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Physiological Responses to Heat Stress Alone or in Combination with Drought: A Comparison between Tall Fescue and Perennial Ryegrass

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Abstract. **Heat and drought are two major factors limiting growth of cool-season grasses during summer. The objective of this study was to compare the effects of heat stress alone (H) or in combination with drought (H+D) on photosynthesis, water relations, and root growth of tall fescue (*Festuca arundinacea* L.) vs. perennial ryegrass (*Lolium perenne* L.). Grasses were exposed to H (35 °C day/30 °C night) or H+D (induced by withholding irrigation) in growth chambers for 35 days. Soil water content declined under H+D for both grasses but to a greater extent for fescue than for ryegrass. Declines in canopy net photosynthetic rate (Pn), leaf photochemical efficiency (Fv/Fm), and leaf relative water content (RWC) and the increase in electrolyte leakage (EL) were much more severe and occurred earlier for ryegrass than fescue subjected to both H and H+D and for both species than under H+D then H. Evapotranspiration (ET) rate increased to above the control level within 3 or 6 days of H and H+D for both species, but fescue had a higher ET rate than ryegrass at 3 and 6 days of H and 6 days of H+D. Root dry weight and viability in all soil layers decreased under H and H+D for both species. However, fescue had higher root dry weight and viability than ryegrass in the 20–40 cm layer under H and in both the 0–20 and 20–40 cm layers under H+D. The results indicated that maintenance of higher Pn, Fv/Fm, ET, RWC, and root growth and lower EL would help cool-season turfgrass survive summer stress, and that their characteristics could be used for selecting stress tolerant species or cultivars.**

Tall fescue and perennial ryegrass are widely used cool-season turfgrasses. The growth of cool-season turfgrasses often is limited by high temperature during summer months in the transitional zone and in warm climates. Moreover, heat stress can also occur in combination with drought under field conditions. The combined heat and drought stresses can be more detrimental than either stress alone (Jiang and Huang, 2000).

Cool-season turfgrass species vary in their tolerance to drought or heat stress. Tall fescue exhibits better drought tolerance than do perennial ryegrass and Kentucky bluegrass (*Poa pratensis* L.) by developing a relatively deep root system (Sheffer et al., 1987) and maintaining higher evapotraspiration (ET) and leaf water potential during drought stress (Aronson et al., 1987). Kentucky bluegrass is more heat-tolerant than is perennial ryegrass (Wehner and Watshke, 1981). Tall fescue generally is considered to have better heat tolerance than either Kentucky bluegrass or perennial ryegrass (Turgeon, 1999). However, Wallner et al. (1982) found that leaves of tall fescue were no more heat tolerance in vitro than leaves of perennial ryegrass as evaluated by a cell electrolyte leakage test. The differential physiological responses of cool-season grass species to heat stress, particularly in combination with drought stress, are not well understood.

The mechanisms of the interactive effects of drought and heat stresses can be intricate and complicated. For example, transpiration often is reduced by drought stress, which decreases the transpirational cooling effect and may result in internal heat stress at high temperatures. Understanding the physiological responses of turfgrass species differing in heat and drought tolerance would help in developing effective breeding and management programs to improve summer performance of cool-season turfgrasses. The objective of this study was to compare the effects of heat stress alone or in combination with drought on photosynthesis, water relations, and root growth of tall fescue vs. perennial ryegrass.

Materials and Methods

Plant materials. Sod pieces of tall fescue ('Mustang') and perennial ryegrass ('Brightstar II') were collected from 4-year-old field plots and placed in polyvinyl chloride tubes (10 cm in diam, 60 cm long) filled with a mixture of 1 sand : 2 soil (v/v) in a greenhouse for 30 d. Tubes were transferred to growth chambers with temperatures of 20 °C day/15 °C night and a 14-h photoperiod, with a photosynthetically active radiation of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the canopy level. Grasses were maintained in the growth chambers for 15 d to allow for adjustment to the environment before stresses were imposed. Root systems of both species were well developed and many roots had reached the bottoms of the PVC tubes when treatments were initiated.

