Differential Effectiveness of Doubling Ambient Atmospheric CO₂ Concentration Mitigating Adverse Effects of Drought, Heat, and Combined Stress in Kentucky Bluegrass

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ABSTRACT. Drought and heat stress can limit the growth of cool-season grass species, whereas doubling ambient CO₂ has been shown to promote plant growth. The objectives of this study were to examine differential responses of shoot and root growth as well as photosynthesis and respiration to doubling ambient CO₂ during drought or heat stress alone or the two stresses combined and to determine the relative effectiveness of doubling ambient CO₂ in mitigating negative effects of drought or heat stress alone and in combination in a cool-season perennial grass species. Kentucky bluegrass (*Poa pratensis* cv. Baron) plants were exposed to ambient CO₂ (400 $\mu\text{L}\cdot\text{L}^{-1}$) or doubling ambient CO₂ (800 $\mu\text{L}\cdot\text{L}^{-1}$) concentrations while subjected to the following stress treatments in growth chambers: drought stress by withholding irrigation, heat stress (35 °C), or the combined two stresses for 28 days. Doubling ambient CO₂ increased root and shoot growth as well as root/shoot ratio under all treatments. Doubling ambient CO₂ enhanced leaf net photosynthetic rate (P_n) to a greater extent under drought or heat alone, whereas it reduced respiration rate (R), to a larger degree under heat and the combined stress, leading to a greater ratio of P_n/R. Doubling ambient CO₂ mitigated adverse physiological effects of drought or heat stress alone, whereas fewer effects were observed under the combined drought and heat stress. The positive effects of doubling ambient CO₂ were associated with the development of roots biomass and the maintenance of a positive carbon balance under either stress alone or the combined drought and heat stress.

Drought and heat stress are two major environmental constraints limiting the growth of cool-season plant species and simultaneous drought and heat can be more detrimental than either stress alone (Albert et al., 2011; Jiang and Huang, 2001a; Mittler, 2006). Major physiological damages under both drought and heat stress include the induction of stomatal closure; the decline in leaf photochemical efficiency, net photosynthetic rate, and leaf relative water content; the increase in electrolyte leakage as well as the induction of oxidative damages (Ebdon and Kopp, 2004; Jiang and Huang, 2001b; Mittler, 2006; Wang and Huang, 2004; Yu et al., 2012; Zhang et al., 2003). In addition to drought and heat stress, plants are exposed to increasing concentrations of atmospheric CO₂, which is predicted to double the current concentration by 2050 (Solomon et al., 2007). Previous studies examined the effects of elevated CO₂ on plant responses to drought or heat stress and concluded that elevated CO₂ may mitigate either drought or heat stress alone as manifested by increased shoot and root growth, improved water use efficiency as well as promoting photosynthesis and decreased respiration and various other physiological activities (Alonso

et al., 2009; Drake et al., 1997; Fleisher et al., 2008; Hamilton et al., 2008; Kirkham, 2011; Long and Drake, 1992; Prasad et al., 2009; Robredo et al., 2007; Wand et al., 1999). However, limited studies have examined the relative effectiveness of the mitigation of stress damages for drought or heat stress, and particularly little is known of the effects of doubling ambient CO₂ under combined drought and heat stress conditions relative to either stress alone.

Shoots and roots may respond differently to elevated CO₂ under drought or heat stress alone or the combined stress. Under ambient CO₂ conditions, roots exhibited better dehydration tolerance than shoots under drought stress (Erice et al., 2006), whereas roots were more sensitive to heat than shoots (Huang and Liu 2003; Lyons et al., 2007; Pote et al., 2006; Xu and Huang, 2000). Elevated CO₂ has been found to increase root-to-shoot ratio in various plant species, mainly under non-stress conditions, but few studies examined the relative sensitivity of shoots and roots to elevated CO₂ under different abiotic stresses (Hunt et al., 1996; Luo et al., 2006; Nie et al., 2013; Wall et al., 2011). Carbon balance involving both photosynthesis and respiration is a determinant factor controlling shoot and root growth (Campbell et al., 2007). Drought and heat stress, applied singly or combined, inhibit photosynthesis, whereas heat stress alone increases respiration, both of which can lead to negative carbon gain or carbon depletion restricting plant growth (Larcher, 2003; Ohashi et al., 2006; Qaderi et al., 2006). The promotive effects of elevated CO₂ on plant growth under

