RESEARCH

Effects of Trinexapac-Ethyl Foliar Application on Creeping Bentgrass Responses to Combined Drought and Heat Stress

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ABSTRACT

Simultaneous drought and heat stress is detrimental to turfgrass growth. Growth regulators may influence plant responses to stresses. The objective of this study was to determine effects of pretreatment with trinexapac-ethyl (TE) on creeping bentgrass (*Agrostis stolonifera* L.) responses to subsequent exposure to combined heat and drought stress. Plants were treated with TE (1.95 mL L^{-1} [v/v], Primo Maxx) every 14 d for 42 d and then exposed to combined drought and heat (35°C) in growth chambers. Application of TE increased turf quality and relative water content and chlorophyll content during 21 d of stress. Trinexapac-ethyl–treated plants had increased growth rate and maintained constant canopy photosynthetic rate (Pn) and photochemical efficiency (Fv/Fm) during the stress period, while these parameters declined for the untreated control. Trinexapac-ethyl–treated plants maintained lower evapotranspiration rate (ET) under nonstressed control conditions, but higher ET at 21 d of stress. Trinexapac-ethyl–treated plants showed no differences from untreated plants in osmotic adjustment, and had lower levels of total nonstructural carbohydrates during the stress period. The study suggests that TE application enhanced turf performance under stress, as demonstrated by maintenance of higher turf quality and growth rate during a prolonged period of com bined stress. The beneficial effects of TE on bentgrass stress responses could be related to the maintenance of photosynthetic activities and greater level of cellular hydration. These and other published data indicate that use of TE on creeping bentgrass that seasonally experiences heat and drought stresses could benefit its performance.

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Abbreviations: ET, evapotranspiration rate; Fv/Fm, photochemical efficiency; Pn, canopy photosynthetic rate; PVC, polyvinyl chloride; RWC, relative water content; TE, trinexapac-ethyl; TNC, total nonstructural carbohydrates; VSG, vertical shoot growth; VWC, volumetric water content.

Drought and heat stress often occur simultaneously during summer months in warm, arid, or semiarid regions. Combined drought and heat stress is detrimental to turfgrasses, particularly for cool-season grasses during summer months (Jiang and Huang, 2002; Wang and Huang, 2005). As such, it is important to understand methods of improving plant tolerance to summer stress and to develop management programs that both reduce overall water consumption and that minimize turf loss during periods of extended summer stress.

Plants have developed various mechanisms in adaptation to drought stress, including avoiding stress by reducing water loss or consumption and slowing plant growth (Huang and Jiang, 2002). Plant growth regulators, such as growth inhibitors, are widely used in turfgrass management to suppress shoot growth and inflorescences (Turgeon, 2002). The growth inhibitor trinexapac-ethyl (TE) blocks the final step in the biosynthesis of the biologically active forms of gibberellins, resulting in slower shoot growth (King et al., 1997). The effects of TE on growth inhibition are well documented in various turfgrass species (Ervin et al., 2002; Ervin and Koski, 1998; McCarty et al., 2004; Pannacci et al., 2004), however, effects of TE on plant responses to environmental stresses are less understood. Heckman et al. (2001a, 2001c) have shown that TE application significantly

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reduced sod heating and injury during storage of Kentucky bluegrass (*Poa pratensis* L.). Jiang and Fry (1998) demonstrated that foliar TE treatments increased turf quality of perennial ryegrass (*Lolium perenne* L.) during dry-down, suggesting that TE may enhance drought tolerance. The application of TE during exposure of plants to salinity enhanced root growth in two cultivars of bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] (Baldwin et al., 2006). Creeping bentgrass (*Agrostis stoloniferia* L.) quality and color were improved by TE application under shaded conditions (Goss et al., 2002). Richardson (2002) reported that TE enhanced fall green color retention but had no consistent effect on growth and development of rhizomes or stolons and did not improve freeze tolerance of rhizomes in bermudagrass.

Limited available data, as reported in the above referred studies, suggest that TE application may be beneficial for plant tolerance to stresses, but the effectiveness varies with turfgrass species, dose and duration of TE treatment, and type of stress. In addition, most studies examined TE effects on plant tolerance to a single stress factor. Whether pretreating plants with TE enhances turfgrass tolerance to subsequent exposure to multiple stresses, such as the combination of drought and heat stress, is unknown. Furthermore, physiological factors involved in changes in turfgrass stress tolerance with TE application are not well understood. Most studies conducted under nonstressed conditions have found that TE application increased chlorophyll content, turf quality, and turf density and reduced shoot extension rate (Beasley et al., 2005; Bingaman et al., 2001; Ervin and Koski, 1998, 2001a; Heckman et al., 2001c; Pannacci et al., 2004). We hypothesized that TE may influence plant tolerance to combined drought and heat stress by regulating photosynthesis and water use. The objectives of this study were (i) to assess the impact of pretreatment of TE on creeping bentgrass responses to subsequent exposure to combined drought and heat stress; and (ii) to examine physiological factors involved in the combined stress tolerance associated with TE application. The information would provide further insight into plant growth regulation of turfgrass tolerance to drought and heat stress.

