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Cold hardiness in bermudagrass cultivars as affected by the sequential trinexapac-ethyl application during growing season

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Abstract

Bermudagrass turfs are widely used in subtropical to tropical regions and in transition zones for golf courses, landscape areas, and athletic fields. Lack of adequate cold tolerance in these grasses causes early winter dormancy and an aesthetically unpleasing surface. This study investigated the effect of sequential Trinexapac-ethyl (TE) applications during the growing season on fall color retention, spring green-up, and physiological responses related to cold hardiness in bermudagrass cultivars. Trinexapac-ethyl (untreated, 6.0, 12.0, and 18.0 mg a.i. m^{-2}) was applied monthly throughout the bermudagrass growing season. According to the results, TE applications enhanced spring green-up and possessed color retention for 20 and 12 days longer in the first and second years of the study, respectively. Higher proline and total carbohydrate contents were observed in the crown of TE-treated as compared to untreated turfs. Hybrid cultivars exhibited higher spring green-up, rhizome survival, proline content, total carbohydrates, and longer color retention than seeded cultivars. The higher proline content of the crown was positively associated with enhanced color retention, rhizome survival, and spring green-up. Finally, these results confirmed that accumulated osmolytes in the crown (resulting from growing-season TE application) minimized the dormancy period in winter *via* improvement of fall color retention and spring green-up.

Key words: Color retention, plant growth regulators, proline, rhizome survival, spring green-up, total carbohydrate.

Introduction

Bermudagrass is the most widely used turf species in subtropical to tropical regions, as well as in transition zones for golf courses, landscape areas, and sports fields (Turgeon, 2005; Musnaw *et al.*, 2006). The most extensively used bermudagrass cultivars are hybrids (*Cynodon dactylon* × *Cynodon transvaalensis*), which have superior quality and dense covering (Turgeon, 2005). Maintaining a high-quality bermudagrass turf requires routine close mowing and aggressive fertilization programs (McCarty, 2005). Further, hybrid bermudagrasses generally lack adequate cold tolerance, and winter injury will lead to dormancy and discoloration in these turfs that created a undesirable surface during winter (Goddard *et al.*, 2008). This problem is one of the few disadvantages of using bermudagrass for intensively managed areas such as golf courses and sports fields in transition zone (Schmidt *et al.*, 1989). Growth of bermudagrass turf is approximately arrested when temperatures dropped below 10 °C, and turfgrass began to discolor when temperatures fall below 0 °C (McCarty, 2001). When warm-season turfgrasses such as bermudagrass expose to low temperatures, membrane dysfunction may occur, resulting in reduced carbohydrate translocation, protein synthesis, respiration, enzyme activity as well as tissue desiccation (McCarty, 2001; Samala *et al.*, 1998).

Turfgrass managers worry about winter survivability of warm-season turfgrasses in the transition zone yearly; therefore any practice to enhance turfs winter survival is of significant importance. Primary strategies for improving cold hardiness of bermudagrasses and to provide aesthetically pleasing turf surface include increasing potassium (K) applications and avoiding late-season nitrogen (N) application (Reeves *et al.*, 1970; Goatley *et al.*, 2005), covering the turf (Goatley *et al.*, 2005), and overseeding warm-season turfgrass with cool-season species (Long, 2006).

Plant growth regulators (PRGs) play an accepted role in turfgrass management programs (Amirikhah *et al.*, 2015). Growth inhibitors, as a group of plant growth regulators, are used extensively in turfgrass management to reduce mowing frequency and suppress inflorescences (Turgeon, 2005). Among that, trinexapac-ethyl (TE) is a gibberellic acid inhibitor that disrupts 3β- hydroxilation of GA_{20} to the physiologically active GA_1, resulting in suppression of laminar cell elongation (Rademacher, 2000). Reduced leaf elongation has been shown to increase chlorophyll concentration and mesophyll cell density that lead to dwarfed shoots and compact plants which are darker green in appearance (Heckman *et al.*, 2005; Amirikhah *et al.*, 2015). Further, the application of TE has been shown to increase photosynthesis rate (Qian and Engelk, 1999) and reduce respiration in the plant (Heckman *et al.*, 2002). Photosynthates, that are not used for cell elongation, are stored or allocated to other sink tissues for growth and maintenance. This would result in increased net carbohydrates (Ervin and Zhang, 2007; Amirikhah *et al.*, 2015). Sequential TE applications maintained fall bermudagrass quality, enhanced spring green-up, and stem carbohydrate levels in root and shoot tissues of ‘Tifway’ hybrid bermudagrass (Waltz and Whitwell, 2005). The increased amount of non-structural carbohydrates in TE treated grasses suggests that TE application may improve tolerance to environmental stress such as drought, shade, and freezing (Qian *et al.*, 1999; Fagerness *et al.*, 2000; Mohammadi *et al.*, 2017). Further, TE application increased levels of proline in leaf tissues of turfgrass (Mohammadi *et al.*, 2017; Flude, 2019). The amino acid proline plays a highly beneficial role in stress tolerance of plants subjected to various stress conditions such as salt stress, water deficient, and low temperature stress (Tatar and Gevrek, 2008; Huang *et al.*, 2013; Huang *et al.*, 2015).

