treatments ended once UDB lost green color, marking the start of winter dormancy. Treatments began on October 4, 2018 (year 1) and October 15, 2019 (year 2) and ended on March 4, 2019 (year 1) and April 14, 2020 (year 2). Treatments ended once green color had been regained and growth commenced in all plots. Treatments (Table 1) included full (0.026 kg a.i. ha^{-1}) and half (0.013 kg a.i. ha^{-1}) labeled rates on weekly and bi-weekly intervals. All treatments were mixed in water and applied using a CO2-pressurized sprayer delivering 842 L ha^{-1} at 276 kPa of pressure via TeeJet TTI 1004 VS spray tips (TeeJet Technologies, Glendale Heights, IL, USA).

Table 1: Field experiment treatments: Evaluation of fall and winter trinexapac-ethyl applications on ultradwarf bermudagrass putting greens.

Treatment		**Abbreviation**
Nontreated		NTC
Trinexapac-ethyl Fall Only Applications		
Trinexapac-ethyl	0.026 kg a.i. ha^{-1} 14 days^{-1}	TEF.HIGH.14
Trinexapac-ethyl	0.013 kg a.i. ha^{-1} 14 days^{-1}	TEF.LOW.14
Trinexapac-ethyl	0.013 kg a.i. ha^{-1} 7 days^{-1}	TEF.LOW.7
Fall and Winter Applications		
Trinexapac-ethyl	0.026 kg a.i. ha^{-1} 14 days^{-1}	TEW.HIGH.14
Trinexapac-ethyl	0.013 kg a.i. ha^{-1} 14 days^{-1}	TEW.LOW.14
Trinexapac-ethyl	0.013 kg a.i. ha^{-1} 7 days^{-1}	TEW.LOW.7

Weekly or bi-weekly applications were made unless weekly high temperatures did not exceed 4.5°C, since UDB putting greens were either covered with turf covers or snow.

Turfgrass quality and color assessments were made at time of first application and every 14 days through the winter. Trials were rated for turfgrass quality and color in accordance with methods outlined by Krans & Morris (2007) on a 1-9 scale with 6 being minimally acceptable and 9 being the maximum score. Turfgrass color (1-9, 6=minimally acceptable) were collected prior to the first application and every 14 days during the study including two additional ratings after the final application. Turfgrass color was assessed as color in the plot, not necessarily green color as the plants were going through different metabolic stages and contained varying levels of chlorophyll.

2.2 Pilot Study

In March of 2020, as UDB was breaking dormancy, sixty 5 cm diameter x 5 cm depth UDB plugs were removed from a UDB putting green in Midlothian, Virginia. The plugs were placed in 6 cm diameter tapered Cone-tainers®, filled with 85/15 sand/peat mixture with physical properties meeting USGA recommendations of methods for putting green construction (United States Golf Association, 2018). The cone-tainers include holes at the bottom for drainage and were prepped with cotton balls to retain the sand/peat mixture. The cone-tainers were placed in a greenhouse and allowed to acclimate for 14 days. The greenhouse was maintained at a daytime temperature of 30°C and nighttime temperature of 18°C with a 12-hour photoperiod supplying an average of 600 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation (PAR). Cone-tainers received supplemental irrigation. After 14 days of acclimation, the UDB plugs had regained green color but were not actively growing. Following acclimation, the cone-tainers were separated into two groups of 28 for cold temperature exposure. Cone-tainers were arranged in a growth chamber in a completely randomized design with 2 runs of 4 replications of 7 treatments. Growth chambers were set to -9.4°C with no PAR and cone-tainers were removed after hours of exposure based on treatment. Treatments included 0, 3, 6, 8, 10, 12, and 18 hours of exposure to -9.4°C. After low-temperature exposure, cone-tainers were placed back in the greenhouse for 7 days for evaluation of potential injury.

2.3 Growth Chamber Evaluation

2.4 Equipment

Two chest freezers were modified for use as growth chambers. The freezer doors were removed and replaced with plexiglass. Spacers were placed under the plexiglass to allow air exchange into and out of the growth chamber. Grow lights (average of 600 µmol m^{-2} s^{-1} of PAR) were installed over the growth chambers and internal temperatures were maintained using Inkbird ITC-308 Digital Temperature Controllers (Shenzhen City, China**)** connected to the growth chamber and to a 500-watt ceramic heater placed inside of the growth chambers. A wire-rack shelf was placed inside of the growth chambers to maximize room for the pots. A small, portable fan was placed under the wire-rack to provide air flow within the growth chambers. Each growth chamber was capable of housing 24, 15.25 cm diameter pots.

2.5 Plant Materials

Two hundred 10.8 cm diameter x 10 cm depth UDB plugs were removed from a nursery putting green in Midlothian, Virginia on February 26, 2020, and planted in 15.25 cm diameter x 14.6 cm depth plastic pots. The UDB plugs were fully dormant at the time of removal with good turfgrass density, khaki-brown in color, and adequate rooting. The pots contained four holes at the base for drainage. Prior to planting, the holes were covered with cotton balls and 4 cm of pea gravel. The UDB plugs were planted on top of the gravel and any remaining volume in the pots was filled with an 85/15 USGA specified sand/peat mixture (United States Golf Association, 2018). The potted plugs were placed in a greenhouse and allowed to acclimate, exit dormancy, and grow for 16 weeks. Plugs were maintained at a daytime temperature of 30°C and nighttime temperature of 18°C with a 12-hour photoperiod with an average of 600 µmol m^{-2} s^{-1} of PAR. Once growth began, plugs received TE applications at 0.026 kg a.i. ha^{-1} and water-soluble 46-0-0 fertilizer at a N rate of 1 g m^{-2} every 14 days. Plugs were irrigated daily and trimmed with electric shears to a height of 3.5mm as necessary. Once the plugs grew radially to the edge of the pots, pots were removed from the greenhouse on June 8, 2020 and placed outside in Midlothian, VA where bi-weekly TE and urea applications continued. Pots with poor turfgrass coverage, density, or quality were removed from the study. The remaining pots were separated into two sets of 24 pots for two runs of three separate experiments.

2.6 Growth Chamber Experimental Design

Three experiments were designed to evaluate the impact of TE applications on cold tolerance of UDB under controlled environments at three different metabolic stages; during CA (fall conditions), during winter dormancy, and during cold de-acclimation (spring green-up). Ultradwarf bermudagrass pots were arranged in a completely randomized design during each experiment with 3 replications of 8 treatments (Table 2).

Table 2: Treatment list for each metabolic stage (cold acclimation, winter dormancy, cold de-acclimation). Trinexapac-ethyl rates were determined by results of field experiment. Minimum temperature and exposure duration were determined by a pilot study to return a range of results.

TRT	**Trinexapac-ethyl Regimen**	**Rate**	**Low-temperature Exposure**
1	Trinexapac-ethyl	0.026 kg a.i. ha^{-1} 14 d^{-1}	4 hours at -9.4°C
2	Trinexapac-ethyl	0.026 kg a.i. ha^{-1} 14 d^{-1}	6 hours at -9.4°C
3	Trinexapac-ethyl	0.026 kg a.i. ha^{-1} 14 d^{-1}	8 hours at -9.4°C
4	Trinexapac-ethyl	0.026 kg a.i. ha^{-1} 14 d^{-1}	10 hours at -9.4°C
5	No Trinexapac-ethyl	n/a	4 hours at -9.4°C
6	No Trinexapac-ethyl	n/a	6 hours at -9.4°C
7	No Trinexapac-ethyl	n/a	8 hours at -9.4°C
8	No Trinexapac-ethyl	n/a	10 hours at -9.4°C

2.7 Cold Acclimation

Beginning in June of 2020, the first two runs of 24 pots were placed into each growth chamber. Daytime temperatures were maintained at 25°C and nighttime temperatures were maintained at 12.75°C. Daytime and nighttime temperatures were dropped 2.78°C every two weeks for 10 weeks until nighttime temperatures were 10°C and 1.67°C respectively for the last two weeks of cold acclimation to mimic fall conditions. Trinexapac-ethyl was applied at 0.026 kg a.i. ha^{-1} every 14 days to treatments 1-4. Pots received 12 hour/day photoperiod with an average of 600 μmol m^{-2} s^{-1} of PAR and were watered as necessary to maintain adequate soil moisture. At the end of 10 weeks, pots underwent exposure to -9.4°C for a pre-determined duration based on treatment.

2.8 Low-temperature Exposure Treatments

At the end of the acclimation phase of each experiment, pots were subjected to -9.4°C for specific hours of duration based on treatment (Table 2). Based on the results of the pilot study, 4, 6, 8, and 10 hours of exposure to -9.4°C were selected to provide a series of predicted outcomes ranging from no damage to complete mortality. Three replications each of TE-treated and nontreated pots were added for each of the listed freeze durations. Prior to low-temperature exposure, UDB pots were removed from the growth chamber and watered to saturation. While the pots drained any excess water, the growth chamber was set to -9.4°C. After two hours of adjusting to -9.4°C, all 24 pots were placed back into the growth chamber with the plexiglass secured down and light off. Treatments were removed from growth chamber after prescribed duration. After thawing, pots were returned to the growth chambers and gradually brought back to 30°C over 72 hours to evaluate potential injury.

2.9 Data Analysis

Field Evaluations

Data were plotted over time for a graphical representation of cold acclimation, winter dormancy, and cold de-acclimation (spring green up) and the impact of TE on these metabolic stages. Data were transformed to standardized area under the progress curve (SAUPC) for the duration of the study. Treatment x location and treatment x year were significant, so data are separated by location (3, 4, 5, 9) and by year. Analysis of variance (JMP Pro 15, SAS Institute, Cary, NC) was used to compare treatment means by location and year. Means separation analyses were made using Fisher's protected LSD ($\alpha = 0.05$).

Growth Chamber Evaluations

Pots were analyzed 1 day prior to and 6 days after growth chamber treatments with digital image analysis (Fiji: ImageJ, 2020) for percent green cover. Percent Green Cover after growth chamber treatments was used to evaluate damage after low-temperature exposure. Data were best modeled using non-linear Gompertz 3 parameter regression (JMP Pro 15, SAS Institute, Cary, NC) for both TE and nontreated UDB over time.

$$y = a\mathrm{Exp}(-\mathrm{Exp}(-b(\mathrm{HLTE}-c)))$$

where y=expected % green cover value as a function of time, a=asymptote, b=growth rate, c=inflection point, and HLTE=hours of low-temperature exposure. This model was selected because of both goodness-of-fit among tested models and biological relevance. The low-temperature exposure duration (time in hours/-9.4C) at which treatments would suffer lethal injury of 50% (LT_{50}) of the UDB was determined using a custom inverse prediction model within the JMP software based on Gompertz 3 parameter regression models:

3 Results

3.1 Field Evaluation

Turfgrass Quality

Turfgrass quality SAUPC was significantly higher in TE treatments when compared to the nontreated control (Tables 3 and 4). Charting turfgrass quality in both years showed trends over time (Figures 2 and 4). In Year 1 (2018-19), there were significant turfgrass quality differences on an individual rating date during cold acclimation (October 30, 2018) and cold de-acclimation (April 3, 2019). Treatments with the low rate (0.013 kg a.i. ha^{-1}) of TE every 14 days (TEF.LOW.14 and TEW.LOW.14) had statistically lower turfgrass quality on October 30 than all other treatments but were still above the minimally acceptable level for quality (≥ 6.0). Turfgrass quality was significantly lower on April 3, 2019 for ‘fall and winter’ applications compared to ‘fall only’ applications due to the fact that plots under ‘fall only’ treatments had broken dormancy. Turfgrass quality ratings were higher in all TE treatments during winter dormancy (Figures 2 and 4). By April 11, 2019 (Year 1) and May 20, 2020 (Year 2) all turfgrass quality ratings were normalized, and all treatments had acceptable quality (≥ 6.0).

Turfgrass Color

There were significant differences in visual turfgrass color in both years during CA, winter dormancy, and cold de-acclimation between the nontreated control, ‘fall only’ treatments, and ‘winter and fall’ treatments (Tables 3 and 4). On individual rating dates in both years, there were significant differences between treatments for individual rating dates during CA and cold-deacclimation. There were significant color differences between treatments for individual rating dates on November 11, 2018 (Year 1), October 12, 2019 (Year 2), and October 26, 2019 (Year 2) during CA. On these rating dates, all TE treated UDB had significantly less color than the nontreated plots and TE treatments at the half label rate of 0.013 kg a.i. ha^{-1} every 7 days (TEF.LOW.7 and TEW.LOW.7) had the lowest color treatment means.