MATERIALS AND METHODS

Growth Conditions

Sod pieces of creeping bentgrass (*A. stolonifera* 'L-93') were transplanted from mature field plots into polyvinyl chloride (PVC) tubes (40-cm length, 10-cm diameter) filled with sterilized sandy loam soil (fine-loamy, mixed mesic Typic Hapludult). Plants were maintained in a greenhouse under seasonal daylight conditions at approximately 20°C for 4 mo, and then moved to growth chambers where treatments were imposed. Growth chambers were set at 20°C (day and night), 12-h photoperiod, with photosynthetically active radiation level of 450 μmol m^{-2} s^{-1} at the canopy level. Grasses were maintained at approximately 2-cm canopy height. Plants were watered three times per week to maintain soil moisture at field capacity. One hundred milliliters of a soluble 20-20-20 (N-P-K) fertilizer was applied weekly at a concentration of 5 g L^{-1} to each container.

Treatments and Experimental Design

Plants were pretreated with 0.8 L ha^{-1} TE (Primo Maxx, Syngenta Professional Products, Greensboro, NC) (1.95 mL L^{-1} [v/v]; ai TE = 11.3%) every 14 d for 42 d and then exposed to drought and heat stress. Final TE treatment was made at 14 d before the initiation of stress, with no additional applications of TE during the stress period. The experiment consisted of three treatments: (i) Stress − TE: plants that were not pretreated with TE were exposed to drought stress and heat stress (35°C, day/night); (ii) Stress + TE: plants pretreated with TE were then exposed to drought and heat stress (35°C, day/night); and (iii) nonstressed control: plants that were not pretreated with TE and maintained under well-watered and optimum temperature (20°C) conditions. Drought stress was imposed by withholding irrigation. Well-watered plants were irrigated to soil moisture reaching field capacity. No TE, fertilizers, or irrigation were applied after initiation of the stress treatments. Plants were rewatered at the end of stress treatment to examine for recuperative ability from drought stress.

High temperature treatments (35°C) were maintained in four growth chambers, containing both TE-treated and untreated plants. A separate growth chamber was used to maintain untreated control plants at a normal temperature (20°C). Temperature treatments were relocated among different chambers weekly to minimize growth chambers effects. Four replicated plants of TE-treated and untreated plants were arranged randomly inside each growth chamber. The experiment was considered as a randomized block design. Statistical significance of data was tested using the analysis of variance procedure (SAS Institute Inc., Cary, NC). Differences between treatment means were separated by Fisher's protected least significance (LSD) test at the 0.05 probability level.

Measurements

Turf quality was visually rated on a scale of 1 to 9 based on color, density, and uniformity (Turgeon, 2002). Plants rated 1 were completely desiccated with a completely necrotic turf canopy. A rating of 9 represented healthy plants with dark green, turgid leaf blades, and a full turf canopy. A rating of 6 was considered the minimal acceptable turf quality. Shoot vertical growth rate was determined by measuring the difference in average canopy height between measurement dates using a ruler.

Water use characteristics were evaluated by measuring leaf relative water content (RWC), osmotic adjustment (data not shown), and soil volumetric water content (VWC). Measurements were taken on a weekly basis. Soil water content in 0- to 20-cm soil depth was measured with the time domain reflectometry method (Soil Moisture Equipment, Santa Barbara, CA) using a 20-cm long probe inserted in the soil. Relative water content was calculated using the formula: 100[(FW − dwt)/(TW − dwt)] where FW is fresh weight, TW is turgid weight, and dwt is dry weight following oven-drying leaf samples for 72 h at 100°C. Turgid weight was determined as the weight of fully turgid leaves after having soaked in distilled water for 24 h.

Canopy net photosynthetic rate (Pn) was measured as CO_2 μmol m^{-2} s^{-1} using the Licor 6400 gas exchange analyzer (Licor

Biosciences, Lincoln, NE). The unit also measured canopy evapotranspiration rate (ET) as mmol H_2O m^{-2} s^{-1}. A clear plexiglass chamber was designed for canopy Pn and ET measurement, which was fitted tightly over the top of the PVC pot, creating a seal from the surrounding atmosphere. Turf canopy was provided constantly with 400 μL L^{-1} CO_2 during the measurement. This design allowed for gas exchange measurement of intact leaves for the entire canopy area with constant CO_2 supply.

Leaf photochemical efficiency was estimated by measuring variable to maximum fluorescence ratio (Fv/Fm) in the nonenergized state accomplished by exposure to darkness. Measurements were made of intact leaves with a fluorometer (ADC BioScientific, Hoddedson, UK) after plants were adapted to dark for 30 min.

Chlorophyll was extracted by placing 0.1 g of fresh leaf tissues in a test tube containing 20 mL dimethyl sulphoxide and left in the dark for 48 h (Hiscox and Israelstam, 1979). The absorbance of the resulting solution was measured at 663 and 645 nm with a spectrophotometer (Spectronic Instruments, Rochester, NY) and total chlorophyll concentration was calculated as described by Arnon (1949).