This reallocation of resources and enhanced proline levels in response to TE application may enhance tolerance to winter injury and accelerate spring green-up in warm-season turfgrasses. The influence of TE application on fall color retention and earlier spring green-up has been investigated in previous studies (Richardson, 2002; Severmutlu *et al.*, 2012). Summer and fall TE applications minimized the dormancy period of 'Tifway' bermudagrass without negatively impacting rhizome freezing tolerance (Richardson, 2002). In contrast, Severmutlu *et al.* (2012) reported that late-season TE application decreased turf quality, fall green color retention up to 3 weeks and delayed spring green-up by 15 to 30 days in warm-season turfgrass species. It is concluded that the effect of TE on winter injury and spring green-up of turfgress may be affected by some factors such as time of application, the concentration of TE, and turfgrass species. Therefore, the present study was aimed at investigating the effect of sequential TE applications during growing season on fall color retention, spring green-up and physiological responses related to cold hardiness in bermudagrass cultivars.

Material and methods

Plant materials and growth conditions: This study was conducted in two years in department of agriculture at Isfahan University of Technology, Isfahan, Iran (32° 38' N, 51° 39' E). The experiment site is located 1500 m above sea level. The climate is cold semiarid with average annual precipitation of 122.8 mm, average annual maximum temperature of 23.4 °C, and average annual minimum temperature of 9.1 °C. The monthly variations in local temperature and precipitation during experimental time are presented in Fig. 1.

Two hybrid cultivars ('Tifway' and 'Tifdwarf') and one seeded cultivar ('Regles') were used in this experiment. 'Tifway' and 'Tifdwarf' cultivars were sprigged by hand at 30 m^3 ha^{-1} and Regles cultivar was cultured at 15 g seeds m^{-2}. After that, bermudagrass plugs were harvested from mature field plots and transplanted into polyvinyl chloride (PVC) pots (dimeter: 16 cm, height: 60 cm) on 27 April in first year. PVC pots filled with mixture of soil: humus: sand (3:2:1) (Table 1). Prior to and throughout the experiment the plugs received general maintenances including mowing and fertilization. Irrigation applied as needed to prevent plant stress.

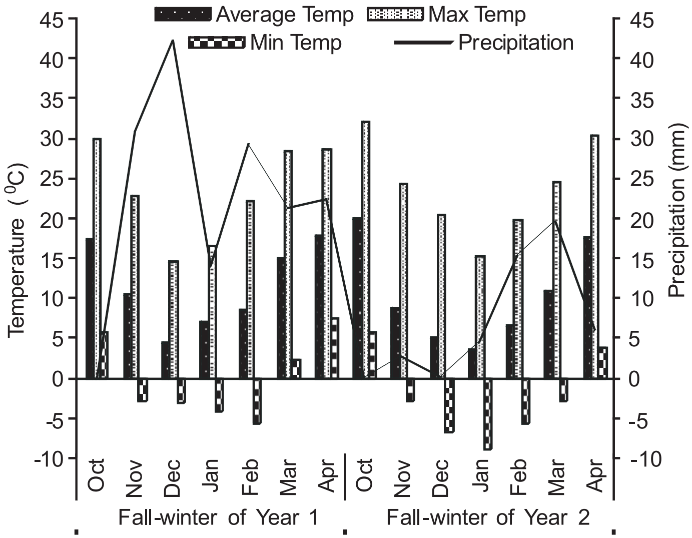

Fig. 1. Monthly average, maximum, and minimum of air temperature (°C) and precipitation at the study site for two experimental growth periods (I – October to April of year 1; II – October to April of year 2).

Table 1. Physical and chemical characteristics of the soil media used for growing cultivars of bermudagrass

Sand (%)	Silt (%)	Clay (%)	Soil texture	Organic matter (%)	pH	EC (dS.m^{-1})
65	18	17	Sandy loam	11.4	7.2	1.2

Experiment design and treatments: This study was arranged in a factorial design based on randomized complete block design with three replications and two pots in each replicate. Trinexapac-Ethyl (TE; Syngenta Crop Protection, Greensboro, NC) at four concentrations (untreated, 6.0, 12.0 and 18.0 mg a.i. m^{-2}) was monthly applied throughout the bermudagrass growing season, from June to September, for a total of four applications. Pots were mowed just before treatments application, with 'Tifway' and Regles mowed at 3.0 cm and 'Tifdwarf' mowed at 1.0 cm, as a recommended mowing height in these cultivars.

