Drought Responses of Kentucky Bluegrass and Creeping Bentgrass as Affected by Abscisic Acid and Trinexapac-ethyl

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ABSTRACT. The plant growth regulators abscisic acid (ABA) and trinexapac-ethyl (TE) may affect turfgrass responses to drought stress through regulating shoot growth and water relations. The objectives of this study were to investigate the effects of foliar application of TE and ABA on turf growth of two cool-season turfgrass species, Kentucky bluegrass (Poa pratensis L.) and creeping bentgrass (Agrostis stolonifera L.) exposed to drought stress, and to examine water relations associated with changes in drought tolerance due to TE or ABA treatment. 'L-93' creeping bentgrass and 'Brilliant' Kentucky bluegrass plants were foliar sprayed with 0.904 mL·ha⁻¹ a.i. TE five times before exposure to drought or with 6.75 mL/week of ABA at 100 µM before and after exposure to drought in growth chambers. Drought stress was imposed by withholding irrigation until plants were permanently wilted. Foliar application of TE or ABA maintained higher soil volumetric water content, leaf relative water content, and turf quality for a longer period of time during 28 days of stress exposure for Kentucky bluegrass and creeping bentgrass compared with the untreated control. Leaves of TE-treated and ABA-treated plants in both species also had lower Δψₘ at 28 days of drought stress than the untreated control. Creeping bentgrass treated with TE or ABA and Kentucky bluegrass treated with TE exhibited significantly lower shoot vertical growth rates at the initiation of drought stress, but maintained higher growth rates during prolonged drought compared with the untreated control. Turf treated with TE or ABA also showed higher levels of photochemical efficiency than the untreated control for both species. Our results suggest that TE or ABA application could prolong the survival of turfgrass under conditions of drought stress by suppressing shoot vertical growth and lowering water use during the early phase of drought and by sustaining growth and photosynthetic activity during prolonged periods of drought stress through osmotic adjustment for retaining cellular hydration.

Drought is one of the most detrimental abiotic stresses for turfgrass growth across a wide range of geographic locations. Most cool-season grass species are not well adapted to extended periods of drought, particularly during the summer months. Decline in turf quality caused by drought stress is a major concern in turfgrass culture. Therefore, developing management practices for improving drought resistance of turfgrasses has become imperative in arid and semiarid regions, especially during periods of water use restriction. One strategy to improve plant drought resistance is to promote drought avoidance by reducing water loss during drought, which may be achieved by slowing the growth rate of shoots and lowering the canopy leaf area to reduce the demand for water (Nilsen and Orcutt, 1996). Another mechanism serving to increase drought tolerance is through osmotic adjustment, which allows plants to maintain leaf cellular hydration and sustain metabolic activities during drought (Nilsen and Orcutt, 1996).

Previous studies have reported that plants with slow-growing shoots may survive more extended periods of drought than faster-growing plants (Kondoh et al., 2006; O'Reagan et al., 1993; Simane et al., 1993). Slow growth may reduce the adverse impact of drought by conserving water and carbon energy, such that plants can use limited water to survive drought for an extended period of time (Kang, 2002). Plant growth regulators such as trinexapac-ethyl (TE) are traditionally used to suppress vertical shoot growth for reducing mowing frequency of turfgrasses (Turgeon, 1999). TE blocks the final step in the biosynthesis pathway of the biologically active forms of gibberellins, results in slower vertical shoot growth (King et al., 1997), enhances superoxide dismutase and photochemical activity (Zhang and Schmidt, 2000), and has no negative impact on root growth (Fagerness and Yelverton, 2001). Reduction in vertical shoot growth may reduce the demand for water, and thus, may reduce the water requirement for plant survival in water-limiting conditions for a prolonged period of time. Jiang and Fry (1998) have shown that foliar TE treatments increased turf quality of perennial ryegrass (Lolium perenne L.) during soil dry-down. In our previous work, exogenous application of TE before plant exposure to stress significantly improved growth and physiological activities of creeping bentgrass subjected to combined heat and drought for 21 d (McCann and Huang, 2007).