In both years, there were dramatic increases in turfgrass color in the nontreated control in February (Year 1) and March (Year 2) (Figures 2 and 4) following unseasonably warm weather during winter dormancy. On the individual rating date, February 19, 2019 (Year 1) the nontreated plots had significantly more color than the TE treated plots ($p<.0001$) following average high temperatures of 20°C for the dates of February 3-8, 2019. For the individual rating date of March 2, 2020 (Year 2), the nontreated plots had significantly more color than the ‘fall only’ TE treated plots ($p<.0001$) which had significantly more color than the ‘fall and winter’ TE treated plots ($p<.0001$) when subjected to ANOVA.

There were significant color differences between treatments for individual rating dates on March 19, 2019 (Year 1), April 3, 2019 (Year 1), March 31, 2020 (Year 2) and April 14, 2020 (Year 2) during cold-deacclimation. In both years, ‘fall only’ treatments had significantly higher turfgrass color ratings on these individual rating dates during cold de-acclimation compared to ‘fall and winter’ treatments. Turfgrass color ratings were significantly lower during winter dormancy than the nontreated control (Figures 2 and 4) and all color ratings were statistically similar following cold de-acclimation in both years.

Table 3: 2018-2019 Field evaluations detailing the impact of trinexapac-ethyl (TE) application rate and timing on turfgrass quality (1-9 scale, 9 = highest quality and 6=minimally acceptable) and turfgrass color (1-9 where 1 = completely brown to 9 = dark green) transformed to standardized area under the progress curve by location. NTC = nontreated control. TEF = fall-only TE applications and TEW = both fall and winter TE applications made at either high (0.026 kg a.i. ha^{-1}) or low (0.013 kg a.i. ha^{-1}) TE levels on either 7- or 14-day intervals.

	'G12' 3		**'TifEagle' 4**		**'G12' 5**		**'TifEagle' 9**	
Treatment	**Quality***	**Color****	**Quality**	**Color**	**Quality**	**Color**	**Quality**	**Color**
NTC	5.60 b***	5.27 a	5.53 b	5.33 a	5.46 b	5.19 a	5.21 c	4.84 a
TEF.HIGH.14	6.26 a	3.74 b	6.24 a	3.79 b	6.22 a	3.87 b	5.66 a	3.61 b
TEF.LOW.14	6.20 a	3.57 c	6.21 a	3.70 bc	6.20 a	3.63 c	5.58 ab	3.46 c
TEF.LOW.7	6.25 a	3.52 c	6.22 a	3.59 c	6.19 a	3.66 c	5.57 ab	3.32 d
TEW.HIGH.14	6.22 a	3.52 c	6.22 a	3.63 c	6.16 a	3.64 c	5.50 b	3.28 d
TEW.LOW.14	6.15 a	3.31 d	6.15 a	3.36 d	6.11 a	3.37 c	5.44 b	3.12 e
TEW.LOW.7	6.17 a	3.25 d	6.16 a	3.32 d	6.12 a	3.34 c	5.52 ab	3.07 e
LSD	0.12	0.15	0.11	0.13	0.11	0.18	0.14	0.13

*Turfgrass quality assessed using a 1-9 scale, 9 = highest quality and 6 = acceptable.

**Turfgrass color assessed using a 1-9 scale, 9=dark green and 6=acceptable.

***Means followed by the same letter within columns are not significantly different according to Fisher's protected LSD (α=0.05).

Table 4: 2019-2020 Field Evaluations, Impact of trinexapac-ethyl application rate and timing *on t*urfgrass *q*uality (1-9) and turfgrass color (1-9) transformed to standardized area under the progress curve by location.

	'G12' 3		**'TifEagle' 4**		**'G12' 5**		**'TifEagle' 9**	
Treatment	**Quality***	**Color****	**Quality**	**Color**	**Quality**	**Color**	**Quality**	**Color**
NTC	3.92 b***	4.67 a	4.29 b	4.68 a	4.32 b	4.83 a	3.27 b	4.24 a
TEF.HIGH.14	5.13 a	3.21 bc	5.40 a	3.64 b	5.50 a	3.74 c	4.31 a	3.19 b
TEF.LOW.14	5.13 a	3.31 b	5.40 a	3.70 b	5.50 a	3.81 b	4.31 a	3.25 b
TEF.LOW.7	5.13 a	3.10 cd	5.40 a	3.49 c	5.50 a	3.72 c	4.31 a	3.08 c
TEW.HIGH.14	5.13 a	3.01 d	5.40 a	3.36 de	5.50 a	3.51 d	4.28 a	2.97 d
TEW.LOW.14	5.13 a	2.98 de	5.40 a	3.44 cd	5.50 a	3.59 d	4.32 a	3.03 cd
TEW.LOW.7	5.13 a	2.87 e	5.40 a	3.28 e	5.50 a	3.41 e	4.26 a	2.83 e
LSD	0.05	0.18	0.50	0.10	0.02	0.08	0.07	0.08

*Turfgrass quality assessed using a 1-9 scale, 9 = highest quality and 6 = acceptable.
**Turfgrass color assessed using a 1-9 scale, 9=dark green and 6=acceptable.
***Means followed by the same letter within columns are not significantly different according to Fisher's protected LSD (α=0.05).

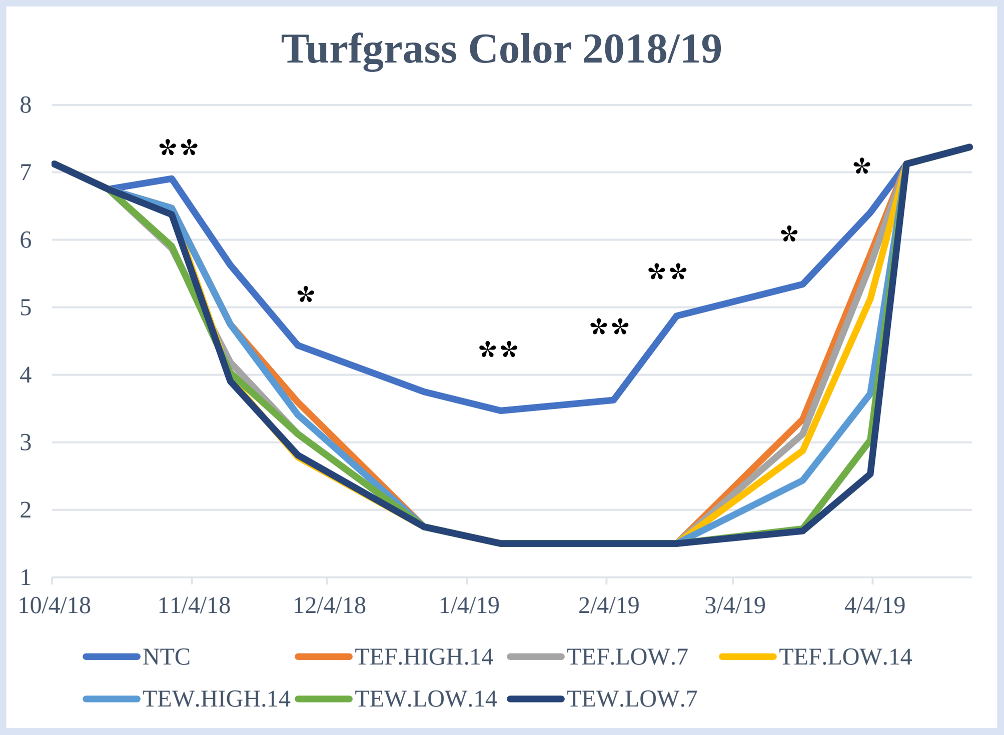

Figure 14: 2018-2019 Field Evaluations, Impact of trinexapac-ethyl (TE) application rate and timing on turfgrass color ratings (scale of 1 to 9 where 1 = completely brown to 9 = dark green) over time. * indicates significant differences between TE treatments for specific rating dates. ** indicates significant differences between non-treated control (NTC) and TE treatments for specific rating dates. TEF = fall-only TE applications and TEW = both fall and winter TE applications made at either high (0.026 kg a.i. ha^{-1}) or low (0.013 kg a.i. ha^{-1}) TE levels on either 7- or 14-day intervals.

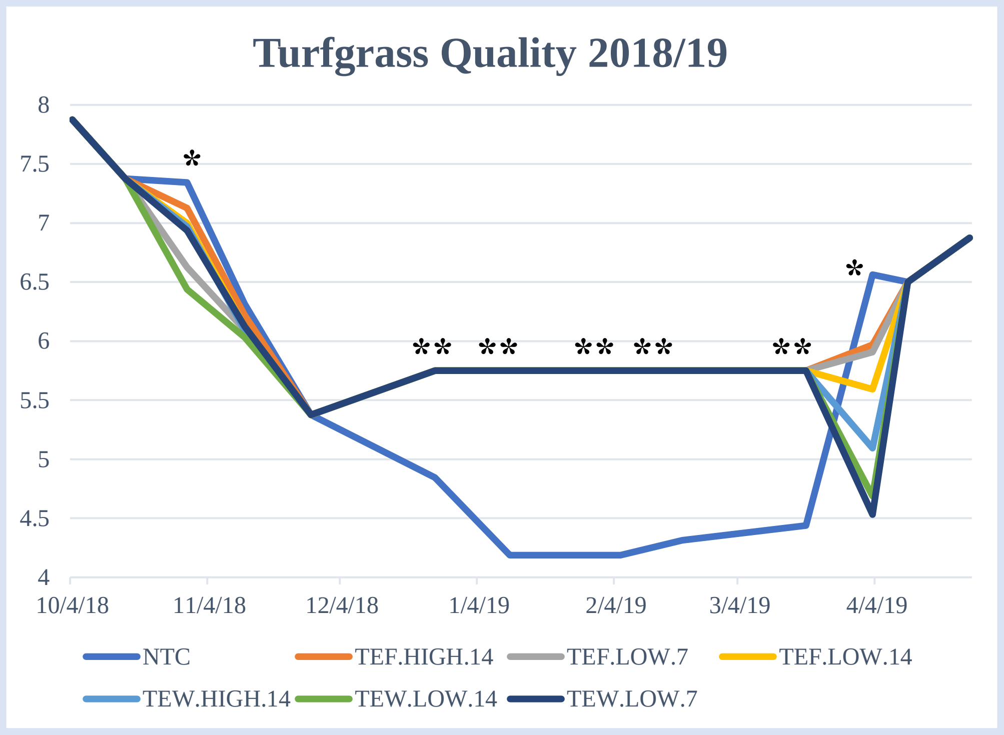

Figure 15: 2018-2019 Field Evaluations, Impact of trinexapac-ethyl (TE) application rate and timing on turfgrass quality ratings (scale of 1 to 9, 9 = highest quality and 6=minimally acceptable) over time. * indicates significant differences between TE treatments for specific rating dates. ** indicates significant differences between non-treated control (NTC) and TE treatments for specific rating dates. TEF = fall-only TE applications and TEW = both fall and winter TE applications made at either high (0.026 kg a.i. ha^{-1}) or low (0.013 kg a.i. ha^{-1}) TE levels on either 7- or 14-day intervals.

