Physiological Adaptation of Kentucky Bluegrass to Localized Soil Drying
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ABSTRACT

This study was designed to investigate effects of surface soil drying (SD) on water relations, gas exchange, growth characteristics, and abscisic acid (ABA) content of leaves and roots for Kentucky bluegrass (Poa pratensis L.), and to examine whether physiological adaptation to SD is associated with hydraulic or chemical regulation. ‘Award’ and ‘Nuglade’ were subjected to three soil moisture treatments in a growth chamber: (i) well-watered control; (ii) SD (0–20 cm); and (iii) full soil profile (0–40 cm) drying (FD). Under SD, turf quality (TQ), relative water content (RWC), photosynthesis, and cell membrane stability remained the same as the controls, but stomatal conductance (g_s) declined by 35 and 45%, and shoot growth rates were reduced by 50 and 40% for Award and Nuglade, respectively. Root DW decreased in 0- to 20-cm dry soil, but increased compared with controls in the 20- to 40-cm wet soil under SD. The ABA content increased by four to sixfold in roots at 0- to 20-cm drying soil and did not change in the 20- to 40-cm wet soil under SD conditions. The ABA content was also higher in leaves of SD plants. The results suggested Kentucky bluegrass adapted to localized soil drying by maintaining TQ, photosynthesis, leaf water status (WS), and root growth using water in the deeper soil profile. Decline in g_s and shoot growth was independent of leaf WS, and could be hormonally controlled, which could help maintain favorable WS in leaves by reducing water loss under SD conditions.

Water deficit is one of the most widespread abiotic stresses limiting plant growth. This issue is becoming increasingly important for turfgrass management as water availability for irrigation in urban areas is declining. Under field conditions, it is common that soil moisture is highly heterogeneous both spatially and temporally, and roots near the soil surface are often exposed to drying soil while water is available deeper in the soil profile. Previous studies found that some warm-season and cool-season turfgrass species were able to adapt to SD without loss of TQ and experiencing water deficit in leaves (Huang et al., 1997; Huang, 1999; Huang and Fu, 2000; Fu and Huang, 2001). Physiological mechanisms for the maintenance of TQ and favorable WS under SD is not well understood. Insights into the mechanisms of turfgrass adaptation to localized drought stress could greatly improve drought resistance and water conservation strategies in turfgrass management.

Plant responses to drought stress involve changes in various morphological, physiological, and metabolic characteristics (Nilsen and Orcutt, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997). Early drought responses include growth inhibition and closure of stomata, which regulate water loss through transpiration. The conventional view is that WS is the major controlling factor regulating shoot physiological responses to drought stress. Hydraulic limitation, as manifested by the decrease in leaf WS, can directly limit growth and cause stomatal closure (Kramer, 1988). Stomatal conductance, however, is not always positively correlated with leaf WS, particularly under mild drought stress. In some species, changes in g_s are observed before changes in leaf WS, particularly during early or mild phases of soil dry-down, and stomatal behavior in these species is more closely correlated with changes in the WS of the soil rather than the leaves (Zhang and Davies, 1989; Ali et al., 1999; Bahrud et al., 2002; Ismail et al., 2002).

As a result, another hypothesis has been proposed on the likelihood of a nonhydraulic, chemical signal moving from roots to shoots in response to localized soil drying that induces stomatal closure and reduces water loss (Blackman and Davies, 1985; Zhang and Davies, 1989; Davies et al., 2002). This has been tested in experiments with split-root systems in which part of the root system is exposed to drying soil while the remaining roots are maintained in moist soil (Zhang and Davies, 1989; Gowing et al., 1990). The split-root technique has shown that restrictions in g_s and growth occur even though roots in the well-watered soil provide sufficient moisture to maintain favorable leaf WS.