Treatments. The treatments included: 1) control plants were grown under the optimum temperature conditions (20 °C day/15 °C night) and maintained well-watered by irrigating every other day until free drainage occurred from the bottom of the tubes; 2) heat stress (H), plants were subjected to 35 °C day/30 °C night and well-watered; and 3) combined heat and drought stresses (H+D), plants were exposed to 35 °C day/30 °C night and not watered. All treatments lasted for 35 d.

Measurements. Measurements were made at 3, 6, 9, 12, 21, and 35 d after stresses were imposed.

Soil water contents at the 0–20 and 40–60 cm layers were monitored to indicate soil dryness during drought stress using the time domain reflectometry method (Soil Moisture Equipment Corp, Santa Barbara, Calif.) (Wraith and Baker, 1991). Leaf relative water content (RWC) was determined according to the method of Barrs and Weatherley (1962);

$$RWC = (FW - DW) / (SW - DW) \times 100,$$

where FW is leaf fresh weight, DW is dry weight of leaves after being dried at 85 °C for 3 d, and SW is turgid weight of leaves after soaking in water for 4 h at room temperature (≈20 °C).

Canopy photosynthetic rate (Pn) and ET rate were measured with an LI-6400 portable gas exchange system (LI-COR, Lincoln, Nebr.). Leaf photochemical efficiency, expressed as chlorophyll fluorescence (Fv/Fm), was determined on five randomly selected leaves in each treatment with a fluorescence induction monitor (Dynamax, Houston).

Electrolyte leakage (EL) of leaves was measured according to the method of Blum and Ebercon (1981) and Marcum (1998) with modifications. Leaves were excised and cut into 2-cm segments. After being rinsed three times with distilled deionized H_2O, 10–15 leaf segments were placed in each test tube containing 10 mL of distilled deionized H_2O. Test tubes were shaken on a shaker for ≈17 to 18 h, and the initial conductivity (C_1) was measured (model 32; Yellow Spring Instrument, Yellow Springs, Ohio). Leaf samples then were killed by autoclaving at 121 °C for 15 min, and the conductivity of killed tissue (C_2) was measured after tubes cooled down to room temperature (C_2). The relative electrolyte leakage was calculated as $(C_1 / C_2)*100$.

At 15 d of stress, half of the plants in each treatment were harvested, and roots were

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washed free of soil and collected separately from the 0- to 20-, 20- to 40-, and 40- to 60-cm layers. Roots were dried at 85 °C for 72 h, and dry weight was determined. Root viability was measured using the method of triphenyltetrazolium chloride (TTC) reduction as described by Joslin and Henderson (1984) and Knievel (1973). Subsamples of 1.5 to 3.0 g fresh roots from each soil depth were incubated in 20 mL of 0.6% TTC solution in 50 mM phosphate buffer (pH 7.4) for 20 h at 30 °C. Formazan, formed from the reduction of TTC by dehydrogenase enzymes in living tissues, was then extracted in 95% (v/v) ethanol at 60 °C for 4 h. The absorbance of the extractants was read at 490 nm with a spectrophotometer (Spectronic Instruments, Rochester, N.Y.). The standard curve was made using different proportions of living and dead roots (killed in an autoclave) to calculate the percentage of living root, which was expressed as proportion of living root dry weight in the total root dry weight.

Experimental design and statistical analyses. The experiment consisted of three treatments and two grasses with four replicates arranged in a completely randomized design. Treatment and grass effects were determined by analysis of variance (ANOVA) based on the general linear model procedure of the Statistical Analysis System (SAS) (SAS Institute, Cary, N.C.). Least significance difference (LSD) $P \leq 0.05$ was used to detect differences among treatment means when F values were significant in ANOVA.

Results and Discussion

Soil moisture. Soil water content declined under H+D in both the 0- to 20- and 40- to 60-cm layers for both species (Fig. 1). A more rapid depletion in soil water content was observed for tall fescue than perennial ryegrass in both soil layers, which could be related to the more extensive root system of tall fescue (Beard, 1989; Qian and Fry, 1997; Sheffer et al., 1987).

