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Leaf Physiological and Water Soluble Carbohydrate Content Responses to Trinexapac-ethyl Application of Sports Turf Grasses Exposed to Water Stress

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JPHR and MH designed the study. Author MHA performed the field experiment and wrote the first draft of the manuscript. Authors JPHR and MH read and edited the draft manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Water stress causes alterations in physiological and metabolic processes in plants and is considered the primary environmental factor affecting the management of sports turf grass species.This glasshouse experiment was conducted to investigate the effect of trinexapac-ethyl (TE) on canopy net photosynthesis (Pn), cell membrane stability (CMS), turf quality (TQ) and water soluble carbohydrate (WSC) accumulation responses of sports turf cultivars [Cv] (100% fescue, Rootzone and Arena sports) subjected to water stress. Commercially obtained sods of turf plants were treated with 2 L/ ha TE and then exposed 7 days after to water stress. The treatments were: (i) water untreated, (ii) water TE-treated, (iii) water stress untreated; and (iv) water stress TE-treated and the experiment was a randomized complete block design with four replicates. Results showed that specifically in Cv. Rootzone, Pn was 50% higher for well water TE treated plants compared to the other treatments during the second and third week of the study. Similarly, at 14 days after application, the effect TE resulted to 35% and 50% reduction in cell membrane leakages

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respectively in well water and water stressed TE-treated Cv. Rootzone plants and this was statistically significant (P=0.05) different from the untreated plants. On a scale of 1-9, all turf types recorded TQ rating of ≥8 at the start of the experiment. By the fourth week of the study, it was observed that all water stress untreated plants had mean TQ (5.75) ratings lower than the minimum acceptable TQ (6). WSC content of well-watered TE-untreated plants was maintained below 60 mg/g DW throughout the study regardless of turf type. After 28 day of water stress duration, the WSC contents obtained in water stress TE-treated plants were 41%, 43% and 50% higher for Cv. Rootzone, 100% fescue and Cv. Arena sports, respectively , than in well water untreated plants. Summer preconditioning of plants with TE can be a possible management tool in alleviating the detrimental impacts of water stress in sport turf species.

Keywords: Water stress; trinexapac-ethyl; net canopy photosynthesis; cell membrane stability; Turf quality; water soluble carbohydrate.

1. INTRODUCTION

Maintaining a lush turf surface in cool-season grasses particularly during the summer months is every turf grass managers' dream but at the same time a very difficult task to achieve. Due to natural changing climatic conditions, turf grasses are constantly exposed to several environmental stresses. Among the environmental stresses, water stress is one of the most serious challenges facing the turf industry [1,2,3]. As such, it is of importance that turf grass managers understand turf responses to water stress to enable them to develop appropriate management schemes for golf courses and athletic fields.

Turf grass globally ranks amongst the most important groundcover plants used extensively in landscaping in cities [4,5]. The main economic importance of turf which drives the turf industry is associated to its use in athletic fields especially golf courses [6]. The turf industry, in the United States for example, generates annual revenue of $35 billion alongside its environmental benefits [7]. Cool season turf grasses, including bent grass (*Agrostis palustris* Huds.), perennial ryegrasses (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Schreb.), kentucky bluegrass (*Poa pratensis* L.); and warm season turf grass zoysiagrass (*Zoysia japonica* Steud.) are the dominant species used in most intensively-managed turf sites [8].

Water stress affects virtually all aspects of plant physiology and metabolism [9]. Turf grass responses to water stress have been reviewed by Huang [1]. Primary deleterious effects of water stress reported in cool-season grasses have included closure of stomata, reduction in photosynthesis, damages to cell membrane structure consequently leading to severe decline in turf quality [10-12]. An important adaptive mechanism and a critical component of water stress tolerance in plants, including turf grass is the maintenance of cell membrane structure [13,14]. This involves the synthesis and accumulation of compatible solutes including water soluble carbohydrates [15-17], which have been used as a physiological measure to evaluate stress tolerance in different plants [12,18,19]. A common cultural practice in turf grass management is the use of plant growth regulators (PGRs) [20]. Trinexapac-ethyl (TE) is one of the most widely use PGR in cool-season and warm-season turf grass management [8,21,22], applied mainly to reduce mowing frequency and improve overall turf quality [23]. TE slows shoot growth by inhibiting the production of the biologically active form of gibberellic acid (GA1) [14,24,25]. Previous studies [26,27] have suggested that the use of PGRs including TE may be accompanied with enhanced turf performance. These studies nonetheless focused more on the effects TE on growth restriction with no elaboration on how TE application affects turf physiological and biochemical responses under water stress conditions. Also, available data on how TE may enhance water stress tolerance in cool-season turf grass species with specific emphasis on WSC accumulation is limited. Understanding how TE can influence WSC accumulation and photosynthesis response would give more insight into water stress tolerance mechanism that may be controlled through plant growth regulation thereby helping to minimize turf loss during extended periods of summer stress.

It has also been reported that preconditioning plants can help to enhance plant tolerance to subsequent exposure to another stress factor. Jiang and Huang [11] demonstrated that prior exposure of kentucky bluegrass (*Poa pratensis* L.) plants to water stress improved heat tolerance. Net photosynthesis rate was shown to be significant higher in creeping bent grass (*Agrostis stolonifera* L.) plants pre-treated with TE compared to untreated plants that exhibited 60% reduction in net photosynthesis when exposed to stress conditions [2]. Bian et al. [15] demonstrated that application of TE improves water use in creeping bent grass (*Agrostis stolonifera*) and suggested that TE treatment may influence the accumulation of membrane solutes associated with increase osmotic adjustment, which is in fact a critical component for water stress tolerance for grasses [28]. From the findings of the above studies, we can postulate that treating plants with TE might influence the tolerance mechanisms of turf grass species to surviving subsequent water stress exposure. In addition, alleviating the detrimental impacts of water stress on turf growth may greatly reduce the cost of irrigation. This study therefore has as objectives (i) to examine the influence of TE pre-treatment on physiological (photosynthesis, cell membrane stability) responses of turf grass species to subsequent water stress exposure. In addition (ii) to evaluate the effects of TE preconditioning on turf quality and status of solute accumulation (specifically water soluble carbohydrate content) associated with stress resistance mechanisms in turf grass species.