Abscisic acid is believed to be involved in hormonal control of plant response to drying soil (Wilkinson and Davies, 2002). Many studies in annual crops and woody species have shown negative correlation of g_s and leaf growth with ABA accumulation (Gowing et al., 1990; Blum et al., 1991; Davies and Zhang, 1991; Tardieu et al., 1992; Bano et al., 1993; Auge et al., 1995; Jackson et al., 1995; Borel et al., 1997; Croker et al., 1998; Stoll et al., 2000; Auge and Moore, 2002), suggesting that chemical signaling is an important factor regulating physiological responses to drought, particularly mild to moderate stress. It is believed that ABA is synthesized in roots exposed to dry soil and transported to shoots, where it triggers a signal transduction cascade, eventually leading to a reduction in guard cell turgor and stomatal closure (McAinsh et al., 1997; Assmann and Shimazaki, 1999; Wilkinson and Davies, 2002). Abscisic acid is also involved in reduction of water loss by inhibiting leaf growth, which reduces transpirational area and, thus, water loss (Bacon et al., 1998; Alves and Setter, 2000).

The objectives of this study were to: (i) examine ef-

Abbreviations: ABA, abscisic acid; DW, dry weight; E, transpiration rate; EL, electrolyte leakage; ELISA, enzyme linked immunosorbent assay; FD, full soil profile drying; FW, fresh weight; g_s, stomatal conductance; P_n, leaf net photosynthetic rate; PVC, polyvinyl chloride; RWC, relative water content; SD, surface soil drying; TDR, time-domain reflectometry; TQ, turf quality; TW, turgid weight; WS, water status.
effects of SD on growth, water relations, gas exchange, and endogenous level of ABA in roots and shoots for Kentucky bluegrass, and (ii) determine whether physiological effects of localized soil drying are related to hydraulic or chemical control for the cool-season grass species. Two cultivars were examined to determine whether the effects are consistent among cultivars.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Sods of Award and Nuglade Kentucky bluegrass were collected from field plots at Rutgers Research Horticulture Farm II, North Brunswick, NJ. These cultivars are ‘Midnight’ types that are commonly used in Kentucky bluegrass blends throughout the country. Sods were washed free of soil and subsequently transplanted into split polyvinyl chloride (PVC) tubes filled with a 1:3 (v/v) sterilized mixture of sand and sandy loam soil (fine-loamy, mixed, mesic, Typic Hapludult). Split PVC tubes consisted of two sections (each of 10-cm diam. by 20-cm length) connected together with duct tape for a total of 48 tubes. The bottom of the lower section for each tube was covered with nylon screen for drainage. For the surface soil dry-down treatment, a hydraulic barrier was inserted between the two soil layers. This consisted of a piece of wax paper sandwiched between two pieces of nylon mesh screen that were coated with Vaseline. The hydrophobic barrier allowed for root penetration while minimizing water movement between the two layers, and permitting examination of plant response to SD under controlled conditions (Huang et al., 1997; Huang, 1999). In these tubes, four drainage holes were drilled near the middle of the PVC columns, immediately above the hydrophobic barrier, to prevent excess water from accumulating in the upper PVC section. For the remaining tubes used for the other treatments, a piece of paper towel was substituted for wax paper to allow water movement between the upper and lower soil layers.

Plants were first grown in a greenhouse for 60 d at 21/15°C (day/night), 14-h photoperiod, and watered three times per week until water ran freely from the bottom of tubes. Turf was hand clipped weekly to approximately 6 cm in height. Controlled release granular fertilizer (16-4-8 N-P-K) was top-dressed twice before the beginning of soil moisture treatments. Plants were then moved to a growth chamber (22/18°C day/night temperature, 14-h photoperiod, and photosynthetically active radiation of 600 μmol m⁻² s⁻¹) and allowed to acclimate for 15 d before irrigation treatments were imposed.