Water relations. Leaf RWC was not affected by H until 12 d for both species, but tall fescue maintained higher RWC than did perennial ryegrass after 12 d of stress (Fig. 2A). The H+D treatment caused a more severe reduction in RWC than H, starting at 3 d for perennial ryegrass and 6 d for tall fescue. Tall fescue had higher RWC than perennial ryegrass within the first 3 d of H+D, but no difference in RWC was observed between the two species after that. The maintenance of higher water content in leaves of tall fescue could be attributed to more rapid water uptake.

The ET rates for both species increased transiently above the control level within 9 d of H and then dropped to below the control level at 21 d (Fig. 2B). Tall fescue had a higher rate of ET than perennial ryegrass during entire period of H except 9 and 12 d. Tall fescue also had a higher rate of ET than perennial ryegrass at 6 d of H+D. Aronson et al. (1987) reported that the better drought resistance of tall fescue was due to its higher

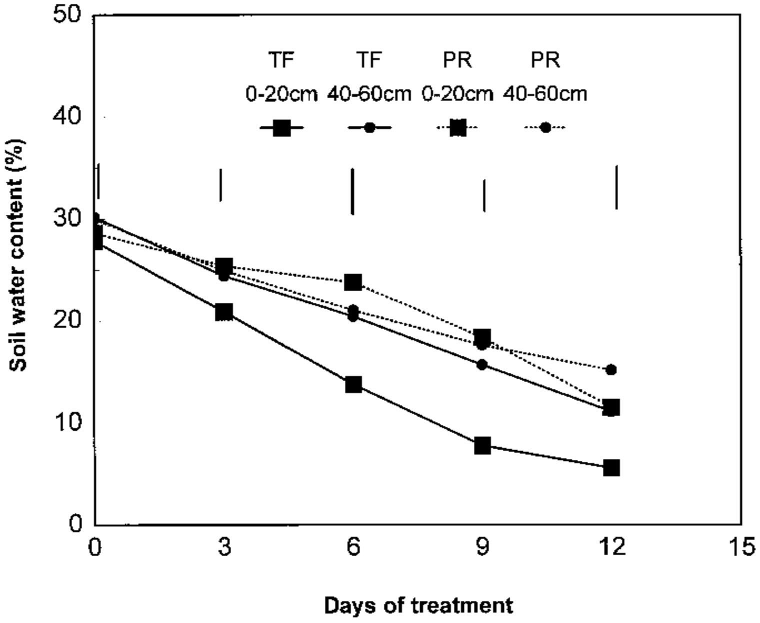

Fig. 1. Soil moisture content under the combined heat and drought stress (H+D) conditions in the 0- to 20- and 40- to 60-cm layers for tall fescue (TF) and perennial ryegrass (PR). Vertical bars indicate LSD ($P \leq 0.05$) for treatment and grass comparisons on a given day of treatment.