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non-stress conditions have been related to the increase in carbon assimilation through promoting photosynthesis and the decline in carbon loss through inhibiting respiration (Ainsworth et al., 2002; Drake et al., 1997; Long et al., 2004; Reddy et al., 2010). Limited information is available on the differential responses of photosynthesis and respiration to doubling ambient CO₂ under drought or heat stress alone and particularly under combined drought and heat stress. Understanding differential responses of shoot and root growth and carbon balance involving photosynthesis and respiration to doubling ambient CO₂ under drought or heat alone and simultaneous stress conditions is essential for improving plant productivity in adverse environmental conditions under the scenarios of global climate change and increasing atmospheric CO₂ concentration.

Kentucky bluegrass is a widely used turfgrass species common to sports fields and home lawns, which exhibits differential responses to drought or heat stress alone or the combined stress of drought and heat (Ebdon and Kopp, 2004; Jiang and Huang, 2000; Su et al., 2007; Wang and Huang, 2004; Zhang et al., 2003). The interactive effects of doubling ambient CO₂ with drought or heat stress alone or with combined drought and heat stress have not been well documented in cool-season perennial grass species. The objectives of this study were to 1) examine differential responses of shoot and root growth and photosynthesis and respiration to doubling ambient CO₂ under drought or heat stress alone and combined drought and heat stress; and 2) determine the relative effectiveness of doubling ambient CO₂ on the mitigation of drought or heat stress alone and the combined stress in a cool-season perennial grass species.

Materials and Methods

PLANT MATERIAL AND GROWTH.

Kentucky bluegrass (cv. Baron) plants were collected from established field plots located at the turfgrass research farm at Rutgers University in New Brunswick, NJ. Plants were propagated in pots (10 cm diameter, 40 cm height) filled with fritted clay and allowed to establish in a greenhouse with average day/night temperatures of 21/18 °C and 12 h natural light at 750 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation (*PAR*). During 38-d plant establishment, plants were trimmed once per week to maintain a canopy height at 10 cm, irrigated every other day, and fertilized every 3 d with half-strength Hoagland's solution (Hoagland and Arnon, 1950). Plants were then transferred to growth chambers (Environmental Growth Chamber, Chagrin Falls, OH) for CO₂, drought, and temperature

treatments. The environmental conditions in the growth chamber were 70% relative humidity, 660 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PAR*, and a 12-h photoperiod.

TREATMENTS AND EXPERIMENTAL DESIGN. The experiment consisted of three factors, including drought, heat, and CO₂ treatments. The treatments were arranged in a split plot design with CO₂ treatment as the main plot and drought and temperature stress as subplots for each treatment. The CO₂ treatments included ambient CO₂ ($400 \pm 20 \mu\text{mol}\cdot\text{mol}^{-1}$) and doubling ambient CO₂ ($800 \pm 20 \mu\text{mol}\cdot\text{mol}^{-1}$). The concentration of CO₂ inside each growth chamber was maintained through an automatically controlled, open-chamber CO₂ system connected to a 100% CO₂ gas tank (Airgas, Piscataway, NJ) using the design described in Yu et al. (2012). The different CO₂ levels were continuously monitored through an infrared gas analyzer (LI-820; LI-COR, Lincoln, NE) and controlled using an automatic system consisting of a programmable logic controller unit, solenoid valves, and a laptop computer with monitoring software accurate to within 20 $\mu\text{mol}\cdot\text{mol}^{-1}$ of the target levels (400 and 800 $\mu\text{mol}\cdot\text{mol}^{-1}$).

Plants exposed to ambient or doubling ambient CO₂ treatments were maintained as a well-watered control or with drought

Fig. 1. Soil water content (SWC) in pots with kentucky bluegrass plants under non-stress control (A), drought stress (B), heat stress (C), and the combined drought and heat stress (D + H) (D) during 28 d of treatment. Vertical bars on the top represent the values of least significant difference at $P \leq 0.05$ for comparison of CO₂ treatment effects at a given day of control, drought, heat, or D + H. Vertical bars on the right side represent the values of least significant difference at $P \leq 0.05$ for comparison between days of treatment.