Turfgrass color retention and spring green-up: Bermudagrass fall color retention rated based on a visual scale of 1 to 9 (with 9 being full retention and 1 completely brown). Color rates were recorded every 10 days following the last treatments application and continued until turf was completely dormant in both years (Richardson *et al.*, 2002).

Turfgrass spring green-up were rated on 1 and 18 March in both years using a visual scale of 0 to 100, with 100 equal to green vegetation over the entire pot, and 0 equal to no green vegetation (Severmutlu *et al.*, 2012).

Photosynthetic pigments assay: Leaves Photosynthetic Pigments concentrations (mg.g^{-1} FW) were determined according to the method of Lichtenthaler and Buschmann (2001) extracting photosynthetic pigments with 10 mL of 100 % acetone. For this, samples composed by 0.5 g of fresh leaf material were collected on 27 November of first and second years from each pot. Light absorbance of the extracts was recorded at 661.6, 644.8, and 470 nm, and concentrations of chlorophyll *a*, chlorophyll *b*, and total carotenoids were calculated, respectively.

Proline and total carbohydrates assay: For proline and carbohydrates determination, samples of crown tissue were taken from treated and untreated turfs on 12 December of first and second years. Proline was extracted from 0.5 g of fresh sample in 10 mL of 5-sulphosalicylic acid (3 %, w/v) and quantified using the acid ninhydrin procedure described by Bates *et al.* (1973). Light absorbance of extracts was recorded at 520 nm with a spectrophotometer (model UV160A, Shimadzu, Kyoto, Japan). Free proline concentration per gram of fresh weight (FW) was determined using L-proline as standard. Total carbohydrates from samples were extracted with 2.5 N hydrochloric acid according to Hedge and Hofreiter (1962).

Freezing tolerance assay: Freezing tolerance was determined following the method described by Richardson (2002). From each pot, five parallel rhizomes with about 10 total nodes were collected on 15 January of second year. The samples were wrapped in moist cheesecloth to prevent desiccation and incubated at 4 °C for 24 h. The incubator was initially set at 0 °C and the temperature was dropped from 0 to -6 °C during a 2-h period. Rhizomes were held at -6 °C for 2 h, thawed in a refrigerator at 4 °C for 24 h. Then, rhizomes were planted in pots and maintained under greenhouse (natural photoperiod and maximum temperature, 30 °C) until regrowth occurred. Rhizome vitality (evaluated as the ability to recover or not) was assessed 20 days after planting. Survival was evaluated on regrowth potential based on the percentage of number of nodes that initiated growth.

Data analysis: Data obtained from each year of this study were separately analyzed including two-way ANOVA (TE rate and cultivars) and correlation analysis using SAS software ver. 9.1. (SAS Institute, 2006). Comparison of means was done by Tukey's test at P=0.05. The biplot analysis was performed with the software package Statgraphics centurion XVI software for the average tester coordination and polygon view, and the visualize correlations between the traits were estimated by using the cosine of the angle between their vectors.

Results

At the first year, there was no remarkable difference in color rates of all treatments till November (for 'Regles' cultivar) or December (for hybrid cultivars). However, with dormant season progress, TE-treated turfs exhibited better color rate than non-treated ones (Fig. 2). During the second year, turf color rate was enhanced by TE application across most fall evaluation dates (Fig. 2). In both years, control plants reached the threshold of 50 % color green-down earlier than TE-treated turfs. Based on the results, turfs treated by sequential TE applications exhibited green color for about 20 and 12 days longer in the first and second years of the study, respectively.

Applications of TE (P < 0.0001) and cultivars (P < 0.0001) significantly affected spring green-up in both years. All TE treatments enhanced spring green-up compared to the controls, though there was no significant difference between 12 and 18 mg a.i. m^{-2} of TE concentrations. The effect of sequential TE application on spring green-up was more pronounced in hybrid cultivars ('Tifway' and 'Tifdwarf') as compared to seeded cultivar ('Regles'). For untreated turfs, however, there was no significant difference between all cultivars during both years of study (Table 2).