2. MATERIALS AND METHODS

2.1 Study Site, Plant Materials and Growth Conditions

The study was conducted at the glasshouse unit of Harper Adams University, Newport, Shropshire, UK (52°46'N, 2°26'W). Glasshouse temperature was set to 25° C day/ 15° C night temperatures. Three commercially cultivated turf sods were examined in this study. Sod pieces of “100% fescue”, “Arena sport” and “Rootzone” turf were collected from Tillers Turf Company farms, North Kelsey, Loncolnshire, UK. The typical species composition of the turf sod mixtures were as follows: 100% fescue (70% Barcrown slender creeping red fescue: *Festuca rubra litoralis* and 30% Bargreen chewings fescue: *Festuca rubra commutata*), Arena sports (70% *Lolium perenne* L. and 25% *Poa pratensis*) and Rootzone [50% bent grass (*Agrostis capillaries*) and 50% fescue (25% *Festuca rubra litoralis;* 25% *Festuca rubra commutata*)]. Sod pieces (10 cm diam and 1 cm thick) were trimmed off, washed free of soil, and transplanted on the 21 May 2016 into polyvinyl chloride (PVC) tubes (10 cm diamenter and 30 cm length) filled with a 9:1 (v/v) soil mixture of sand and peat based soil (Levington M3)[ph range 5.3-6.0; particle size:0-10mm; conductivity:355-435s; nutrient added 204N 10P 339K]. A plastic mesh screen held in place with a ducting clamp covered the bottom of each PVC tube to restrain the soil and allow water to drain freely from the tubes. Plants were maintained under natural light conditions and 25° C day/ 15° C night temperatures in glasshouse to fully establish root system for 8 weeks before treatments were imposed. During this time, all plants were watered three times a week until water drained from the bottom of the tubes and grasses were maintained weekly at 2 cm canopy height. The mean relative humidity was 52.8%.

2.2 Treatment and Experimental Design

Plants were pretreated on 27 July 2016 with 2 L/ ha TE (Primo Maxx, Syngenta Professional Products, Greensboro, NC) (10 mL / L [v/v] TE in water), using a Precision pot sprayer (KV Ltd Milton Keynes, England) with two Lurmark Flat Fan nozzles (F110 02) that delivered 200 L/ha of solution at a pressure of 2 bars. Water stress was initiated by withholding irrigation 7 days after TE application. No additional application of TE was made after plants were exposed to water stress. The experiment consisted of four treatments: (i) well-watered plant not pretreated with TE (water untreated; control), (ii) well-watered plants pretreated with TE (water TE-treated), (iii) water stress plants not pretreated with TE (water stress untreated); and (iv) water stress plants pretreated with TE (water stress TE-treated). Well-watered plants in all three turf types included in the overall experiment were watered three times a week until water drained from the bottom of each PVC tube while water stress was maintained by withholding irrigation in stress tubes. Each treatment was replicated four times and the experimental treatments arranged in a randomized complete block design.

2.3 Measurements

Water use characteristics were evaluated weekly by measuring soil volumetric water content (SWC) at the 0 - 8 cm soil depth with an ML3 ThetaProbe soil moisture sensor meter type HH2 (Delta-T Devices Ltd, Cambridge, England). Standard deviation and standard errors based on means of six replicates of sample data for each set of irrigation regime was estimated using Microsoft Office Excel 2007 software version to determine whether withholding irrigation had an effect on SWC between irrigation regimes.

Plant physiological responses of photosynthesis, cell membrane stability and turf quality data were collected weekly for four weeks, starting 7 days after TE application, corresponding to the day in which water stress was initiated.

Canopy net photosynthetic rate (Pn) was measured weekly (between 9 am and 3 pm) as CO_2 µmol m^2 s^{-1} using an infrared gas analyser (CIRAS 1 Li 6400, Portable Photosynthesis (P.P) System). The system was connected to a CPY-4 Canopy Assimilation Chamber (Amesbury, MA 01913 U.S.A) which was fitted tightly over the turf surface in the PVC tube. A seal to enclose the leaf canopy in the PVC tubes from the surrounding atmosphere was created by a flat rubber surface board adapted on the CPY-4 chamber. Maximum photosynthesis rate was recorded after two minutes of signal stabilization and the value registered was considered to be the difference in concentration of carbon dioxide in air entering and that of air exiting the CPY-4 chamber.

Cell membrane stability (CMS) was determined weekly by measurement of the electrolyte leakage (EL) of leaf samples following the method described by DaCosta et al. [29] with some modifications. For EL, clipped leaf samples (0.3 g) were rinsed three times with distilled water and then immersed in 20 mL distilled water in 25 ml glass vials. The initial conductivity ($C_{initial}$) of the solution was read with a conductivity meter (Janway 4510, Bibby Scientific Ltd, Staffordshire, UK), after allowing the leaf sample in bathing solution for 18 hours. Leaves were next autoclaved at 121 ^oC for 15 minutes, to completely kill plan tissue and achieve total membrane permeability. Final conductivity (C_{final}) of the kill tissue was measured when the solution had cooled to room temperature. The relative EL was estimated as a percentage according to the formula: ($C_{initial}$ / C_{final}) x 100.

Turf grass quality was visually assessed based on colour, uniformity and density of leaves on a scale of 1 to 9 [30]. A rating of 9 corresponded to an optimum green-up healthy plant with uniform turf canopy and plants rated 1 were brown or dead turf. Minimum accepted quality of turf was considered to have a rating of 6.

Water soluble carbohydrate was determined using the colorimetric anthrone procedure specified in MAFF [31] with slight modifications as described in Maness [32]. For the assay, 0.2 g of leaf samples were oven dried at 70 ° C for 72 hours. The leaf samples were ground to fine powder and put in 250 ml shaking bottle; and 200 ml of distilled water added and shaken for one hour. The solution was filter through Whatman 1 filter paper and approximately 50 ml of the extract was retained for water soluble carbohydrate determination. A 545 ml solution of 12.5 M H_2SO_4 was made by adding 380 ml of sulphuric acid 98% (18.4 M H_2SO_4) to 165 ml of distilled water. Anthrone reagent was made by dissolving 0.5 g. of anthrone and 0.5 g of thiourea in the 12.5 M H_2SO_4 solution. The reagent was stirred at two minutes interval until it was perfectly clear and left to stand for 30-40 minutes. Upon cooling, the reagent was stored in a tightly stoppered bottle at 4°C. Fresh reagent was prepared for each day of carbohydrate analysis of sample.

A stock standard solution of 0.004 M $C_6H_{12}O_6$ was prepared by dissolving 0.4 g of D-glucose in 10 ml of distilled water and making up the volume to 500 ml with distilled water. This glucose base stock solution was prepared immediately before use. To ensure a constant weight of sugar was used to prepare stock standard, the sugar used, was first oven dried at 105°C for 5 minutes to free it of any water. Working standards containing 0.0, 0.04, 0.08, 0.16 and 0.20 mg/ml glucose were prepared from the stock solution with distilled water and stored at 4^0C. The stock and working standards were used within a week of preparation.