For carbohydrate analysis, 200 mg leaf tissue was oven-dried for 72 h at 100°C. The tissue was finely ground and hydrolyzed with 2.5 mL amylase for 24 h at 37°C. A 0.5 mL of 0.6 mol L^{-1} HCl (1:1 v/v) was added and slowly shaken for 18 h at 37°C. The solution was neutralized with approximately 0.31 mL of 10 mol L^{-1} NaOH to a pH between 5 and 7, diluted to 50 mL, and filtered through no. 40 Whatman filter paper. One milliliter of the filtered solution was transferred to a 20-mL volumetric tube and 1.5 mL of ferricyanide reagent was added. The solution was boiled in a water bath for 10 min and then cooled in running water. Three milliliters of 1 mol L^{-1} H_2SO_4 was added to partially neutralize the solution. After neutralization, the solution in the tube was shaken until gas evolution ceased, and 1.2 mL of arsenomolybdate solution was added. The solution was again shaken and diluted to a volume of 25 mL. The absorbance of the solution was measured at 515 nm using a spectrophotometer (Spectronic Genesys 2, Spectronic Instruments Inc., Rochester, NY). The reducing sugar content was calculated as milligrams of total nonstructural carbohydrates (TNC) per gram of dry tissue.

At the end of the stress period (21 d), roots were carefully removed from PVC columns and gently washed with water to remove all soil material but to keep roots intact. Rooting depth (longest length of roots) was measured to determine overall length. Root dry weight was measured to determine root mass after drying roots in an oven at 85°C.

RESULTS

Before initiation of stress (0 d), TE-treated plants had significantly higher quality ratings than other treatments following 42 d of TE application (Fig. 1). During 21 d of stress exposure, quality ratings for nontreated plants dropped while TE-treated plants maintained significantly higher turf quality than the nontreated plants, and was still equivalent to the nonstressed control plants. After 10 d of rewatering (irrigated, 35°C), turf quality of TE-treated plants fully recovered, while that of nontreated turf improved to some extent, but was still below minimal acceptable turf quality (quality rating 6).

Both TE-treated plants exposed to stress and nonstressed control treatment maintained leaf RWC between 85 and 90% during the entire treatment period (Fig. 2). Relative water content dropped significantly during stress in the nontreated turf and was significantly lower than TE-treated turf at 10, 18, and 21 d of stress. Relative water content of nontreated turf was below 50% by 21 d of stress. Relative water content quickly recovered in nontreated plants and was similar to other treatments after 10 d of rewatering.

Soil VWC remained at field capacity (approximately 30%) in the nonstressed control treatment (Fig. 3). Soil VWC in both TE-treated and nontreated treatments decreased during the stress, beginning at 10 d. The decline in VWC was 3% in the TE treatment and 21% in the non-TE treatment at 10 d of stress. After 18 d of stress, decline in VWC was 42% in the TE treatment and 59% in the non-TE treatment.

Vertical shoot growth (VSG) was significantly reduced following 42 d of TE treatment before stress was imposed (0 d) (Fig. 4). During the stress period, TE-treated plants exhibited increasing VSG from 0.12 cm d^{-1} at 0 d to 0.21 cm d^{-1} at 21 d of stress, whereas the rate of VSG in nontreated plants steadily decreased from 0.25 cm d^{-1} at 0 d to 0.08 cm d^{-1} at 21 d of stress and fell below TE-treated plants at 18 and

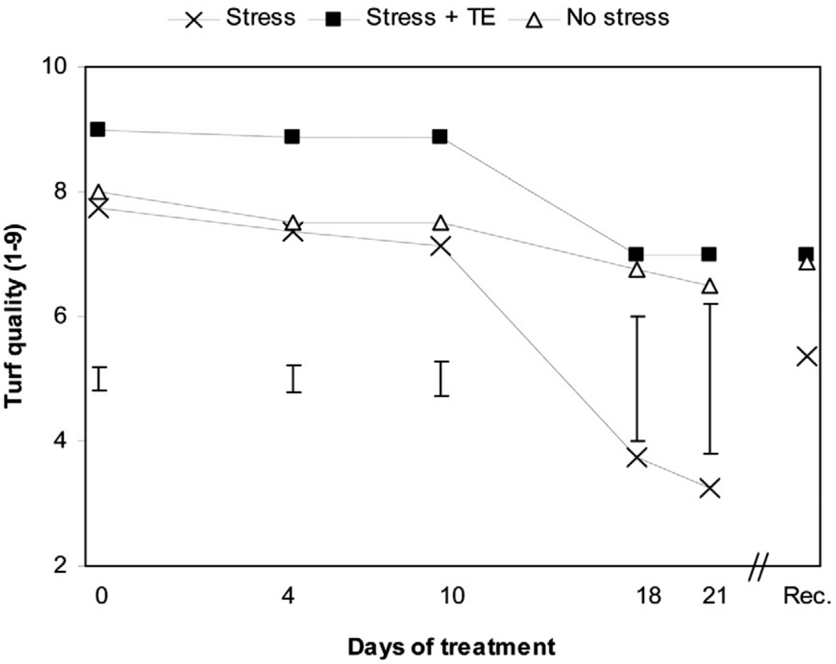

Figure 1. Effects of foliar application of trinexapac-ethyl (TE) on turf quality during combined heat and drought stress (stress). Turf quality was expressed on a scale of 1 to 9 based on turf color, density, and uniformity. Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (P = 0.05) for treatment comparisons at a given day of treatment.