Treatments

The experiment consisted of three soil moisture treatments: (i) well-watered control, irrigated three times per week until water ran freely from the bottom of the tubes to maintain soil moisture at field capacity; (ii) SD, irrigation withheld from the upper 20 cm soil while lower layer (20–40 cm soil) was watered three times per week by subirrigating through the bottom of the PVC tube; and (iii) FD, irrigation withheld from the entire soil profile (0–40 cm). The treatments were terminated when the majority of the plants in the FD treatment were completely desiccated and brown (32 d).

Measurements

Turf quality was rated visually based on color, density, and uniformity of turf on a 1-to-9 scale [1 = brown, dry, dead; 9 = fully turgid, green, and dense]. A rating of 6 indicates minimum acceptable TQ. Shoot extension rate was measured every 2 to 3 d by measuring the difference in canopy height.

Volumetric soil water content was measured with the time-domain reflectometry (TDR) method (Topp et al., 1980) by three pronged buriable waveguide TDR probes (20 cm long) installed vertically in the 0- to 20- and 20- to 40-cm soil layers (Soil Moisture Equipment, Santa Barbara, CA). The cable from TDR probes in the 20- to 40-cm soil layer was taped up against the inner wall of the PVC tube and projected out above the surface of the soil in the upper PVC section. Soil water content in the both upper and lower layers of control and the lower layer of SD tubes was maintained close to field capacity (approximately 30 ± 2.9%, mean of eight replications ± standard error) throughout the experimental period. Moisture content in the 0- to 20-cm soil layer of SD and FD treatments decreased to approximately 5 to 7% (v/v) after 32 d of treatment, corresponding to the permanent wilting point of the soil.

Leaf RWC was determined weekly with 10 to 15 first and second fully expanded leaves per pot. Leaves were clipped and weighed (fresh weight, FW), then placed into small Petri dishes filled with water. They were soaked in water for approximately 18 h at 4°C and then weighed immediately after excess moisture was removed with paper towels (turgid weight, TW). The leaves were then dried in an oven at 75°C for 72 h to determine DW. Leaf RWC was calculated as (FW − DW)/DW.

Cell membrane stability was estimated by calculating percentage of electrolyte leakage (EL) from cells. Approximately 1 first and second fully expanded leaves per pot were carefully excised and cut into 2-cm pieces. They were rinsed three times with distilled water and placed into vials containing 20 mL distilled water. After shaking for 6 h, initial conductivity (Cᵢ) of the bathing solution containing fresh leaves was measured with a conductivity meter (YSI, Inc., Yellow Springs, OH). Leaves were then killed in an autoclave, and placed on a shaker for 24 h before final conductivity (Cₒ) of the bathing solution was measured. The EL was calculated as Cᵢ/Cₒ × 100.

Single-leaf photosynthetic rate, gₛ, and transpiration rate (E) were measured with a portable gas exchange system (LI-6400, LI-COR, Inc., Lincoln, NE). Three to four fully expanded leaves per pot were placed within a 6-cm² leaf chamber which was suspended over the top of the pots with a camera tripod. While still attached, individual leaves were enclosed in the leaf chamber at a PAR of 800 μmol m⁻² s⁻¹ and temperature of 23°C and allowed to equilibrate for approximately 1 min before data were recorded.

On the same day as gas exchange measurements, approximately 0.3 g of shoots (FW) were harvested for leaf ABA analysis. Leaves were excised and wrapped in aluminum foil, and then immediately submerged into liquid nitrogen. Samples were stored at −80°C until further quantification. Abscisic acid extraction and analysis were performed with the indirect enzyme linked immunosorbent assay (ELISA) method as described by Alves and Setter (2000) and modified by Wang et al. (2003). Leaves were extracted in 80% [v/v] methanol with 1% glacial acetic acid [v/v] and 10 mg L⁻¹ butylated hydroxytoluene (sample to extraction = 1:10). Supernatants were vacuum dried and redissolved in 20% methanol. For purification, aliquots were applied onto C₁₈ chromatography columns (Supelco, Bellefonte, PA). These ABA-containing fractions were eluted with 50% methanol [v/v] containing 1% glacial acetic acid, and vacuum dried, and redissolved with Tris-buffered saline solution (pH 7.5). Abscisic acid was quantified with anti-ABA monoclonal antibodies (Agdia, Inc., Elkhart, IN) by indirect ELISA.