stress (D), heat stress (H), or the combination of drought and heat stress (D + H). Plants in the control treatment were irrigated every 2 d to replace 100% evapotranspirative (ET) water loss at 20 °C day/17 °C night. Plants under D were irrigated every 2 d to replace 50% ET water loss. Plants under H were exposed to 35 °C day/32 °C night temperature and irrigated same as the control treatment. The D + H treatment was set at 35/32 °C and irrigated every 2 d to replace 50% ET. Control plants and D plants were placed in four growth chambers controlled at ambient or doubling ambient CO₂ with temperature set at 21/18 °C. H and D + H plants were maintained in four growth chambers controlled at ambient or doubling ambient CO₂ concentration with temperature set at 35/32 °C. The two CO₂ treatments were imposed sequentially over time with ambient CO₂ treatment conducted during 2 Sept. to 2 Oct. 2013 and doubling ambient CO₂ treatment applied during 5 Oct. to Nov. 2013. A total of eight growth chambers was used for each CO₂ concentration at one time.

MEASUREMENTS. Leaf relative water content (RWC) of fully expanded leaves was measured once per week and determined based on initial fresh weight (FW), turgid fresh weight (TW), and dry weight (DW) using the following formula: $RWC (\%) = [(FW - DW)/(TW - DW)] \times 100$. Leaf samples were detached from plants and immediately weighed for FW. Leaves were then soaked in deionized water for 24 h in the dark at 4 °C and then weighed immediately to determine TW. Leaf DW was obtained by measuring DWs after leaves were dried in the oven at 87 °C for at least 72 h (Barrs and Weatherly, 1962).

Soil volumetric water content (SWC) in the 0- to 20-cm soil layer of each pot was monitored every 2 d using time domain reflectometry with a Trase TDR system (Soil Moisture Equipment Corp., Santa Barbara, CA) (Topp et al., 1980).

Turf quality (TQ) was used to estimate overall turf performance and was rated visually as a combination of density of shoots and leaves, uniformity, health, texture, and color on a scale of 1 (the lowest rating representing completely desiccated and brown leaves) to 9 (fully turgid and green turf canopy) (Beard, 1973; Turgeon, 2011).

Shoot and root biomass were harvested after 28 d at the end of stress treatments. Roots were washed free of fritted clay and then immediately frozen in liquid nitrogen and stored at -80 °C for further analysis. Biomass was determined from total DW after sample lyophilization for 12 h. Root-to-shoot ratio was calculated as root weight divided by shoot weight per individual plant.

Leaf photochemical efficiency was estimated by measuring chlorophyll fluorescence [the ratio of variable to maximum fluorescence (F_v/F_m)] after dark-adapting leaves for 20 min using the Plant Efficiency Analyzer (Bioscientific, Hoddesdon, U.K.).

Single-leaf Pn and dark R rates were measured weekly with a portable infrared gas exchange system (LI-6400; LI-COR) in open flow mode connected to standard 2 × 3-cm chambers. Six second and third fully expanded leaves from the top of the plant were randomly selected in each pot and measured at ≈8 h into the daily light cycle. Light-saturated Pn, or light saturation point, was determined at the light level of 1000 μmol·m⁻²·s⁻¹ using a built-in red/blue light-emitting diode light source of the LI-6400. Plants were exposed to dark for 2 h before R measurement. R was measured using the infrared gas analyzer (Bruhn et al., 2002) with leaves enclosed in the chamber without light supply and an opaque cloth was used to cover the leaf chamber to prevent light penetration from outside. The analyzer was set at 500 μmol·s⁻¹ flow rate and 70% relative humidity. The CO₂ and temperature of the analyzer for Pn and R measurements were set at the level corresponding to the level of CO₂ and temperature treatment of the plants.

Fig. 2. Effects of doubling ambient CO₂ on relative water content (RWC) in kentucky bluegrass under non-stress control (A), drought stress (B), heat stress (C), and the combined drought and heat stress (D + H) (D) during 28 d of treatment. Vertical bars on the bottom represent the values of least significant difference at $P \leq 0.05$ for comparison of CO₂ treatment effects at a given day of control, drought, heat, or D + H. Vertical bars on the right side represent the values of least significant difference at $P \leq 0.05$ for comparison between days of treatment.