Abscisic acid (ABA) is a plant hormone and growth regulator known to be involved in plant adaptation to drought stress. Exogenous application of ABA has been reported to improve drought tolerance in various plant species such as maize (Zea mays L.; Bochicchio et al., 1991), pepper (Capsicum annum L.; Leskovar and Cantiliffe, 1992), old jack pine (Pinus banksiana L.; Rajasekaran and Blake, 1999), and Tradescantia virginiana L. (Franks and Farquhar, 2001). Foliar application of ABA improved the growth of Kentucky bluegrass (Wang et al., 2003) and tall fescue (Festuca arundinacea Schreb.; Huang and Jiang, 2002) under drought stress. ABA-induced plant tolerance to water deficit has been associated with...
changes in various physiological processes, including inhibition of leaf growth or transpirational area for water loss (Alves and Setter, 2000; Bacon et al., 1998), induction of stomatal closure (Kirkham, 1983; Wilkinson and Davies, 2002), and enhancement of osmotic adjustment (Kirkham, 1983; LaRosa et al., 1987).

Although there is evidence that TE or ABA application may promote drought tolerance of turfgrass plants (Huang and Jiang, 2002; Jiang and Fry, 1998; McCann and Huang, 2007; Wang et al., 2003), the information on how TE and ABA may regulate turfgrass responses to drought stress is still limited. In addition, the relative effects of TE and ABA on drought tolerance for different turfgrass species are not well documented. We have hypothesized that treatment of turfgrass plants with TE or ABA may allow plants to survive a prolonged period of drought stress with greater tolerance than controls by regulating shoot growth and water relations. Therefore, the objectives of this study were to investigate the effects of exogenous application of TE and ABA on the responses of two cool-season turfgrass species, Kentucky bluegrass and creeping bentgrass, to drought stress, and to examine changes in water relations associated with improved drought tolerance from TE or ABA treatment.

Materials and Methods

Plant Materials. Sod pieces of ‘L-93’ creeping bentgrass and ‘Brilliant’ Kentucky bluegrass were transplanted from field plots into PVC tubes (10-cm diameter and 40-cm length) filled with sterilized sandy loam soil (fine-loamy, mixed mesic Typic Hapludult). Plants were maintained in a greenhouse under natural light conditions with temperatures of ≈21 °C day/15 °C night for 2 months in Fall 2006 (approximate 12-h photoperiod), and were then moved to a walk-in growth chamber where treatments were imposed. The growth chamber (3 × 2.5 m) was set at 20 °C day/15 °C night, 12-h photoperiod, with a photosynthetically active radiation level of 450 μmol·m⁻²·s⁻¹ at the canopy level. Plants in each container were watered three times per week to maintain soil moisture at field capacity and were fertilized weekly with 100 mL of a soluble fertilizer of 20N–8.8P–16.6K [Peter’s General Purpose 20-20-20 with panacea NPK; Westink, Inc., Santa Barbara, CA] using a 20-cm-long probe inserted in the soil. The rate of leaf turgor (Ψₛ) at full turgor (Ψₛ100) was determined according to the formula: 100 × [(fresh weight – dry weight)/(turgid weight – dry weight)] after oven-drying leaf samples for 72 h at 100 °C. Turgid weight was determined as fresh weight of fully turgid leaves after soaking leaves in distilled, refrigerated water for 24 h. Leaves for RWC measurement were a random mix of old and new leaves and were cut from uniform and representative areas of the canopy. ET was determined by the gravimetric mass balance method. This was accomplished by weighing pots to calculate the total amount of water lost by comparing differences in pot weight between two measurement dates.

Osmotic potential (Ψₛ100) was determined according to the rehydration method, where Ψₛ100 of leaves was determined after soaking in water for full rehydration (Blum, 1989; Blum and Sullivan, 1986). Turgid leaf samples were frozen in liquid nitrogen and were subsequently stored at −20 °C until analysis of leaf Ψₛ. Frozen tissue samples were thawed and cell sap was pressed from leaves, which was subsequently analyzed for osmolality [C (millimoles per kilogram)] using a vapor pressure osmometer (Vapro model 5520; Wescor, Logan, UT). Osmolarity of cell sap was converted from millimoles per kilogram to Ψₛ (megapascals) using the formula: MPa = –C × 2.58 × 10⁻³.
Results

SOIL AND PLANT WATER RELATIONS. The initial soil VWC under well-watered conditions averaged 27.4% in all treatments for both grass species (Table 1). During the 26-d period of drought, soil VWC of all treatments decreased to between 2.8% and 4.4%. Kentucky bluegrass with TE treatment had significantly higher soil VWC than the untreated control at 13 d of drought, but no differences in VWC were observed between treatments at 26 d of drought stress. For creeping bentgrass, TE and ABA treatments maintained significantly higher soil VWC than the untreated control by 13 and 26 d of drought. Compared with ABA treatment, TE treatment had the same VWC at 0 and 26 d of drought, but higher VWC at 13 d of drought.

