CHAPTER I\*

# EFFECTS OF DROUGHT OR HEAT STRESS ALONE AND IN COMBINATION ON KENTUCKY BLUEGRASS

\*This chapter has been published in Crop Sci. 2000. 40: 1358-1362

#### ABSTRACT

Cool-season turfgrasses are frequently subjected to heat and drought stresses during summer months. This study was conducted to determine physiological responses of Kentucky bluegrass (Poa pratensis L.) to drought and heat alone or together, and effects of drought preconditioning on plant responses to subsequent heat stress. Kentucky bluegrass (cv. Mystic) was subjected to drought and/or heat stress (35°C/30°C, day/night) in growth chambers for 40 d. Canopy photosynthetic rate (Pn) and leaf photochemical efficiency (Fv/Fm) decreased under drought and heat stress. The decline in Pn was more severe under heat than under drought stress during the first 12 d of treatment. The reduction in Fv/Fm ratio was more severe under drought stress than under heat stress after 20 d of treatment. The combined drought and heat stresses (DH) caused more dramatic reductions in Pn and Fv/Fm than either heat or drought alone, starting at 3 d and 9 d after treatment, respectively. Drought or heat alone, or DH, significantly reduced root dry weight. However, reduction was more severe under heat alone than under drought stress, particularly in the top 20 cm of soil. Drought preconditioning enhanced plant tolerance to subsequent heat stress but had no influence on plant tolerance to DH. Drought-preconditioned plants maintained higher water status, stomatal conductance, and transpiration rate, and had significantly higher Pn and

root dry weight than non-preconditioned plants during subsequent heat stress. No significant difference in Fv/Fm was observed between drought-preconditioned and nonpreconditioned plants under either heat alone or DH. The results indicated that simultaneous drought and heat stresses were more detrimental than either stress alone. Drought preconditioning could improve Kentucky bluegrass tolerance to subsequent heat stress.

# ABBREVIATIONS:

Fv/Fm, photochemical efficiency or chlorophyll fluorescence;  $\psi_w$ , leaf water potential;  $\psi_{\pi}$ , osmotic potential;  $\psi_p$ , turgor pressure;  $g_s$ , stomatal conductance; LSD, least significance difference; Pn, canopy photosynthetic rate.

Kentucky bluegrass is a cool-season grass widely used for home lawns and commercial landscapes in temperate climates. The optimum temperature for shoot growth of cool-season grasses is 15 to 23 °C (Beard, 1973). However, temperatures in the transition zone often approach 30°C or higher during summer months. In conjunction with heat stress, drought often lasts for prolonged periods. Drought or heat stress alone causes a severe decline in turf quality of cool-season grasses (Wehner and Watschke, 1981; Huang et al., 1998a and 1998b). Physiological changes associated with performance of cool-season grasses in response to drought or heat vary with species or cultivars (Aronson et al., 1987; Howard and Watschke, 1991; Huang et al., 1998a and 1998b). Drought stress reduced root dry weight, leaf water potential, evapotranspiration, and photochemical efficiency in tall fescue (Festuca arundinacea L.), Kentucky bluegrass, and perennial ryegrass (Lolium perenne L.) (Aronson et al., 1987; Carrow, 1996; Perdomo et al., 1996; Huang et al., 1998a). Heat stress alone caused reductions in photosynthetic rate, chlorophyll content, cell membrane stability, and carbohydrate accumulation in many species, including creeping bentgrass (Agrostis palustris L.), Kentucky bluegrass, and perennial ryegrass (Wehner and Watschke, 1984; White et al., 1988; Howard and Watschke, 1991; Huang et al., 1998b).

Heat and drought stresses often occur simultaneously during summer months, limiting plant growth. Simultaneous heat and drought stresses reduced the rates of CO<sub>2</sub> uptake and O<sub>2</sub> evolution in bean (*Phaseolus vulgaris* L.) (Yordanov et al., 1997); considerably inhibited leaf growth in sorghum (*Sorghum bicolor* (L) Moench.) (Kaigama, 1986); increased cell membrane permeability of perennial ryegrass (Chen et al., 1988) and reduced leaf water content, water potential and osmotic potential in wheat (*Triticum aestivum* L.) (Shah, 1992).