Table 2. Turfgrass spring green-up following growing-season trinexapac-ethyl (TE) applications for bermudagrass cultivars

Cultivars	TE treatments (mg a.i.m^{-2})	Evaluation dates			
		Year 1		Year 2	
		1-Mar	18-Mar	1-Mar	18-Mar
		SGU (%)^{y}		SGU (%)	
'Regles'	0.0	4.00d	18.67f	0.00d	18.33e
	6.0	22.00c	38.67e	22.00c	38.67d
	12.0	34.67b	52.33bcd	25.00bc	44.00cd
	18.0	34.67b	50.33d	39.67a	52.33abc
'Tifway'	0.0	6.00d	17.67f	0.00d	17.00e
	6.0	35.00b	51.67cd	31.00abc	51.00abc
	12.0	40.00ab	60.67abc	37.00ab	54.67abc
	18.0	42.00ab	62.33ab	37.33a	56.67ab
'Tifdwarf'	0.0	6.00d	17.33f	0.00d	17.33e
	6.0	38.00ab	55.33abcd	40.33a	56.33ab
	12.0	43.00a	65.33a	38.33a	59.67a
	18.0	36.00ab	53.33bcd	32.33abc	47.33bcd

SGU, Spring Greenup; In each column, Means followed by the same letter are not significantly different as determined by Tukey's multiple-range test at P<0.05. * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level. ‡ NS = Not Significant. y, Turfgrass spring green-up was rated using a visual scale of 0 to 100, with 100 = green vegetation over the entire Tube, and 0 = no green vegetation.

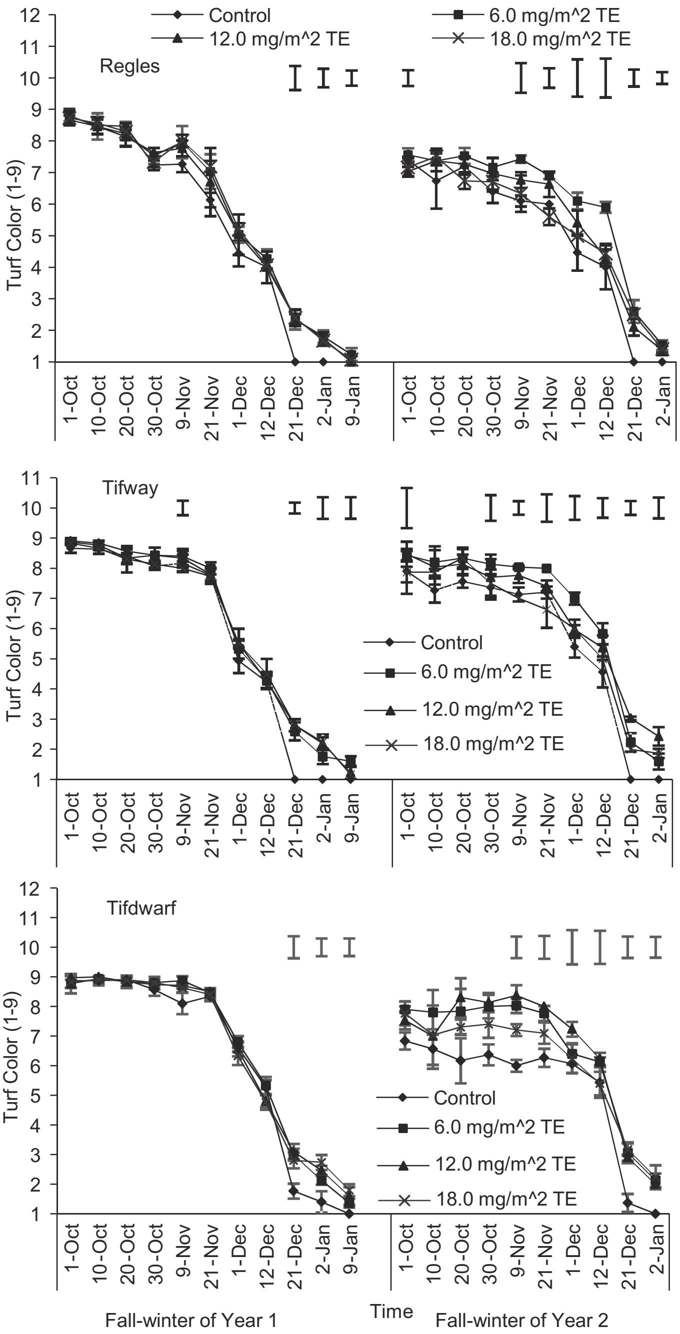

Fig. 2. Fall color retention of Regles (A), Tifway (B), and Tifdwarf (C) cultivars of bermudagrass as affected by growing-season applications of trinexapac-ethyl (TE). Bars represent critical value for comparison based on Tukey's multiple-range test (P<0.05). Turf color retention rated based on a visual scale of 1 to 9 (with 9 being full retention – 1 completely brown) and expressed as percentage. Data represent the mean±standard error (SE) (n=3).