To determine the amount of non structural carbohydrates, 2 ml of each working standard and 2 ml of each extract were pipetted into 50 ml culture tubes and allowed to stand in an ice bath for 10 min. Ten millilitres (10 ml) of anthrone reagent was carefully added down the side of each tube. The tubes were loosely stopper with cling film and the content in the tube thoroughly mixed. The tubes were next placed in water bath (100°C) for 20 minutes. After 20 minutes, the tubes were rapidly cooled for approximately 5 minutes in an ice bath and the cling films were removed. Once cool, absorbance of solution was read at 620 nm using a spectrophotometer (Genesys 2, Spectronic Instruments Inc.,Rochester, NY) within 30 minutes. The spectrometer was zeroed with the 0.0 mg/ ml glucose standard. A calibration curve using the working standards were prepared for each set of samples analysed. The formula for the line of best fit of the standard curve of working standards of known concentrations was used to calculate the value of the total soluble carbohydrate content in the sample.

2.4 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using general linear model procedure of the Genstat 16th edition (VSN international, Hemel Hemstead UK). Data were tested for normality and homogeneity of variance. Anova residual plots showed that data were normally distributed. Data were analysed with factorial ANOVA (variety x irrigation x TE) to evaluate the main effect of treatment on turf response at specific post treatment timings (week 1, 2. 3 and 4 following water stress initiation) For conciseness, results of each sod turf type to experimental treatments were presented individually. Differences between means for treatments were considered significant by standard error of means (SEM) at 0.05 probability level.

3. RESULTS

Volumetric soil water content (SWC) was maintained at ≥ 30% in the well-watered tubes throughout the duration of experiment. Withholding irrigation resulted to significant decline in SWC. At 28 days of water stress, SWC was 6% in the upper 10 cm soil depth of the water stress PVC tubes (Fig. 1).

In Cv Rootzone, under well water conditions Pn response was significantly *(P=0.05)* higher for TE treated plants than the untreated plants from the second to fourth week after water stress induction. No significant differences in Pn were detected between TE-treated and untreated water stress plants which both maintained low Pn levels (<1.4 μmol$CO_2m^{-2}s^{-1}$) throughout this study (Fig. 2). Under water stress conditions, Pn of TE treated 100% fescue plants was significantly *(P=0.05)* higher (1.85 μmol$CO_2m^{-2}s^{-1}$) compared to the water stress untreated plants (0.55 μmol$CO_2m^{-2}s^{-1}$) only at one week after water stress induction. From the second to the fourth week after water stress induction, there was no significant difference in Pn between the TE treated and untreated plants, and Pn of both treatments steadily declined with the progression of water stress. Contrary, in well water conditions, Pn increased with the progression of the experiment in both TE-treated and untreated 100% fescue plants. Pn was 51% and 32% higher in TE-treated plants than the untreated plants at 14 days and 21 days after plant growth regulator application respectively (Fig. 2). This suggests the progression of water stress limit the effect of TE on Pn. For Cv. Arena sports turf, there was no difference in Pn response between TE-treated and untreated plants at any rating day under water stress irrigation regime (Fig. 2).

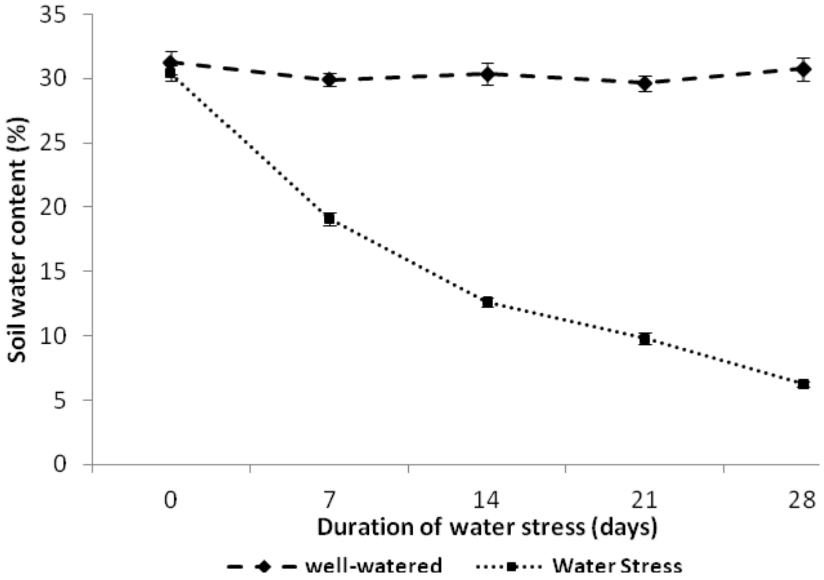

Fig. 1. Changes in volumetric soil water content (%) in the 0- to 10 cm top soil depth of well-watered and water stress PVC tubess