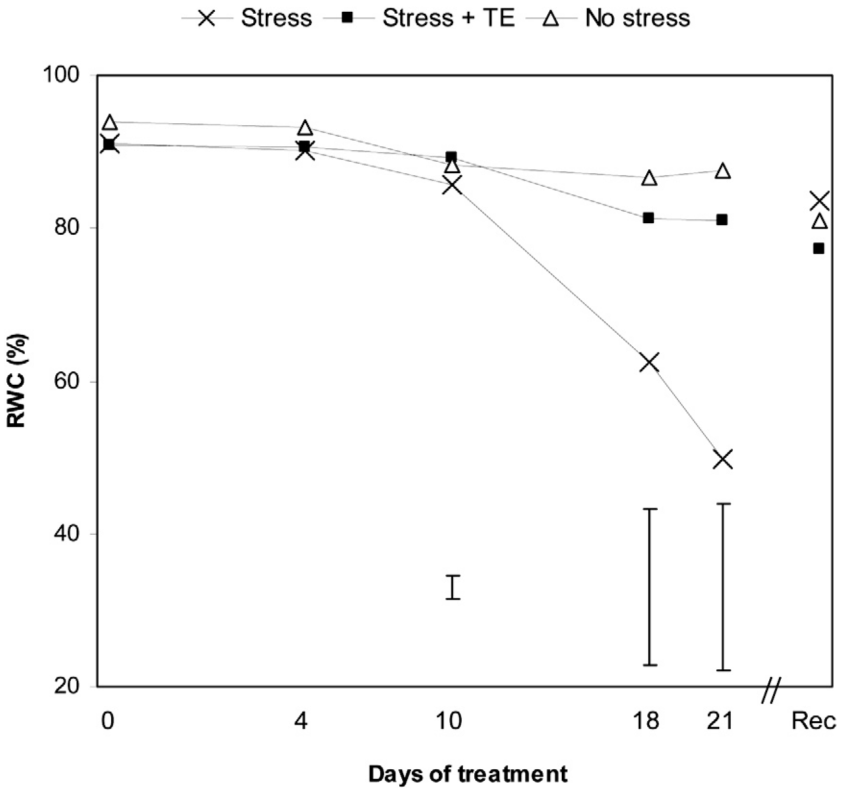

Figure 2. Effects of foliar application of trinexapac-ethyl (TE) on relative water content (RWC) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (*P* = 0.05) for treatment comparisons at a given day of treatment.

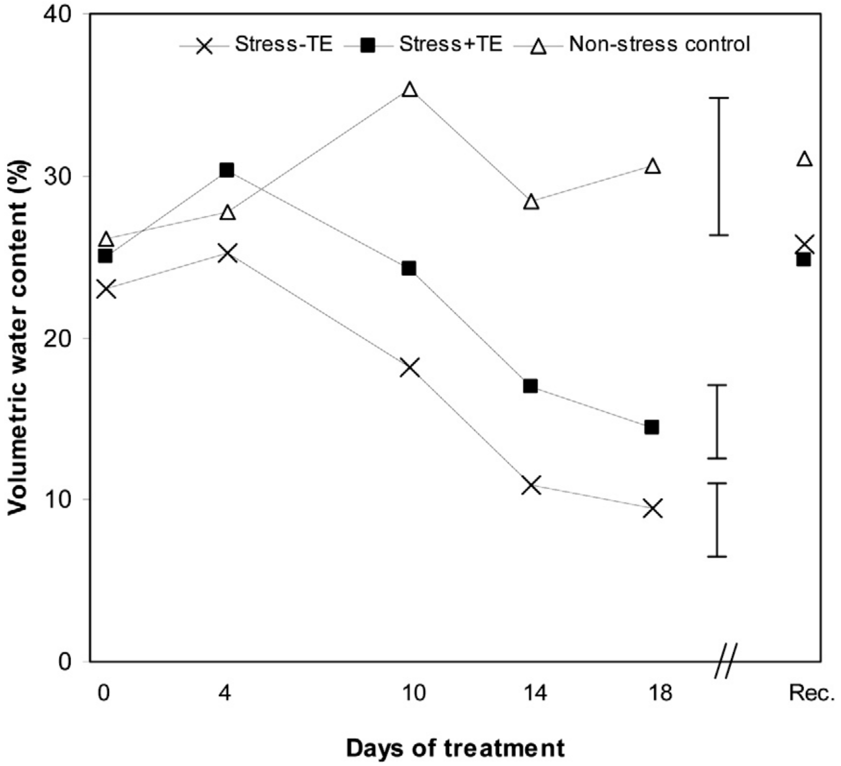

Figure 3. Changes in soil volumetric water content (%) in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (*P* = 0.05) for comparison of changes over treatment period.

21 d of stress. Control plots maintained a rate of growth at about 0.25 cm d^{-1}.

Canopy net photosynthetic rates (Pn) were not significantly different between TE-treated and nontreated plants at 0 and 10 d of stress (Fig. 5). At 21 d of stress exposure, TE-treated turf had significantly higher Pn than nontreated turf. Net photosynthetic rates of TE-treated plants remained unchanged throughout the stress period while that of nontreated plants declined by 60% by 21 d of stress.