At the end of the experimental period, roots were harvested
separately from each soil layer. Roots were placed in test tubes containing extraction solution for ABA immediately after brushing clean of soil. Approximately 0.3 g root FW was used for ABA analysis. The remaining root portions were washed free of soil and dried in an oven at 75°C for DW determination.

Experimental Design and Statistical Analysis

The experiment consisted of two factors (two cultivars and three soil moisture treatments) with eight replications for each treatment in a completely randomized block design. Treatment effects were determined by ANOVA according to the general linear model procedure of the Statistical Analysis System V.8.2 (SAS Institute, Cary, NC). Differences among means for treatments for each cultivar were determined by the LSD test at the 0.05 probability level.

RESULTS

Both cultivars maintained TQ near 8 throughout the experimental period under well-watered control conditions (Fig. 1). After 10 d of FD, TQ decreased to below the control level for both cultivars, and declined to below the minimal acceptable level (6.0) by 14 d. Plants under SD had significantly higher TQ than fully dried plants, but did not show significant differences compared with well-watered plants. Surface-dried plants maintained good color and density even though soil moisture declined from approximately 30 to 5% in upper soil layers by the end of the experimental period (data not shown), which corresponds to >80% reduction in soil moisture content in upper soil layers. This trend was observed for both cultivars.

Well-watered plants generally maintained leaf RWC around 90% (Fig. 2). For the first 10 d, no significant differences were detected in RWC among the three treatments. Full drying led to rapid decline of leaf RWC for both cultivars; RWC declined to 46% for Award, and 57% for Nuglade at 15 d of treatment. Plants under SD maintained the level of RWC equivalent to control levels for the entire duration of the experiment, even though the upper soil layer contained only approximately 5% water.

No significant differences in EL were detected among the three treatments at 0 and 7 d (Fig. 3). By 23 d of treatment, cell EL of fully dried plants significantly increased to above the levels of control plants and surface-dried plants. Drying of the upper soil layer alone for 23 d did not cause any significant change in EL compared with control plants.

Shoot growth rate was significantly lower for surface-dried plants than the control plants, starting at 23 d for Award, and 21 d for Nuglade (Fig. 4). Surface-dried plants maintained lower growth rates throughout the remainder of the treatment, which corresponded to approximately a 50% decrease in growth rate for Award and 40% decrease for Nuglade.

Full drying caused rapid declines in both leaf net photosynthetic rate (Pn) and g, for both cultivars (Fig. 5). A significant decline in g, was detected at 9 d of SD, with approximately 35% reduction for Award and 45% reduction for Nuglade. However, at this time there were no differences in Pn between SD and the control.
Fig. 3. Response of cell membrane stability, as indicated by electrolyte leakage (EL) from cells in response to drought stress. Vertical bars are LSD values \((p = 0.05)\) within a cultivar for treatment comparisons at a given day of treatment.

After 7 d of FD, significant increases in ABA content of shoots were detected, and these continued to amplify throughout the remainder of the drought stress (Fig. 6). Under SD, significant increases in ABA shoot content were observed at 4, 8, 29, and 32 d for Award and 4, 8, and 15 d for Nuglade.

DISCUSSION

Drying of entire soil profile led to severe declines in all measured parameters for both cultivars. The physiological injuries of FD appeared predominantly because of water deficit or hydraulic limitation. Turf quality, RWC, \(P_n\), and cell membrane stability for both cultivars of Kentucky bluegrass, however, were not affected by SD, even with \(>80\%\) reductions in soil moisture in the upper layer. Split-root experiments with annual crops reported that partial drying of the root system had no adverse effects on leaf WS and \(P_n\) (Zhang and Kirkham, 1995). This indicated that roots in the well-watered lower layer could supply adequate water to shoots for prevention of leaf water deficit and maintenance of physiological and metabolic functions, and that SD did not impose hydraulic limitation to these growth and physiological parameters.