Vertical bars indicate standard error of means of six replicates

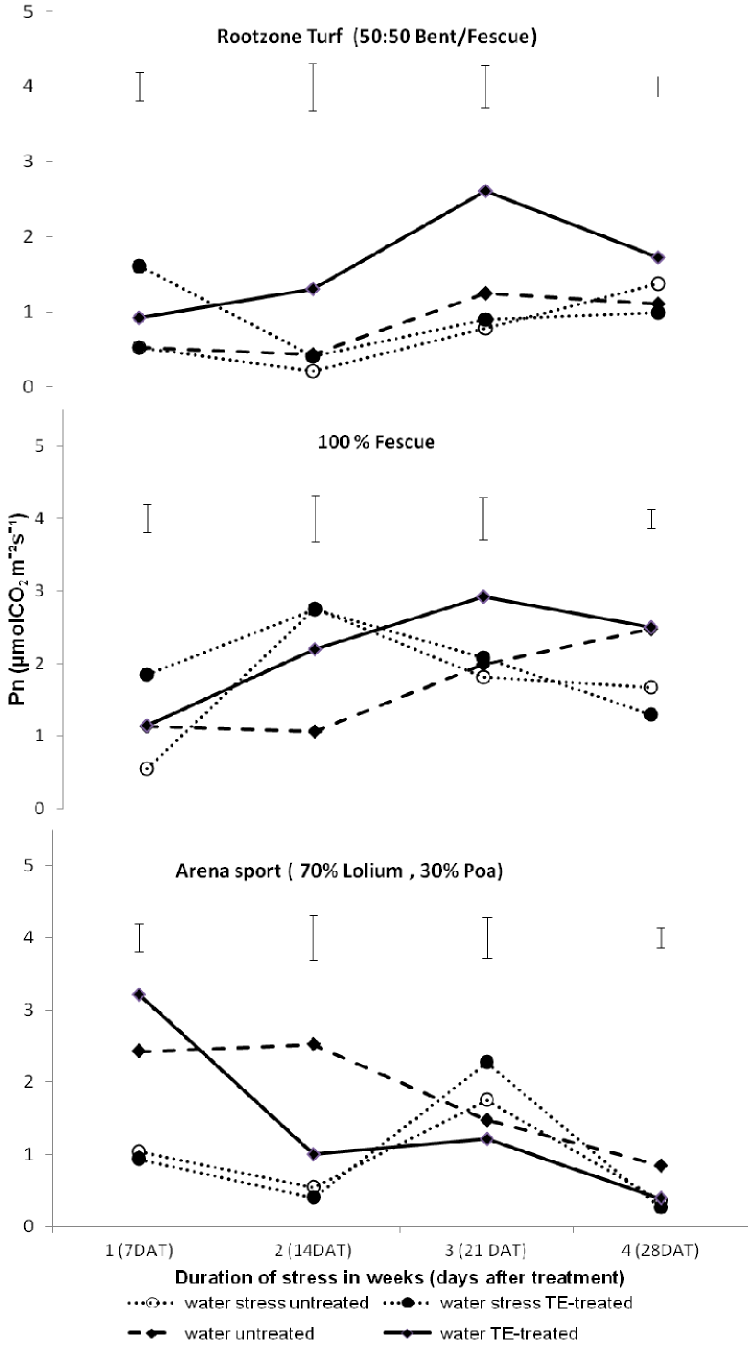

Fig. 2. Effect of trinexapac-ethyl (TE) application (2 L/Ha) on net canopy photosynthetic response (Pn) in Cv. Rootzone, Cv. 100% fescue, and Cv. Arena sports turfgrass under well-watered and water stress conditions

Vertical bars indicate standard error of mean for treatment comparison at given days of measurements

For all turf types, electrolyte leakage (EL) values showed an increasing trend over time. There were no significant differences in EL between well water and water stress plants in both Cv 100% fescue and Cv Arena sports throughout the study. For Cv Rootzone significant differences *(P=0.05)* in mean EL between well water (29.4%) and water stress (45.46%) plants were only detected at one week of withholding irrigation. At 14 days after TE application, corresponding to week one of sampling, there was 24% lesser membrane damage in well water

TE-treated plants (25.35%) compared to the untreated plants (33.46%). Similarly, significant *(P=0.05)* reductions in membrane injury were detected at 14days after TE application, with a 35% and approximately 50% decline in cell EL in TE-treated plants compared to the untreated plants under the well-watered and the water stressed irrigation regimes respectively (Fig. 3). No significant effect TE was detected from the third week (21 days after TE application) of water stress in any of the turf type used; an indication the potency of the TE on plant EL was diminished.

At one week of water stress initiation, turf quality (TQ) rating for all treatment for all turf types was ≥ 8. After four weeks of water stress duration, TQ of water stress control plants for all three turf types declined to around 6, which was significantly *(P=0.05)* lower than TQ of all other treatments (Fig. 4). TQ rating of water stress plants compared to well water plants were observed to be significantly *(P=0.05)* lower on all measurement dates throughout the entire study period specifically in Cv. 100% fescue plants. Well water TE treated and well water untreated Cv. 100% fescue plants however showed no clear difference when visually compared at 21 days after TE application (Fig. 5). For Cv. Arena sports TQ rating were maintained above 7 throughout the study particularly TE-treated well water plants. By the end of the water stress duration (28 d), TQ of water stress plants which did not received TE-treatment declined to levels (5.75) below that of the minimum acceptable turf quality (quality rating 6).

Water soluble carbohydrate (WSC) content increased with initiation and progression of water stress. TE-treated plants of Cv. Rootzone, Cv. 100% fescue and Cv. Arena sports turf under both well-watered and water stressed conditions exhibited higher levels of WSC compared to untreated plants throughout the experimental period (Fig. 6). Regardless of turf type, WSC content of well-watered untreated plants were generally maintained under 60 mg/g DW throughout the study duration. WSC content at 21 d following water stress induction was significantly *(P=0.05)* higher in TE-treated plants for Cv. Rootzone, Cv. 100% fescue, and Cv. Arena sports compared to untreated plants. By 28 day of water stress duration, WSC content levels in water stress TE-treated plants were 41%, 43% and 50% higher for Cv. Rootzone, 100% fescue and Cv. Arena sports, respectively , than well water untreated plants (Fig. 6).

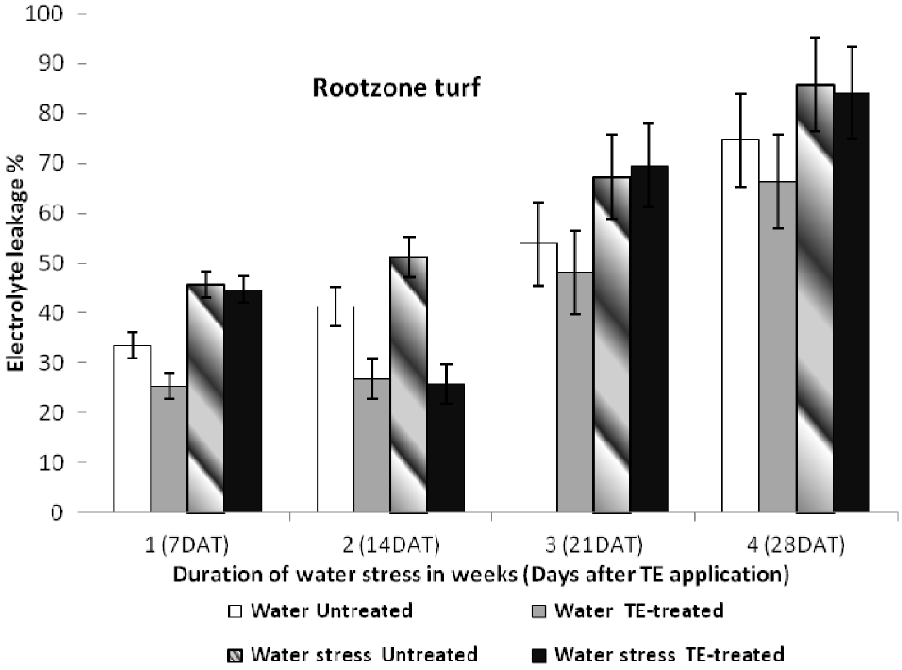

Fig. 3. Effect of trinexapac-ethyl (TE) application (2 L/Ha) on leaf electrolyte leakage (EL) response in Rootzone turf under well-water and water stress conditions

Vertical bars represent standard errors of means of three replications for treatment comparisons at a given day treatment