Significant difference was observed between bermudagrass cultivars in terms of leaf chlorophyll *a* and chlorophyll *b* contents, during the first year (Table 3). Regles cultivar had significantly lower photosynthetic pigments than 'Tifdwarf'. Sequential

Table 3. Effect of Trinexapac-ethyl (TE) on late-season leaf photosynthetic pigments of bermudagrass cultivars

Cultivars	TE (mg a.i. m^{-2})	Years under review					
		Year 1			Year 2		
		Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Car (mg g^{-1} FW)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Car (mg g^{-1} FW)
'Regles'	0.0	1.03±0.05	0.28±0.04	0.41±0.02	1.04±0.11	0.37±0.14	0.28±0.04
	6.0	0.92±0.12	0.26±0.05	0.43±0.06	1.18±0.07	0.42±0.09	0.43±0.02
	12.0	0.79±0.16	0.19±0.05	0.38±0.07	1.09±0.06	0.39±0.08	0.48±0.01
	18.0	0.57±0.03	0.16±0.04	0.31±0.01	1.18±0.07	0.44±0.09	0.49±0.02
	Means	0.83 b	0.23 b	0.38 b	1.12 a	0.41 a	0.42 a
'Tifway'	0.0	1.18±0.22	0.33±0.09	0.44±0.08	1.08±0.16	0.39±0.24	0.30±0.05
	6.0	0.99±0.01	0.25±0.03	0.38±0.04	1.27±0.13	0.47±0.19	0.46±0.04
	12.0	1.06±0.13	0.27±0.05	0.44±0.05	1.16±0.08	0.42±0.11	0.52±0.02
	18.0	1.36±0.07	0.38±0.04	0.60±0.01	0.99±0.09	0.37±0.13	0.46±0.04
	Means	1.14 a	0.31 ab	0.47 ab	1.12 a	0.40 a	0.44 a
'Tifdwarf'	0.0	1.16±0.25	0.40±0.14	0.48±0.11	1.10±0.09	0.40±0.14	0.29±0.03
	6.0	1.02±0.13	0.32±0.04	0.46±0.02	1.36±0.08	0.50±0.11	0.49±0.02
	12.0	0.94±0.12	0.33±0.07	0.44±0.05	1.10±0.14	0.40±0.19	0.51±0.04
	18.0	1.50±0.15	0.64±0.12	0.68±0.07	1.06±0.09	0.39±0.09	0.43±0.02
	Means	1.16 a	0.43 a	0.51 a	1.16 a	0.42 a	0.43 a
Source of variation							
TE		NS	NS	NS	NS	NS	**
Cultivar		**	**	*	NS	NS	NS
TE×Cultivar		NS	NS	NS	NS	NS	NS

Chl a, chlorophyll a; Chl b, chlorophyll b; Car, total carotenoids. Means followed by the same letter are not significantly different as determined by Tukey's multiple-range test at $P<0.05$. * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level. ‡ NS = Not Significant. Data represent the mean±standard error (SE) (n=3).

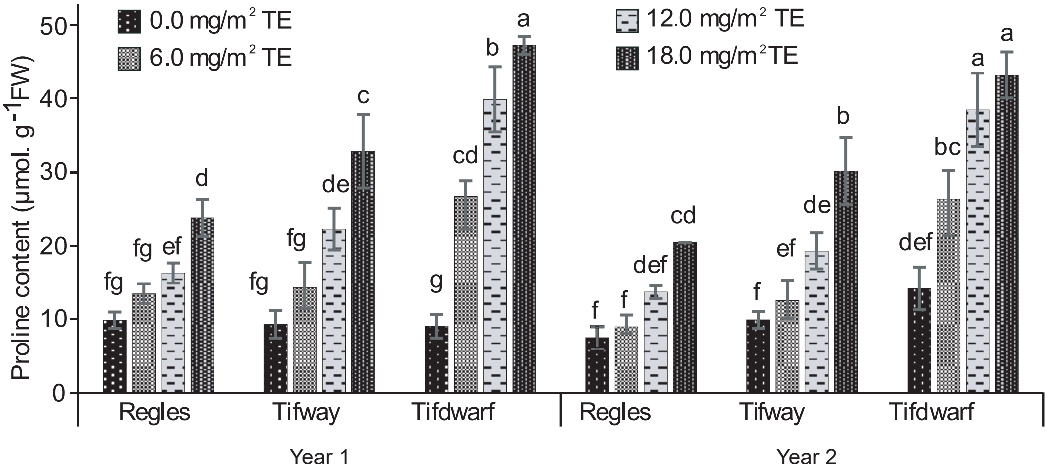

Fig. 3. Proline concentration of bermudagrass leaves in December of first and second years of experiment, as affected by growing-season applications of trinexapac-ethyl (TE). In each year, means followed by a different letter indicate significant difference according to Tukey's multiple-range test at $P<0.05$. Data represent the mean±standard error (SE) (n=3).