Fig. 3. Effects of doubling ambient CO_2 on turf quality (TQ) in Kentucky bluegrass under non-stress control (A), drought stress (B), heat stress (C), and the combined drought and heat stress (D + H) (D) during 28 d of treatment. TQ was rated on the scale of 1 to 9 (1 = the lowest rating representing completely desiccated and brown leaves and 9 = fully turgid and green turf canopy). Vertical bars on the bottom represent the values of least significant difference at $P \leq 0.05$ for comparison of CO_2 treatment effects at a given day of control, drought, heat, or D + H. Vertical bars on the right side represent the values of least significant difference at $P \leq 0.05$ for comparison between days of treatment.

STATISTICAL ANALYSIS. Treatment effects were determined using analysis of variance procedure of SAS statistics software (Version 9.0; SAS Institute, Cary, NC). Significant differences between the treatment means were separated by using the least significant difference test at the 0.05 P level.

Results

SOIL WATER CONTENT AND LEAF HYDRATION STATUS. Volumetric soil water content was maintained at field capacity ($\approx 20\%$) for 28 d in both the control and H under ambient and doubling ambient CO_2 (Fig. 1). SWC decreased rapidly during D, and particularly under D + H; by the end of the treatment period (28 d), SWC dropped to 5% and 2% under D and D + H, respectively. SWC had no significant difference between under ambient and doubling ambient CO_2 .

Leaf RWC did not change significantly under control and H treatments for plants exposed to ambient or doubling ambient CO_2 , whereas D and D + H caused significant decreases in RWC under both ambient and doubling ambient CO_2 conditions (Fig. 2). D + H induced more significant decline (58%) in RWC

than D alone (34%). Doubling ambient CO_2 suppressed RWC decline induced by D alone (45%) or D + H (25%).

SHOOT AND ROOT GROWTH. Turf quality remained constant in control plants under both ambient and doubling ambient CO_2 during the experimental period but declined during D, H, and D + H (Fig. 3). TQ of plants exposed to ambient CO_2 decreased below the minimum acceptable level (6.0) by 28 d in all stress treatments and the decline was most pronounced under D + H (88% reduction) than either H (44% reduction) or D (31% reduction) when compared with the control. Under doubling ambient CO_2 , TQ declined by 78%, 33%, and 28% at 28 d of D + H, H, and D, respectively, compared with the control. TQ of plants exposed to doubling ambient CO_2 remained above 6.0 under D or H alone but decreased to below 6.0 at 28 d of D + H treatment. Plants exposed to doubling ambient CO_2 exhibited significantly higher TQ than plants at ambient CO_2 under control, D, H, and D + H conditions.

Total shoot DW (Fig. 4A) and root DW (Fig. 4B) were highest under the control treatment and decreased by 45% and 25% under D, by 78% and 84% under H, and by 85% and 91% under D + H, respectively. The lowest shoot and root DW were found in plants exposed to D + H under both ambient and doubling ambient CO_2 conditions. Root/shoot ratio was greater under

D when compared with the control treatment but decreased to significantly lower values under H or D + H for plants in both ambient and doubling ambient CO_2 conditions when compared with the control (Fig. 4C). Shoot and root DW increased by 38% and 65%, respectively, and root/shoot ratio increased by 46% under doubling ambient CO_2 compared with those under ambient CO_2 .

LEAF PHOTOCHEMICAL EFFICIENCY AND SINGLE-LEAF NET PHOTOSYNTHETIC RATE. F_v/F_m ratios under control treatment was maintained above 0.805 throughout the experiment under both ambient and doubling ambient CO_2 (Fig. 5). D did not have significant effects on F_v/F_m ratio under ambient or doubling ambient CO_2 conditions. H and D + H stress caused a significant reduction in F_v/F_m after 8 d of H and after 4 d of D + H, and the decline was more severe under D + H than under H alone. The decline in F_v/F_m under H alone and D + H stress was less pronounced under doubling ambient CO_2 than under ambient CO_2 . At a given day of H or D + H stress, plants exposed to doubling ambient CO_2 had significantly greater F_v/F_m than those under ambient CO_2 , beginning at 14 d of treatment.

Fig. 4. Effects of doubling ambient CO_2 on shoot dry weight (A), root dry weight (B), and root/shoot dry weight ratio (C) in Kentucky bluegrass under drought stress, heat stress, and drought and heat stress at 28 d of treatment. Vertical bars represent the values of least significant difference at $P \leq 0.05$ for comparison of CO_2 treatment effects at a given treatment. Different letters on columns indicate significant differences between CO_2 treatments.