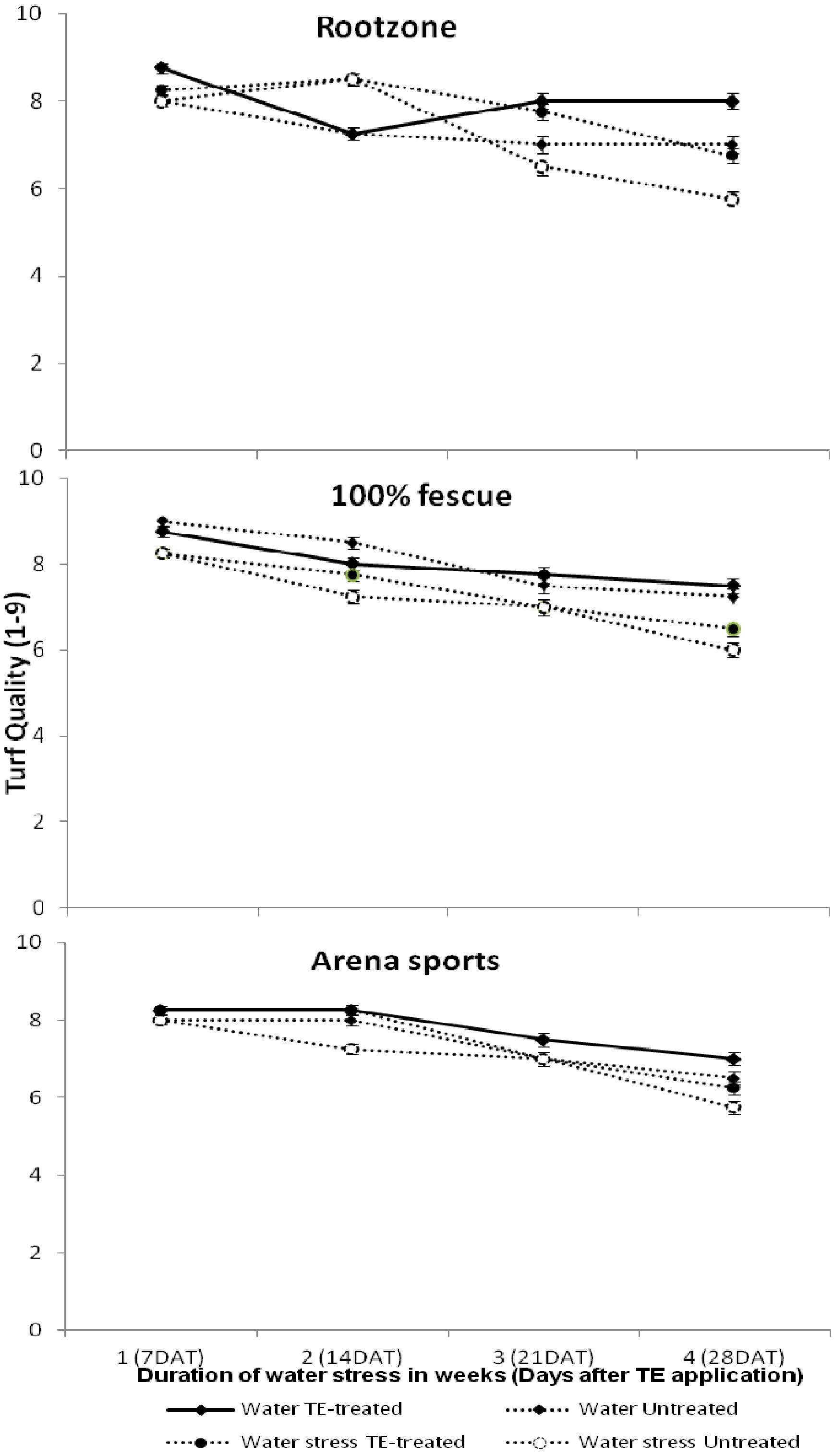

Fig. 4. Effect of trinexapac-ethyl (TE) application (2 L/ ha) on turf quality of Cv. Rootzone, 100% fescue and Arena sports turfgrass under well water and water stress conditions

Vertical bars are standard errors of mean for treatment comparisons at given days of water stress

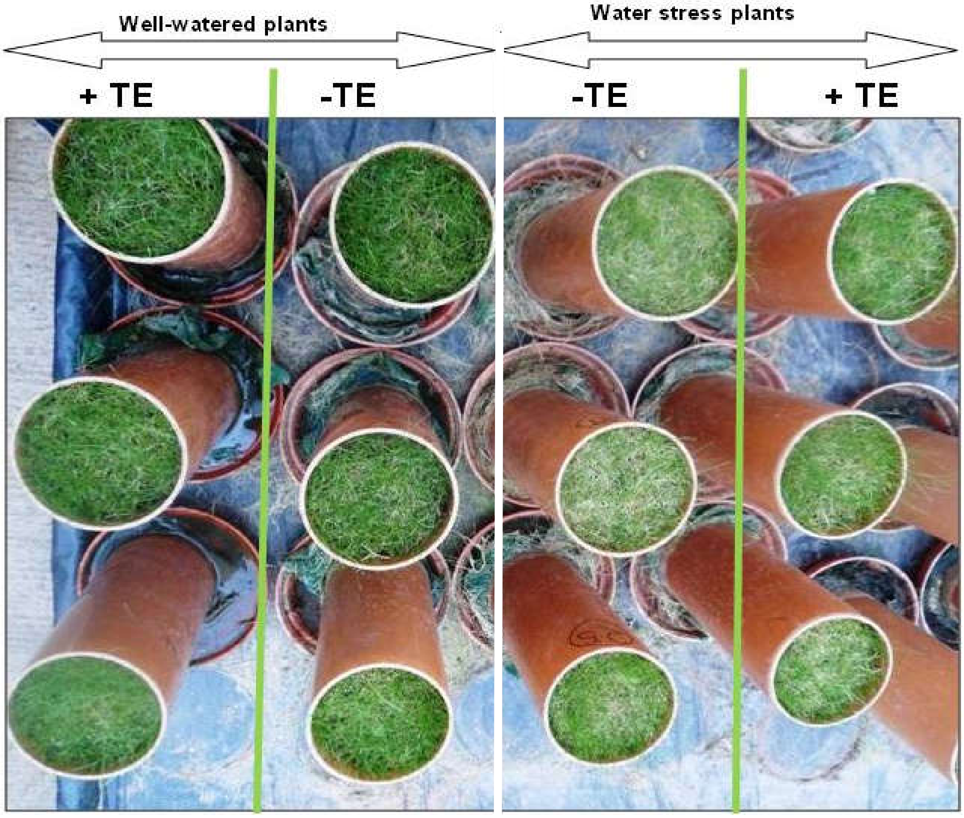

Fig. 5. Visual quality comparison of trinexapac-ethyl treated (+TE) and untreated plants (-TE) of Cv. 100% fescue 28 days after TE application under well watered and water stress conditions (i.e at three weeks after initiation of water stress)

4. DISCUSSION

4.1 Water use Characteristics and Canopy net Photosynthetic Rate (Pn)

In this study, withholding irrigation and the progression of water stress had a negative influence on various physiological processes in turf plants. Overall Pn, leaf membrane stability and TQ were substantially lower in water stress plants compared to well-watered plants. TE application enhanced Pn in Cv Rootzone and lessened the impact of water stress on Pn for the first seven days in Cv 100% fescue plants following water stress induction. Our findings suggest the maintenance of Pn under mild water stress conditions was influenced by TE application and further indicated that the effect of TE on Pn diminishes as the severity of the stress increases. Similar studies by McCann and Huang [2] reported that Pn in TE treated creeping bent grass (*Agrostis stolonifera*) plants remain unchanged under 21 days of water stress conditions, while Pn in untreated plants declined by 60%. The maintenance photosynthetic activity for longer stress duration in this study could be due the used of three TE applications before exposing plants to water stress compared to our study which used just one TE application. Other studies have reported similar beneficiary influence of TE treatment on Pn related to better stress tolerance. TE application increased photosynthetic activity [24] and enhanced antioxidant activity for increased turf performance [10] under water stress conditions in creeping bent grass species and tall fescue (*Festuca arundinacea*) [14].

