HortScience 59(3):355–361. 2024. https://doi.org/10.21273/HORTSCI17519-23

Evaluation of Fall and Winter Trinexapac-ethyl Applications on Ultradwarf Bermudagrass Putting Green Color, Quality, and Green Cover

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Keywords. turfgrass, bermudagrass, cold tolerance, plant growth regulator, golf

***Abstract.* Ultradwarf bermudagrass (UDB) putting greens grown in subtropical and temperate climates can face elevated risk of winter injury from cold temperatures. Trinexapac-ethyl (TE) inhibits UDB growth potentially reducing spring green-up and overexertion of carbohydrate reserves for UDB during the cold de-acclimation period. A field study was conducted to determine the effect of fall and winter TE applications on the visual quality and color of UDB putting greens in Virginia from the cold acclimation phase through the cold de-acclimation phase. A second controlled-environment study was conducted to determine how TE applications to UDB during cold acclimation affected UDB cold tolerance. In the first study, plots were treated with 0.026 kg·ha^{-1} a.i. every 14 days, 0.013 kg·ha^{-1} a.i. every 14 days, or 0.013 kg·ha^{-1} a.i. every 7 days either in the fall only or in the fall and winter. A nontreated control was included for comparison. For the second study, cup-cutter plugs (10.8-cm diameter) of UDB were treated with 0.026 kg·ha^{-1} a.i. every 14 days from the time growth resumed after green-up through cold acclimation or not treated with TE. Plugs were then exposed to −9.4 °C for 4, 6, 8, or 10 hours and placed into a greenhouse to green up. The GC_{50} values (exposure time to reduce bermudagrass green cover by 50% 6 days after exposure to −9.4 °C) for the treatments were then calculated based on exposure time and percent green-up. In the first study, TE applications improved UDB quality >3.8%. However, TE applications reduced UDB color, and trends exhibited this reduction in color particularly during the late cold acclimation, winter dormancy, and early cold de-acclimation phases. In the second study, TE applications reduced GC_{50} values by >10.9% compared with nontreated plugs, suggesting TE reduces UDB cold tolerance during the cold acclimation phase.**

Lack of cold tolerance is one of the limiting factors reducing expansion of UDBs [*Cynodon dactylon* (L.) *Pers.*× *Cynodon transvaalensis* Burtt-Davy] into the US transitional climate zone and farther north (Anderson et al. 2002; Goatley et al. 2007; Zhang et al. 2008). Compared with creeping bentgrass (*Agrostis stolonifera* L.), UDB has 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 turfgrass in the southern United States, and improved management practices have fostered UDB use well into the transition zone since the early 2010s (Richardson et al. 2014). Although 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 used to reduce winter mortality of UDB is turfgrass covering and moisture management during cold stress, but integration of additional strategies is needed in colder climates (DeBoer et al. 2019; Goatley et al. 2007; Richardson and Booth 2023). Research has sought to better understand factors associated with cold acclimation and turfgrass response to cold stress in other types of turfgrass, and some of these approaches may have applicability to UDB.

Cold acclimation is the combination of metabolic and physiological changes to prevent ice formation and low-temperature injury in turfgrasses (Chalmers and Schmidt 1979; Davis and Gilbert 1970; Fry and Huang 2004; Gatschet et al. 1994). Although bermudagrasses vary in cold tolerance between cultivars (Anderson et al. 1993, 2002; Gatschet et al. 1994), bermudagrasses have the capability of going through cold acclimation 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 (Fry and Huang 2004; Gatschet et al. 1994). During cold acclimation, changes in plant hormone levels slow turfgrass growth, alter cell membrane properties, reduce cell water content, and induce accumulation of these cryoprotectants (Davis and Gilbert 1970; Fry and Huang 2004; Gatschet et al. 1994; Zhang et al. 2006). Plant growth regulators are frequently applied to UDB putting greens (Reasor et al. 2018) and they can alter plant hormone production (McCullough et al. 2004), suggesting they may affect UDB cold tolerance. Mixed results have been reported on the influence of plant growth regulators on cold tolerance and cold de-acclimation in cool-season grasses (DaCosta et al. 2021; Laskowski and Merewitz 2020), but they could potentially increase cold tolerance in the warm-season UDB. Plant growth regulators that alter plant hormone levels, increase stress responses, and/or reduce carbohydrate expenditure could potentially improve UDB tolerance to cold stress like what has been shown by Steinke and Stier (2004) in supina bluegrass.