TE applications did not significantly affect chlorophyll *a* and chlorophyll *b* contents in both years under review (Table 3), though in some cases, chlorophyll contents were improved for TE-treated plants compared with the untreated control. In second year, TE treatments enhanced total carotenoids compared with controls (Table 3).

In both years, applications of TE ($P < 0.0001$) and cultivar type ($P < 0.0001$) significantly affected proline content. Leaf proline content considerably increased by application of TE during both years of the study. Proline content increased significantly as TE concentration rose, so that the maximum proline content was observed in plants treated with the highest concentration of TE. In all levels of TE concentrations, cultivar 'Tifdwarf' exhibited maximum proline content, followed by cultivar 'Tifway' (Fig. 3).

TE treatments ($P < 0.05$), but not bermudagrass cultivars, significantly affected total carbohydrates of leaves in both years of the study. All TE treatments enhanced total carbohydrates compared with the untreated controls. However, there was no significant difference between higher concentrations (*i.e.* 12 and

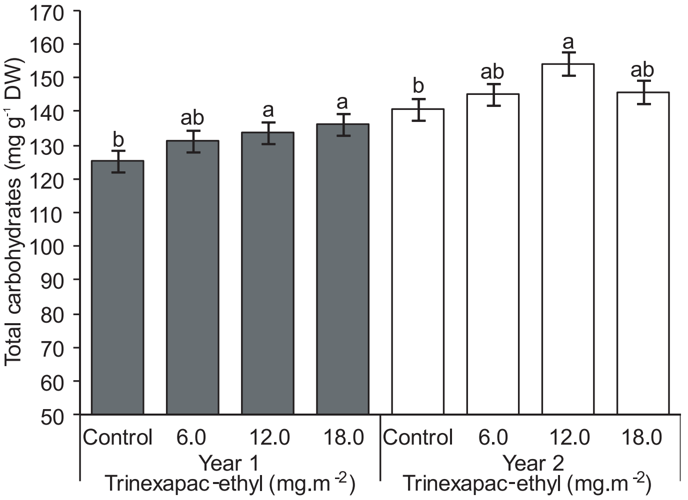

Fig. 4. Total carbohydrates of bermudagrass cultivars in December of first and second years, as affected by growing-season applications of trinexapac-ethyl (TE). In each year, means followed by a different letter indicate significantt difference according to Tukey's multiple-range test at $P<0.05$. Data represent the mean±standard error (SE) (n=3).

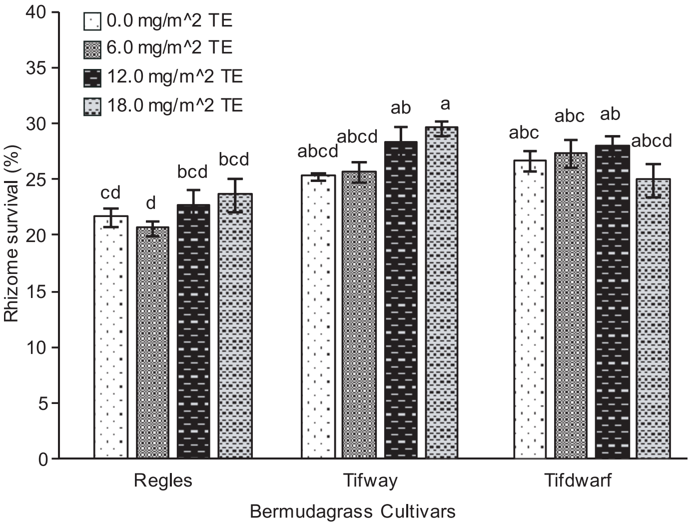

Fig. 5. Rhizomes survival of bermuda grass cultivars under freezing temperature (-6 ºC), as affected by growing-season application of trinexapac-ethyl (TE). Rhizomes were sampled on 15 Jan. of second year. Error bars represent standard error of the mean. Means followed by a different letter indicate significant difference according to Tukey's multiple-range test at $P<0.05$