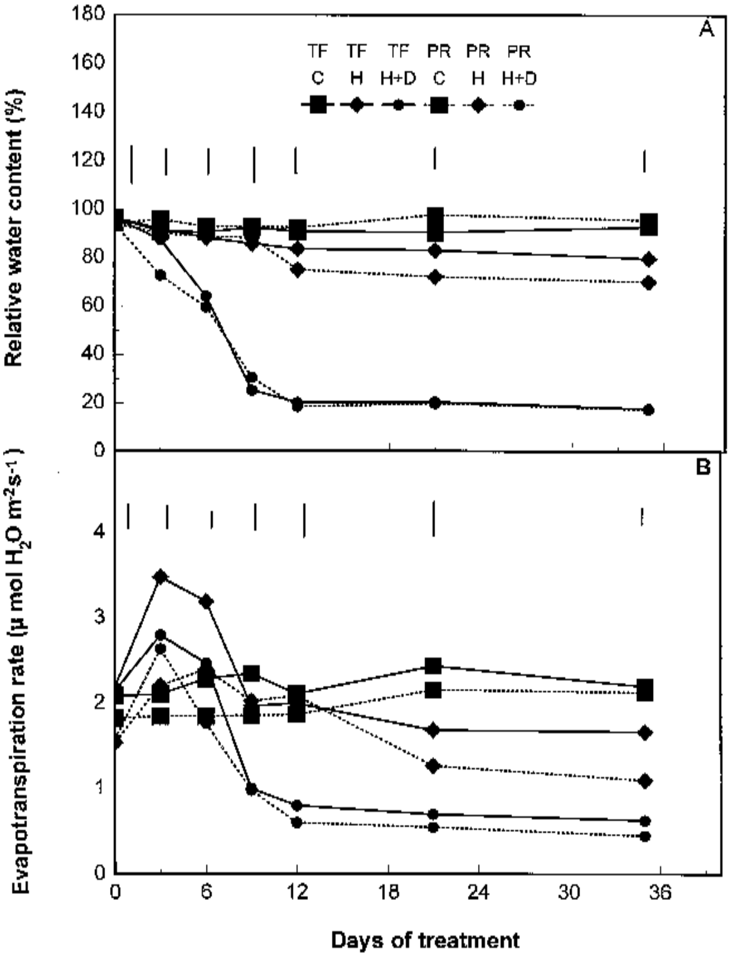

Fig. 2. (**A**) Leaf relative water content (RWC) and (**B**) evapotranspiration rate (ET) as affected by heat stress (H) and combined heat and drought stresses (H+D) for tall fescue (TF) and perennial ryegrass (PR). C = control plants. Vertical bars indicate LSD ($P \leq 0.05$) for treatment and grass comparisons at a given day of treatment.

rate of ET. Higher rate of ET, especially under H+D, could provide more effective cooling effects and attenuate internal heat injury. Seedlings of ponderosa pine (*Pinus ponderosa* Dougl. Lawson) that survived dry and hot conditions had significantly higher stomatal conductance and transpiration rates than did seedlings that failed to survive (Kolb and Robberecht, 1996).

Photosynthesis. Huang et al. (1998) reported that a reduction in Pn rate under heat stress has been found to be related closely to a decline in turf quality. The H significantly reduced Pn rate below the control level, beginning at 3 d for perennial ryegrass and 6 d for tall fescue, and the decline in Pn rate was more severe for perennial ryegrass (Fig. 3A). Canopy Pn rate dropped to near zero at 21 d of H for perennial ryegrass and 34 d of H for tall fescue. The reduction in Pn rate was more severe with H+D than with H for both species; however, the rate was higher in tall fescue than in perennial ryegrass at 3 d of H and 6 d of H+D. The combined stresses also significantly decreased the rate of CO_2 uptake in bean (*Phaseolus vulgaris* L.) (Yordanov et al., 1997) and Kentucky bluegrass (Jiang and Huang, 2000), and grain yield in barley (*Hordeum vulgare* L.) (Savin and Nicolas, 1996).

The photosynthetic apparatus, particularly photosystem II (PSII), is sensitive to heat and drought stresses (Berry and Bjorkman, 1980; Faria et al., 1998). The leaf photochemical efficiency of PSII often is expressed as cholorophyll fluorescence (Fv/Fm) (Epron, 1997; Havaux, 1992; Lu and Zhang, 1999). The Fv/Fm ratio was reduced by H alone, starting at 6 d for perennial ryegrass (Fig. 3B). Tall fescue maintained higher Fv/Fm than did perennial ryegrass after 21 d of H. The decline in Fv/Fm was earlier and more severe with H+D than H for both species, but Fv/Fm was higher in tall fescue than in perennial ryegrass at 6 and 9 d of H+D. These results strongly indicate the importance of maintaining photosynthetic activity and the integrity of PSII in the tolerance of cool-season grasses to heat, drought, or both.

Cell membrane stability-EL. The EL is an indicator of cell membrane stability and can be used to screen for drought- or heat-tolerant species or cultivars (Blum and Ebercon, 1981; Bouslama and Schapaugh, 1984; Marcum, 1998; Sapra and Anaele, 1991). The EL increased with H alone to above the control level, starting at 12 d for perennial ryegrass and 21 d for tall fescue (Fig. 4). The EL was higher in perennial ryegrass than in tall fescue after 21 d of H. Increases in EL were more severe and earlier under H+D than under H in both species. Perennial ryegrass had higher EL than did tall fescue from 6 d to 12 d of H+D, but no difference in EL was observed between the two species after 21 d. The lower EL of tall fescue under heat alone or the combined stresses indicated that tall fescue was able to maintain cell membrane stability, which could contribute to its higher Pn and Fv/Fm during stresses.