Trinexapac-ethyl–treated turf had similar rates of canopy ET before initiation of stress (0 d) when compared to nontreated turf (Fig. 6). However, at 10 d of stress, nontreated plants had significantly (11%) higher ET than TE-treated plants. At 21 d of stress, ET of nontreated plants dropped and was significantly lower (53%) than TE treatments; TE-treated plants maintained a consistent ET (4.9 mmol H_2O m^{-2} s^{-1}) throughout the stress period (10 and 21 d).

Nontreated plants showed a steady decline in Fv/Fm values, while TE-treated plants exhibited a constant Fv/Fm during the stress period, which was at levels similar to the nonstressed control plants (Fig. 7). However, Fv/Fm data were not statistically different between treatments at a given day of stress due to large variation between replicated plants. Measurements taken 10 d after rewatering showed that Fv/Fm of nontreated plants increased to the control level.

Chlorophyll content of TE-treated plants was significantly higher than both nontreated plants and control plants at 0 and 10 d of stress (Fig. 8). At 21 d of stress, chlorophyll in TE treatments (16.3 mg g^{-1} dwt) was still maintained at the level of the nonstressed control and was significantly higher than that of nontreated turf (5.0 mg g^{-1} dwt).

Before stress initiation, no differences existed in levels of TNC between treatments following 42 d of TE application. However, TNC in TE-treated plants were significantly lower than nontreated plants at 10 and 21 d of stress (Fig. 9).

Rooting depth of TE-treated and non–TE-treated plants at the end of the stress period was 33 and 35 cm, respectively, and no significant differences in rooting depth between the treatments were detected. Similar to rooting depth, root mass had no statistically significant difference between the two treatments following the 21-d combined stress (data not shown).

DISCUSSION

Preconditioning creeping bentgrass with TE before exposure of plants to combined drought and heat stress helped maintain higher turf quality, representing overall turf performance, during the exposure to the combined stress for a period of 21 d. Plants treated with TE also had better recuperative ability from the combined stress than nontreated plants. Our data suggested that preconditioning of plants with TE had a positive impact on creeping bentgrass survival of combined drought and heat stress.

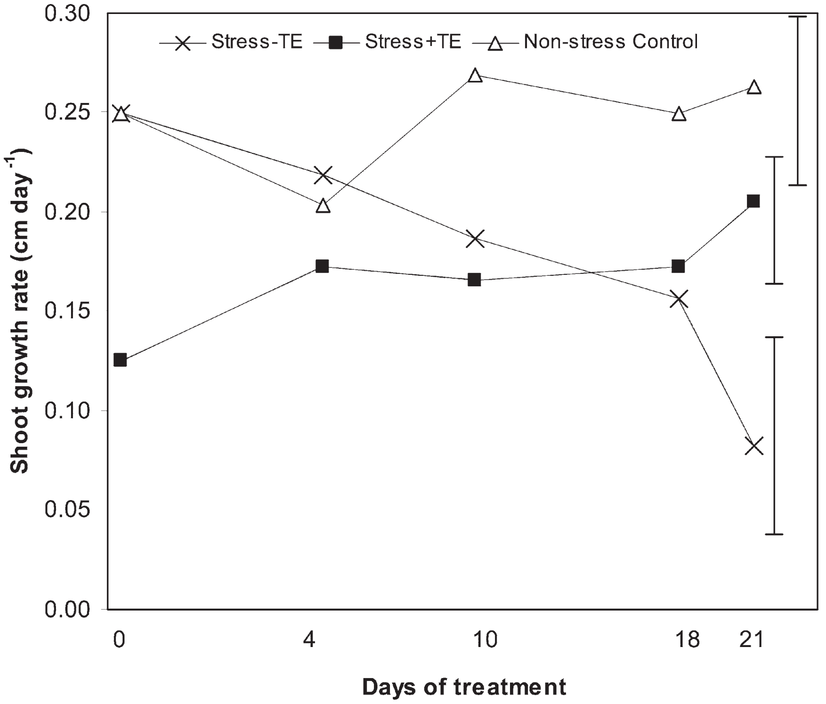

Figure 4. Changes in vertical shoot growth in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Vertical bars indicate LSD values ($P = 0.05$) for comparison of changes over the treatment period.

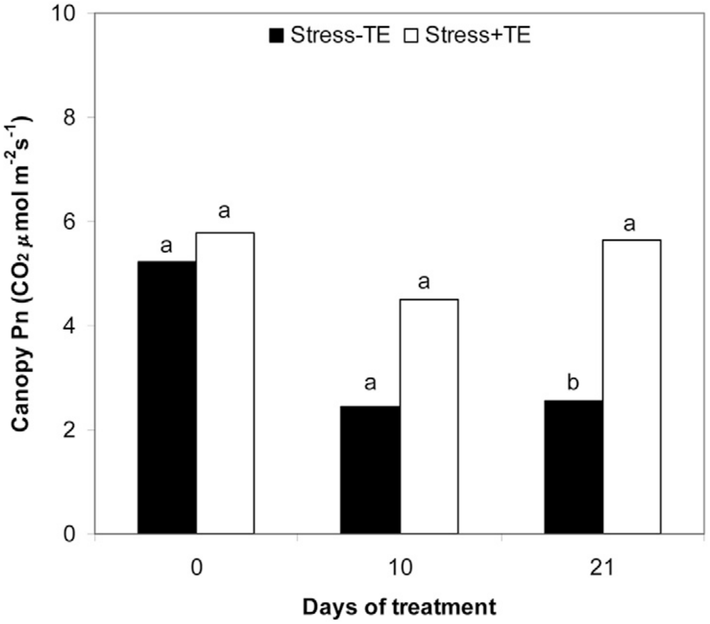

Figure 5. Effects of foliar application of trinexapac-ethyl (TE) on canopy photosynthesis rate (Pn) (CO$_2$ mmol m^{-2} s^{-1}) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values ($P = 0.05$).