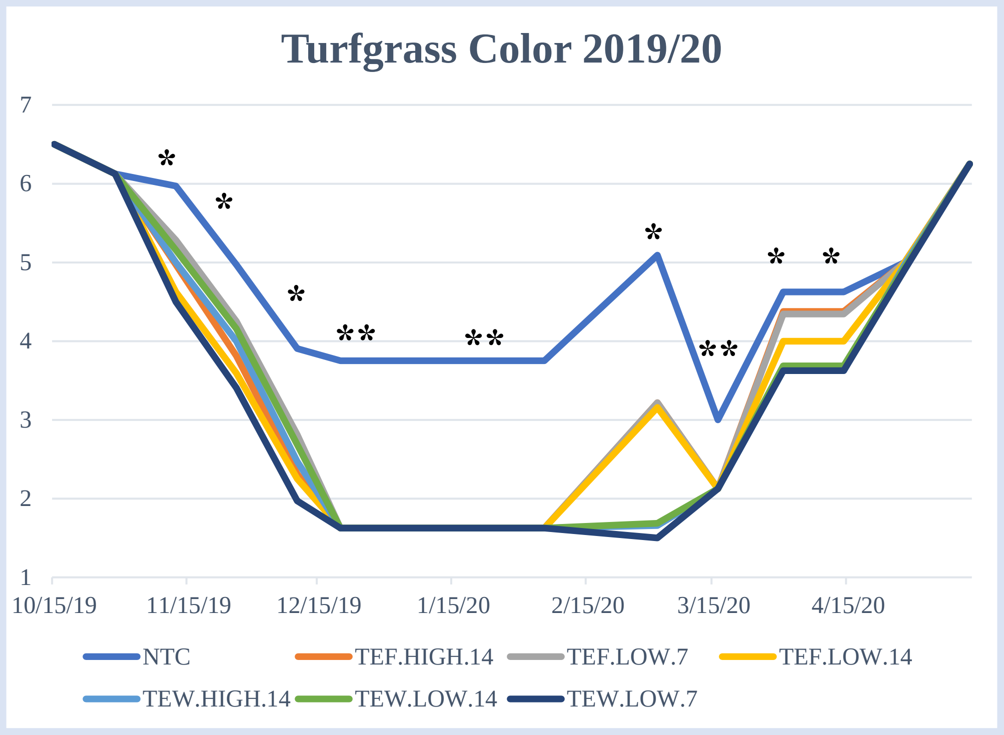

*Figure 16: 2019-2020 Field Evaluations, Impact of trinexapac-ethyl Application Rate and Timing. Turfgrass Color Ratings over Time. * indicates significant differences between treatments for specific rating dates. ** indicates significant differences between UTC and TE treatments for specific rating dates.*

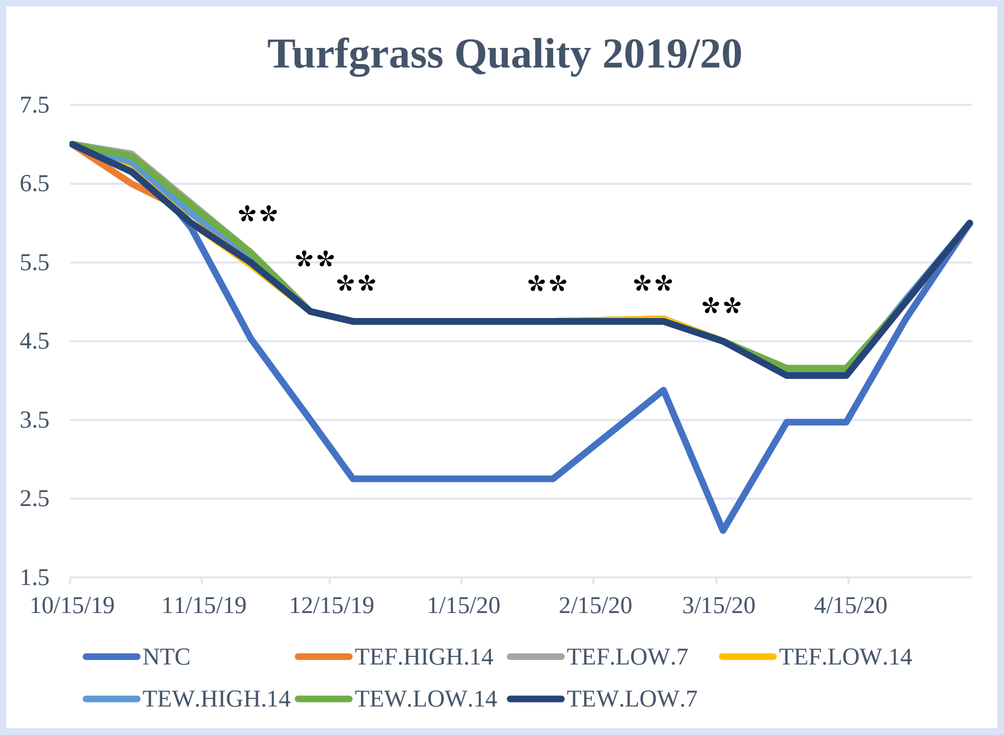

*Figure 17: 2019-2020 Field Evaluations, Impact of trinexapac-ethyl Application Rate and Timing. Turfgrass Quality Ratings over Time. ** indicates significant differences between UTC and TE treatments for specific rating dates.*

3.2 Growth Chamber Evaluations

<u>Pilot Study</u>

The pilot study revealed that varying levels of low-temperature injury occurred between 4-10 hours of exposure to -9.4°C. All treatments over 8 hours of exposure to -9.4°C experienced lethal injury (Figure 5). Our treatment levels of 4, 6, 8, and 10 hours of exposure to -9.4°C were chosen based on the results of this pilot study.

Figure 18: Impact of low-temperature exposure during pilot study. Plugs were re-acclimated to warm conditions in greenhouse after low-temperature exposure treatments. This image was taken 6 days after low-temperature x time treatments ended. Treatments with longer exposure to -6.7°experienced greater injury.

<u>Low-temperature Exposure during Cold Acclimation</u>

Percent green cover 6 days after treatment was used to evaluate low-temperature injury differences between treatments. Data were best modeled using non-linear Gompertz 3 parameter regression (JMP Pro 16). During both runs of this experiment, the LT_{50} was higher for the nontreated pots (11.52 and 11.19 hours at -9.4°C) than the pots treated with TE (9.91 and 9.97 hours at -9.4°C) (Figures 6-9). These data suggest that TE has a negative impact on cold tolerance of ultradwarf bermudagrass during cold acclimation.

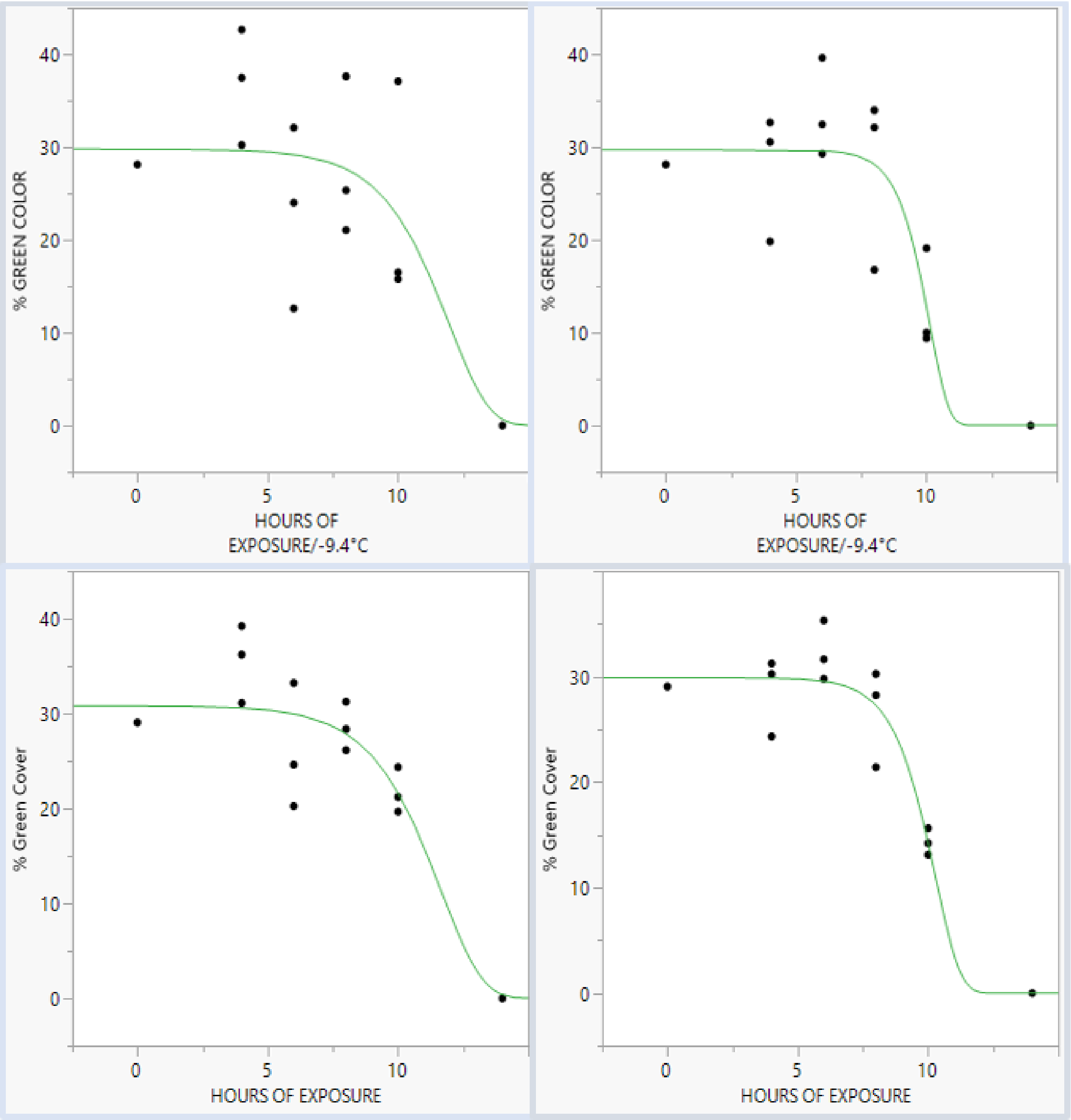

Figure 6: Impact of exposure time at -9.4°C on percent green cover of 'Champion' ultradwarf bermudagrass (UDB) under controlled conditions, modeled using non-linear Gompertz 3 regression ($r \geq 0.82$) without trinexapac-ethyl TE (top and bottom left) and with TE at 0.026 kg a.i. ha^{-1} every 14d (top and bottom right). The lethal temperature required to reduce bermudagrass green cover by fifty percent (LT_{50}) was 11.2 to 11.5 hr for nontreated UDB and 9.9 to 10.0 hr for TE-treated UDB.

4 Discussion

The assessment of turfgrass color and quality in field evaluations indicated that TE applications had an impact on color and quality during CA, winter dormancy, and cold de-acclimation. Plots under TE treatments lost color prior to the nontreated control plots during CA. Plots treated with TE on a 7-day interval lost color at a faster rate than other TE treatments. This loss of color or discoloration may be viewed as a positive or a negative but it indicates a change in the plant under TE treatments during CA. There was higher turfgrass quality in plots under TE treatments compared to the nontreated plots during winter dormancy.

Ultradwarf bermudagrass is most susceptible to low-temperature injury during de-acclimation or during extended periods of dormancy following low-temperature exposure (Chalmers & Schmidt, 1979). When warm temperatures occurred in February of 2019 and February/March of 2020, the nontreated control plots began to break dormancy and even had significant growth in Year 2 (2019/20). Our observations and data confirmed that TE prevented early dormancy break in the field evaluation. 'Fall Only' treatments also began to break dormancy in Year 2, indicating that winter treatments are both impactful and important to preventing pre-mature cold de-acclimation. All treatments broke dormancy at different times but did reach acceptable turfgrass quality and color once soil temperatures increased in the spring of the year. Sustained winter dormancy during is one of the plant's best defenses until consistent growing conditions arrive and entering and exiting dormancy multiple times causes unnecessary expenditure of carbohydrate reserves. This may be more critical in more temperature regions where UDB rarely if ever goes fully dormant.

Turfgrass color and density was improved under TE treatments during CA in Growth Chamber evaluations. We did observe greater low-temperature injury for plants under TE treatments during cold acclimation. This is likely less important as extreme, low temperatures are rare in the fall of the year in areas with UDB putting greens and the use of covers will likely prevent UDB putting greens from experiencing these drastic temperatures. An increase in low-temperature sensitivity during CA may predict an improved cold tolerance during winter dormancy. Unfortunately, our growth chamber experiments did not yield results across the three metabolic stages.

5 Conclusions

Trinexapac-ethyl applications improved UDB color and quality in the early stages of CA. Ultradwarf bermudagrass plots treated with TE had reduced color while improving UDB quality in the later stages of CA and the entirety of winter dormancy. Winter TE treatments were more impactful at delaying cold de-acclimation, compared to fall TE treatments, indicating a benefit to both fall and winter treatments. Treatments of TE prevented premature cold de-acclimation during winter dormancy in both the field and controlled environment studies. Growth chamber studies indicated a change in cold tolerance of UDB treated with TE during CA indicating that UDB CA was altered by TE. Further research is needed to evaluate the impact of TE on cold tolerance of UDB during winter dormancy and cold de-acclimation.