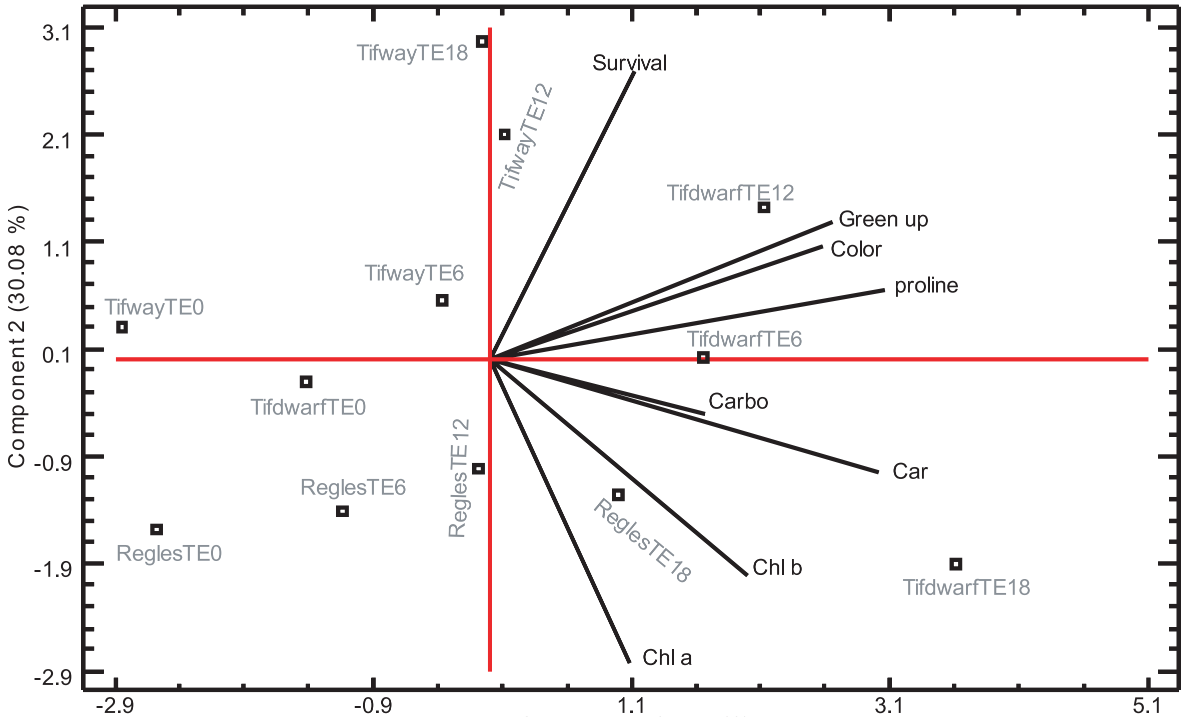

Fig. 6. The biplot display the traits relationships across Bermudagrass cultivars and treatment levels. (*Survival*: rhizome survival percentage, *Green up*: spring green up, *Color*: color retention, *Proline*: proline content, *Carbo*: total carbohydrate, *Chl a*: chlorophyll *a*, *Chl b*: chlorophyll *b, Car*: total carotenoid). TE0, TE6, TE12, and TE18 represent 0.0, 6.0, 12.0, and 18.0 mg.m^{-2} levels of trinexapac-ethyl, respectively.

18 mg a.i. m^{-2}) and 6 mg a.i. m^{-2} of TE application (Fig. 4). The significant impact of TE treatments was more pronounced during the second year as compared to the first year.

In the rhizome freeze tests, no significant difference was observed in the overall rhizome survival between TE- and non TE-treated plants; although a slightly higher node regrowth was recorded in rhizomes of TE-treated turfs (Fig. 5). Rhizome survival was affected by bermudagrass cultivars ($P<0.05$). Hybrid bermudagrass cultivars ('Tifway' and 'Tifdwarf') had significantly higher survival than seeded cultivar 'Regles'.

Associations between traits are displayed in the biplot figure (Fig. 6). Principle component analysis (PCA) revealed that 44.81 % and 30.08 % of the total variation explained by the first (PC1) and second (PC2) components, respectively (Fig. 6). According to the biplot results, spring green up plus color retention had high and positive correlation (an acute angle) with proline content. Further, a positive correlation (acute angles) exhibited between spring green up plus color retention and total carbohydrate, and rhizome survival had positive association with color retention plus proline content. These associations were highly consistent with the numerical Pearson correlation coefficients (data not shown). The biplot analysis in Fig. 6 also exhibited that Tifdwarf treated by 12.0 mg. m^{-2} of TE had high values of spring green up, color retention, and proline content. Further, Tifdwarf turf treated by 6.0 mg. m^{-2} of TE had high value of carbohydrate content.