TE is a type II, class A, late-stage gibberellic acid inhibitor (Rademacher 2015). Turfgrass color, quality, rooting, shade tolerance, photosynthesis, and drought tolerance improved after applications of TE (Ervin and Koski 1998; Fry and Huang 2004; Qian and Engelke 1999; Rademacher 2015; Reasor and Brosnan 2020). TE is also reported to bolster certain plant stress defenses such as traffic tolerance and salt tolerance (Sattar et al. 2019; Williams et al. 2010). Total soluble carbohydrates increase in turfgrasses following TE applications, perhaps by reduced carbohydrate consumption associated with leaf growth (Qian and Engelke 1999; Steinke and Stier 2004). This enhancement of plant stress defenses and the increased soluble carbohydrates by TE could potentially extend to enhancing tolerance to cold stress in UDB. TE is reported to increase cold tolerance of hybrid bermudagrass and *Poa supina* Schrad. (Fagerness et al. 2002; Richardson 2002; Steinke and Stier 2004). Although there is documented evidence that TE improves cold tolerance of 'Tifway' hybrid bermudagrass during the fall in Raleigh, NC, USA (Fagerness et al. 2002), and during the winter in Fayetteville, AR, USA (Richardson 2002), little is understood about the influence of TE on cold tolerance of UDB.

Received for publication 29 Sep 2023. Accepted for publication 22 Dec 2023.
Published online 12 Feb 2024.
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Gibberellins antagonize abscisic acid, a stress defense hormone. Although not evaluated in turfgrasses, abscisic acid increases as gibberellic acid decreases in other plant species (Shu et al. 2018). Likewise, gibberellic acid is negatively associated with cold tolerance, whereas abscisic acid increases during cold acclimation and is positively associated with enhanced cold tolerance (Fry and Huang 2004; Zhang et al. 2008) and lower lethal temperatures in bermudagrasses (Zhang et al. 2008). Decreased carbohydrate loss and increased abscisic acid may both promote freezing tolerance by increasing dehydration tolerance (Fry and Huang 2004). The reduction of gibberellic acid through TE applications before, during, and after cold acclimation may lead to increased levels of abscisic acid and stored carbohydrates, resulting in improved cold tolerance in turfgrasses under TE treatments (Zhang et al. 2006). We hypothesized that TE would increase UDB cold acclimation and/or winter stress tolerance. Because neither of these factors have been previously investigated, studies were designed to 1) evaluate the influence of fall and winter TE applications on UDB visual color and quality when the turfgrass is not actively growing, and 2) evaluate the influence of fall and winter TE applications on cold tolerance of UDB during cold acclimation.

Materials and Methods

Field study

Studies were conducted in Midlothian, VA, USA, on UDB putting greens built to US Golf Association (USGA) specifications at the Virginia Tech Research Short Course at Independence Golf Club (US Golf Association 2018). Four total greens that were newly planted in the summer of 2018 were used each year (two 'TifEagle' and two 'G12') for a total of eight site-years. The 2018–19 trial was conducted from 4 Oct 2018 until 25 Apr 2019 and the 2019–20 trial was conducted from 15 Oct 2019 until 13 May 2020. The putting greens were maintained at 3.25-mm height of cut with a reel mower (2500A Triplex Mower; Deere and Co. Moline, IL, USA) in the growing season (approximately May to September) and the height of cut was raised to 4 mm 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 (Primo; Syngenta Crop Protection, Greensboro, NC, USA) applications at a rate of 0.026 $kg \cdot ha^{-1}$ a.i. every 14 d during the growing season. Vertical mowing followed by sand topdressing occurred every 2 weeks during the growing season. Plots were 1.2 m × 1.8 m and arranged in a randomized block design with four replications of seven treatments at each location. The "Fall Only" applications began on 4 Oct 2018 (Year 1) and 15 Oct 2019 (Year 2) after traditional TE applications ended during the UDB active growing season (Table 1). The "Fall Only" applications ended on 27 Nov 2018 (Year 1) and 26 Nov 2019 (Year 2) when UDB lost green color, marking the start of winter dormancy. The "Fall and Winter" applications began on 4 Oct 2018 (Year 1) and 15 Oct 2019 (Year 2) and ended on 4 Mar 2019 (Year 1) and 14 Apr 2020 (Year 2). Applications ended once green color had been regained and growth commenced in all plots. Treatments (Table 1) included full (0.026 $kg \cdot ha^{-1}$ a.i.) and half (0.013 $kg \cdot ha^{-1}$ a.i.) labeled TE rates on weekly and biweekly intervals. All treatments were mixed in water and applied using a CO_2-pressurized sprayer delivering 842 $L \cdot ha^{-1}$ at 276 kPa of pressure via TeeJet TTI 1004 VS spray tips (TeeJet Technologies, Glendale Heights, IL, USA). Weekly or biweekly applications were made unless UDB putting greens were covered by turf covers or snow.