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Chapter 3: Evaluating the Impact of Fall Fungicide Programs and Acibenzolar-S-Methyl on Winter Quality of Bermudagrass Putting Greens in the Transition Zone

1 Introduction

Fungicides have been used to control fungal diseases of golf putting greens in the United States for over 100 years (Latin, 2011) and widely prescribed since at least 1932 (Monteith & Dahl, 1932). Over the last 20 years, ultradwarf bermudagrass (UDB; *Cynodon dactylon* (L.) *Pers.*× *C. transvaalensis* Burtt-Davy) has become the prevalent turfgrass used on golf putting greens in the Southeastern United States (McCullough et al. 2007; Hartwiger, 2009; Inguagiato & Martin 2015). Ultradwarf bermudagrasses gained popularity due to their heat and traffic tolerance and the ability to provide excellent golf putting greens (Goatley et al. 2007; Hartwiger, 2009; Inguagiato & Martin 2015). Ultradwarf bermudagrass putting greens have been widely adopted throughout the warm/humid zone and southern transition zone and are now being grown in the upper transition zone including Virginia (Hartwiger, 2009; Richardson & Booth, 2021). These cooler regions of adaptation have fewer days of active growth and greater risk of exposure to sublethal temperatures, so winter management is critical to long term success (Goatley et al. 2007; O'Brien & Hartwiger, 2007; Richardson & Booth, 2021).

Fungal diseases present challenges during the UDB active growing season but are arguably more concerning when UDB is not actively growing preceding, during, and exiting winter dormancy (Inguagiato & Martin 2015). Fungicide use increases on many UDB putting greens during the winter to combat fungal diseases including leaf spot (*Bipolaris cynodontis*), Pythium blight (*Pythium* spp.), Microdochium patch (*Microdochium nivale*), and cream leaf blight (*Limonomyces roseipellis)* (Smiley et al. 2005; Inguagiato & Martin 2015). Successful management of the various diseases is traditionally accomplished using a combination of active ingredient tank mixtures or rotations (Latin, 2011; Inguagiato & Martin 2015).

While fungal diseases are one component of winter UDB challenges, abiotic stress including low-temperature exposure and desiccation can lead to decreased turfgrass quality and density (Goatley et al. 2007; O'Brien & Hartwiger, 2007; DeBoer et al. 2019; Richardson & Booth 2021.) Beneficial, nontarget effects of certain fungicides have been documented including improved rooting in controlled environments, improved turfgrass quality, and increased turfgrass density (Dernoeden & McIntosh, 1991; Brosnan et al., 2010). However, research has also demonstrated that fungicides may not have an impact on turfgrass quality or rooting in the absence of disease (Benelli et al 2016; Schiavon et al. 2021).

Phosphonate fungicides have been shown to improve stress tolerance of turfgrasses in the absence of disease (Huang & Liu, 2009; McCarty et al. 2013). Several commercially available phosphonate fungicides contain a green pigment. Phosphonate fungicides have been shown to improve turfgrass quality in the absence of disease independent of pigment (Huang & Liu, 2009; McCarty et al. 2013).

Acibenzolar-S-methyl (ASM) is an active ingredient combined with a fungicide in several commercially available products. While the exact mechanisms are unknown, ASM is labeled as a plant defense activator and has been shown to reduce disease symptoms in creeping bentgrass (*Agrostis stolonifera* L.) (Lee et al. 2003; Fry et al. 2006) and induce limited transpiration rate leading to water conservation and drought tolerance (Shekoofa et al. 2016). Acibenzolar-S-methyl has also been shown to improve heat and drought tolerance in creeping bentgrass when applied as pre-formulated products with chlorothalonil (Jespersen & Huang 2017). However, chlorothalonil + ASM resulted in less root area in creeping bentgrass trials and treatments with

ASM resulted in lower turfgrass quality under drought conditions compared to the nontreated control (Schiavon et al. 2021).

Turf covers constructed from woven polypropylene are commonly used to buffer soil and canopy temperatures of UDB putting greens during periods of cold weather (Goatley et al. 2007; O'Brien & Hartwiger, 2007; Richardson & Booth, 2021). Turf cover thresholds have been developed and widely implemented to guide covering decisions based of forecasted low air temperatures. (O'Brien & Hartwiger, 2007; DeBoer et al. 2019; Richardson & Booth, 2021). Until recently, the recommended threshold for forecast minimum temperature was -3.9°C (O'Brien & Hartwiger, 2007). Despite research showing UDB tolerance to -9.4°C in field situations (DeBoer et al. 2019), the current conservative recommendation of -6.7°C accounts for most site-specific variability and potential differences in UDB cultivar cold tolerance (Richardson & Booth, 2021).

Little is known about how the interactions between fungicides, ASM and turf covers impact UDB winter quality. The objective of this study was to evaluate the impact of late fall fungicide programs, ASM, and turf cover thresholds on UDB quality.

2 Materials and Methods

This experiment was performed during the winters of 2019/2020 (Year 1) and 2020/2021 (Year 2) on mature stands of UDB putting greens at the Virginia Tech Research Short Course at Independence Golf Club in Midlothian, Virginia ((37.55 N, -77.69 E)The study was conducted on the experimental UDB variety 'JK 110521' in Year 1 and 'MiniVerde' UDB in Year 2. These greens were not painted or overseeded with a cool-season turfgrass for winter color/playability during the course of the experiment and were maintained at a height of 4mm heading into and throughout winter dormancy.

The study was repeated each year under two turf cover regimes matching recommendations by O'Brien & Hartwiger (2007) of -3.9°C and Richardson & Booth (2021) of -6.7°C. Observed air temperature data for winter 2019-2020 and 2020-2021 are presented in Tables 3 and 4. Each trial consisted of 1.8m x 1.8m plots arranged in a randomized complete block design with four replications of four treatments. Trial 1 in each year was covered with a black, woven polypropylene turfgrass cover (S and S Turf Covers, Covington, GA) whenever forecast lows were below -3.9°C. The second set of replicated treatments (Trial 2) was covered when forecast lows were below -6.7°C. If forecast lows were below -9.4°C, both studies were covered with two black, woven polypropylene turfgrass covers.

In Year 1, a turf cover was applied over Trial 1 (-3.9°C) on November 13-14, 2019 (Figure 1); December 19-22, 2019; February 14-17, 2020; February 28-March 2, 2020. Cover treatments were applied to both Trial 1 and Trial 2 (-6.7°) on January 20-23, 2020 and February 20-23, 2020.

***Figure 1**: Black, woven polypropylene turf cover applied over Trial 1 on November 14, 2019.*

In Year 2 (‘MiniVerde’), a turf cover was applied over Trial 1 (-3.9°C) on December 24-31, 2020; January 22-25, 2021; February 19-22, 2021; March 5-8, 2021. Cover treatments were applied to both Trial 1 and Trial 2 (-6.7°) on January 29-February 1, 2021.

Fungicide program treatments were initiated on October 1, 2019 in Year 1 and October 28, 2020 in Year 2 (Table 1). In each year, initial treatments were followed by three subsequent applications every 14 days. In both years, two applications of products containing azoxystrobin were made 28 days apart and four applications of products containing chlorothalonil or potassium phosphite were made every 14 days apart. All treatments were applied using a CO_2-pressurized sprayer delivering solution at 276 kPa of pressure with a water carrier volume of 842

L ha^{-1}. The fungicide treatments were Appear ® II, Daconil® Action™, Heritage®Action™, Daconil Weatherstik®, and Heritage® (all produced by Syngenta Crop Protection, Greensboro, NC, USA, and a.i.'s described in Table 1).

Table 1: Treatment application information for fungicides applied to ‘JK 110521’ in 2019-2020 and ‘MiniVerde’ in 2020-2021 ultradwarf bermudagrass putting greens during fall and winter months in Midlothian VA.*Application interval as weeks after initial application (WAIA). Initial applications were made on October 1, 2019 and October 28, 2020.

Treatment	**Active ingredient**	**Rate (g ai$^{-ha)}$**	**Trade Name, Manufacturer**	**WAIA***
NTC	Nontreated Control			
HA	Azoxystrobin + acibenzolar-S-methyl	0.12 g/m^2	Heritage Action, Syngenta Crop Protection	0,4
DA	Chlorothalonil + acibenzolar-S-methyl	1.12 mL/m^2	Daconil Action, Syngenta Crop Protection	0,2,4,6
PP	Potassium phosphite	1.92 mL/m^2	Appear II, Syngenta Crop Protection	0,2,4,6
DA	Chlorothalonil + acibenzolar-S-methyl	1.12 mL/m^2	Daconil Action, Syngenta Crop Protection	0,2,4,6
HA	Azoxystrobin + acibenzolar-S-methyl	0.12 g/m^2	Heritage Action, Syngenta Crop Protection	0,4
DW	Chlorothalonil	1.15 mL/m^2	Daconil WeatherStik, Syngenta Crop Protection	0,2,4,6
H	Azoxystrobin	0.12 g/m^2	Heritage WG, Syngenta Crop Protection	0,4

Turfgrass quality and color assessments were made at time of application and monthly through the winter and then again bi-weekly in early spring. Trials were rated for turfgrass quality in accordance with methods outlined by Krans & Morris (2007) on a 1-9 scale with 6 being minimally acceptable and 9 being the maximum score. Turfgrass color (1-9, 6=minimally acceptable) and turfgrass coverage (%) was also recorded.

2.1 Experimental Design and Data Analysis

Treatments were arranged in a completely randomized design with four replications of four treatments. Turf covers were applied to all four treatments and replications based on forecasted air temperatures.

Turfgrass quality and color data were collected and transformed to Standardized Area Under the Progress Curve (SAUPC). Turfgrass quality SAUPC and turfgrass color SAUPC data were subjected to analysis of variance (ANOVA) and means were separated to compare treatments using Fisher's protected LSD ($\alpha = 0.05$) (JMP Pro, SAS Institute, Cary, NC).

3 Results

There were no statistical or biological differences in bermudagrass performance metrics between trials covered at -3.9°C and trials covered at -6.7°C ($p \geq 0.226$).

Turfgrass color

There was no treatment by year, trial, or trial by year interactions with regard to turfgrass color ($p \geq 0.860$). Therefore, all treatment data were combined by site-year (Table 2). Plots treated with Appear II + Heritage Action + Daconil Action (DA+HA+PP) had higher turfgrass color ratings than the nontreated control (NTC), Heritage Action + Daconil Action (DA+HA), and Heritage +

Daconil Weatherstik (DW+H) for Turfgrass Color SAUPC. There were no significant differences between DA+HA and DW+H in both trials of each year, but both provided superior turfgrass color compared to the nontreated control (NTC).

Table 2: Impact of fall fungicide programs on ultradwarf bermudagrass color (1-9 scale, 9=dark green and 6=acceptable) and quality (1-9 scale, 9=highest quality and 6=minimally acceptable) transformed using the standardized area under the progress curve. Means with the same letters within columns are not significantly different according to Fisher's protected LSD test (α = 0.05). DA= Daconil Action, DW = Daconil Weatherstik, H = Heritage, HA = Heritage Action, PP = phosphanate, and NTC = nontreated control.

Treatment	**Color***	**Quality****
NTC	3.61 c***	4.02 c
DA+HA+PP	6.31 a	6.50 a
DA+HA	4.83 b	5.73 b
DW+H	4.87 b	5.64 b
LSD	0.38	0.26

[*] Turfgrass color assessed using a 1-9 scale, 9=dark green and 6=acceptable.
[**] Turfgrass quality assessed using a 1-9 scale, 9 = highest quality and 6 = acceptable.
[***] Means followed by the same letter within columns are not significantly different according to Fisher’s protected LSD (α=0.05).

Turfgrass quality

There was no treatment by year, trial, or trial by year interactions with regard to turfgrass quality (p≥0.277). Therefore, all treatment data were combined by site-year (Table 2). Plots treated with Appear II + Heritage Action + Daconil Action (DA+HA+PP) had higher turfgrass quality SAUPC than the nontreated control (NTC), Heritage Action + Daconil Action (DA+HA), and Heritage + Daconil Weatherstik (DW+H) for Turfgrass Color SAUPC. There were no significant differences between DA+HA and DW+H in both trials of each year, but both provided superior turfgrass quality compared to the nontreated control (NTC).