Fig. 5. Effects of doubling ambient CO_2 on leaf photochemical efficiency (F_v/F_m) in Kentucky bluegrass under non-stress control (A), drought stress (B), heat stress (C), and the combined drought and heat stress (D + H) (D) during 28 d of treatment. Vertical bars on the bottom represent the values of least significant difference at $P \leq 0.05$ for comparison of CO_2 treatment effects at a given day of control, drought, heat, or D + H. Vertical bars on the right side represent the values of least significant difference at $P \leq 0.05$ for comparison between days of treatment.

Leaf P_n of the control plants remained stable during the experimental period, but it decreased during D, H, or D + H under either ambient or doubling ambient CO_2 with the most severe decline under D + H (Fig. 6). Under ambient CO_2 levels,

P_n was reduced by 63%, 81%, and 92% under D, H, and D + H, respectively, compared with the control averaged over the 4 sampling days. Under doubling ambient CO_2 , the corresponding reductions under D, H, and D + H were 13%, 50%, and 68%, respectively. Doubling ambient CO_2 resulted in significantly higher P_n in all treatments compared with the ambient CO_2 level. Compared with ambient CO_2 , doubling ambient CO_2 increased P_n by 32%, 206%, 250%, and 440%, respectively, under control, D, H, and D + H treatments when the data were averaged over 7, 14, 21, and 28 d of treatment (Fig. 6).

LEAF DARK RESPIRATION RATE. Leaf R increased above the control level during D, H, and D + H under ambient and doubling ambient CO_2 (Fig. 7). Under ambient CO_2 , the R was 37%, 87%, and 102% higher under D, H, and D + H, respectively, than the control when the data were averaged over 7, 14, 21, and 28 d of treatment; the corresponding increases were 21%, 56%, and 57% under doubling ambient CO_2 .

Doubling ambient CO_2 significantly suppressed R under all treatments (Fig. 7). Leaf R decreased by 19%, 27%, 32%, and 37% under control, D, H, and D + H, respectively, under doubling ambient CO_2 when compared with the corresponding treatments under ambient

CO_2 for the data averaged over the course of the treatment period (Fig. 7).

SINGLE-LEAF P_n/R RATIO. The P_n/R ratio was greatest at 7 d of treatment and decreased with increasing duration of D, H,

Fig. 6. Effects of doubling ambient CO₂ on single-leaf photosynthetic rate (Pn) in kentucky bluegrass under non-stress control (A), drought stress (B), heat stress (C), and the combined drought and heat stress (D + H) (D) during 28 d of treatment. Vertical bars on the bottom represent the values of least significant difference at $P \leq 0.05$. Vertical bars on the right side represent the values of least significant difference at $P \leq 0.05$ for comparison between days of treatment.

and D + H treatments under both ambient and doubling ambient CO₂ conditions (Fig. 8). Under ambient CO₂, the ratio decreased to below 1.0×28 d of D (Fig. 8B), from 14 d of H (Fig. 8C), and from 7 d of D + H (Fig. 8D). The Pn/R ratio was maintained above 1.0 in all treatments under doubling ambient CO₂ and significantly greater than under ambient CO₂ in all treatments.

Discussion

DIFFERENTIAL EFFECTS OF DROUGHT, HEAT, OR THE COMBINED STRESSES ON KENTUCKY BLUEGRASS WATER RELATIONS, SHOOT AND ROOT GROWTH, AND CARBON BALANCE. The combination of the two stresses was more detrimental to plants than either stress alone in kentucky bluegrass in this study. Similar results were reported in other turfgrass species such as tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*) (Jiang and Huang, 2001a, 2001b; Su et al., 2007) and non-turfgrass species (Mittler, 2006). Compared with D alone, the decline caused by H for growth and physiological parameters, except RWC, was more severe, suggesting that H at 35 °C was a more significant stress than D stress for kentucky bluegrass. Heat-induced decline in TQ and shoot and root growth could be mainly associated with leaf senescence (Pote et al., 2006), as reflected by