Discussion

The results of the present study clearly demonstrate that sequential TE applications during growing season have significant impact on improving spring green-up, fall color retention and consequently better tolerance of bermudagrasses to low temperatures. TE applications exhibited green color for about 20 and 12 days longer in the first and second years of the study, respectively. It is noteworthy that slight differences were observed in maximum and minimum monthly temperature patterns during the two years of this study (Fig. 1). In general, minimum monthly temperatures during fall and winter seasons of first year were lower than second year. This may be the reason for longer green color retention in the first year of study as compared to the second year. These results are consistent with findings of Richardson (2002) that reported that sequential TE applications caused 15 days longer fall color retention and 10 days earlier spring green-up compared to untreated 'Tifway' bermudagrass. However, Severmutlu *et al.* (2012) found 7 to 21 days shorter fall color retention and a 15 to 30 days delayed spring green-up with two TE treatments during fall season. Further, fall TE application decreased acclimation status for annual bluegrass (Flude, 2019). The difference compared with our result could be due to the use of different concentrations, time of TE application, cultivars/species and environment conditions.

According to results of this study, although applications of TE slightly enhanced rhizome survival but there was no significant differences between TE treatment and control. This is in agreement with previous study by Fagerness *et al.* (2000) who reported a slight enhance in freezing tolerance of bermudagrass stolons when TE was applied at late growing season. Further, Flude (2019) showed that plant growth regulators such as trinexapac-ethyl were beneficial for maintaining the acclimation status of creeping bentgrass, while reducing the acclimation status of annual bluegrass. Also, results of the present study suggest a cultivar-specific response for cold hardiness of bermudagrass species. Hybrid cultivars ('Tifway' and 'Tifdwarf') had significantly higher color retention, rhizome survival, and spring green-up than seeded cultivar 'Regles'. The biplot analysis also exhibited that TE-treated Tifdwarf cultivar had high values of spring green up, color retention, proline, and carbohydrate content. Cold hardening is a process associated with several morphological, biochemical and physiological responses which adjust plant cells metabolism to prevent freezing of inter- and extra cellular water (Hsiao, 1973; Flude, 2019). During cold hardening process, major osmatic adjustments such as soluble carbohydrates and amino acids increase (Richardson, 2002). In this study, turfs treated with TE had higher proline content than untreated turfs. Also, in the previous reports, increase in proline content during cold acclimation was observed and this increase had positive correlation with freeze tolerance (Cai *et al.*, 2004; Munshaw *et al.*, 2004; Patton *et al.*, 2007). Further, the biplot analysis exhibited that rhizome survival and color retention had positive correlation with proline content (Fig. 6). Proline is a non-polar amino acid that accumulates in plants during dehydrative stress (Matysik *et al.*, 2002). During dehydration, caused by freezing stress, it is suggested that proline protects the cell through helps to stabilize membrane proteins (Schmidt *et al.*, 2016), as an osmotic adjuster limit dehydration stress (Huang *et al.*, 2015), and scavenging free radicals (Kaul *et al.*, 2008). Results of this study showed that proline content in crown was positively correlated with color retention, rhizome survival, and spring green-up (Fig. 5), suggesting that proline accumulation would be an important adjustment metabolic response to cold stress.

Carbohydrates are known as osmotic compounds that have non-colligative functions in freezing resistance, especially in perennial grasses. These compounds have been shown to increase during cold acclimatization (Patton *et al.*, 2007; Pompeiano *et al.*, 2011; Zhang *et al.*, 2011). Based on the results of the present study, turfs treated with TE produced more carbohydrates than untreated turfs. However, the total carbohydrates exhibited poor correlation with color retention and rhizome survival as compared to proline content (Fig. 6). Dunn and Nelson (1974) reported very little change in the total carbohydrates pool of three bermudagrass cultivars during the dormancy period. They also found only a slight shift in the soluble carbohydrate fractionation. Collectively, these data suggest that proline may be a more important osmolyte than carbohydrates in bermudagrass.

Reduction in the dormancy period can confer several benefits including reduce winter injury and increase in days of functional pleasing green surface available to the user (Richardson, 2002). Schmidt *et al.* (1989) demonstrated that earlier break of dormancy in warm-season turfgrasses will result in more effective recovery from damages caused by winter traffic or low temperature injury. This is especially important when this turfgrass is used for fall activities such as football, where injury from traffic can be severe in certain areas of the turf field (Richardson, 2002).

In conclusion, our data demonstrated that sequential TE applications aimed at reducing mowing frequency and enhancing turf quality during growing season, resulted in increased proline and carbohydrate contents of bermudagrass in the wintering period. Greater osmolyte (especially proline) contents in crown of TE treated turfgrass minimized the dormancy period *via.* enhancement of fall color retention and spring green-up.

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