Drought stress also often occurs prior to heat stress in the summer either due to deficit irrigation or insufficient precipitation. Several studies have reported that prior exposure of plants to water deficit (drought preconditioning) reduces osmotic potential and enhances stomatal opening and photosynthetic rate during subsequent drought and, thus, increases plant resistance to the stress (Thomas et al., 1976; Bennett and Sullivan, 1981; Abrams, 1988). Wehner and Watschke (1981) reported that infrequent irrigation increased heat tolerance in Kentucky bluegrass, perennial ryegrass, and annual bluegrass (Poa annua L.). Becwar et al. (1983), however, found that drought preconditioning did not increase in vitro heat tolerance in four turfgrass species. How drought and heat stresses interact and influence growth of cool-season turfgrasses is not well understood. Knowledge of this interaction would

help to identify physiological factors involved in drought and heat tolerances to improve summer performance of coolseason turfgrass.

Two experiments were conducted to investigate the interactive effects of drought and heat stresses on Kentucky bluegrasss. The objective of the first experiment was to compare the effects of drought, heat, or the combined stresses on turf growth and physiological activities of Kentucky bluegrass. The objective of the second experiment was to determine the effects of drought preconditioning on Kentucky bluegrass responses to subsequent heat stress.

### MATERIALS AND METHODS

## Experiment 1

Sods (10 cm in diameter. and approximately 1 cm thick) of Kentucky bluegrass (cv. Mystic) were collected from field plots at the Rocky Ford Turfgrass Research Center, Kansas State University, Manhattan, KS in September 1998. Sods were washed free of soil and then planted in polyvinyl chloride (PVC) tubes (10 cm in diameter, 60 cm long) filled with a mixture of sand and topsoil (fine, montmorillonitic, mesic, aquic arguidolls) (1:2, v:v) in the greenhouse for 30 d and then transferred to growth chambers with a temperature of 20°C/15°C (day/night), 14-h photoperiod, 65% relative humidity, and photosynthetically active radiation of 600  $\mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}.$  Grasses were maintained in growth chambers for 15 d before drought and heat treatments were imposed. Controlled-release fertilizer (17N-6P-10K) was topdressed twice prior to dry-down to provide a total of 17g N m<sup>-2</sup>. Turf was hand-clipped twice weekly at about 4-cm height.

The experiment included two temperature and two soil moisture treatments. Temperature treatments were optimum temperature (20°C/15°C, day/night) and heat stress (35°C /30°C). Soil moisture treatments included: a) well-watered control, grasses in eight containers (replicates) irrigated every other day until water drained from the bottom of the PVC tubes and b) drought stress, irrigation withheld until permanent leaf wilting occurred (leaves were no longer

rehydrated at night or early mornings) for plants in eight containers. The treatments combining soil moisture and temperature were: Control (well watered, optimum temperature); drought (D-unwatered, optimum temperature); heat (H-high temperature, well watered); and the combined drought and heat (DH). Plants in four of the drought-stress containers were rewatered when permanent leaf wilting occurred in the drought and DH treatment and allowed to recover. The potential of growth recovery was evaluated for drought-stressed and drought-and heat-stressed plants.

Soil moisture in 0 to 20 and 40 to 60 cm soil layers was monitored to indicate soil dryness during drought stress using time domain reflectometry (TDR) (Soil Moisture Equipment Corp., Santa Barbara, CA). The field capacity of the sand and topsoil mix was 30  $\pm$  2 % (mean of four replications  $\pm$  standard error), which was measured in four PVC tubes with the TDR when drainage ceased following watering the soil to complete saturation.

Several physiological measurements were made at various times during the experiment. Canopy photosynthetic rate (Pn) was measured with an LI-6400 portable gas exchange system (Li-COR Inc., Lincoln, NE), and rates were expressed as CO<sub>2</sub> uptake per unit turf canopy area. Leaf photochemical efficiency expressed as chlorophyll fluorescence (Fv/Fm ratio) was determined on five randomly selected leaves in each container using a fluorescence induction monitor (Dynamax, Houston, TX).

Plants in four containers in drought stress and wellwatered treatments under optimum temperature and heat conditions were harvested when leaves of drought-stressed plants permanently wilted and when volumetric soil moisture dropped to 5 % (about the minimal level, 17 % of field capacity) in the 0 to 20-cm layer of soil in the drying treatment. At harvest, roots were separated from shoots; collected separately from 0 to 20-, 20 to 40-, and 40 to 60cm soil layers; and washed free of soil. Root dry weight was determined after samples were dried in an oven at 85°C for 3 d.