The mechanisms of TE regulation of stress tolerance are not well understood. Some studies have demonstrated that TE application promotes tiller production (Bingaman et al., 2001; Beasley et al., 2005; Ervin and Koski, 1998) and increases in chlorophyll content (Heckman et al., 2001b; Ervin and Koski, 2001c). Improved turf color and chlorophyll content have also been observed in different turfgrass species exposed to shade (Goss et al., 2002; Stier and Rogers, 2001). In the present study, TE application increased canopy Pn and leaf chlorophyll content and suppressed the decline in Fv/Fm ratio during the stress period, as compared to non-TE treatment. These results indicated that TE application may improve canopy photosynthesis capacity and single-leaf photochemical efficiency, which could lead to increased turf quality during summer stress. Increased tiller density with TE treatment (Bingaman et al., 2001; Beasley et al., 2005; Ervin and Koski, 1998) may also contribute to the higher canopy Pn due to increased leaf area available for light absorption. In the present study, turf canopy treated with TE appeared to be denser than untreated turf, but this parameter was not quantified. Previous studies reported "rebound" effects of TE treatment, that means plant growth rate resumes following a period of growth inhibition (Fagerness and Yelverton, 2000). Rebound effects were not observed during the stress period in this study, but TE-treated plants maintained higher growth rate than untreated plants during prolonged period of the combined stress. How rebound effects of TE may be related to turfgrass tolerance to prolonged period of stress was not evaluated in this study or previously reported by others, which may deserve investigation.

The accumulation of total nonstructural carbohydrates (TNC) has been associated with a plant's ability to tolerate stresses and assists in recuperation following stress damage (Huang and Jiang, 2002). In the present study, the positive effects of TE on Pn and turf quality, however, did not seem to be related to leaf TNC accumulation associated with TE application. Plants treated with TE for 42 d had a similar amount of TNC in leaves as nontreated plants before stress treatment. Furthermore, TE-treated plants had lower TNC content in leaves than nontreated plants at 10 and 23 d of stress. Total nonstructural carbohydrate accumulation is the result of the balance between carbohydrate production and consumption (Huang and

Jiang, 2002). The lower TNC content may be the result of active shoot growth (VSG rate increased from 0.12 to 0.2 cm d^{-1} during 21 d of stress) for TE-treated plants that continued carbohydrate consumption during prolonged period of summer stress. In contrast, growth rate of nontreated plants declined from 0.25 to 0.08 cm d^{-1} during 21 d of stress, which had lower demand on carbohydrates. The growth decline of nontreated plants may have resulted in TNC accumulation during the stress period. Previous research has also shown that TE applications had no effects on TNC accumulation or reduced TNC content (Han et al., 1998; Richie et al., 2001). It is also possible that more carbohydrates are partitioned to crowns and rhizomes due to TE application. Our measurement of leaf TNC does not account for a possible reallocation of carbohydrates from leaves to crowns, but could help to explain why leaf TNC was lower in TE-treated plants even when Pn rates were higher. If more carbohydrates were allocated to the crown, it would also explain increased tillering that has been observed in previous research. Currently, no research information is available in these aspects of TE effects, which deserve investigation. Regardless, the current results suggest that TE effects on creeping bentgrass tolerance to the combined stress was not associated with leaf TNC accumulation, but could be mainly due to growth regulation and water conservation.