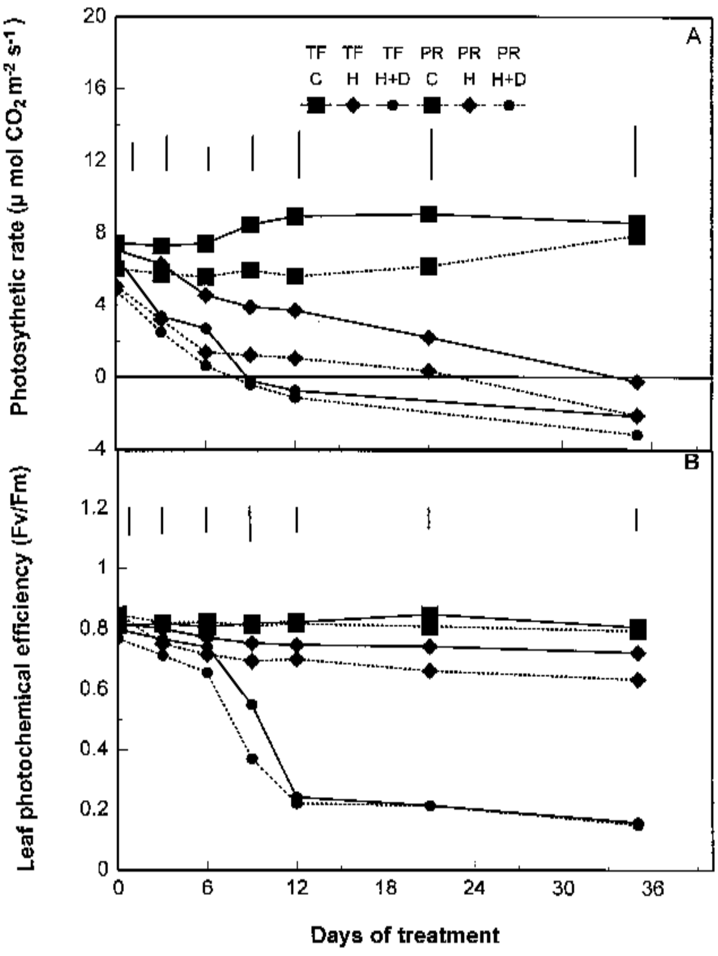

Fig. 3. Canopy photosynthetic rate (Pn) (**A**) and leaf photochemical efficiency (Fv/Fm) (**B**) as affected by heat stress (H) and combined heat and drought stresses (H+D) for tall fescue (TF) and perennial ryegrass (PR). C = control plants. Vertical bars indicate LSD ($P \leq 0.05$) for treatment and grass comparisons at a given day of treatment.

Root dry weight and viability. Both H and H+D dramatically reduced root dry weight for both species in all three soil layers, but to a greater extent in the 0- to 20-cm layer (Fig. 5). Tall fescue had greater root dry weight than did perennial ryegrass in the 20- to 40-cm layer under H and in both the 0- to 20- and 20- to 40-cm layers under H+D. The more extensive roots in the deeper soil layers could facilitate water uptake (Fig. 1) and contribute to the higher RWC (Fig. 2A) in tall fescue. Deep rooting has been considered as an important mechanism of drought resistance (Huang et al., 1998). Engelke et al. (1985) reported that creeping bentgrass (*Agrostis palustris* L.) cultivars that produced more and deeper roots were better able to survive intensive heat because they could use a large amount of the soil moisture reservoir for transpirational cooling. Bonos and Murphy (1999) found that heat-tolerant cultivars of Kentucky bluegrass had 19% more roots at the 15- to 30-cm depth and 65% more roots at the 30- to 40-cm depth than did intolerant cultivars; the extensive root system of the former allowed them to have significantly lower stomatal resistance and maintain canopy temperature 5 °C cooler than the latter. Our results are consistent with the data collected on mature turf in other studies (Aronson et al., 1987; Bonos and Murphy, 1999), suggesting that extensive and deep roots contribute to turfgrass tolerance to heat stress alone or in combination with drought.