4 Discussion

The winters of 2019-2020 and 2020-2021 were relatively mild based on seasonal averages for Midlothian, Virginia. Temperatures were never forecast nor reached the double cover threshold of -9.4°C when low-temperature UDB would be likely to occur. This study was designed to mimic ‘real-world’ scenarios, so an uncovered control was not used. No low-temperature injury was found in either year and all treatments maintained 100% turfgrass coverage. This supports the covering threshold of -6.7°C as a viable option for UDB putting greens as outlined by Richardson & Booth in 2021.

Many golf courses use paints, pigments, or overseed with cool season grasses to maintain green color on putting greens during the winter dormant season (McCarty et al. 2013; Inguagiato & Martin 2015). These strategies can mask disease and winter UDB decline. While there were no paints or overseed used in this study, the presence of pigment in Appear II, certainly had an impact on turfgrass color in Treatment 2. With that said, turfgrass quality was significantly improved in this treatment, indicating an impact greater than pigment alone. The presence of the pigment seemed to have an impact on spring green up in treatment 2, despite having been applied multiple months prior (Figure 2). This would indicate both improved turfgrass quality and spring green up using Appear II and potentially other pigmented products.

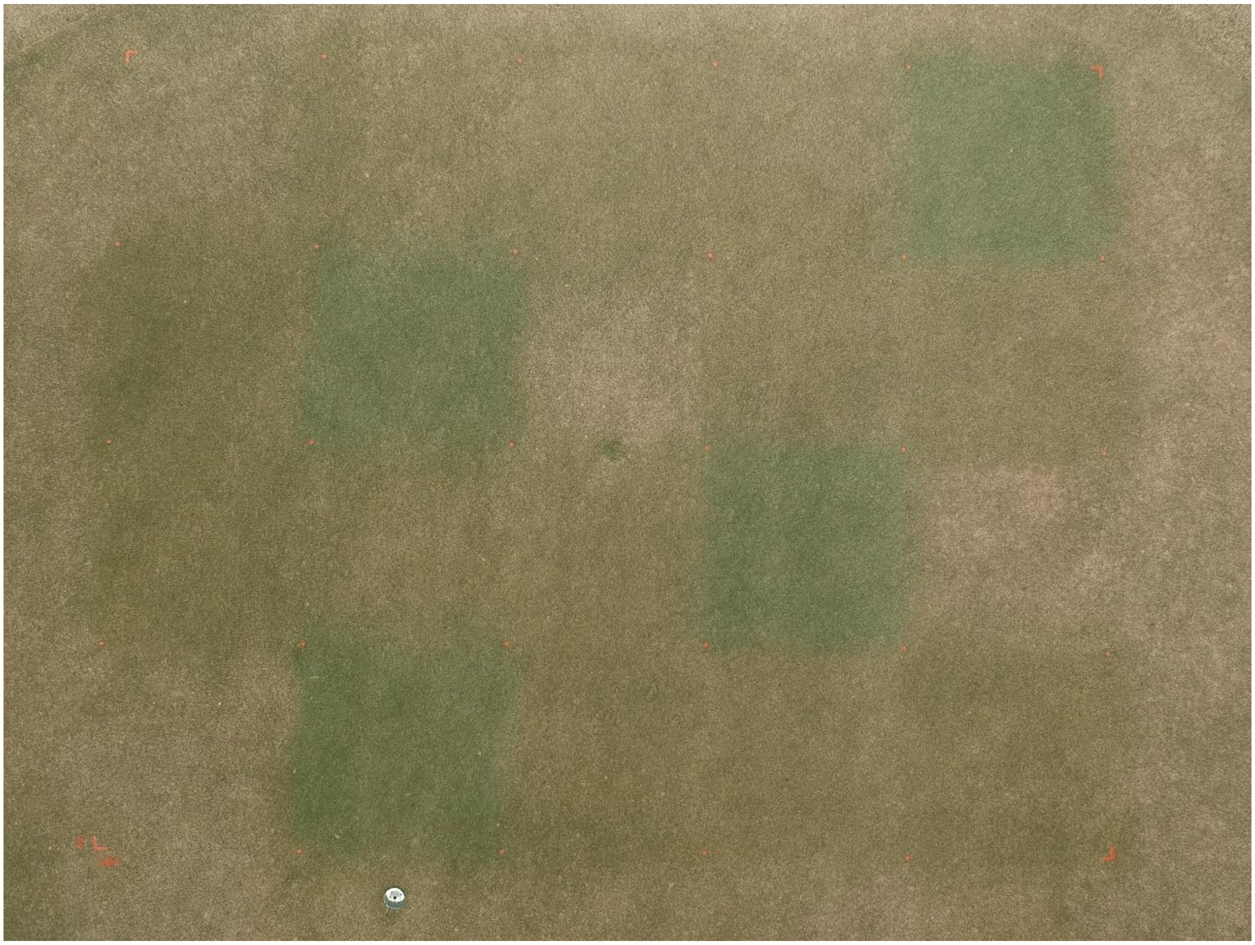

Figure 19: Aerial image of Trial 1, Year 2 ('MiniVerde') March 30, 2021. This trial was covered when air temperatures were forecast below -6.7°C. Treatments included an untreated control, and various fungicide programs, with and without acibenzolar-s-methyl.

There were no consistent differences between treatments containing ASM and treatments without ASM in turfgrass quality or color. Symptoms of cream leaf blight (*Limonomyces roseipellis)* were observed in Year 2 (Figure 2 and 3) on the nontreated control plots (Treatment 1) and areas surrounding the trials, but this pathogen was not confirmed through microscopy. All fungicide treatments (2-4) controlled these disease symptoms for the extent of the study.

Figure 20: Cream leaf blight (*Limonomyces roseipellis*) symptoms on 'MiniVerde' nontreated control plot on January 6, 2021.

The late covering of Trial 1 in Year 2 on March 5-8, 2021 led to visual increases in color of the entire Trial 1 compared to Trial 2 but these changes did not lead to statistical differences or long-lasting effects. This supports the value of covering during marginal cold temperatures (<-3.9°C) during the metabolic phase of cold de-acclimation of UDB resulting in spring green up, but further research is needed to confirm theses suspicions.

Two applications of Velista® (Penthiopyrad, Syngenta Crop Protection, Greensboro, NC) followed by post-application irrigation were applied in the fall of 2019 and 2020 respectively for the prevention of spring dead spot (*Ophiosphaerella* spp.). Increases in visual winter turfgrass quality has been observed following applications of Velista® which is a Group 7, succinate dehydrogenase inhibiting (SDHI) fungicide. Further research is needed to evaluate the impact of this fungicide group on winter quality and spring green up of UDB putting greens.

5 Practical Implications of this Research

The results of this research imply a clear benefit of rotating applications of contact, systemic, and pigmented phosphonate fungicides on UDB putting greens leading into winter dormancy. This type of fall fungicide programming protected UDB from fungal pathogens, improved winter and spring turfgrass quality, and improved spring green up. This research does not support the need for acibenzolar-s-methyl in fungicide programs for winter management of UDB.

The results of this research also supported the covering threshold recommendations of -6.7°C.

Winter 2019-2020 Observed Air Temperatures °C

	November 2019			**December 2019**			**January 2020**			**February 2020**			**March 2020**		
	MAX	AVG	MIN	MAX	AVG	MIN	MAX	AVG	MIN	MAX	AVG	MIN	MAX	AVG	MIN
1	14.4	9.8	5.6	10.0	7.6	5.0	12.2	6.8	2.2	10.0	5.9	3.3	11.7	3.8	-5.6
2	15.0	7.4	1.7	10.0	7.3	5.6	15.6	8.3	0.0	14.4	8.7	0.6	19.4	12.1	2.2
3	14.4	7.6	1.7	10.6	6.6	2.8	14.4	10.8	7.2	21.7	14.7	8.9	17.8	14.0	8.9
4	17.2	9.2	0.6	11.1	6.7	0.6	17.2	13.4	7.2	21.7	17.5	13.9	17.2	12.8	5.0
5	20.6	13.2	7.8	12.2	6.2	1.1	9.4	5.7	1.7	17.2	10.1	5.0	12.2	8.9	3.9
6	16.1	10.6	6.1	13.9	6.6	-2.8	13.3	5.9	0.0	10.6	7.7	5.0	13.9	8.2	4.4
7	18.9	11.9	3.3	8.9	4.4	-2.2	6.7	3.4	-1.1	16.1	11.1	2.2	12.2	7.5	3.3
8	11.1	4.9	-1.1	10.6	4.9	-3.3	11.7	4.6	0.0	7.2	3.2	-0.6	16.7	7.8	-1.7
9	8.9	2.7	-2.8	15.6	9.4	6.7	6.7	1.6	-3.9	10.6	4.2	-2.2	22.2	13.4	5.0
10	17.8	8.2	-0.6	20.0	15.2	9.4	16.1	9.1	0.6	13.3	9.1	3.3	21.1	17.2	13.9
11	21.1	12.3	4.4	8.9	3.2	-1.7	22.2	17.6	12.8	16.1	14.2	11.1	20.0	15.0	10.6
12	13.9	5.1	-2.2	5.0	0.6	-3.9	20.6	17.2	8.9	10.6	8.4	6.7	17.2	12.2	8.3
13	3.3	-1.5	-5.0	5.0	2.8	-0.6	15.0	12.5	11.7	16.7	10.7	6.1	26.7	19.4	10.6
14	6.7	2.1	-6.1	12.8	7.3	5.0	12.2	11.1	10.0	7.8	3.9	-2.2	16.7	12.8	8.9
15	9.4	6.3	4.4	13.9	8.1	4.4	12.2	9.9	8.3	3.3	-0.9	-6.1	11.1	8.5	6.7
16	7.8	5.8	4.4	10.0	5.8	3.9	16.1	11.3	3.3	11.1	5.2	-1.1	13.9	9.2	5.6
17	8.3	5.7	3.9	11.1	6.3	3.3	5.0	1.3	-1.7	13.3	8.0	3.3	19.4	12.4	5.6
18	11.1	7.3	5.0	7.8	3.2	-1.7	5.0	1.7	-1.7	16.7	8.8	1.1	15.0	12.0	10.0
19	10.6	7.7	6.1	2.8	-1.7	-5.6	11.1	6.4	-0.6	12.2	8.2	1.1	27.8	16.8	10.0
20	13.9	8.4	5.0	6.7	0.6	-4.4	2.2	-1.7	-3.9	4.4	1.2	-0.6	31.1	25.1	20.0
21	12.8	8.2	2.8	6.1	0.9	-2.2	2.8	-2.2	-5.6	3.9	-0.7	-5.0	18.9	12.6	9.4
22	16.1	11.4	6.1	8.9	2.6	-3.9	5.6	-0.8	-6.1	11.7	2.4	-6.7	12.8	9.5	7.2
23	8.3	5.3	-0.6	12.2	5.9	1.7	6.7	1.9	-3.9	17.2	7.1	-3.3	9.4	7.4	6.1
24	13.3	8.3	4.4	13.9	6.7	1.1	15.6	8.2	0.6	12.8	8.2	3.9	14.4	9.6	4.4
25	13.9	6.5	-0.6	12.2	4.2	-1.1	16.1	11.7	6.1	15.6	10.6	8.3	10.0	8.5	7.2
26	18.3	9.7	1.7	17.2	4.8	-1.1	11.1	6.1	1.1	12.8	11.1	8.9	13.9	8.7	5.0
27	15.0	12.0	8.9	15.6	8.0	5.0	11.7	5.9	-0.6	10.0	6.1	1.1	21.1	16.4	10.0
28	13.3	9.2	5.0	18.9	11.9	6.1	8.3	5.2	0.0	10.0	4.7	-1.7	25.0	17.6	13.3
29	12.2	7.3	2.8	17.2	13.1	8.3	7.2	2.7	-2.2	6.1	3.1	-0.6	30.0	18.1	12.2
30	7.2	5.9	3.9	22.2	18.8	11.1	6.7	1.7	-2.8	-	-	-	22.8	18.3	12.8
31	-	-	-	13.3	8.9	6.1	7.8	2.7	-2.2	-	-	-	13.3	9.3	6.7

Table 3: Observed Air Temperature Data °C 2019-2020, Midlothian, VA.