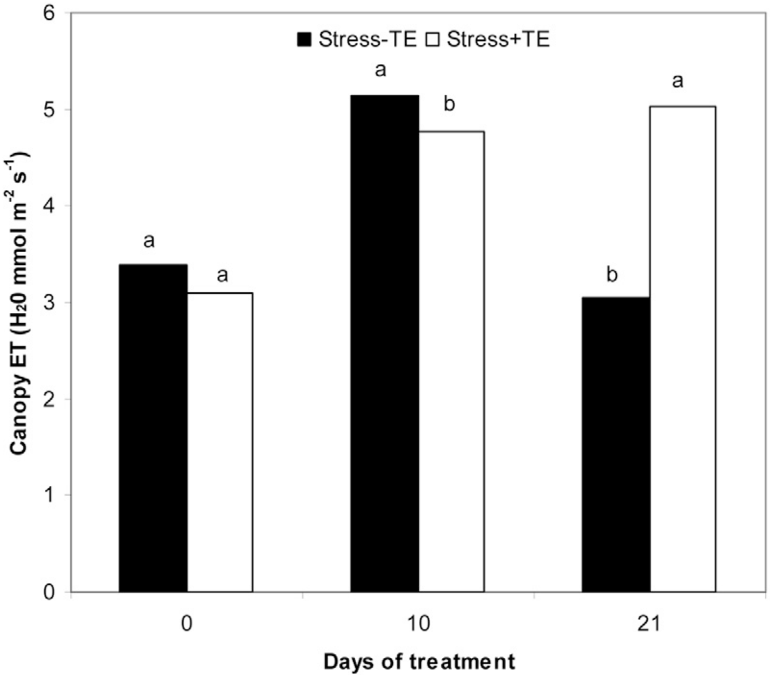

Figure 6. Effects of foliar application of trinexapac-ethyl (TE) on canopy evapotranspiration (ET) (H_2O mmol m^{-2} s^{-1}) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (P = 0.05).

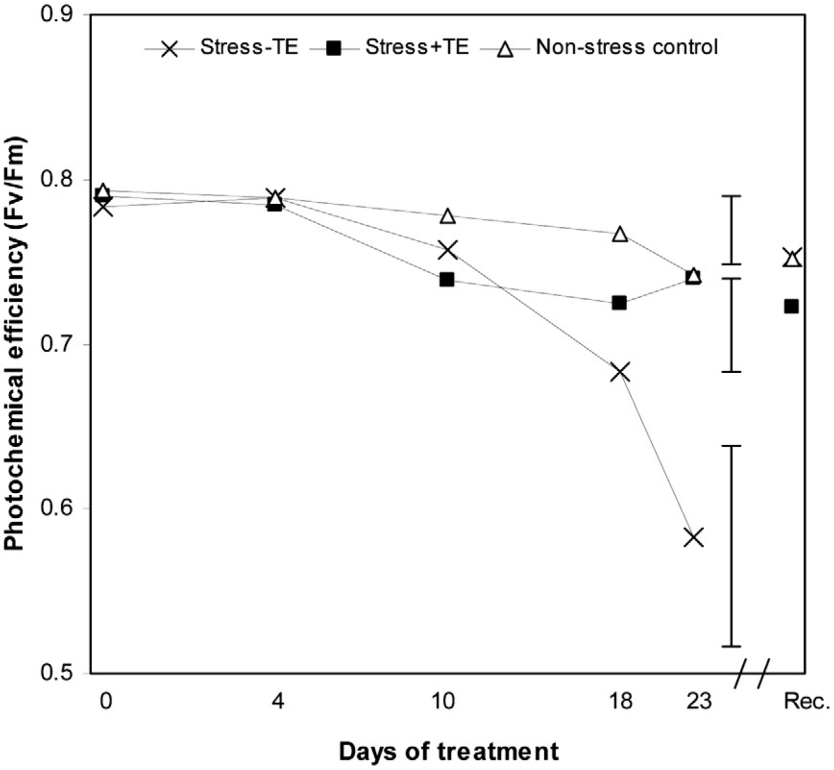

Figure 7. Changes in photochemical efficiency (Fv/Fm) in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (P = 0.05) for comparison of changes over treatment period.

Research has shown that application of TE may reduce water consumption of plants due to its growth-inhibiting effects. Studies evaluating both Kentucky bluegrass and tall fescue (*Festuca arundinacea* Schreb.) have found lower ET when TE was applied under nonstressed conditions (Ervin and Koski, 2001b; Marcum and Jiang, 1997). In the present study, under nonstressed conditions (0 d of stress), TE-treated turf had similar canopy ET, but by 10 d stress exposure TE treatments were significantly lower (11%) than nontreated plants. However, following an extended period of stress (21 d), TE-treated plants maintained higher canopy ET and higher shoot growth rate than nontreated plants. These data suggested that TE-treated plants continued transpiring following a prolonged period of stress, which may facilitate transpirational cooling and shoot growth under combined drought and heat conditions, and, therefore, the maintenance of higher canopy ET with TE application may help plants survive prolonged period of heat stress. Trinexapac-ethyl–treated plants did not exhibit water deficit during the entire stress period, while nontreated plants had severe water deficit as shown by lower RWC. High leaf RWC may be the result of low water loss from leaves through transpiration and/or water retention through osmotic adjustment (Nilsen and Orcutt, 1996). The better hydration of TE-treated plants during the stress period may not be related to root water uptake, since no significant differences in root growth were found between TE-treated and untreated plants at the end of the stress period. In addition, TE application had no significant effects on osmotic adjustment, suggesting that TE effects on the maintenance of cellular hydration was not due to osmotic regulation. In addition, water use efficiency was calculated using the data of net photosynthetic rate and transpiration rate measured with the gas exchange analyzer and found no effects of TE on water use efficiency under the combined drought and heat stress (data not shown). Other physiological factors, beside water relations, may be involved in the promotive effects of TE on of plant tolerance to the combined stress.

Maintenance of turf quality and physiological functions in TE-treated plants could have also resulted from increased production of antioxidants, and reduced levels of reactive oxygen species. Increased levels of antioxidants (such as superoxide dismutase) have been associated with increased drought tolerance in grasses (Price and Hendry, 1989). Previous studies have shown TE application to be partially associated with increased levels of endogenous superoxide dismutase (Zhang and Schmidt, 2000). It has also been shown to improve cell membrane thermostability of leaf tissues in Kentucky bluegrass (Heckman et al., 2002). The observed maintenance of Pn, RWC, and quality through the stress period could be related to the effects of TE on antioxidant enzyme activities and cell membrane thermostability, as reported in other studies (Zhang and Schmidt, 2000; Heckman et al., 2002).