4.2 Cell Membrane Stability (CMS)

The preservation of favourable water status is vital for water stress tolerance in plants. Various studies [10,29,33] have used EL from leaf tissues as an indicator of CMS associated with the evaluation water stress tolerance. PGRs including TE have been shown to influence hormone activity responsible for protecting membrane integrity of leaf tissues resulting to increase stress tolerance [3,24] in cool season grass species. The effectiveness of the TE

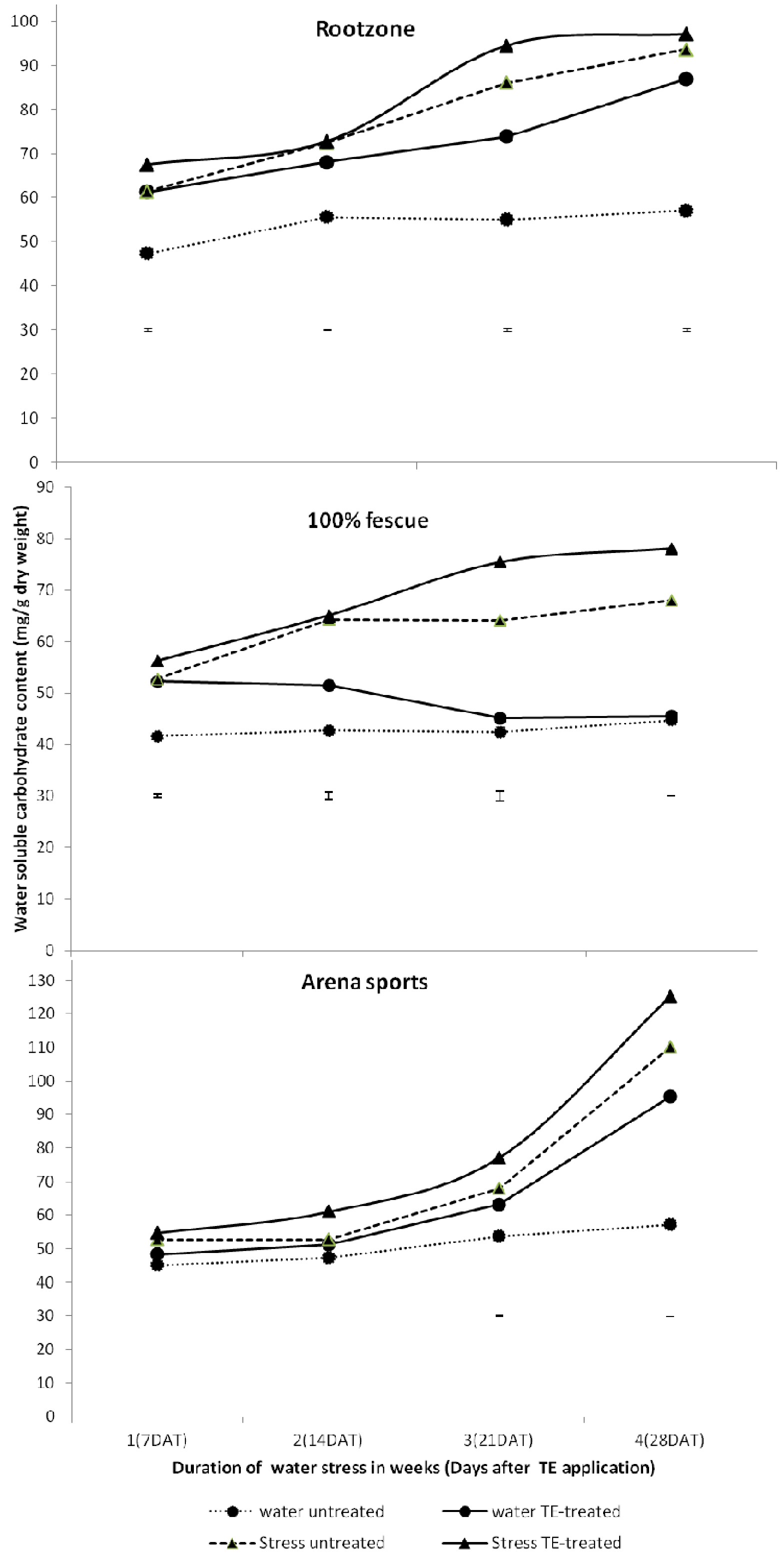

Fig. 6. Effects of trinexapac-ethyl (TE) application (2 L/ ha) on water soluble carbohydrate content in different commercial sports turfgrass (Cv. Rootzone, Cv. 100% fescue, and Cv. Arena sports) under well water and water stress irrigation regimes

Vertical bars are standard errors of means for treatment comparison at given days of measurement treatment however have been shown to vary depending on factors such as plant species, dose and type of stress [2,8,21]. In this study, progression of water stress alone increased EL indicative membrane injury had occurred. TE treatment significantly improved membrane stability at two weeks of water stress in Cv. Rootzone with maximum decline in EL observed in TE treated plants under water stress conditions. This decline in EL did not continue into the third week after TE application. This observation is in line with the product specification information which indicated that the potency of the TE on plants diminishes after 14 days. TE application did not improve membrane stability in 100% fescue or Arena sports turf. Reasons for lack of response were not clear but may be due to cultivar differences. Previous studies have shown some cultivars have slow growth rate and tend to metabolize TE slowly than other cultivars [34].