RWC of Kentucky bluegrass averaged 82% within 14 d of drought, and then declined rapidly after 14 d of drought in all treatments (Fig. 1A). The decline in RWC was more severe in untreated control plants than in TE- or ABA-treated plants. Significantly higher leaf RWC was observed in ABA-treated Kentucky bluegrass than in the untreated control at 20 and 27 d of drought stress. For creeping bentgrass, leaf RWC of untreated plants dropped sharply after 7 d of drought and was significantly lower than TE- or ABA-treated plants at 14 and 20 d of drought stress (Fig. 1B). No differences in RWC were observed between TE- and ABA-treated plants for Kentucky bluegrass or creeping bentgrass under well-watered conditions (0 d) or during drought stress.

ET rates of Kentucky bluegrass did not differ among treatments within 15 d of drought, but were significantly higher in TE-treated plants than the untreated control during 15 to 19 d of drought and were significantly higher in ABA-treated plants than the untreated control during 19 to 26 d of drought (Fig. 2A). TE- or ABA-treated creeping bentgrass had significantly lower rates of ET than the untreated control from 0 to 13 d of stress, but higher ET rates from 13 to 26 d of drought (Fig. 2B). No differences in ET rates were detected between TE and ABA treatments during the entire drought period.

Osmotic potential at full turgor ($\Psi_{t,100}$) was not impacted by TE or ABA treatment in Kentucky bluegrass before exposure to drought (0 d; Fig. 3A). However, $\Psi_{t,100}$ in Kentucky bluegrass was significantly lower with ABA treatment at 21 d of drought and with TE and ABA treatments at 28 d of drought compared with the untreated control. For creeping bentgrass, $\Psi_{t,100}$ was significantly lower with TE treatment at 21 d of drought and with TE and ABA treatments at 28 d compared with the untreated control (Fig. 3B).

TURF GROWTH. Kentucky bluegrass treated with ABA or TE maintained turf quality at the same level as the untreated control under well-watered conditions (0 d of drought; Fig. 4A). However, TE-treated Kentucky bluegrass exhibited significantly higher turf quality than untreated turf at 7, 21, and 28 d of drought stress. ABA-treated Kentucky bluegrass had significantly higher turf quality than untreated turf at 28 d of drought stress. At 21 and 28 d of drought, Kentucky bluegrass treated with TE had higher turf quality than turf treated with ABA. For creeping bentgrass, TE-treated plants had lower turf quality than the untreated control at 0 and 7 d of drought, but maintained turf quality above the minimum acceptable level (6.0) and the untreated control level from 14 to 28 d of drought (Fig. 4B). No difference in turf quality was detected between ABA and untreated creeping bentgrass at 0 and 7 d of drought, but ABA-treated plants had significantly higher turf quality

### Table 1. Soil volumetric water content in treatments with abscisic acid or trinexapac-ethyl during drought stress of ‘Brilliant’ Kentucky bluegrass and ‘L-93’ creeping bentgrass.

| Species             | Compound       | Days of treatment | Soil volumetric content (%) |
|---------------------|----------------|-------------------|-----------------------------|
|                     |                | 0     | 13   | 26   |                 |
| Kentucky bluegrass  | Control        | 26.1 a* | 5.3 b | 3.8 a |
|                     | Abscisic acid  | 28.0 a | 6.1 b | 3.8 a |
|                     | Trinexapac-ethyl| 27.2 a | 7.9 a | 4.1 a |
| Creeping bentgrass  | Control        | 28.0 a | 3.8 c | 2.8 b |
|                     | Abscisic acid  | 27.4 a | 6.4 b | 4.4 a |
|                     | Trinexapac-ethyl| 27.7 a | 8.3 a | 3.8 a |

*Any two means within a column and plant species not followed by the same letter are significantly different by Duncan’s multiple range test at $P = 0.05$. 