## Experiment 2

Growth conditions were the same as described above, ` except the tubes were 40 cm long. Plants in eight containers were well watered (non-preconditioning). Plants in another eight containers were subjected to two cycles of soil drying and rewatering (drought preconditioned) before being exposed to heat stress. When volumetric soil moisture reached about 5 % in each drying period, grasses were rewatered and turf quality was allowed to recover to the same level as the well-watered plants. The first cycle of drought stress lasted for 17 d and the second cycle for 28 d. After the second cycle of rewatering, drought preconditioned and nonpreconditioned plants in eight containers were exposed to  $35^{\circ}C/30^{\circ}C$  (day/night) in growth chambers for 35 d. Plants in

four containers in heat, preconditioned treatment or heat, non-preconditioned treatment were well-watered, and plants in the other four containers were not watered to induce drought stress.

The Pn and Fv/Fm were measured as described in Exp.1. Leaf water potential ( $\psi_w$ ) was measured using a thermocouple psychrometer (Decagon Devices Inc., Pullman, WA). Osmotic potential ( $\psi_\pi$ ) was measured using a vapor pressure osmometer (Wescor, Inc., Logan, UT) after cell sap were expressed from frozen leaves with a hydraulic press. Turgor pressure ( $\psi_p$ ) was determined by calculating the difference between  $\psi_w$  and  $\psi_{\pi}$ . Stomatal conductance ( $g_s$ ) and transpiration rate were measured using a steady state porometer (Li-1600, Li-Cor Inc., Lincoln, NE). At the end of the experiment, roots were separated from shoots and washed free of soil. Total root length was measured using an image analysis system (Decagon Devices, Inc., Pullman, WA). Root dry weight was determined as described in Exp. 1.

The experiments involved two factors (temperature and soil moisture) arranged in a split-plot design (temperature as main plots and soil moisture as sub-plots) with repeated measurements in four replicates (containers) (Kempthorne, 1952). Each temperature regime was replicated four times in four growth chambers. Soil moisture treatments were arranged randomly within each temperature regime. Temperature treatments were rearranged in different growth chambers once

during the experiment. Plants in four replicates were randomly sampled from four growth chambers for each measurement. Analysis of variance was based on the general linear model procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC). Interactions among temperature, soil moisture, and treatment duration occurred for all parameters examined. Effects of temperature and soil moisture treatments were analyzed by comparing them with the control at a given measurement time. Least significance difference (LSD) at a 0.05 probability level was used to detect the differences between treatment means.

#### RESULTS

#### Experiment 1

Soil water content in the 0 to 20- and 40 to 60-cm layers decreased rapidly under turf subjected to drought and heat stresses (Fig. 1-1). Water content declined to about 5 %, which was about the minimum level of soil moisture, in the 0 to 20-cm layer after 12 d of the combined drought and heat stress (DH), but not until 25 d after drought alone. At 12 d of drought stress, soil water content declined to about 10 % in the 0 to 20-cm layer and 20 % in the 40 to 60-cm layer. At 12 d of DH, the corresponding figures were 5 % and 13 %, respectively.

Canopy Pn declined significantly below the control level beginning at 3 d under heat stress and at 6 d under drought conditions (Fig. 1-2). The decline in Pn was more severe under heat than under drought during the first 10 d of treatment. However, no significant differences in Pn were observed between heat and drought at 12 and 20 d. The combination of heat and drought caused more rapid and severe reduction in Pn than either stress alone. Canopy Pn dropped to about 0 at 10 d of DH, 20 d of drought, and 34 d of heat stress. The Pn of drought-stressed plants increased after rewatering; it recovered to the control level at 14 d of rewatering. However, Pn of drought- and heat-stressed continued to decline and never recovered after rewatering.

Leaf photochemical efficiency decreased to below level of the control at 20 d of drought and 34 d of heat stress

(Fig. 1-3). The reduction in Fv/Fm was more severe under drought than heat at 20 d of treatment. The DH stress caused an earlier and more dramatic decline in Fv/Fm than either stress alone, dropping below the control level after 9 d of treatment. The Fv/Fm of plants subjected to drought alone returned to the control level after 14 d of rewatering. For plants exposed to DH, Fv/Fm recovered to some extent but did not return to the control level 28 d after rewatering.

The majority (85 %) of roots of both control and stressed plants were distributed in the top 0-20 cm of soil (Fig. 1-4). Drought, heat or DH significantly reduced root dry weight in that layer, compared to the control. The reduction in root dry weight caused by heat alone did not differ significantly from the DH stress. Root dry weights in 20-40 and 40-60 cm soil did not differ between treatments.