Table 1. Field experiment treatments: Evaluation of fall and winter trinexapac-ethyl (TE) applications on ultradwarf bermudagrass putting greens. NTC = nontreated control; TEF = fall-only TE applications and TEW = both fall and winter TE applications made at either high (0.026 $kg \cdot ha^{-1}$ a.i.) or low (0.013 $kg \cdot ha^{-1}$ a.i.) TE levels on either 7- or 14-d intervals.

Treatment		Abbreviation
Nontreated		NTC
Fall only applications		
TE	0.026 $kg \cdot ha^{-1}$ a.i./14 d	TEF.HIGH.14
TE	0.013 $kg \cdot ha^{-1}$ a.i./14 d	TEF.LOW.14
TE	0.013 $kg \cdot ha^{-1}$ a.i./7 d	TEF.LOW.7
Fall and winter applications		
TE	0.026 $kg \cdot ha^{-1}$ a.i./14 d	TEW.HIGH.14
TE	0.013 $kg \cdot ha^{-1}$ a.i./14 d	TEW.LOW.14
TE	0.013 $kg \cdot ha^{-1}$ a.i./7 d	TEW.LOW.7

Turfgrass quality was assessed at the time of the first application throughout the duration of the trial. Trials were visually assessed for turfgrass quality in accordance with methods outlined by Krans and Morris (2007) on a 1 to 9 scale, with 6 being minimally acceptable and 9 being the maximum score. Dormant turfgrass quality was also assessed in which plots with a lighter tan color had higher dormant turfgrass quality than plots that appeared darker tan (Fig. 1). Turfgrass color (1–9, 6 = minimally acceptable and 9 = optimal dark green color) was assessed before the first application throughout the duration of the study. Plots were assessed on overall greenness, which often included both semidormant and actively growing plants within the same canopy.

Pilot study

A pilot study was conducted to determine the levels of injury sustained by cold-acclimated 'Champion Dwarf' UDB to −9.4 °C exposure for various lengths of time to determine exposure time treatments for the full-scale growth chamber study. Sixty 5-cm diameter × 5-cm depth UDB plugs were removed from a Champion Dwarf UDB putting green in Midlothian, VA, USA, while breaking dormancy in March 2020. The plugs were placed in 6-cm-diameter tapered cone-tainers (Stuewe and Sons, Inc, Tangent, OR, USA), filled with 85%/15% sand/peat mixture with physical properties meeting USGA recommendations of methods for putting green construction (US Golf Association 2018). The cone-tainers included 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 d. 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 $\mu mol\ m^{-2} \cdot s^{-1}$ of photosynthetically active radiation (PAR). Cone-tainers received supplemental irrigation as needed. After 14 d of acclimation, the UDB plugs had regained green color. Following acclimation, the cone-tainers were separated into two groups of 28 for cold temperature exposure. Cone-tainers were arranged in a freezer in a completely randomized design with four replications of seven treatments, and the study was repeated. The freezer was 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 h of exposure to −9.4 °C. After low-temperature exposure, cone-tainers were placed back in the greenhouse for 7 d for evaluation of percent green cover.

Growth chamber study: equipment

Two chest freezers, different from the freezer used in the pilot study, 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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-W 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

Fig. 1. Nontreated controls outlined in white demonstrate lower dormant turfgrass quality (i.e., darker tan color) than the higher turfgrass quality (i.e., lighter tan color) surrounding plots treated with trinexapac-ethyl.

Table 2. Treatment list for the 2 × 4 factorial study with no trinexapac-ethyl (TE) application vs. TE application as the first factor and 4, 6, 8, and 10 h of exposure to −9.4 °C as the second factor.

Treatment	TE regimen	Rate	Low-temp exposure
1	TE	0.026 kg·ha^{-1} a.i./14 d	4 h at −9.4 °C
2	TE	0.026 kg·ha^{-1} a.i./14 d	6 h at −9.4 °C
3	TE	0.026 kg·ha^{-1} a.i./14 d	8 h at −9.4 °C
4	TE	0.026 kg·ha^{-1} a.i./14 d	10 h at −9.4 °C
5	No TE	n/a	4 h at −9.4 °C
6	No TE	n/a	6 h at −9.4 °C
7	No TE	n/a	8 h at −9.4 °C
8	No TE	n/a	10 h at −9.4 °C

n/a = no TE applied.

plastic pots (15.25-cm diameter × 14.6-cm depth).