lower F_v/F_m and Pn, but was not the result of leaf dehydration or water deficit, because of lack of differences in RWC between heat-stressed plants and the control. In addition, our results demonstrated that root growth was more sensitive to H than shoot growth, as reflected by the decreased root/shoot ratio under H or D + H in kentucky bluegrass. Growth inhibition and heat-induced decline in TQ resulting from H was not caused by leaf water deficit but may be related to heat inhibition of other functions of roots such as hormone synthesis and nutrient uptake. Liu and Huang (2005) have shown in creeping bentgrass (*Agrostis stolonifera*) that root cytokinin synthesis was most sensitive to H and inhibition of cytokinin production in roots was a primary factor leading to heat-induced leaf senescence, although impairment of other root physiological functions such as decreased water and nutrient uptake and increased abscisic acid could all contribute to the decline in shoot and root growth under H. Changes in root hormone profiles for creeping bentgrass under doubling ambient CO₂ and exposed to D, H, or D + H deserves investigation.

Adverse effects of D or H alone or D + H were also reflected in changes in Pn and R and the balance of the two processes. The balance of Pn and R or a positive carbon gain of a plant is critically important for promoting plant growth and adaptation to stresses, but imbalanced carbon relations or carbon deficit can be detrimental for cool-season grass adaptation to H (Jiang and Huang, 2000; Xu and Huang, 2000). Carbon gain is associated with greater Pn than R or a Pn/R ratio greater than 1.0 when carbon assimilation rate exceeds carbon consumption rate. H and D caused severe decline in Pn, but enhanced R, leading to reduction in Pn/R ratio below 1.0 or carbon deficit, and the extent of carbon deficit was more pronounced under H alone and D + H than D alone in kentucky bluegrass. The decline of Pn/R ratio with increasing temperatures has also been reported in other species, mainly woody plants (Centritto et al., 2011; Fares et al., 2011; Way and Sage, 2008). Results in our study suggested that either H or D alone or in D + H leads to greater rate of carbon consumption than carbon assimilation, particularly under H and D + H, which ultimately could cause the decline in total shoot and root biomass accumulation.

DIFFERENTIAL EFFECTS OF DOUBLING AMBIENT CO₂ ON KENTUCKY BLUEGRASS WATER RELATION, SHOOT AND ROOT GROWTH, AND CARBON BALANCE UNDER DROUGHT OR HEAT ALONE AND THE COMBINED STRESS. Enhanced plant growth by elevated CO₂ has been reported in other plant species exposed to either D or H alone (Hamerlynck et al., 2000; Kirkham, 2011; Xu and Zhou, 2006), but few examined the relative sensitivity

Fig. 7. Effects of doubling ambient CO₂ on single-leaf respiration rate (R) in kentucky bluegrass under non-stress control (A), drought stress (B), heat stress (C), and the combined drought and heat stress (D + H) (D) during 28 d of treatment. Vertical bars on the bottom represent the values of least significant difference at $P \leq 0.05$ for comparison of CO₂ treatment effects at a given day of control, drought, heat, or D + H. Vertical bars on the right side represent the values of least significant difference at $P \leq 0.05$ for comparison between days of treatment.

of shoots and roots to doubling ambient CO₂ under different stress conditions; in particular little is known on the effects of doubling ambient CO₂ on plants subjected to the D + H (Yu et al., 2012). This study confirmed the positive effects of doubling ambient CO₂ on the mitigation of H or D damages, but it is worthy to point out that there were significant interactions between CO₂ and stress treatments, and the positive effects of doubling ambient CO₂ were more pronounced for promoting TQ, RWC, Pn, shoot and root biomass as well as root/shoot ratio under D than under H alone. Furthermore, the positive effects of doubling ambient CO₂ were to a lesser extent under the D + H compared with individual stress alone, suggesting plants exposed to dry and hot conditions were less sensitive to doubling ambient CO₂ than under either D or H.