## Experiment 2

Canopy Pn for both drought-preconditioned and nonpreconditioned plants decreased from their initial levels when they were subjected to heat or DH (Fig.1-5). However, drought-preconditioned turf exposed to heat alone had a higher canopy Pn than non-preconditioned turf starting after 9 d of treatment. No differences in Pn were observed between drought-preconditioned and non-preconditioned plants when exposed to DH and drought stresses. Drought preconditioning

had no effect on Fv/Fm during subsequent stress from heat alone or DH (Fig. 1-6)

Drought-preconditioned plants had significantly higher  $\psi_w$  and  $\psi_p$  than non-preconditioned plants at 28 d of heat stress (Table 1-1). They also had a higher  $\psi_w$ ,  $g_s$ , and transpiration rate than non-preconditioned plants at 35 d of heat stress. No differences in  $\psi_p$  were observed between preconditioned and non-preconditions plants at 35 d of heat stress.

Root length density expressed as root length per unit volume soil and dry weight of drought-preconditioned plants were about two times those of the non-preconditioned plants when exposed to 35 d of heat stress (Table 1-2). Both variables did not differ significantly between preconditioned and non-preconditioned plants when exposed to the combined stresses (data not shown).

## DISCUSSION

Heat stress had a more detrimental effect on canopy Pn than drought during the early periods of treatment. However, prolonged periods of drought could be more detrimental than heat stress. The decline in canopy Pn is closely related to turf quality decline under conditions of heat or drought stress (Huang et al., 1998b; Huang and Fry, 1998). Studies have demonstrated that photosynthesis of plants in general is particularly sensitive to heat stress with increased photoinhibition of photosystem II (Weis and Berry, 1988; Georgieva and Yordanov, 1993), but is relatively resistant to water deficits (Kaiser, 1987; Cornic and Briantais, 1991). Drought stress alone did not completely damage the photosynthetic apparatus, as evidenced by the quick recovery in Pn after rewatering. However, drought combined with heat caused rapid leaf desiccation and permanent damage to the photosynthetic system. The strong interaction between drought and heat suggested that these two stresses have additive effects on Pn in cool-season turfgrass species when they occur simultaneously. Ludlow et al. (1990) reported that the combined stresses of heat and drought caused a dramatic decline in grain yield of sorghum in the field.

Reduction in leaf Fv/Fm under drought and heat alone occurred at 10-12 d and 30 d later than that of Pn, respectively, suggesting that Pn was more sensitive to these individual stresses. Under the combined drought and heat stresses, the reduction in Fv/Fm was correlated with that of

Pn. The interaction of drought and heat caused more rapid and severe damage to the photochemical efficiency of PSII than either stress alone, similar to the effects on Pn. Unlike canopy Pn, Fv/Fm of some leaves exposed to the combined stresses recovered to some extent after rewatering, although they did not reach to level of the control. The discrepancy between the responses of Fv/Fm of individual leaves and Pn to drought or heat and rewatering following exposure to stresses suggested that factors other than Fv/Fm contributed to the decline in canopy Pn during the early periods of stress and during recovery for Kentucky bluegrass.

An extensive, deep root system is an important characteristic of drought-resistant plants (Sheffer et al., 1987; Marcum et al., 1995; Carrow, 1996; Huang and Fry, 1998). Such a root system facilitates water uptake and, in turn, affects plant tolerance to drought and heat. These stresses and their combined influence reduced root growth in the surface soil layer. A strong combined effects of drought and heat on root dry weight was observed, but the reduction was similar to that from heat stress alone. The more severe inhibition of root dry weight by heat stress than drought could have been related to the more significant reduction in Pn during the early periods of treatment.

Drought preconditioning enhanced canopy Pn during subsequent heat stress. This could have been associated with improved root growth and water relations. Drought-

preconditioned plants developed more extensive root systems than non-preconditioned plants, which could facilitate water uptake under heat stress. Drought-preconditioned plants had significantly higher g, and transpiration rate under heat, which could lead to greater cooling effects than in nonpreconditioned plants. High g, and transpiration rate are related positively to seedling survival under high temperatures (Kolb and Robberecht, 1996). Our results also demonstrated that drought-preconditioned grasses maintained higher  $\psi_w$  and  $\psi_p$  during subsequent heat stress, which could have been due to solute accumulation during drought. Ashraf et al. (1994) found that a heat-tolerant cultivar of cotton (Gossypium hirsutum L.) accumulated significant amounts of organic osmotica such as soluble sugars and proline under heat stress. Drought stress also might induce accumulation of solutes in cells, which would enhance turgor maintenance during heat stress. This deserves further investigation.