Growth chamber study: plant materials

A total of 200, 10.8-cm diameter × 10-cm depth UDB plugs were removed from a Champion Dwarf UDB nursery putting green in Midlothian, VA, USA, on 26 Feb 2020, and planted in 15.25-cm diameter × 14.6-cm depth plastic pots. The UDB plugs were fully dormant at the time of removal with good turfgrass density, khaki-brown color, and adequate rooting. The pots contained four holes at the base for drainage. Before 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 (US 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·ha^{-1} a.i. and water-soluble 46N–0P–0K urea fertilizer at an N rate of 1 g·m^{-2} every 14 d using a CO_2-pressurized sprayer delivering 842 L·ha^{-1} at 276 kPa of pressure with TeeJet TTI 1004 VS spray nozzles. Urea applications were applied separately from TE applications; moreover, urea applications were immediately irrigated in whereas TE applications were not. Plugs were irrigated daily and trimmed with electric shears to a height of 3.5 mm when necessary. Once the plugs grew radially to the edge of the pots and outdoor weather conditions were conducive for UDB growth, pots were removed from the greenhouse on 8 Jun 2020 and placed outside in Midlothian, VA, USA, where biweekly 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.

Growth chamber study: cold acclimation

An experiment was designed to evaluate the influence of TE applications on cold tolerance of UDB under controlled environments during the cold acclimation metabolic stage (fall conditions). UDB pots were arranged in a completely randomized design with three replications of eight treatments (Table 2). Beginning in Jun 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 13 °C. Daytime and nighttime temperatures were reduced incrementally every 2 weeks for 10 weeks until daytime and nighttime temperatures were 10 °C and 2 °C, respectively, for the last 2 weeks of cold acclimation to mimic fall conditions. Plots receiving TE were treated on a 14-d interval at 0.026 kg·ha^{-1} a.i. with a CO_2-pressurized sprayer delivering 842 L·ha^{-1} at 276 kPa of pressure via TeeJet TTI 1004 VS spray nozzles. Pots received a 12-h daily 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

Table 3. The 2018–19 field evaluations detailing the influence of trinexapac-ethyl (TE) application rate and timing on turfgrass quality (1–9 scale, where 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·ha^{-1} a.i.) or low (0.013 kg·ha^{-1} a.i.) TE levels on either 7- or 14-d intervals.

	‘G12’ 3		‘TifEagle’ 4		‘G12’ 5		‘TifEagle’ 9	
Treatment	Quality	Color	Quality	Color	Quality	Color	Quality	Color
NTC	5.60 b[i]	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

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

Table 4. The 2019–20 field evaluations detailing the influence of trinexapac-ethyl (TE) application rate and timing on turfgrass quality (1–9 scale, where 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·ha^{-1} a.i.) or low (0.013 kg·ha^{-1} a.i.) TE levels on either 7- or 14-d intervals.

	‘G12’ 3		‘TifEagle’ 4		‘G12’ 5		‘TifEagle’ 9	
Treatment	Quality	Color	Quality	Color	Quality	Color	Quality	Color
NTC	3.92 b[i]	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	0.05	0.10	0.02	0.08	0.07	0.08

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

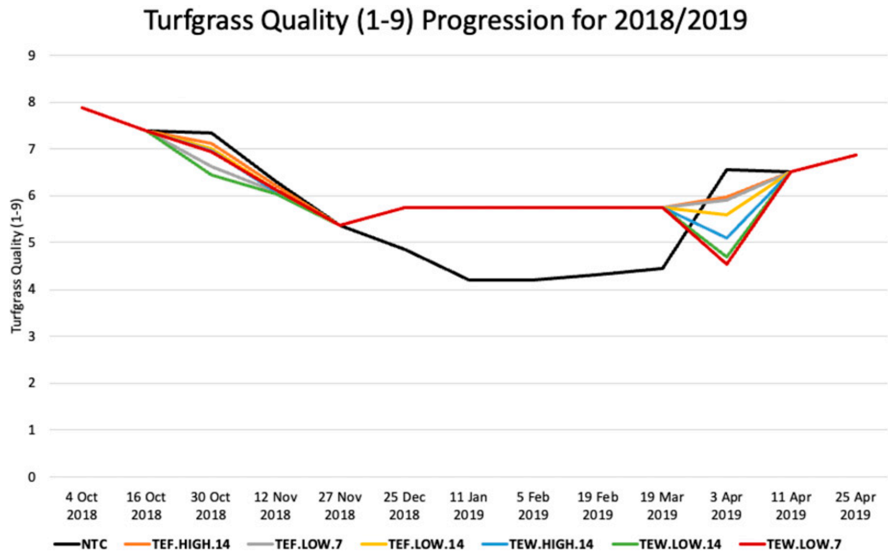

Fig. 2. Influence of trinexapac-ethyl (TE) application rate and timing on turfgrass quality over time in 2018–19 averaged across all locations. NTC = nontreated control; TEF = fall-only TE applications and TEW = both fall and winter TE applications made at either high (0.026 kg·ha^{-1} a.i.) or low (0.013 kg·ha^{-1} a.i.) TE levels on either 7- or 14-d intervals.

of 10 weeks, pots underwent exposure to −9.4 °C for a predetermined duration based on the preliminary pilot study.