Winter 2020-2021 Observed Air Temperatures °C															
	November 2020			**December 2020**			**January 2021**			**February 2021**			**March 2021**		
	MAX	AVG	MIN	MAX	AVG	MIN	MAX	AVG	MIN	MAX	AVG	MIN	MAX	AVG	MIN
1	16.7	11.1	5.6	8.9	5.4	2.2	6.1	4.2	2.2	0.6	-0.1	-1.1	15.6	11.5	6.7
2	11.1	6.9	2.8	10.6	4.3	0.6	18.3	8.8	2.8	4.4	2.1	0.6	7.8	3.9	0.0
3	16.7	9.7	4.4	14.4	5.3	-2.8	9.4	6.5	4.4	9.4	3.9	0.0	15.6	6.6	-1.1
4	19.4	11.2	3.9	15.0	10.8	5.6	8.3	4.6	1.7	11.7	4.8	-3.3	16.1	9.2	3.3
5	21.7	12.9	5.0	13.3	9.2	5.0	8.9	5.5	3.9	11.1	7.7	3.9	8.9	3.7	-1.7
6	22.8	14.1	9.4	9.4	4.5	-0.6	8.3	4.4	0.6	13.3	7.3	0.0	11.1	4.4	-3.3
7	25.0	15.1	8.9	2.2	0.9	-0.6	8.9	2.5	-2.8	6.1	3.0	0.6	9.4	3.1	-2.8
8	24.4	16.7	10.0	8.9	2.3	-2.2	6.1	3.1	0.0	6.7	1.2	-3.3	13.9	5.0	-3.3
9	25.0	17.9	11.1	9.4	2.3	-3.9	8.3	3.0	-1.1	13.9	5.2	-0.6	21.7	11.2	0.0
10	23.9	20.9	17.2	12.8	4.9	-1.7	10.6	3.6	-2.2	6.1	4.2	1.7	23.9	14.2	3.3
11	21.1	16.5	11.1	17.2	9.3	1.7	7.8	2.6	-3.3	3.9	1.9	-0.6	25.6	16.7	7.8
12	17.8	12.3	9.4	17.2	12.1	8.3	11.1	4.7	-0.6	-0.6	-1.2	-1.7	22.8	17.2	12.2
13	16.7	10.6	4.4	21.7	14.2	8.9	11.7	3.3	-2.8	0.0	-0.6	-1.7	17.2	12.2	6.1
14	23.3	16.5	7.2	8.9	5.8	0.6	13.3	5.4	1.1	1.1	-0.1	-1.1	18.3	11.2	1.1
15	16.1	11.3	5.6	6.1	1.8	-1.7	7.2	7.2	7.2	3.3	2.3	1.1	10.0	6.8	2.2
16	14.4	8.9	4.4	2.8	1.4	-2.2	7.8	4.6	0.6	11.7	5.9	0.6	5.6	4.1	1.1
17	8.9	3.5	-1.7	6.7	2.6	-1.1	9.4	4.6	-1.7	5.0	0.3	-3.3	11.7	8.3	5.6
18	12.2	4.4	-2.2	5.6	2.8	-0.6	8.9	4.3	0.0	0.6	-0.3	-1.1	13.9	11.1	8.3
19	19.4	10.9	2.8	5.0	0.9	-3.3	13.3	4.8	-2.2	2.8	0.4	-1.1	10.6	6.4	1.7
20	22.2	13.4	5.0	5.6	3.8	0.0	10.0	4.4	-2.2	3.9	-0.4	-3.9	12.2	5.6	-1.1
21	19.4	14.4	10.0	8.3	4.9	2.8	13.9	5.6	-3.3	3.9	-0.6	-6.1	18.3	8.8	1.1
22	15.0	12.7	3.9	11.7	6.6	-0.6	12.8	6.8	1.1	7.2	2.9	-1.1	19.4	11.3	3.3
23	11.7	6.8	0.6	10.6	4.3	-2.8	4.4	-0.1	-3.9	17.2	8.6	2.2	18.9	13.3	7.8
24	16.1	9.7	3.9	17.8	15.2	6.1	5.0	0.1	-6.1	20.0	10.0	0.0	15.6	13.2	11.7
25	22.8	16.4	11.7	14.4	3.6	-3.3	6.7	3.6	1.1	15.0	10.4	3.3	23.3	16.3	10.0
26	20.0	14.2	10.6	3.3	-1.6	-5.6	6.7	4.0	1.7	8.3	4.8	0.6	29.4	23.4	17.8
27	17.8	11.5	7.2	6.7	0.6	-5.0	8.9	5.6	2.8	11.7	7.2	4.4	22.8	15.9	10.0
28	16.1	9.6	2.8	14.4	6.4	-0.6	3.9	0.5	-2.8	10.6	9.9	8.9	25.0	17.3	12.8
29	20.6	16.1	8.3	8.3	3.9	-1.1	1.7	-2.2	-5.6	-	-	-	17.2	11.7	7.8
30	22.8	18.3	12.8	9.4	3.4	-4.4	3.9	-1.4	-7.8	-	-	-	21.7	13.9	5.0
31	-	-	-	13.9	9.5	5.6	1.1	-0.3	-1.1	-	-	-	22.8	16.8	15.0

Table 4: Observed Air Temperature Data °C 2020-2021, Midlothian, VA.

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Chapter 4: Impact of Core Aeration Programs on Performance of Bermudagrass Putting Greens with Sand-Based Rootzones in Virginia

1 INTRODUCTION

Hybrid bermudagrasses (*Cynodon dactylon* (L.) *Pers.*× *C. transvaalensis* Burtt-Davy) are warm-season turfgrasses adapted to tropical, subtropical, and warm temperate regions (Turgeon & Kaminski, 2019). Hybrid bermudagrass has been used on golf putting greens since the 1950's (O'Brien, 2012; Baxter & Schwartz, 2018). Since 1995, the term 'ultradwarf' has been used to describe the lower growth habit and finer leaf texture of modern hybrid bermudagrasses commonly used on putting greens including cultivars 'Champion', 'MiniVerde', and 'TifEagle' (Reasor et al. 2016). Since the early 2000's, ultradwarf bermudagrass (UDB) has become the predominant turfgrass used on golf course putting greens in the Southeastern United States (Hartwiger, 2009, Lyman et al. 2007). With the success of UDB in the Southeast, golf courses have replaced creeping bentgrass with UDB on putting greens well into the transition zone (Hartwiger, 2009; O'Brien & Hartwiger, 2011; Richardson et al 2014). While UDB offers improved heat and drought tolerance over creeping bentgrass, it is more susceptible to winter injury (Richardson et al. 2014, Richardson & Booth, 2021).

Ultradwarf bermudagrass is a prolific thatch and organic matter producer and research has been focused on this topic for many years in Florida and the Southeast (Carrow, 2004; Rowland, 2008; Rowland et al. 2009; Craft et al. 2016; Fidanza et al. 2017; Vines et al. 2017). Thatch is a layer of interwoven organic residue in the upper zone of soil, directly below turfgrass shoots and stems (McCarty et al. 2007; Turgeon & Kaminski, 2019). Thatch is beneficial for turfgrass moisture retention and traffic tolerance but can be detrimental in large quantities (Carrow, 2004; McCarty et al. 2007; Vines et al. 2017; Turgeon & Kaminski, 2019). Too much thatch can lead to soft, wet putting greens prone to mower scalping, disease, and insect damage (Carrow, 2004;

McCarty et al. 2007; Turgeon & Kaminski, 2019). Thatch accumulates in UDB putting greens when production of organic matter is greater than the rate of decomposition or removal (Carrow, 2004; McCarty et al. 2007; Turgeon & Kaminski, 2019). Turfgrass growth, duration of growing season, and plant stress impact organic matter production (Carrow, 2004). Natural decomposition of organic matter in putting greens is influenced by temperature, moisture, pH, aeration, and microbial health in the soil (Turgeon & Kaminski, 2019). Cultural practices including sand topdressing, vertical mowing, and core aeration impact thatch levels by physically removing or diluting the organic matter that resists decomposition in the soil (White & Dickens, 1984; Carrow, 2004; Schmid et al. 2014).

The United States Golf Association (USGA) has developed widely-adapted specifications for putting green rootzone construction (United States Golf Association, 2018). These specifications, originally developed in 1960, have been routinely updated to standardize the materials and methods used to construct putting greens with the goal of improving consistency across golf courses (Fidanza et al. 2017; United States Golf Association, 2018). While the USGA specifications include recommended physical soil properties and organic matter source and content during construction, organic matter rapidly accumulates in the first two years after planting (Carrow, 2004). Since organic matter accrues faster than it can decompose, thatch accumulates at an undesirably high rate and must be managed through cultural practices.

Many golf courses chose to convert their older bermudagrass or creeping bentgrass putting greens to UDB using the no-till method (Hartwiger, 2007; O’Brien and Hartwiger 2011). The no-till method results in a planting bed developed by chemical eradication of the existing turfgrass followed by aggressive aeration to minimize disruption of the putting surface, downtime to golf course closure, and unintentionally changing the contours of the putting surface (Hartwiger, 2007). Some golf courses chose to remove the thatch and organic matter from the previous putting greens during no-till renovations while some opted to plant UDB directly into this thatch/mat layer (Hartwiger, 2007). The existence of organic matter accelerates the grow-in process of UDB through increased nutrient and moisture retention but can lead to complications in the long-term health of the UDB putting greens. Excess thatch can exacerbate disease and pest severity in UDB putting greens as well as lead to soft, inconsistent playing conditions (Carrow, 2004; Turgeon & Kaminski, 2019).

Ultradwarf bermudagrass putting greens have been planted in Virginia since the early 2010's (Bevard, 2013). Latitude, longitude, elevation, weather patterns, and micro-environments influence growing season duration, minimum and maximum temperature exposure, growth rates, and organic matter decomposition rates that provide challenges with UDB use for putting greens in this area. These factors vary widely between locations in the Commonwealth, let alone the United States. Thatch and organic matter management is critical to successful UDB putting green performance in tropical and sub-tropical environments. To what extent this is true in temperate regions is unknown. With more UDB putting greens being grown in temperate regions, adjustments may need to be made to traditional thatch and organic matter management programs tailored from regions with warmer, longer growing seasons.

We hypothesize that organic matter and thatch accumulation rate in UDB putting greens in Virginia will vary from those grown in tropical and subtropical locations. The shorter growing season in the transition zone of Virginia not only influences growth patterns but also influences when and how much surface disruption can take place from cultural practices. Ultradwarf bermudagrass putting greens are also more prone to cold-temperature exposure in Virginia, so low-temperature injury is a greater concern for turfgrass managers. This research aims to evaluate the impact of core aeration and sand topdressing by percent surface disruption on the performance of UDB putting greens in Virginia, as assessed by measuring surface firmness, turfgrass quality, spring green up, and soil organic matter.

2 Materials and Methods

2.1 Experimental Design

This experiment was designed to evaluate the impact of core aeration programs on performance of UDB putting greens in Virginia over multiple growing seasons. The experiment was performed on UDB putting greens with USGA specified rootzones(United States Golf Association, 2018) at the Virginia Tech Research Short Course at Independence Golf Club in Midlothian, Virginia (37.55 N, -77.69 E). Two ‘TifEagle’ UDB putting greens and two ‘G12’ UDB putting greens were used in this study. Each ‘TifEagle’ and ‘G12’ location differed by construction method. Locations and their abbreviations include:

1. Location 3: ‘G12CR’: Complete Renovation 2018
2. Location 4: ‘TifEagleNT’: No-Till Renovation 2017
3. Location 5: ‘G12NT’: No-Till Renovation 2017
4. Location 9: ‘TifEagleCR’: Complete Renovation 2018

Locations TifEagleNT and G12NT were planted in 2017 using a “no-till” renovation method that included chemical eradication of the existing creeping bentgrass putting greens followed by aggressive core aeration and sand topdressing prior to vegetatively planting the bermudagrass. Locations G12CR and TifEagleCR were renovated following drastic winter injury in 2018. All of the existing turfgrass, organic matter, and thatch was removed from these greens with a sod cutter and replaced with an 85/15 sand/peat mixture conforming to USGA recommended particle size and distribution for a golf putting green (United States Golf Association, 2018). The ‘G12’ and ‘TifEagle’ sprigs were planted directly into the new greens mix on Location G12CR and TifEagleCR respectively with no existing thatch present. 2 m x 4 m plots were installed across all four locations with 3 replications of 6 treatments. All greens were irrigated as needed to provide adequate soil moisture for both establishment and maintenance and received routine fungicide and fertility applications as needed to maintain acceptable turfgrass growth. All four locations received monthly vertical mowing and sand topdressing in the growing season. Core aeration treatments were applied with a Toro ProCore™ 648 aerator (The Toro Company, Bloomington, MN) equipped with 12.7mm inner diameter coring tines spaced 3.8cm apart with a depth of ~7.8cm. After aeration treatments, the debris was removed, and holes were backfilled with USGA specified topdressing sand at ~5.85 kg/m2. Sand was applied with a John Deere JD100 drop topdresser mounted to a John Deere ProGator™ (John Deere, Moline, IL) and brushed into the aeration holes with a push broom to fill the holes.