4.3 Turf Grass Quality

Leaf green-up is a common characteristic associated with TE application on turf. Maintenance of high TQ associated with TE under non stressed conditions has been reported in Kentucky bluegrass [35] and bermunda grass [22]. Similarly, the application of TE has been shown to improve turfgrass colour and quality of perennial ryegrass (*Lolium perenne* L.) [21], with higher rates of TE application reported to have significantly influence on the visual quality and biomass production [36]. Our study demonstrates that TQ of the turf grass species were significantly influenced by TE application. From 21 days after TE application, treated plants under non stress conditions exhibited best TQ compared to untreated plants. This result of significant higher TQ under well-watered condition was expected as label instructions confirmed TE performs best on none-stress plants. TE treated plants also showed better TQ than untreated plants for all turf types under prolong water stress. Improved TQ for TE treatment at 21 and 28 days of water stress induction were more pronounced in Cv. Rootzone whose canopy composition is 50% made of creeping bent grass. These results are similar to those reported by McCann and Huang [2] in creeping bent grass that showed that TE treatment can substantially maintained high TQ rating (7) after 21 days of exposing plants to water and heat stress compared to untreated plants. Although TE-treated and untreated plants had TQ values below the minimum acceptable level (6) by 21 days of stress in our study, these data have similar trend as those reported by Bian et al. [15] in creeping bent grass who observed higher TQ values maintained for TE-treated plants at 21 days of stress compared to control plants. According to Stienke and Stier [37] increasing rates of TE application may increase the duration of growth regulation in plants although this might also increase the phytotoxicity potential of the growth regulator used. Two to three applications of 0.8 L/ ha TE was used to obtained the TE-induced response in TQ seen in above mentioned studies while only a single higher dose, 2.0 L/ ha TE was applied in our study to obtained similar results.

4.4 Water Soluble Carbohydrate

The ability of plants to withstand dehydration-related stress including water stress has been associated with osmolytes accumulation within plant cells [2]. Soluble sugars including water soluble carbohydrates accumulation (WSC) increase osmotic adjustment [12], a physiological mechanism use by plant to regulate the impact of water stress through maintenance of cell turgor. In this study, regardless of the fact that water stress initiation alone triggered increase in WSC in the plants, TE treated plants contained significantly more WSC content than untreated plants in Cv. Arena Sports under both irrigation regimes and at 21 and 28 d of stress for Cv. Rootzone and Cv. 100% fescue water stressed plants. Similar results of positive effects of TE application associated with higher WSC content accumulation have been reported in other cool-season grass species [38,39]. Etemadi et al., [14] investigating the effect of TE and water stress in turfgrasses reported that water stress increased soluble sugar content in control tall fescue (*Festuca arundinacea*) plants and suggested that the use of TE enhanced water stress resistance by improving soluble sugar content in TE treated plants. Under water stress conditions, Han et al., [38] showed that TE increased the levels of total non-structural carbohydrate content in creeping bent grass after application but these increases were transient and observed only between the second and fourth week after the PGR treatment. Our studies showed similar observations of TE induced increases in leaf WSC content in Cv.100% fescue plants that were evident only for the first two weeks after TE application. By the fifth week after TE application, the effect of TE treatment had diminished and no clear differences in the amount WSC content existed between TE-treated (45.15 mg/g^{-1}) and untreated (42.47 mg/g^{-1}) well water Cv. 100% fescue plants. Similar results have been reported in tall fescue plants in studies by Richie et al. [40]. The author found that at 6 weeks after TE application, there was no significant difference in the quantity of total non-structural carbohydrate content in leaves of TE-treated (41. 8 mg/g^{-1}) and non-treated (44.5 mg/g^{-1}) well watered tall fescue plants.

McCann and Huang [2] reported no positive effect of TE on total non structural carbohydrate content in creeping bent grass and have associated the improved turf performance by TE to maintenance of photosynthetic activity. However levels of carbohydrate content accumulation in cool-season grass have been shown to vary depending on plant cultivar, TE treatment interval and dose rate applied [2,15,38,41]. Nonetheless, despite these variations in results, no negative effects of TE application on overall turf performance were observed in this study as well as in previous literature.

5. CONCLUSION

In the present study, commercial turfs (100 fescue, Rootzone, Arena sports) were subjected to single dose TE (2 L/ ha) pre-treatment application and the effects of treatment on net canopy photosynthesis (Pn), turf quality (TQ), cell membrane stability (CMS), and water soluble carbohydrate (WSC) responses to subsequent water stress exposure were evaluated under glasshouse conditions. The study showed that withholding irrigation (initiation of water stress and its progression) for 28 days significantly reduced the soil water content to 6% in 10 cm soil dept exploited by the roots on the turf plants. Similarly values of net canopy photosynthesis, turf quality, cell membrane stability, and water soluble carbohydrate (WSC) accumulation were generally lower in water stressed plants compared to well water plants. Application of trinexapac-ethyl did not significantly enhance photosynthesis of plants under water stress condition however under well watered conditions, the application of TE resulted to >40% increase in Pn but this was only observed in Rootzone. Under water stress conditions, TE application resulted in significant increases in water soluble carbohydrate accumulation, improved membrane stability, and enhanced turf quality in TE treated plants compared to untreated plants. These beneficial effects of TE on turf plants performance were noticed often during the third and fourth week after its application. Thus preconditioning plants with TE can be used as a possible management tool in alleviating the detrimental impacts of water stress on turf growth and performance. However, to make a conclusive commercial recommendation, this study needs to be replicated under natural field conditions. Further research to evaluate if multiple dose application of TE may favour improved drought tolerance mechanisms in turf species is warranted.

The results of this study however have to be seen in light of some potential limitation. The duration of measuring maximum photosynthesis rate by the infrared gas analyser was set for two minutes and any sudden weather fluctuation during this time may have influenced our photosynthesis readings estimated. It is possible that similar estimates of photosynthesis will not be obtained if measurement were estimated for a shorter during and under fixed artificial lighting condition.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Huang B. Recent advances in drought and heat stress physiology of turfgrass-a review. Acta Horticulturae. 2004;661:185-192.
2. McCann SE, Huang B. Effects of trinexapac-ethyl foliar application on creeping bent grass responses to combined drought and heat stress. Crop Science. 2007;47:2121-2128.
3. Nabati DA, Schmidt RE, Khaleghi ES, Parrish DJ. Assessment of drought stress on physiology growth of *Agrostis Palustris* Huds. as affected by plant bioregulators

and nutrients. Asian Journal of Plant Sciences. 2008;7:717-723.