Growth chamber study: 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 h of exposure to −9.4 °C were selected to provide a series of predicted outcomes ranging from no damage to complete mortality. Minimum exposure time in the pilot study was 3 h; however, in this study, the minimum exposure time was 4 h to have equal 2-hour incremental changes between exposure time treatments. Three replications each of TE-treated and nontreated pots were added for each of the listed freeze durations (i.e., 4, 6, 8, and 10 h). Before 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 2 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 the growth chamber after prescribed duration. After thawing, pots were returned to the growth chambers and brought back to 30 °C over a 72-h period to evaluate percent green cover.

Data analysis

Field study. Data were plotted over time for a graphical representation of cold acclimation, winter dormancy, and cold deacclimation (spring green-up) and the effect of TE on these metabolic stages. Data were transformed to standardized area under the progress curve (SAUPC) for the duration of the study. Treatment × location and treatment × year interactions were significant, so treatment data were separated by location and year. Significance was determined from analysis of variance (JMP Pro 15; SAS Institute, Cary, NC, USA) and means were compared using Fisher's protected least significant difference ($\alpha = 0.05$).

Growth chamber studies. Light box images of pots were analyzed 1 d before and 6 d after growth chamber treatments using digital image analysis with the software ImageJ (Rasband 1997) for percent green cover (%). There were no percent green cover differences before growth chamber treatments. Percent green cover after growth chamber treatments were analyzed, and data were best modeled using nonlinear Gompertz 3-parameter regression (JMP Pro 15; SAS Institute) for both TE and nontreated UDB over time.

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

where y = expected percent 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. For each treatment, the low-temperature exposure duration (time in hours/−9.4 °C) at which 50% of the UDB had green cover (GC_{50}) was determined using a custom inverse prediction model within the JMP software based on Gompertz 3-parameter regression models.

Results

Field study

Turfgrass quality. All TE treatments provided >3.8% greater turfgrass quality SAUPC values than the nontreated control in the 2018–19 study year (Table 3). Similarly, but to a greater extent in the 2019–20 study year, TE-treated plots resulted in >23.2% greater turfgrass quality SAUPC values than nontreated plots (Table 4). When averaged across all locations, the turfgrass quality trends in Fig. 2 for 2018–19 and Fig. 3 for 2019–20 indicate that TE-treated plots had consistently greater turfgrass quality throughout most of the study period in both years, particularly during winter dormancy. Plots treated with TE had different turfgrass quality SAUPC values in only one site-year (‘TifEagle’ 9 in 2018–19), with TEF.HIGH.14 having 2.8% and 3.9% higher turfgrass SAUPC values than TEW.HIGH.14 and TEW.LOW.14, respectively (Table 3).

Turfgrass color. Turfgrass color differences were more variable than turfgrass quality differences (Tables 3 and 4). The nontreated

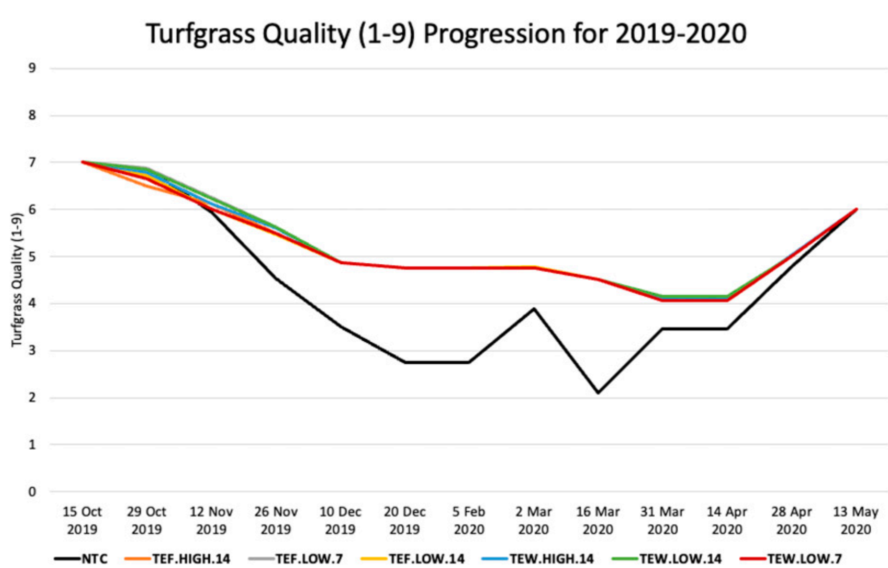

Fig. 3. Influence of trinexapac-ethyl (TE) application rate and timing on turfgrass quality over time in 2019–20 averaged across all locations. NTC = nontreated control; TEF = fall-only TE applications and TEW = both fall and winter TE applications made at either high (0.026 kg·ha^{-1} a.i.) or low (0.013 kg·ha^{-1} a.i.) TE levels on either 7- or 14-d intervals.