The highest proportion of root biomass typically occurred in the upper 20 cm of soil. Under SD, however, the proportion of roots in the 20- to 40-cm soil layer increased compared with corresponding layers under well-watered and FD conditions. Deep rooting is considered to be an important mechanism for drought avoidance in many species, including turfgrasses, which allows
water uptake from the deeper soil profile when surface soil is dry and avoids or delays tissue dehydration (Qian et al., 1997; Huang, 1999; Volaire and Lelievre, 2001). Keeley and Koski (2002) and Bonos and Murphy (1999) reported drought-tolerant Kentucky bluegrass cultivars had greater percentages of root systems in deeper soil layers compared with susceptible cultivars. Enhanced rooting may be due to increased carbon reallocation from shoots to roots at deeper soil depths. Huang and Fu (2000) investigated carbon metabolic responses to SD for Kentucky bluegrass and tall fescue and found that both species exhibited decreased respiration rates in shoots and roots in the upper drying layer, but enhanced carbon allocation to roots in the lower, wet soil layer.

Stomatal conductance was inhibited by 35 to 45% when part of the root system was exposed to dry soil, even though TQ, EL, RWC, and Pn were not affected by SD. These reductions are similar to those found in other split-root experiments: 25 to 35% in sorghum (sor-

Table 1. Changes in root dry weight (DW), root proportion, and root abscisic acid (ABA) accumulation in response to surface and full drying.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>0–20 cm</th>
<th>20–40 cm</th>
<th>% total DW</th>
<th>Root ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g</td>
<td></td>
<td>pmol g⁻¹ DW</td>
<td></td>
</tr>
<tr>
<td>Award</td>
<td>well watered</td>
<td>0.69a</td>
<td>0.32a</td>
<td>63.5a</td>
<td>12.86a</td>
</tr>
<tr>
<td></td>
<td>surface dry</td>
<td>0.54b</td>
<td>0.30a</td>
<td>61.5a</td>
<td>73.21b</td>
</tr>
<tr>
<td></td>
<td>full dry</td>
<td>0.31c</td>
<td>0.21b</td>
<td>65.9a</td>
<td>143.3c</td>
</tr>
<tr>
<td>Nuglade</td>
<td>well watered</td>
<td>0.62a</td>
<td>0.49ab</td>
<td>62.2a</td>
<td>19.36a</td>
</tr>
<tr>
<td></td>
<td>surface dry</td>
<td>0.57a</td>
<td>0.60a</td>
<td>54.2b</td>
<td>70.01b</td>
</tr>
<tr>
<td></td>
<td>full dry</td>
<td>0.46b</td>
<td>0.40b</td>
<td>58.0ab</td>
<td>128.5c</td>
</tr>
</tbody>
</table>

† Values followed by the same letter within a column are not significantly different (F = 0.05) based on a LSD test.
Fig. 6. Leaf ABA accumulation in response to drought stress. Vertical bars are LSD values ($p = 0.05$) within a cultivar for treatment comparisons at a given day of treatment.

glycine max (L.) (Auge et al., 1995); 50% in rice (Oryza sativa L.) (Bano et al., 1993); and 50% in maize (Zea mays L.) (Zhang and Davies, 1989). Similarly, $E$ values were reduced by 30 to 40% for both cultivars without reduction in $P_n$ under SD conditions, suggesting that surface-dried plants were able to maintain carbon assimilation with less water. It was evident that the extent of stomatal closure was not sufficient to have an effect on carbon fixation rates in Kentucky bluegrass exposed to SD soil. Other studies also demonstrated that $g_s$ could be substantially reduced while photosynthesis rates remained unaffected under drought stress (Nguyen et al., 1997). The reduction in $g_s$ in both cultivars occurred in leaves that did not experience water deficit under SD conditions, indicating that stomatal closure was not caused by the lack of water or hydraulic limitation, but could be chemically controlled.