Heat stress significantly reduced root viability in the 0- to 20-cm layer for tall fescue and in all layers for perennial ryegrass (Fig. 6). The H+D caused more reduction in root viability than did H alone in all soil layers and both species. Tall fescue had higher root viability than perennial ryegrass in the 20- to 40- and 40- to 60-cm layers under H and in all layers under H+D. Root viability is positively related to drought resistance (Huang et al., 1997). Higher root viability in all soil layers for tall fescue may result in more water uptake under the combined stresses and thus could favor transpirational cooling and maintenance of higher leaf water content and photosynthetic activity.

In summary, tall fescue exhibited better tolerance to heat stress, both alone and in combination with drought stress, by maintaining higher Pn, Fv/Fm, ET, leaf RWC, cell membrane stability, and root growth and lower EL than did perennial ryegrass. These physiological responses could be used as stress

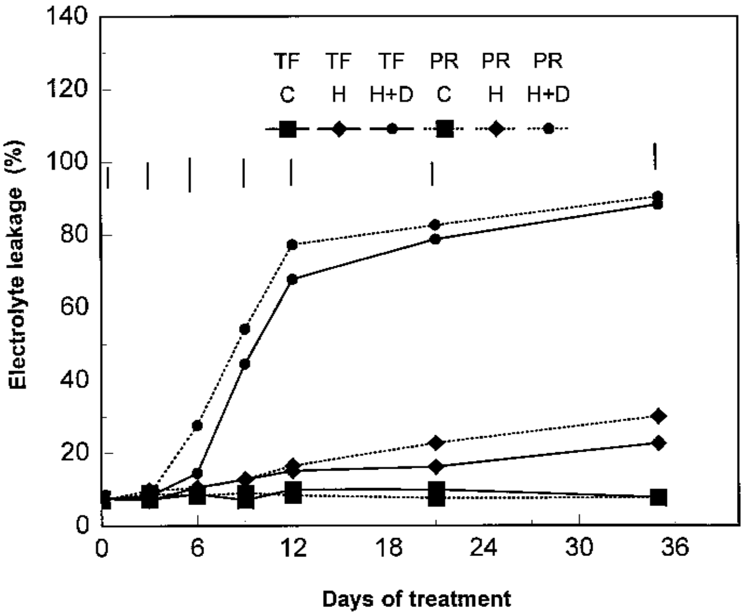

Fig. 4. Leaf electrolyte leakage (EL) as affected by heat stress (H) and combined heat and drought stresses (H+D) for tall fescue (TF) and perennial ryegrass (PR). (C), Control plants. Vertical bars indicate LSD ($P \leq 0.05$) for treatment and grass comparisons at a given day of treatment.