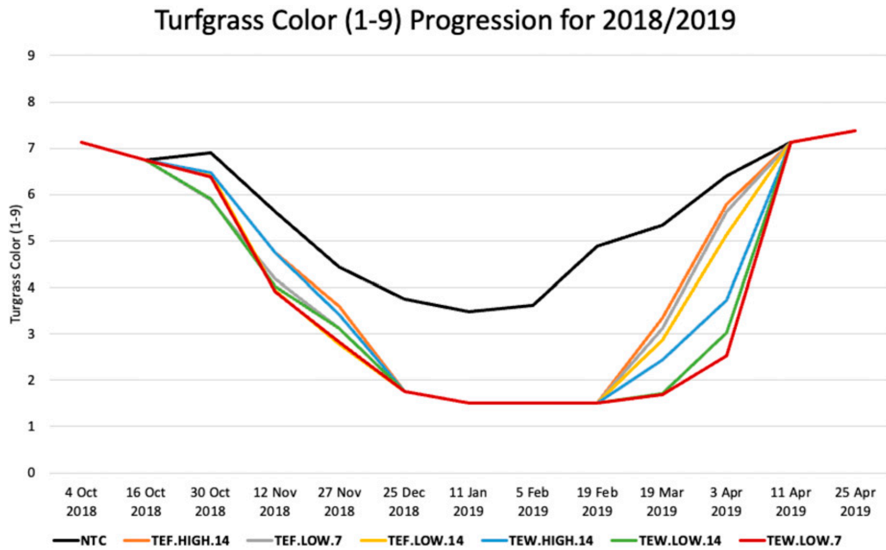

Fig. 4. Influence of trinexapac-ethyl (TE) application rate and timing on turfgrass color over time in 2018–19 averaged across all locations. NTC = nontreated control; TEF = fall-only TE applications and TEW = both fall and winter TE applications made at either high (0.026 kg·ha^{-1} a.i.) or low (0.013 kg·ha^{-1} a.i.) TE levels on either 7- or 14-d intervals.

control consistently had greater turfgrass color SAUPC values (>25.4%) than TE treatments across all locations in 2018–19. Similar differences were evident in 2019–20 with nontreated plots having >20.9% greater turfgrass color SAUPC values than TE-treated plots across all locations (Table 4). Nontreated plots generally exhibited a trend of darker green plots throughout the duration of the trial in both 2018–19 and 2019–20, particularly during the late stages of cold acclimation, winter dormancy, and the early stages of cold de-acclimation (Figs. 4 and 5).

Turfgrass color SAUPC values differed between TE treatments in both years across all locations (Tables 3 and 4). Generally, "Fall Only" treatments had greater turfgrass SAUPC values than "Fall and Winter" treatments likely due to the "Fall and Winter" treatments having a delayed green-up during cold de-acclimation, as evidenced in Figs. 4 and 5. In addition, TEF.LOW.7 generally had lower turfgrass color SAUPC values than TEF.HIGH.14 treatments, and TEW.LOW.7 treatments generally had lower turfgrass color SAUPC values than TEW.HIGH.14 treatments (Tables 3 and 4). Although plots treated with the high rate of TE at 14-d intervals were receiving equivalent amounts of TE as plots treated with the low rate of TE at 7-d intervals, turfgrass color differences were common across locations and years.

Growth chamber evaluations

Pilot study. The pilot study revealed that varying levels of low-temperature injury occurred between 3 and 18 h of exposure to −9.4 °C. All treatments with more than 8 h of exposure to −9.4 °C experienced complete lethal injury (Fig. 6). Our treatment levels of 4, 6, 8, and 10 h of exposure to −9.4 °C were chosen based on the results of this pilot study with 0% injury at 0 and 3 h of exposure and complete UDB death at 10, 12, and 18 h of exposure occurring in the pilot study (data not shown).

Low-temperature exposure during cold acclimation. Percent green cover 6 d after exposure to −9.4 °C for various lengths of time was used to evaluate differences between treatments. Data were best modeled using nonlinear Gompertz 3-parameter regression (r^2 ≥0.67) (JMP Pro 16). During both runs of this experiment, the GC_{50} was higher for the nontreated pots (11.52 h for the first run and 11.19 h for the second run at −9.4 °C) than the pots treated with TE (9.91 h for the first run and 9.97 h for the second run at −9.4 °C) (Fig. 7). These data suggest that TE has a negative effect on cold tolerance of UDB during cold acclimation.