An initial survey of 25 golf course superintendents from Virginia and North Carolina revealed cultural practice programs with surface disruption targets between 5% and 20%. One specific model of 20% every other year was adapted from a program aimed to reduce disruption of play at the golf course. Treatments included.

1. ~20% annual surface disruption (3.8 cm x 3.8 cm tine spacing) in two directions. Abbreviation: (20A)
2. ~15% annual surface disruption (3.8 cm x 5 cm spacing) in two directions. (15A)
3. ~10% annual surface disruption (3.8cm x 3.8 cm spacing) in one direction. (10A
4. ~5% annual surface disruption (3.8 cm x 6.4cm spacing) in one direction (5A)
5. ~20% surface disruption every other year (20EO)

6. No aeration (N)

Aeration treatment timing was based on turfgrass maturity in the first year and the golf calendar at Independence Golf Club in the following years. Aeration events occurred on 28 Aug 2018, 1 Jul 2019, 26 Aug 2020, and 10 Aug 2021. Plots receiving 20% surface disruption every other year were aerated on 01 July 2019 and 10 Aug 2021.

2.2 Data Collection

Trials were rated for turfgrass quality and color in accordance with methods outlined by Krans & Morris (2007) on a 1-9 scale with 6 being minimally acceptable and 9 being the maximum score. Turfgrass color (TC) (1-9, 9=darkest green color, 6=minimally acceptable) ratings were collected annually on 11 Apr 2019, 13 May 2020, and 5 May 2021, during spring green up, when UDB was transitioning from dormancy to active growth. Spring green up occurs at different times throughout the years due to differences in environmental conditions.

Turfgrass quality (TQ) ratings (1-9, 6=minimally acceptable), soil volumetric water content (VWC%) (FieldScout® TDR 350 with 3.8 cm probes, Spectrum Technologies, Inc., Aurora, IL), and surface firmness (TRUFIRM) (FieldScout® TruFirm, Spectrum Technologies, Inc., Aurora, IL) (Linde et al. 2011) data were recorded 29 Aug 2018, 16 Jun 2019, 12 Aug 2020, and 28 July 2021 while UDB was actively growing but before aeration treatments occurred for that growing season. Volumetric water content represents the amount of moisture in the top 3.8 cm of the soil profile. TRUFIRM data represent a turf penetration value when a hemisphere-shaped impact hammer is dropped from a consistent height. The lower the turf penetration value (TRUFIRM), the firmer the surface. The penetration values were generated as inches and converted to centimeters. For both VWC% and TRUFIRM, three measurements were taken per plot and averaged to account for variance and plot size.

Following aeration treatments in 2019 and 2020, UDB recovery (% UDB Coverage) was visually rated for four weeks (1 Jul – 29 Jul 2019 and 26 Aug -24 Sep 2020).

Two 11.4 cm diameter plugs were taken from each plot of G12CR and G12NT on 28 Jul 2021 to evaluate soil organic matter (OM). These locations were selected based on available resources for testing, similar growing environments, and difference in construction methods. Each plug's soil profile was separated by depth in 2.5 cm increments to represent the soil organic matter in the top 2.5 cm, 2nd 2.5 cm, and 3rd 2.5 cm zone of the soil. Each 2.5 cm zone of the two plugs for each plot were combined for representative samples. Soil samples were dried, ground, blended with a Waring blender for 30 seconds, and sorted into 10 cc representative samples. All samples were blended for consistency and necessity to break up OM and thatch in top 2.5 cm. Samples were then dried to 150°C and %OM was determined through loss on ignition at 360°C.

$$\%OM = 100 \times \frac{(\text{wt. at } 150°C - \text{wt. at } 360°C)}{(\text{wt. at } 150°C)}$$

where wt. = weight.

2.3 Data Analysis

For TC during spring green up, data were subjected to analysis of variance (ANOVA) (JMP Pro 16, SAS Institute, Cary, NC) to compare treatment differences for each year. Treatment means were separated using Fisher's protected LSD ($\alpha = 0.05$). TRUFIRM, VWC%, and TQ ratings were subjected to ANOVA to compare treatment and location differences by year. Recovery after aeration treatments was best modeled with logistic 3 parameter modeling (JMP Pro 16 (SAS Institute, Cary, NC) for %green cover over time by treatment.

$$y = c / ((1+\exp(-a(DAT-b)))$$

where y = expected % green cover value as a function of time, a=asymptote, b=growth rate, c=inflection point, and DAT=days after treatment. This model was selected because of both goodness-of-fit among tested models and biological relevance. The number of days after treatment when treated plots would recover to 75% and 100% green cover was determined using a custom inverse prediction model ($\alpha = 0.05$) within the JMP software based on Logistic 3 parameter regression models for each treatment in both 2019 and 2020.

Due to a significant location effect ($p=0.03$), OM data were separated by location and subjected to ANOVA. Means were separated when appropriate using Fisher's protected LSD ($\alpha = 0.05$). Organic matter data were analyzed for correlation with TRUFIRM and VWC% using bivariate fit modeling (JMP Pro 16, SAS Institute, Cary, NC).

3 Results

Turfgrass color during spring green up was not significant by location ($p=0.98$) but was by year ($p=0.0003$). Therefore, TC data were pooled by location and separated by year (Table 1). Treatments had a significant impact on TC for each year ($p \leq 0.0001$). Plots receiving no OM removal did not have acceptable TC (≥ 6) during spring green-up and had among the lowest color assessments of all treatments each year. Initial spring TC was always highest in plots receiving 20% annual surface removal, with a consistent decline in TC as the percent surface removal also declined. Plots receiving 15% annual removal had more green color in 2019 than plots receiving 10% but not in 2020 or 2021. Plots receiving only 5% annual removal consistently had lower TC at green-up than plots receiving greater annual removal. Plots receiving 20% surface removal every other year compared similarly to the nontreated control in 2019 but had higher TC at green-up in 2020 and 2021. The lack of differences between no surface removal and the 20% surface removal every other year is logical as no cores had been removed from these plots prior to the 2019 data collection. In subsequent years, TC in plots receiving 20% removal every other year compared favorably with plots receiving 5% annual surface removal.

Table 1: Impact of varying % summer surface removal by core aeration annually (A) or every other year (EO, or none (N) on ultradwarf bermudagrass putting green color during spring green up by year in Midlothian VA. Data were pooled across two locations for each year.

Spring Turfgrass Color *			
% surface removal	2019	2020	2021
20A	7.5 a**	6.75 a	7.17 a
15A	7.0 b	6.38 b	6.63 b
10A	6.58 c	6.17 b	6.54 b
5A	6.08 d	5.58 c	6.17 c
20EO	5.88 e	5.67 c	6.21 c
N	5.92 de	5.08 d	5.67 d
LSD	0.20	0.22	0.28

[*] Turfgrass color assessed using a 1-9 scale, where 9 = darkest, desirable green and 6 = minimally acceptable.

[**] Means followed by the same letter within columns are not significantly different according to Fisher’s protected LSD (α=0.05).

[***] Surface removal occurred every other year (2019 and 2021) rather than annually.

Turfgrass quality was not significant by location (p=0.11) but was by year (p=0.0001). Therefore, TQ data were pooled by location and separated by year (Table 2). Treatments had a significant impact on TQ for each year (p≤0.0001). Turfgrass quality improved as percent surface disruption increased. All treatments led to acceptable levels of TQ besides 5A and N (Table 2).

Table 3: Impact of varying % summer surface removal by core aeration annually (A) or every other year (EO, or none (N) on ultradwarf bermudagrass in-season turfgrass quality by year in Midlothian VA. Data were pooled across two locations for each year.

In-Season Turfgrass Quality*

TRT	2019	2020	2021
20A	7.67 a**	7.38 a	6.96 a
15A	7.42 a	6.83 b	6.46 b
10A	7.04 b	6.42 c	6.08 c
5A	6.67 c	6.04 d	5.70 d
20EO	6.83 bc	6.67 bc	6.17 c
N	6.63 c	5.83 d	4.92 e
LSD	0.36	0.27	0.25

[*] Turfgrass quality assessed using a 1-9 scale, where 9 = highest quality and 6 = minimally acceptable.

[**] Means followed by the same letter within columns are not significantly different according to Fisher's protected LSD (α=0.05).

[***] Surface removal occurred every other year (2019 and 2021) rather than annually.

Treatment differences were not detected for TRUFIRM and VWC% data in any year. However, TRUFIRM and VWC% data did confirm significant differences between locations in each year (Table 3), indicating that surface firmness and soil moisture may be more dependent on location than core aeration treatments. On average, locations G12CR and TifEagleCR were drier and firmer than locations TifEagleNT and G12NT, indicating that original construction method may be a significant driver of soil moisture retention and surface firmness. This was also evident in 2018 prior to aeration treatments (Table 3).

Table 4: Impact of location on surface firmness and soil volumetric water content (VWC) of ultradwarf bermudagrass putting greens by year in Midlothian, VA.

Surface Firmness and Volumetric Water Content (VWC)				
	2018	2019	2020	2021

Location	Firmness[z]	VWC[y]	Firmness	VWC	Firmness	VWC	Firmness	VWC
G12CR[x]	570.56 c[w]	21.59 b	469.78 b	25.35 c	517.89 b	16.13 b	206.39 b	29.67 a
TifEagleNT	660.78 a	28.90 a	490.94 a	26.67 b	553.83 a	15.3 bc	248.50 a	27.06 b
G12NT	595.33 b	29.31 a	494.28 a	28.61 a	508.83 a	19.6 a	255.28 a	28.28 ab
TifEagleCR	498.00 d	21.40 b	434.55 c	25.25 c	518 b	14.43 c	181.39 c	18.95 c
LSD	4.11	0.68	20.5	1.15	11.79	1.14	13.64	2.04

[z] Surface firmness measured using a FieldScout TruFirm device.

[y] Soil volumetric water content measured using a FieldScout TDR 350 with 3.8 cm probes.

[x] Ultradwarf bermudagrass varieties of ‘G12’ or ‘TifEagle’ established either as a complete renovation (CR) or through no-till (NT) conversion.

[w] Means followed by the same letter within columns are not significantly different according to Fisher’s protected LSD (α=0.05).

Treatments varied in days to recover to both 75% and 100% green turfgrass cover in both 2019 and 2020 (Table 4). Despite aeration treatments occurring in different months (July 1, 2019 vs. August 26, 2020) due to turf maturity and the golf calendar for the course, recovery was very similar in both years. 100% recovery was predicted to occur between 27 and 36 days after aeration in both years, and it is evident that it takes much less time to see 75% recovery in treatments with lower surface disruption. While days to recover to 75% was similar in both years, it took longer for treated plots to reach 100% recovery in 2020, likely due to aeration treatment timing. Many factors influence UDB recovery including sunlight duration, temperature, soil moisture, and fertility.