In summary, preconditioning plants with TE was beneficial for plant survival of extended period of combined drought and heat stress, as manifested by improved turf quality and shoot growth rate under stress conditions. The effects of TE on plant tolerance to combined drought and heat stress could be related to its effect on the promotion of photosynthetic capacity associated with increased chlorophyll content and photochemical efficiency, and on the maintenance of cellular hydration. Our results imply that TE application could be utilized as a preconditioning treatment for turf that seasonally experiences heat and drought stress. Such a management program could result in reduced turf loss, quicker recovery, and reduced need for irrigation. However, the use of TE in alleviating stress injury under field conditions deserves investigation.

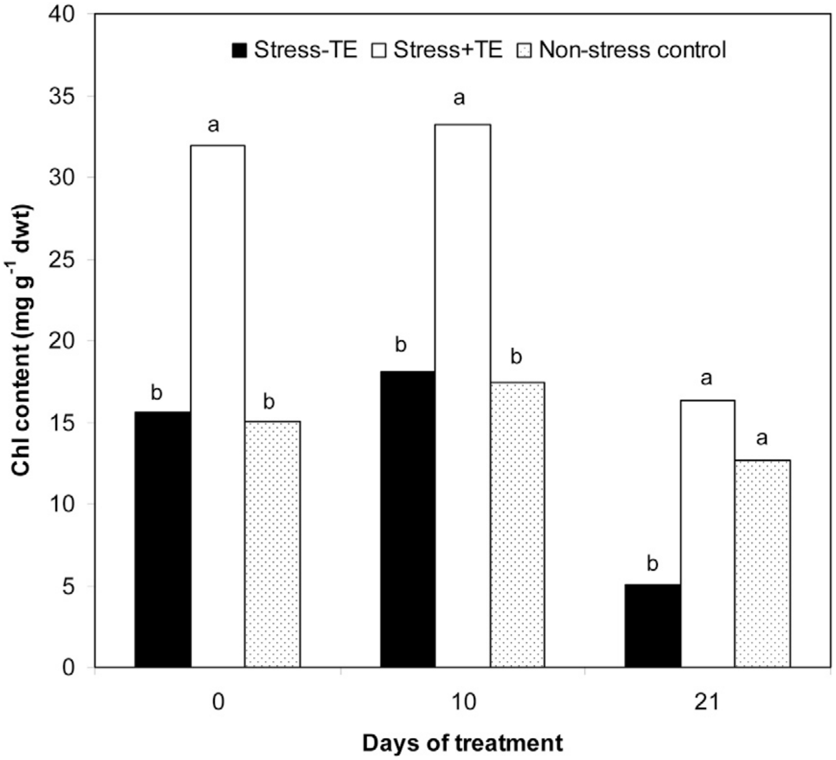

Figure 8. Effects of foliar application of trinexapac-ethyl (TE) on leaf chlorophyll content (mg g^{-1} of dry weight [dwt]) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (P = 0.05).

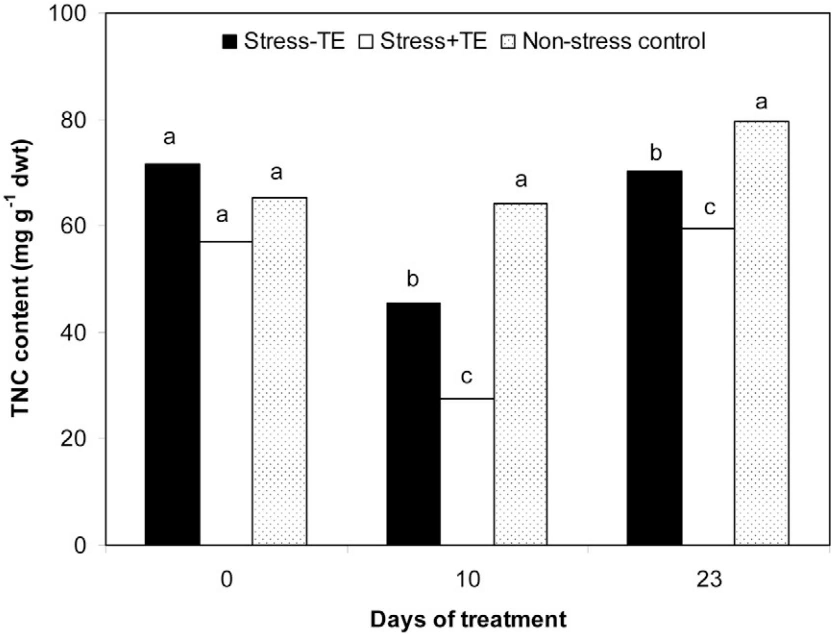

Figure 9. Effects of foliar application of trinexapac-ethyl (TE) on total nonstructural carbohydrates (TNC) (mg g^{-1} of dry leaf tissue [dwt]) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (P = 0.05).

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