Shoot extension was also significantly inhibited by SD relative to that of controls. These differences manifested themselves after approximately 2 wk of withholding irrigation from the upper soil layer. Shoot extension rate of surface-dried plants was approximately 40 to 50% lower than well-watered plants, but based on visual observation, leaves of those plants still maintained ample turgidity. Similarly, split-root experiments with other species also found that reductions in shoot growth occurred without any obvious decrease in leaf turgor (Michelena and Boyer, 1982; Passioura, 1988; Bacon et al., 1998; Ismail et al., 2002). While it has generally been assumed that leaf turgor plays a predominant role in controlling leaf expansion during drought conditions, our study and previous split-root studies indicate other factors than water may be involved in the growth inhibition. Taken together, our results suggested that a regulatory mechanism other than hydraulic limitation controlling shoot physiological responses in Kentucky bluegrass adaptation to SD. Since leaf WS could not account for the conductance and growth inhibition responses, evidence pointed toward a potential chemical constraint as a function of soil moisture availability, as discussed in the introduction.

Studies on chemical signaling have found that ABA is the primary hormonal regulator associated with changes in leaf physiology under localized soil drying. The role of ABA in regulating stomatal behavior and shoot growth is well recognized, even though the actual receptors that trigger the signal transduction events have not yet been identified (Assmann and Shimazaki, 1999; Wilkinson and Davies, 2002) and the mechanistic basis for how ABA might directly inhibit leaf expansion is not known. Bacon et al. (1998) hypothesized that an increase of ABA in the vicinity of elongation zones may decrease leaf extension rates by reducing cell wall extensibility and/or turgor of these cells. More recent work suggests there may be other interacting factors that may depend on the degree of soil drying, and so the exclusive role of ABA here is less certain (Feng, 1996; Sharp, 2001; Roberts et al., 2002; Sharp and Le-Noble, 2002).

In the present study, ABA content of roots in dry soil increased substantially under SD even though roots in the lower soil profile did not change relative to that
in well-watered plants. Increased ABA accumulation was detected in shoots of plants under SD, although the magnitude of change was not consistent across all days of treatment and was to a lesser extent than that in roots. This has also been shown in other species (Stoll et al., 2000). In leaves, the biosynthesis of ABA generally increases only when leaf water relations are significantly disturbed, such as when leaf turgor approaches zero (Hartung et al., 2002). This could account for the large increases in ABA content in leaves of fully dried plants. However, under conditions of localized soil drying, import of ABA from roots in dry soil would likely be responsible for increased accumulation of ABA in leaves because there was no leaf water deficit. The increases in ABA content in shoots, even though small in magnitude in some cases, may be sufficient to have effects on shoot physiological functions. Significant changes in g, can occur with only slight changes in leaf ABA content (Loveys, 1984; Stoll et al., 2000). On the basis of evidence from this and other studies, it is reasonable to assume that the increases in ABA content found in root and shoots of Kentucky bluegrass cultivars could be associated with the decline in g, and shoot growth during SD.

In summary, Kentucky bluegrass plants were able to adapt to localized soil drying by maintaining TQ, photosynthesis, leaf WS, and root growth with water in the deeper soil profile. The inhibition of g, and shoot growth, independent of WS, could be because of hormonal control, which ultimately helped maintain WS by reducing water loss under localized drought conditions. Enhanced accumulation of ABA in roots and shoots seem to play a role in this response, although a direct association for ABA as the primary chemical signal responsible for changes in shoot physiology was not determined in this study, requiring further investigation. The capability of exploiting such chemical signaling in turfgrasses would benefit the development of water-saving grasses and efficient irrigation programs. This would be an important strategy for conserving water and modifying growth characteristics without sacrificing TQ and persistence.

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