Discussion

TE treatments clearly influenced turfgrass color and quality in our studies, and trends indicated that turfgrass color and quality were influenced during cold acclimation, winter dormancy, and cold de-acclimation. Plots under TE treatments lost color before the nontreated control plots during cold acclimation, which could be due to phytotoxic effects of TE on bermudagrass. Plots treated with TE on a 7-d interval lost color at a faster rate than other TE treatments, likely due to a more consistent load of TE in the plant with 7-d application intervals rather than the fluctuations (suppression/rebound cycle) of TE in the plant from biweekly application intervals as described by Reasor et al. (2018). 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 cold acclimation. There were higher turfgrass quality SAUPC values in plots under TE treatments compared with the nontreated plots, which was largely due to TE-treated plots having higher turfgrass quality during winter dormancy. The TE-treated plots exhibited a whiter or cleaner look during winter dormancy, which is a desirable appearance as opposed to a tan, water-soaked appearance during dormancy. This cleaner look to TE-treated plots led them to have higher dormant turfgrass quality than nontreated plots.

UDB is most susceptible to low-temperature injury during cold de-acclimation or during extended periods of dormancy following low-temperature exposure (Chalmers and Schmidt 1979). When warm temperatures occurred in Feb 2019 and Feb/Mar 2020, the nontreated plots began to break dormancy and even began to substantially grow in Year 2 (2019–20). Our observations suggest that TE prevented early dormancy break in the field evaluation. The "Fall Only" treatments also began to break dormancy

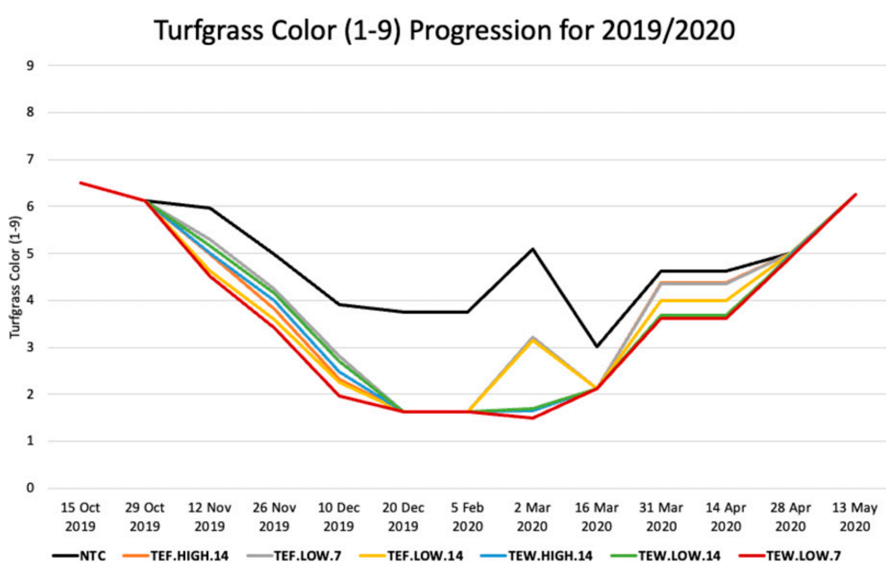

Fig. 5. Influence of trinexapac-ethyl (TE) application rate and timing on turfgrass color over time in 2019–20 averaged across all locations. NTC = nontreated control; TEF = fall-only TE applications and TEW = both fall and winter TE applications made at either high (0.026 kg·ha^{-1} a.i.) or low (0.013 kg·ha^{-1} a.i.) TE levels on either 7- or 14-d intervals.

Fig. 6. Influence of various durations of low-temperature exposure during the pilot study. Plugs were re-acclimated to warm conditions in the greenhouse after low-temperature exposure treatments. This image was taken 6 d after treatments ended. The study was a completely randomized design, but plugs were arranged in the image for organized visualization.

early in Year 2, indicating that winter treatments are important for preventing premature cold de-acclimation as suggested by DaCosta et al. (2021) on annual bluegrass (*Poa annua* L.). This prevention of premature cold de-acclimation could potentially help reduce late frost injury to UDB. All treatments broke dormancy at different times but eventually reached acceptable turfgrass quality and color once soil temperatures increased in the spring of the year. Winter dormancy is one of the plant's best defenses, as entering and exiting dormancy multiple times could cause unnecessary expenditure of carbohydrate reserves (Munshaw 2004). This may be most critical in more temperate regions where UDB rarely goes fully dormant.