4. Riaz A, Younis A, Hameed M, Kiran S. Morphological and biochemical response of turf grass to water deficit conditions. Pakistan Journal of Botany. 2010; 42(5):3441-3448.
5. Shooshtarian S, Salehi H. Physiological and ecological investigation on adoption of some groundcover plants as turfgrass alternatives in arid landscape region of Kish Island during cool season. African Journal of Agricultural Research. 2012; 7(4):546-554.
6. Alshehhi AMH, Khan IA, Alsaid FA, Deadman ML, Al-Khanjari, Ahmad T. Evaluation of warm season turf grass under different irrigation regimes in arid regions. Notunlae Scienta Biologicae. 2010;2(3):30-38.
7. Zhou S, Abaraha A. Response to heat stress in warm season and cool season turf grass cultivars. Scientific Research and Essay. 2007;2(4):95-100.
8. Richardson MD. Turf quality and freezing tolerance of Tifway bermundagrass as affected by late-season nitrogen and trinexapac-ethyl. Crop Science. 2002; 42:1621-1626.
9. Samieiani E, Ansari H. Drought stress impact on some biochemical and physiological traits of 4 groundcovers (*Lolium perenne, Potentilla spp, Trifolium repens* and *Frankinia spp*) with potential landscape usage. Journal of Ornamental Plants. 2014;4(1):53-60.
10. Huang B, Gao H. Root physiological characteristics associated with drought resistance in tall fescue cultivars. Crop Science. 2000;40:196-203.
11. Jiang Y, Huang B. Osmotic adjustment associated with drought-preconditioning enhanced heat tolerance in Kentucky bluegrass. Crop Science. 2001;41:1168-1173.
12. DaCosta M, Huang B. Osmotic adjustment associated with variation in bent grass tolerance to drought stress. Journal of the American Society for Horticultural Sciences. 2006;131(3):338-344.
13. Lui J, Xie X, Du J, Sun J, Bai X. Effects of simultaneous drought and heat stress on kentucky bluegass. Scientia Horticulturea. 2008;115:190-195.
14. Etemadi N, Sheikh-Mohammadi MH, Nikbakht A, Sabzalian MR, Pessarakli M. Influence of trinexapac-ethyl in improving drought resistance of wheatgrass and all fescue. Acta Physiologiae Plantarum. 2015;37:53-60.
Available:https://doi.org/10.1007/s11738-015-1799-6
15. Bian X, Merewitz E, Huang B. Effects of trinexapac-ethyl on drought responses in creeping bent grass associated with water use and osmotic adjustment. Journal of the American Society for Horticultural Science. 2009;134:505-510.
16. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: Effects, mechanisms and management. Agronomy for Sustainable Development. 2009;29: 185-212.
17. Méndez AM, Castillo D, del Pozo A, Matus I, Morcuende R. Differences in stem soluble carbohydrate contents among recombinant chromosome substitution lines (RCSLs) of barley under drought in a mediterranean-type environment. Agronomy Research. 2011;9:433-438.
18. Xu Q, Huang B. Seasonal changes in carbohydrate accumulation for two creeping bentgrass cultivars. Crop Science. 2003;43:266-271.
19. Qian YL, Fu JM. Response of creeping bentgrass to salinity and mowing management: Carbohydrate availability and ion accumulation. Hort Science. 2005;40(7):2170-2174.
20. Tilhou NW, Nave RL. Improving nutritive value of native warm-season grasses with the plant growth regulator Trinexapac-Ethyl. Agronomy Journal. 2018;110:1836-1842.
21. Heckman NL, Horst GL, Gaussoin RE, Tavener BT. Trinexapac-ethyl influence on cell membrane thermostability of Kentucky bluegrass leaf tissue. Scientia Horticulturae. 2002;92(2):183-186.
22. McCullough PE, Liub H, McCarty LB, Whitwell T, Toler JE. Bermudagrass putting green growth, colour, and nutrient partitioning influenced by nitrogen and trinexapac-ethyl. Crop Science. 2006; 46:1515-1525.
23. Brosnan JT, Thoms AW, Breeden GK, Sorochan JC. Effects of various plant growth regulators on the traffic tolerance of ‘Riviera’ Bermudagrass (*Cynodon dactylon* L.) Horticultural Science. 2010;45(6):966-970.
24. Zhang X, Schmidt RE. Application of trinexapac-ethyl and propiconazole enhances superoxide dismutase and

photochemical activity in creeping bent grass (*Agrostis stoloniferous* var. *palustris*). Journal of the American Society for Horticultural Science. 2000;125(1):47-51.

25. Glab T, Szewczyk W, Gondek K, Knaga J, Tomasik M, Kowalik K. Effect of plant growth regulators on visual quality of turfgrass. Scientia Horticulturae. 2020;267: 1-10.
Available:https://doi.org/10.1016/j.scienta.2020.109314
26. Ervin EH, Ok CH, Fresenburg BS, Dunn JH. Trinexapac-ethyl restricts shoot growth and prolongs stand density of'Meyer'zoysiagrass fairway under shade. Hort Science. 2002;37(3): 502-505.
27. Ervin EH, Koski AJ. Trinexapac-ethyl increases Kentucky bluegrass leaf cell density and chlorophyll concentration. Hort Science. 2001;36(4):787-789.
28. Volaire F, Lelievre F. Drought survival in *Dictylis glol. Merata* and *Festuca arundinacea* under similar rooting conditions in tubes*.* Plant Soil. 2001;229(2):225-234.
29. DaCosta M, Wang Z, Huang B. Physiological adaptation of Kentucky bluegrass to localized soil drying. Crop Science. 2004;44(4):1307-1314.
30. Turgeon AJ. Turfgrass management. 6th ed. Prentice Hall, Englewood, Cliffs, NJ; 2002.
31. Ministry of Agriculture, Fisheries and Food (MAFF). London; 1986.
32. Maness N. Extraction and analysis of soluble carbohydrates In: Methods in molecular biology (Clifton N. J). 2010;639: 341-370.
33. Liu X, Huang B. Heat stress injury of creeping bent grass in relation to membrane lipid peroxidation. Crop Science. 2000;40:503-510.
34. Qian YL, Engelke MC. Influence of trinexapac-ethyl on Diamond zoysiagrass in a shade environment. Crop Science. 1999;39(1):202-208.
35. Stier JC, Rogers JN. Trinexapac-ethyl and iron effects on supina and Kentucky bluegrasses under low irradiance. Crop Science. 2001;41(2):457-465.
36. Pornaro C, Fiorio S, Macolino S, Richardson MD. Growth and quality responses of low-maintenance turfgrasses to trinexapac-ethyl. Crop Protection. 2017; 98:236-242.
Available:https://doi.org/10.1016/j.cropro.2017.04.007
37. Steinke K, Stier JC. Nitrogen selection and growth regulator applications for improving shaded turf performance. Crop Science. 2003;43(4):1399-1406.
38. Han SW, Fermanian TW, Juvik JA, Spomer LA. Growth retardant effects on visual quality and nonstructural carbohydrates of creeping bentgrass. Hort Science. 1998;33:1197-1199.
39. Spak DR, DiPaola JM, Lewis WM, Anderson CE. Tall fescue sward dynamics: II. Influence of four plant growth regulators. Crop Science. 1993;33:304-310.
40. Richie WE, Green RL, Merino F. Trinexapac-ethyl does not increase total non-structural carbohydrate content in leaves, crowns, and roots of tall fescue. Hort Science. 2001;36(4):772-775.
41. Han SW, Fermanian TW, Juvik JA, Spomer LA. Total nonstructural carbohydrate storage in creeping bentgrass treated with trinexapac-ethyl. Hort Science. 2004;39(6):1461-1464.

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