Table 4. *Estimated recovery time (in days) following varying % summer surface removal by core aeration annually (A) or every other year (EO), or none (N) of ultradwarf bermudagrass putting greens in Midlothian VA using the inverse prediction function from logistic 3-parameter regression models.*

Days to Recovery by Treatment (Logistic Regression, Inverse Prediction α = 0.05)										
	2019 (July 1: TRT Date)						2020 (August 26: TRT Date)			
% surface removal	r^2	75%	Std Error	100%	Std Error	r^2	75%	Std Error	100%	Std Error
20A	0.97	19.62	0.35	31.36	1.04	0.95	19.61	0.49	35.87	4.28
15A	0.95	17.24	0.44	29.63	0.83	0.93	16.63	0.49	32.21	2.37
10A	0.95	15.40	0.43	29.12	0.75	0.94	13.17	0.35	30.41	1.58
5A	0.96	9.18	0.24	27.88	1.20	0.95	7.74	0.28	28.10	1.57
20EO	0.92	19.65	0.58	30.95	1.32	NA	NA	NA	NA	NA
N	NA	0	0	0	0	NA	0	0	0	0

Soil OM data were analyzed, and ANOVA revealed significant differences in OM% between treatments. Treatment 20A had significantly lower OM compared to all other treatments besides treatment 10A, and 10A had lower OM% than the nontreated plots (N). After OM analysis, OM was compared to TRUFIRM and VWC% data taken on the same day. Organic matter in the top 2.5 cm was moderately correlated with TRUFIRM data for G12CR (Figure 1). Organic matter in the top 2.5 cm accounted for 76-80% of the OM in the top 7.5 cm on G12CR (data not shown).

Table 5. Influence of varying % summer surface removal by core aeration annually (A) or every other year (EO, or none (N) on organic matter accumulation in the top 7.5 cm of ‘G12’ ultradwarf bermudagrass putting green rootzones in Midlothian VA constructed from complete renovation (CR) and no-till (NT) establishment methods.

Organic Matter % in Top 7.5cm 2021		
	G12CR	**G12NT**
% surface removal	**p-value = 0.016**	**p-value = 0.2355**

20A	6.73 c*	*8.71*
15A	9.18 ab	*10.05*
10A	8.41 bc	*9.64*
5A	10.02 ab	*10.6*
20EO	10.34 ab	*10.72*
N	10.84 a	*12.8*
LSD	2.2	

[*] Means followed by the same letter within columns are not significantly different according to Fisher's protected LSD (α=0.05).

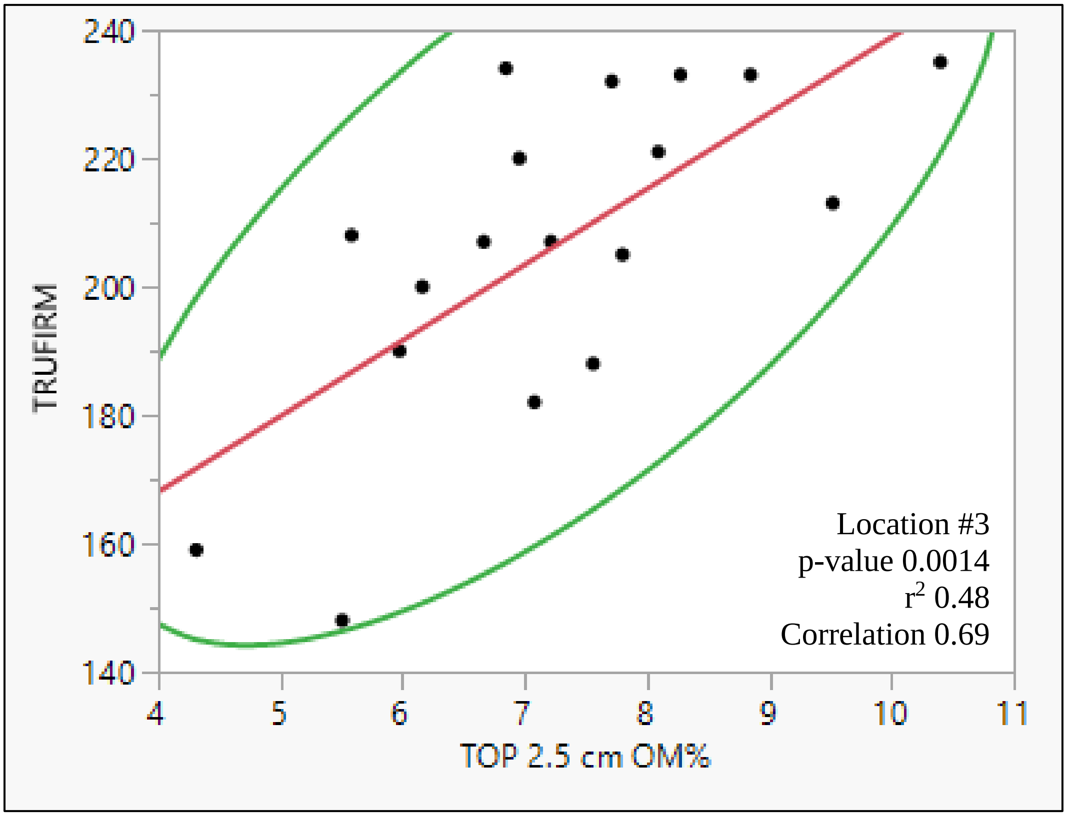

Figure 21: Bivariate correlation analysis between surface firmness (TRUFIRM) and organic matter (OM) percentage in the top 2.5 cm on July 28, 2021.

4 Discussion

Turfgrass color during spring green up (Table 1) was improved incrementally as surface disruption increased in all three years. Annual surface removal treatments of 20, 15, and 10% produced statistically higher bermudagrass color ratings than treatments with lower surface disruption. Spring green up and TC is critical during shorter UDB growing seasons in Virginia and the rest of the Transition Zone. Turfgrass quality (Table 2) for annual surface disruption treatments 20, 15, and 10% was also statistically higher than annual surface removal treatments of 5% or no removal in the optimal growing season. The surface removal treatment of 20% every other year is appealing to golf course operators. Further analysis may reveal challenges or positive attributes of this method on TQ. It was especially evident in plots with 20EO that TQ was significantly higher vs nontreated plots (N) after 2019. It would be preferable from a playability and labor perspective to aerate putting greens every other year as long as there are no long-term, detrimental effects. Turfgrass quality continued to decrease year over year in the nontreated plots and in the 5% annual treatment, confirming the need for core aeration in management programs.

Many factors influence putting green surface firmness including turfgrass variety, soil moisture, and thatch (Linde et al. 2009). High organic matter is a concern for both surface firmness and excess water retention on sand-based putting greens (Schmid et al. 2014). With soil cultivation and core aeration being a key strategy for OM management in golf course putting greens (Schmid et al. 2014), it is surprising that surface firmness and moisture content were not significantly different between treatments in any year. However, it is not surprising that they were different between locations (Table 3). Locations with no-till construction were softer and held more moisture compared to greens where all existing thatch and organic matter had been removed. TifEagleCR was overall the driest and firmest green across all ratings, so it is not surprising that firmness was moderately correlated with soil moisture in the top 3.8 cm on TifEagleCR in 2021 (Figure 2). This confirms conclusions made by Linde et al. 2009 that surface firmness can be manipulated through soil organic matter and moisture management.

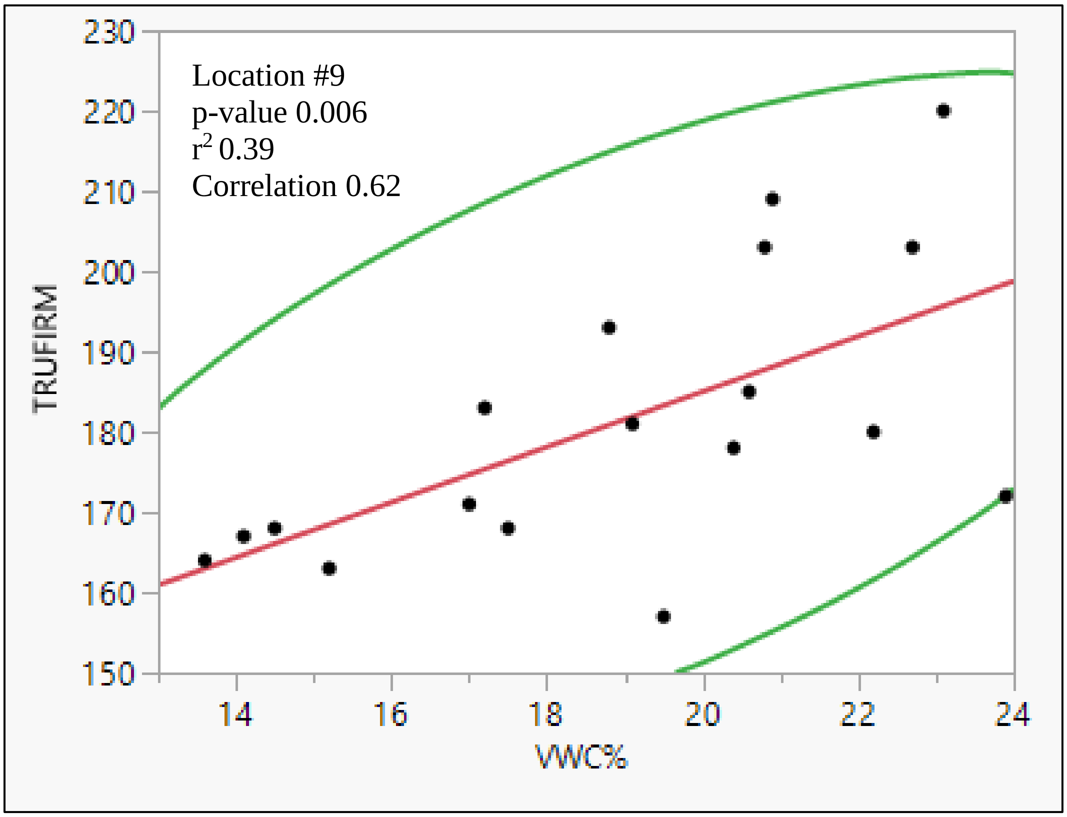

Figure 22: Bivariate correlation analysis between surface firmness (TRUFIRM) and volumetric water content (VWC%) on 28 July 2021.

Similar to findings by Ervin & Nichols (2011) on creeping bentgrass, recovery from aeration with higher levels of surface disruption took more time to heal compared to lower levels of surface disruption. What didn’t show up in the data was the damage experienced in 20A and 15A due to loss of surface firmness and mower scalping following aeration in two directions. Based on these observations, it would not be ideal to aerate in two directions without adjusting methods.

Treatments had a significant impact on OM of G12CR. Treatments with higher levels of surface disruption resulted in lower levels of OM. It is evident based on the OM data that 15A may not have impacted as much surface percentage as we had calculated. This could be due to overlap from 2nd aeration direction or inaccuracy of machine speed. Organic matter was higher in 15A treatment than 10A treatment, but this could certainly be an anomaly in the data.

Thatch thickness was measured in July of 2021 (data not shown) and the entirety of the thatch layer was found in the top 2.5 cm when sampled from locations G12CR and G12NT. While there were no significant treatment differences on G12NT, the OM% in the top 7.5 cm was numerically higher than G12CR across all treatments. 76-80% of the overall OM% in the top 7.5 cm was found in the top 2.5 cm on G12CR and 66-73% of the OM% in the top 7.5 cm was found in the top 2.5 cm on G12NT (data not shown). This indicates more OM is lower in the profile on G12NT due to age and existing OM remaining from no-till construction method. Moreover, the OM in the no-till treatments is better distributed throughout the profile, possibly causing future challenges in management. Therefore, OM% and thatch management may be easier in a green with a complete removal of existing OM from the surface vs. no-till method because the top 2.5 cm has more of an impact on the total OM%. This may be the reason that TRUFIRM was correlated to the OM% in the top 2.5 cm of G12CR but was not correlated in G12NT (Figure 1). Based on these data, it can be assumed that turfgrass managers will have greater impacts on OM% in newly constructed greens and in turn, surface firmness compared to no-till constructed UDB putting greens.

5 Conclusion

With a shorter growing season and lower maximum temperatures, UDB OM management programs need to be adjusted for golf courses in Virginia as compared to more southern locations. Based on the data from the last four years, treatment 10A (10% surface disruption annually, 7.8 mm coring tines 3.8cm x 3.8 cm spacing) appears to provide the greatest benefits of acceptable TC during spring green up and TQ during the growing season. Moreover, 10A had shorter recovery time compared to higher levels of surface disruption without the challenges associated with aerating in two directions. Treatment 20EO (20% every other year) may provide the same benefits of OM reduction, TQ, and TC during spring green up with the benefit of biennial aeration. However, there may be challenges associated with aerating in two directions and longer recovery times in the given year of aeration.

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