Our results agree with those of Wehner and Watschke (1981), who also reported drought preconditioning increased heat tolerance of cool-season turfgrasses by observing plant recovery after a 30-min exposure to 47°C. However, our results refuted those of Becwar et al. (1983), who found that drought did not enhance tolerance to a short-term heat shock (48°C) using excised leaves.

In summary, heat and drought stresses significantly reduced canopy Pn, Fv/Fm, and water uptake, which would

result in a decline in turf quality during hot and dry summers. The detrimental effects of the combined stresses were significantly greater than those of either stress alone. However, drought preconditioning increased tolerance of Kentucky bluegrass to subsequent heat stress, which suggested that water deficit or infrequent irrigation during spring could be used to encourage root growth and enhance hardiness to drought or heat stress during summer.

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Table 1-1. Leaf water potential  $(\psi_w)$ , turgor pressure  $(\psi_p)$ , stomatal conductance  $(g_s)$ , and transpiration rate (Tr) of drought-preconditioned and non-drought preconditioned Kentucky bluegrass exposed to 28 and 35 d of heat stress.

	28 d Heat stress			35 d Heat stress			
Treatment	Ψ₩	$\Psi_{\mathbf{p}}$	_	Ψ₩	ψ <sub>p</sub>	gs	Tr
		1	MPa -			— mmol m	<sup>-2</sup> s <sup>-1</sup> —
Preconditioned	-1.65a*	0.21a		-1.76a	0.11a	31.58a	1.42a
Non-preconditioned	-2.02b	0.03b		-2.13b	-0.02a	18.84b	0.85b

\* Means followed by the same letters within a column were not significantly different based on LSD test (P = 0.05). Table 1-2. Root length density expressed as root length per unit soil volume and dry weight of drought-preconditioned and non-drought preconditioned Kentucky bluegrass exposed to 35 d of heat stress.

Treatment	Root length density	Root dry weight		
	— mm·cm <sup>-3</sup> —	— g —		
Preconditioned	32.5a*	1.05 a		
Non-Preconditioned	17.7b	0.52 b		

\* Means followed by the same letters within a column were not significantly different based on LSD test (P = 0.05).



Fig.1-1. Soil water content of Kentucky bluegrass exposed to drought (D) and the combination of two stresses (DH) at 0 to 20- and 40 to 60-cm soil layers.



Fig. 1-2. Canopy photosynthetic rate  $(P_n)$  of Kentucky bluegrass under optimum temperature and well-watered conditions (Control), drought (D), heat (H), and the combination of two stresses (DH). Vertical bars indicate LSD values (P = 0.05) for treatment comparisons at a given day of treatment. Arrows indicate when plants were rewatered.



Fig. 1-3. Photochemical efficiency (Fv/Fm) of Kentucky bluegrass under optimum temperature and well-watered conditions (control), drought (D), heat (H), and the combination of two stresses (DH). Vertical bars indicate LSD values (P = 0.05) for treatment comparisons at a given day of treatment. Arrows indicate when plants were rewatered.



Fig. 1-4. Root dry weight of Kentucky bluegrass under optimum temperature and well-watered conditions (control), drought (D), heat (H), and the combination of two stresses (DH) in 0 to 20-, 20 to 40-, and 40 to 60-cm soil layers at 25 d of D or H alone and 12 d of the combined stresses. Means followed by the same letters within each soil layer were not significantly different based on LSD test (P = 0.05).



Fig. 1-5. Effects of drought preconditioning on canopy photosynthetic rate (Pn) of Kentucky bluegrass under subsequent heat stress. Pre + H, drought preconditioning under subsequent heat stress; Non + H, non-preconditioning under heat stress; Pre + HD, drought preconditioning under the combination of heat and drought stresses; Non + HD, nonpreconditioning under the combination of heat and drought stresses. Vertical bars indicate LSD values (P = 0.05) for treatment comparisons at a given day of treatment.



Fig. 1-6. Effects of drought preconditioning on Photochemical efficiency (Fv/Fm) of Kentucky bluegrass under subsequent heat stress. Pre + H, drought preconditioning under subsequent heat stress; Non + H, non-preconditioning under heat stress; Pre + HD, drought preconditioning under the combination of heat and drought stresses; Non + HD, nonpreconditioning under the combination of heat and drought stresses. Vertical bars indicate LSD values (P = 0.05) for treatment comparisons at a given day of treatment.