Fig. 1. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on leaf relative water content during drought stress for (A) ‘Brilliant’ Kentucky bluegrass and (B) ‘L-93’ creeping bentgrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparisons at a given day of treatment.
than untreated control from 14 and 28 d of drought. ABA-treated creeping bentgrass had higher turf quality than TE-treated plants at 0 and 7 d of drought, but the difference diminished after 14 d of drought. TE-treated plants had a significantly lower vertical shoot growth (VSG) rate than the respective untreated control for Kentucky bluegrass and creeping bentgrass during 1 to 4 d of drought treatment (Table 2). Creeping bentgrass treated with ABA also had lower VSG than the untreated control within the first 4 d of drought. During 4 to 15 d of drought, VSG of the untreated Kentucky bluegrass decreased by 87%, whereas TE-treated plants maintained the same level of VSG as well-watered plants, and the VSG of TE-treated plants was three times greater than that of the untreated control and ABA-treated plants. For creeping bentgrass, a 91% reduction in VSG was detected in the untreated control plants from 4 to 15 d of drought compared with well-watered conditions, whereas TE- or ABA-treated plants did not exhibit a significant decline in VSG when comparing well-watered conditions to 4 to 15 d of drought. In addition, the VSG for ABA- and TE-treated creeping bentgrass was two and three times greater, respectively, than the untreated control during 4 to 15 d of drought. TE treatment in Kentucky bluegrass regulated greater growth inhibition than ABA treatment under well-watered conditions, but maintained higher VSG than the ABA treatment during 15 d of drought. TE and ABA treatments had similar effects on VSG for creeping bentgrass under well-watered or drought conditions.

**Photochemical efficiency.** Untreated plants showed a more rapid decline in leaf photochemical efficiency than TE- or ABA-treated plants during 27 d of drought for both species (Fig. 5). Higher Fv/Fm ratios were detected in ABA- and TE-treated Kentucky bluegrass at 21 d of drought and in ABA-treated bluegrass at 27 d of drought compared with the untreated control (Fig. 5A). TE- and ABA-treated creeping bentgrass exhibited higher Fv/Fm than the untreated control during 15 d of drought (Fig. 5B). TE treatment and ABA

Fig. 2. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on evapotranspiration (mm·d⁻¹) during drought stress for (A) ‘Brilliant’ Kentucky bluegrass and (B) ‘L-93’ creeping bentgrass. Treatments with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (P = 0.05).

Fig. 3. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on Ψs during drought stress for (A) ‘Brilliant’ Kentucky bluegrass and (B) ‘L-93’ creeping bentgrass. Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (P = 0.05).
treatment had similar effects on $F_v/F_m$ ratio for both species during the entire drought period.

**Discussion**

TE or ABA application before turf exposure to drought stress helped maintain higher turf quality and shoot growth rate for Kentucky bluegrass and creeping bentgrass during prolonged periods of drought compared with the respective untreated controls. TE application at the manufacturer’s recommended rate was more effective than ABA application in improving turf quality and maintaining active shoot growth rate for Kentucky bluegrass, but was equally effective as ABA treatment for creeping bentgrass. Maintenance of higher turf quality and active shoot growth of TE- or ABA-treated plants under long-term drought stress could be associated with the modification of morphological and physiological traits associated with drought avoidance and tolerance. These included slower shoot growth rate and reduced ET during the early phase of drought and improved plant water status and photochemical efficiency during prolonged drought in TE- or ABA-treated plants compared with untreated plants.

Foliar application of TE or ABA may promote drought avoidance in both species at the beginning of drought stress. The slower shoot growth rate during the early phase of drought may reduce demand for water, and thus sustain plant growth for a longer period of drought. In fact, creeping bentgrass treated with TE or ABA exhibited a significantly lower ET rate than the untreated control during the first 13 d of drought. Soil water content was also higher under TE-treated Kentucky bluegrass and TE- and ABA-treated creeping bentgrass at 13 d of drought, suggesting that TE or ABA treatment may result in lower water depletion rates due to growth inhibition during the early phase of drought. Previous studies evaluating Kentucky bluegrass and tall fescue have found lower rates of ET when TE was applied under nonstress conditions, possibly resulting from effects on growth inhibition (Ervin and Koski, 2001; Marcum and Jiang, 1997). ABA is also known to induce stomatal closure, leading to reduction in water loss (Finkelstein et al., 2002; Nambara and Marion-Poll, 2005). Stahnke and Beard (1981) reported that exogenous application of ABA reduced transpiration of creeping bentgrass by 59% compared with untreated plants under well-watered conditions. However, after a prolonged period of drought (19 d), ET rates in Kentucky bluegrass and creeping bentgrass treated with TE or ABA were significantly higher than the untreated control. This could be related to the maintenance of higher turf quality and normal shoot growth in TE- and ABA-treated plants at this time, and thus, a greater demand for water.