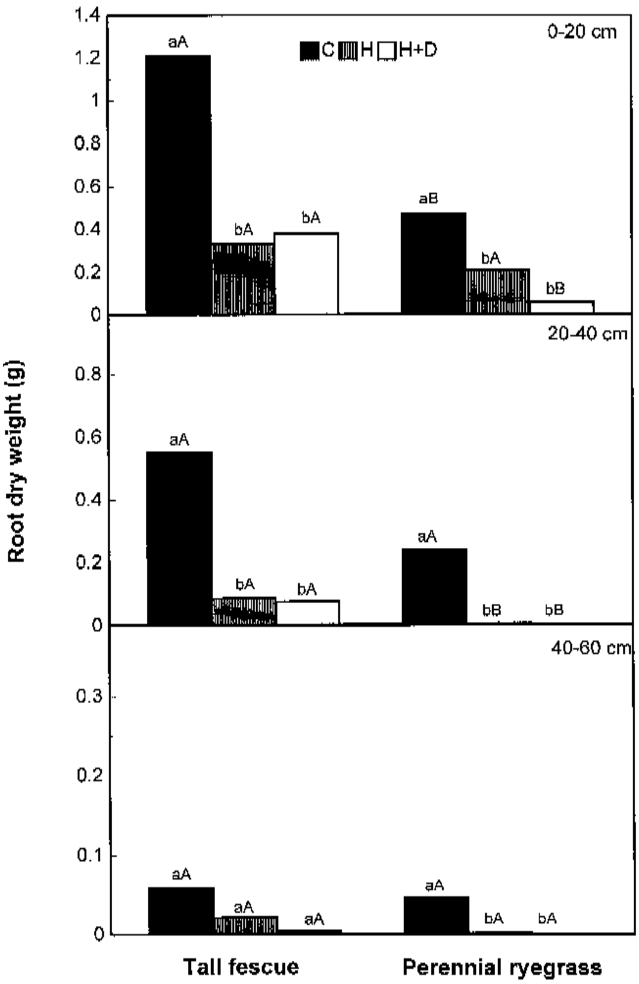

Fig. 5. Root dry weight in the 0- to 20-, 20- to 40-, and 40- to 60-cm soil layers following 15 d of heat stress (H) and combined heat and drought stresses (H+D) for tall fescue (TF) and perennial ryegrass (PR). C = Control plants. Columns at a given soil depth marked with the same letters are not significantly different based on an LSD test ($P \leq 0.05$). The lowercase letters are for treatment comparisons within a soil depth for one grass, and the uppercase letters are for grass comparisons for a given treatment within a soil depth.

indicators in selecting for heat- and drought-tolerant species or cultivars of cool-season grasses. Simultaneous drought and heat stress were more detrimental than was heat stress alone for both of these cool-season species, suggesting that sufficient irrigation during hot summers could help turfgrass to better survive heat stress by maintaining favorable leaf water status and photosynthesis.

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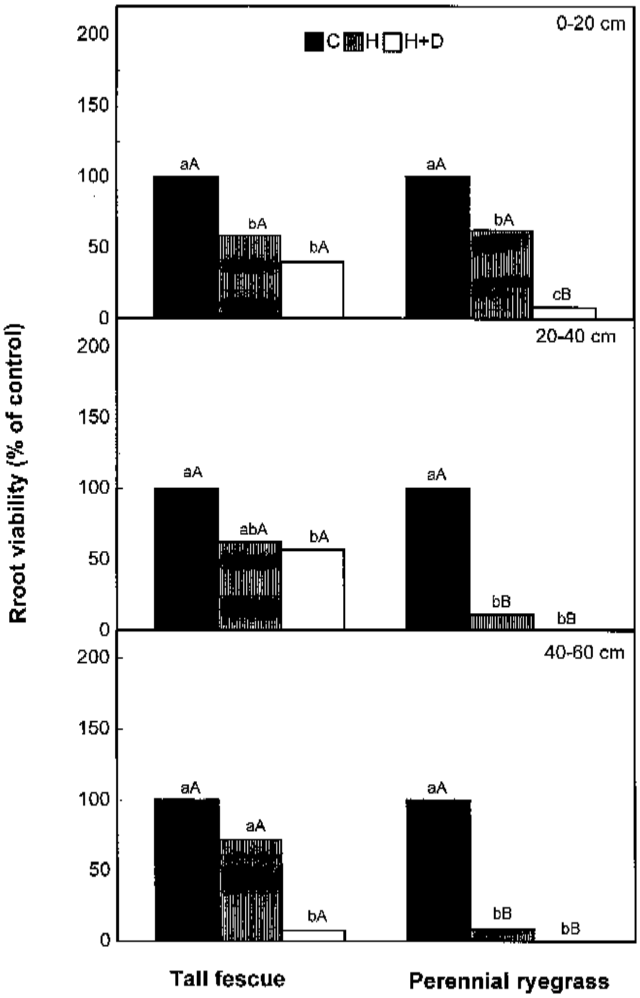

Fig. 6. Root viability in the 0- to 20-, 20- to 40-, and 40- to 60-cm soil layers following 15 d of heat stress (H) and combined heat and drought stresses (H+D) for tall fescue (TF) and perennial ryegrass (PR). C = control plants. Columns at a given soil depth marked with the same letter are not significantly different based on an LSD test ($P \leq 0.05$). The lowercase letters are for treatment comparisons within a soil depth for one grass and the uppercase letters are for grass comparisons for a given treatment within a soil depth.

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