In growth chamber evaluations, we observed greater low-temperature injury for plants under TE treatments during cold acclimation, which aligns with what DaCosta et al. (2021) found on annual bluegrass. 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. More research is needed in the future on the effect of TE on UDB cold tolerance during the metabolic stages of winter dormancy and cold de-acclimation.

Conclusions

TE applications improved UDB quality, yet reduced color with trends suggesting that color reduction was predominantly in the later stages of cold acclimation, the entirety of winter dormancy, and early stages of cold de-acclimation. Winter TE treatments were more effective at delaying cold de-acclimation compared with fall TE treatments, indicating a benefit to both fall and winter treatments. Treatments of TE prevented premature cold de-acclimation during winter dormancy which could be beneficial for golf course superintendents managing UDB putting greens in temperate climates where large temperature fluctuations during winter and early spring are common. Growth chamber studies indicated a decrease in cold tolerance of UDB treated with TE during cold acclimation, indicating that UDB cold acclimation was altered by TE. This research suggests that fall and winter TE applications on UDB may be beneficial for turfgrass practitioners managing UDB in the transition zone. Although this is currently not a common practice, our results suggest that TE applications may benefit UDB by increasing dormant turfgrass quality and preventing premature cold de-acclimation. However, further research is needed to evaluate the effect of TE on cold tolerance of UDB during winter dormancy and cold de-acclimation.

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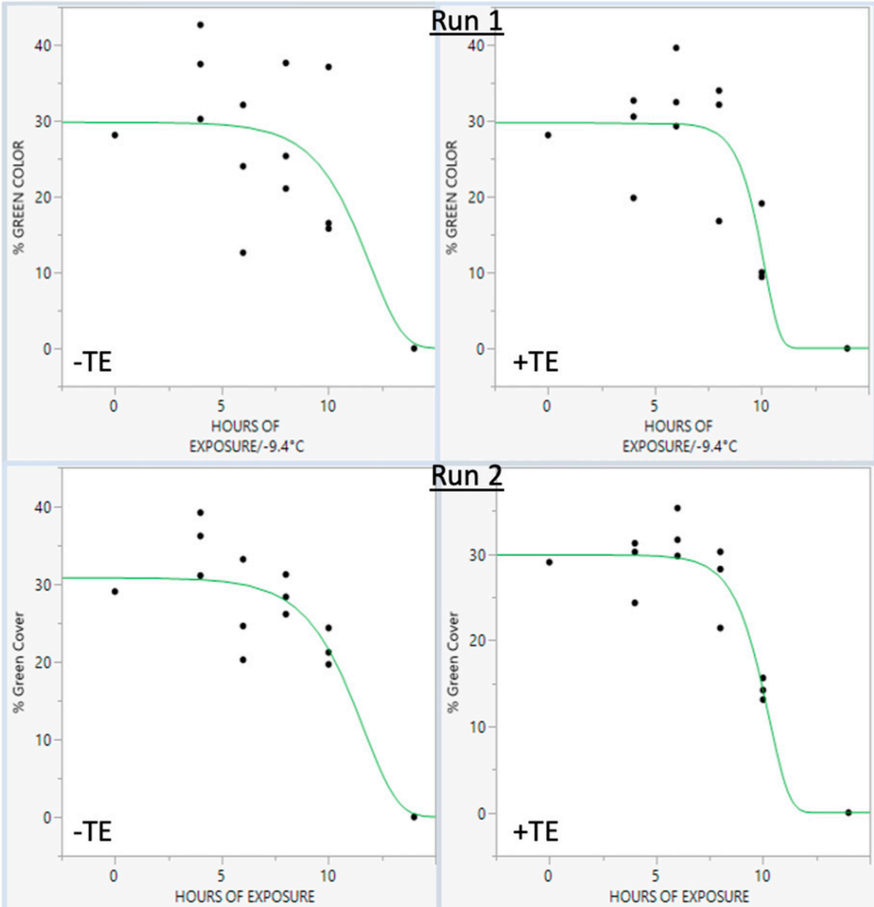

Fig. 7. Effect of exposure time at −9.4 °C on percent green cover (%) of 'Champion Dwarf' ultradwarf bermudagrass (UDB) as measured 6 d after freezing. Data were modeled using nonlinear Gompertz 3-parameter regression ($r^2 \geq 0.67$) without trinexapac-ethyl (TE) (top and bottom left) and with TE at 0.026 kg·ha^{-1} a.i. every 14 d (top and bottom right). The time required to reduce bermudagrass green cover by 50% (GC_{50}) was 11.2 to 11.5 h for nontreated UDB and 9.9 to 10.0 h for TE-treated UDB for experimental runs 1 and 2, respectively.

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