Improved water relations by TE or ABA treatment during prolonged periods of drought suggest that both treatments may also promote drought tolerance, which could contribute to the higher turf quality under prolonged periods of drought for Kentucky bluegrass and creeping bentgrass. Leaf RWC of the untreated control for Kentucky bluegrass and creeping bentgrass drop-

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**Table 2. Vertical shoot growth as affected by application of abscisic acid or trinexapac-ethyl during drought stress of ‘Brilliant’ Kentucky bluegrass and ‘L-93’ creeping bentgrass.**

| Species                  | Compound       | Vertical shoot growth (cm d–1) | Days of treatment |
|--------------------------|----------------|-------------------------------|------------------|
|                          |                | 0–1 | 1–4 | 4–15|
| Kentucky bluegrass       | Control        | 0.375 aA | 0.375 aA | 0.047 bB |
|                          | Abscisic acid  | 0.406 aA | 0.406 aA | 0.047 bB |
|                          | Trinexapac-ethyl | 0.167 bA | 0.167 bA | 0.156 aA |
| Creeping bentgrass       | Control        | 0.331 aA | 0.331 aA | 0.031 bB |
|                          | Abscisic acid  | 0.156 bA | 0.156 bA | 0.063 abA |
|                          | Trinexapac-ethyl | 0.125 bA | 0.125 bA | 0.094 aA |

*Any two means within a column and plant species not followed by the same lowercase letter are significantly different by Duncan’s multiple range test at $P \leq 0.05$. Any two means within a row not followed by the same uppercase letter are significantly different by Duncan’s multiple range test at $P \leq 0.05$. 

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Fig. 4. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on turf quality during drought stress for (A) ‘Brilliant’ Kentucky bluegrass and (B) ‘L-93’ creeping bentgrass. Turf quality was expressed on a scale of 1 to 9 (1 = completely desiccated, brown turf canopy; 9 = healthy plants with dark green, turgid leaf blades, and dense turf canopy). Vertical bars indicate LSD values ($P = 0.05$) for treatment comparisons at a given day of treatment.
ped sharply during drought stress, whereas RWC of TE- and ABA-treated plants exhibited less severe decline and was significantly higher in ABA-treated Kentucky bluegrass and TE- or ABA-treated creeping bentgrass after 14 d of drought. This finding is consistent with our previous results of improved photochemical efficiency with TE treatment for creeping bentgrass exposed to combined heat and drought (McCann and Huang, 2007) and with ABA treatment for Kentucky bluegrass exposed to drought stress (Wang et al., 2003). TE or ABA treatment helped maintain the integrity of photosystem II for photosynthesis. Rajasekaran and Blake (1999) reported that old jack pine seedlings treated with ABA maintained a higher photosynthetic rate, which was related to the protective action of ABA on membrane integrity during drought stress.

Although the results here are encouraging for the development of a field trial, the complex interactions of environmental factors can diminish or negate findings from controlled-environment experiments. Jiang and Fry (1998) have previously found that the improved drought tolerance of ryegrass treated with ethephon or TE in greenhouse trials did not result in improved drought tolerance under field conditions. Regardless, the data warrant further research to evaluate plant impact under field conditions.

In summary, our data suggested that foliar application of TE or ABA improved turf quality and growth for creeping bentgrass and Kentucky bluegrass exposed to drought stress. TE and ABA treatments promoted drought avoidance during the early phase of stress, as suggested by an initial reduction in shoot growth rate and ET rate, and improved drought tolerance during prolonged stress, as manifested by the maintenance of higher RWC, lower $\psi_S$, and higher photochemical efficiency later in the process of drought stress. The combination of avoidance and tolerance mechanisms could result in an improvement in turf performance after TE or ABA application.

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