In this study, differential responses of shoots and roots to doubling ambient CO₂ were also observed. The ratio of root to shoot biomass increased by 65% to 115% under doubling ambient CO₂ across all treatments with the greatest increase under D, indicating that CO₂ enrichment caused a greater increase in root growth in kentucky bluegrass, particularly under D. Previous studies also reported increased root/shoot biomass ratio of plants under elevated CO₂ in other plant species, but mainly under non-stress conditions (Luo et al., 2006; Nie et al.,

2013). The increased root/shoot ratio could contribute to the improved leaf water status under D. RWC was significantly higher in plants treated with doubling ambient CO₂ than those treated with ambient CO₂ under D alone or in combination with H in this study. Studies in *Panicum cloratum* (Seneweera et al., 2001) and barley [*Hordeum vulgare* (Robredo et al., 2007)] also observed higher RWC in plants exposed to elevated CO₂ than those with ambient CO₂ under D conditions. The increased root/shoot ratio and RWC with doubling ambient CO₂ suggested that high CO₂ may enhance the capacity of water uptake by the root system, supplying water to maintain leaf hydration, which is critically important for plant survival of D and the D + H.

The growth-promoting effects of doubling ambient CO₂ on kentucky bluegrass could be associated with the maintenance of carbon balance between Pn and R, as manifested by greater Pn/R ratio under control, D or H alone, and the D + H in kentucky bluegrass. The positive carbon gain under doubling ambient CO₂ was the result of both increases in Pn rate and suppression of R rate. Leaf Pn increased by 32% to 440% with doubling ambient CO₂ in this study and increased to the greatest extent under D alone. Some previous studies also found enhanced Pn with increasing CO₂ concentra-

tion relative to ambient CO₂, but the magnitude of increases varied under different environmental conditions, which is from 12% to 212% under non-stress conditions (Albert et al., 2011; Erice et al., 2006; Fleisher et al., 2008; Ge et al., 2011; Qaderi et al., 2006; Robredo et al., 2010), 33% under higher temperature (Vu, 2005), and 162% under D + H (Yu et al., 2012). In contrast to Pn responses to doubling ambient CO₂, a significant decline (by 18% to 37%) in leaf R rate under different treatments was observed in this study with the greatest suppression under the D + H. Others also reported inhibition of respiration by elevated CO₂ in other plant species, mainly under non-stress conditions; for example, the reduction in respiration rate was $\approx 18\%$ in various woody species (Curtis and Wang, 1998), 25% to 30% in *Rumex crispus* (Amthor et al., 1992), and 30% to 40% in soybean [*Glycine max* (Bunce and Ziska, 1996)]. The increase in carbon assimilation and the decline in R carbon loss could contribute to improved growth under elevated CO₂ conditions (Ainsworth et al., 2002; Drake et al., 1997; Long et al., 2004; Reddy et al., 2010).

The mechanisms imparting the photosynthesis-promoting and respiration-inhibiting effects of increased CO₂ concentrations have been examined under non-stress environmental conditions (Baker et al., 1992; Drake et al., 1997, 1999; Farrar and

Fig. 8. Effects of doubling ambient CO₂ on single-leaf photosynthetic rate (Pn) to single-leaf respiration rate (R) ratio (Pn/R) in kentucky bluegrass under non-stress control (A), drought stress (B), heat stress (C), and the combined drought and heat stress (D + H) (D) during 28 d of treatment. Vertical bars on the top represent the values of least significant difference at $P \leq 0.05$ for comparison of CO₂ treatment effects at a given day of control, drought, heat, or D + H. Vertical bars on the right side represent the values of least significant difference at $P \leq 0.05$ for comparison between days of treatment.

Williams, 1991; Gifford, 1995). The promotive effects of elevated CO₂ have been associated with reduced photorespiration and increased Rubisco carboxylation as well as a reduction in stomatal conduction caused by increased substomatal CO₂ concentrations (Leakey et al., 2006; Long and Drake, 1992; Reddy et al., 2010; Wand et al., 1999). The suppression of R rate has been attributed to various factors, including inhibition of mitochondrial electron transport enzymes, cytochrome c oxidase, and succinate dehydrogenase during short-term exposure to elevated CO₂ (Drake et al., 1997, 1999) and declines in carbohydrate status and adenylate supply in plants exposed to elevated CO₂ treatments (Baker et al., 1992; Farrar and Williams, 1991; Gifford, 1995). However, which metabolic factors or processes contribute to the differential effects of doubling ambient CO₂ on Pn and R under D or H alone and particularly under the D + H are largely unknown and deserve further investigation. Understanding such mechanisms will facilitate the development of cool-season grass cultivars or germplasm that can maintain more active growth and be more productive in the scenario of global climate changes and increasing CO₂ concentration.

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