CHAPTER II*

OSMOTIC ADJUSTMENT AND ROOT GROWTH ASSOCIATED WITH DROUGHT

PRECONDITIONING-ENHANCED HEAT TOLERANCE IN KENTUCKY

BLUEGRASS

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ABSTRACT

Prior exposure to drought stress (drought preconditioning) affects turfgrass tolerance to subsequent heat stress. The study was designed to examine whether these effects for Kentucky bluegrass (*Poa pratensis* L.) are associated with osmotic adjustment and root growth. Plants were subjected to two cycles of drying and rewatering, and turf quality was then allowed to recover to the well-watered control level before being exposed to 21 d of heat stress (35 °C/30 °C) in growth chambers. Drought-preconditioned plants had higher turf quality than non-preconditioned plants during heat stress; and higher leaf relative water content at 14 and 21 d of heat stress, whereas osmotic adjustment was about 0.90 and 0.42 (MPa) in drought-preconditioned plants, and 0.75 and 0.22 (MPa) in non-preconditioned plants, respectively. Total ion (K^+, Ca^{2+}, Na^+, Ma^{2+}, Cl^-, and P) concentration of cell sap increased during heat stress, but drought-preconditioned plants had about 11-16 % higher of this concentration than non-preconditioned plants. The concentration of K^+ accounted for about 59-65% of total ion solutes in both groups of plants during heat stress. Soluble carbohydrate content (WSC) of leaves increased during heat stress, but was about 21 % and 44 % higher in drought-preconditioned plants than non-preconditioned plants at d 14 and 21, respectively. Heat stress decreased root dry weight (DW) and WSC, but

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significant higher DW and WSC content of roots in the 40-60 cm soil layer were observed for preconditioned plants than non-preconditioned plants before and after heat stress. The results demonstrated that drought preconditioning enhanced heat tolerance in Kentucky bluegrass, which could be related to the maintenance of higher osmotic adjustment associated with accumulation of ion solutes and water soluble carbohydrates and development of extensive roots deeper in the soil profile.

ABBREVIATIONS:

RWC, relative water content; WSC, water soluble carbohydrate; LSD, least significance difference.

Plants are constantly subjected to changing and interactive environmental stresses. Previous growing conditions can influence responses and adaptation of plants exposed to subsequent environmental stresses (Ackerson, 1980; Bennett and Sullivan, 1981; Eamus, 1987). For example, prior exposure of plants to drought stress (drought preconditioning) because of either insufficient irrigation or precipitation increased subsequent heat tolerance in various species including Kentucky bluegrass (Jiang and Huang, 2000a); perennial ryegrass (*Lolium perenne* L.) and annual bluegrass (*Poa annua* L.) (Wehner and Watschke, 1981); and cotton (*Gossypium hirsutum* L.) (Brown and Thomas, 1980). Jiang and Huang (2000a) found that drought-preconditioned Kentucky bluegrass had higher canopy photosynthesis and turgor potential than non-preconditioned plants during subsequent heat stress. Brown and Thomas (1980) reported that drought-preconditioned plants had a lower dark respiration rate.

The mechanisms by which heat tolerance is enhanced by drought preconditioning are not well understood. Heat stress injury involves water deficit and cell turgor loss (Ahmad et al., 1989). Maintenance of favorable water status is essential for plant tolerance to heat stress (Graves et al., 1991; Lehman and Engelke, 1993; Jiang and Huang, 2000b). A heat-tolerant cultivar of cotton was able to survive heat stress by accumulating solutes to maintain cell turgor

(Ashraf et al., 1994). Drought preconditioning-enhanced heat tolerance may be related to the maintenance of plant water relations by reducing water loss and/or increasing water uptake capacity. Osmotic adjustment is well known to be an important physiological mechanism of water retention and cell turgor maintenance (Turner and Jones, 1980; Morgan, 1984). The accumulation of solutes such as amino acids, organic acids, ions, and soluble sugars is associated with active osmotic adjustment during drought stress (Ranney et al., 1991; Zhang and Archbold, 1993; Premachandra et al., 1995; Guicherd et al, 1997; Bussis and Heneke, 1998). Deep and extensive root systems contribute positively to water uptake (Sheffer, 1987; Huang and Fry. 1998; Bonons and Murphy, 1999). Infrequent irrigation promotes deep rooting (Qian and Fry, 1997). Whether drought preconditioning - enhanced heat tolerance in Kentucky bluegrass as reported by Jiang and Huang (2000a) involves osmotic adjustment or stimulation of root growth or both mechanisms has not been examined. Furthermore, the major solutes contributing to osmotic adjustment in Kentucky bluegrass are not known. Understanding the physiological mechanisms of drought-induced heat tolerance would help to develop effective irrigation practices for Kentucky bluegrass and identify physiological traits for improving summer performance of cool-season turfgrasses.

Therefore, the objectives of this study were to determine whether effects of drought preconditioning on

subsequent heat tolerance could be related to osmotic adjustment and root growth in Kentucky bluegrass and to determine the relative contributions of various solutes to osmotic adjustment.

MATERIALS AND METHODS

Plant Materials

Sods of Kentucky bluegrass (cv. Mystic) were collected from field plots at the Rocky Ford Turfgrass Research Center, Kansas State University. Plants were grown in polyvinyl chloride tubes (40 cm long, 20 cm in diameter) filled with a mixture of sand and loamy soil (fine, montmorillonitic, mesic, aquic arquidolls) (1:2, v:v) in a greenhouse. Plants in each tube were watered daily and fertilized weekly with liquid fertilizer of N-P-K (20-10-20) (Scotts-Sierra Horticultural Products Comp. Marysville, OH) for 40 d before being moved to growth chambers and exposed to stress treatment.

Drought Preconditioning and Heat Stress Treatments

Plants in eight tubes were kept well-watered by daily irrigation (non-preconditioned, control). Plants in another eight tubes were subjected to two 14-d cycles of soil drying (drought preconditioned) and rewatering. Conditions in the growth chambers were temperatures of 20°C/15°C (day/night), a 14-h photoperiod, 65 % relative humidity and a photosynthetically active radiation of 400 μmol m^{-2} s^{-1}. When volumetric soil moisture in each tube reached about 5% (about 15 % of field capacity) during each drying period, preconditioned plants were rewatered and turf quality was allowed to recover to the same level as that of non-

preconditioned plants. Soil moisture was monitored using a time domain reflectometer (Soil Moisture Equipment Corp., Santa Barbara, CA). After the second cycle of rewatering, both drought-preconditioned and non-preconditioned plants were exposed to heat stress (35°C/30°C, day/night) in the growth chambers for 21 d.

Measurements

Various measurements were made weekly during the heat stress period. Turf quality was rated visually as an integral of grass color, uniformity, and density on the scale of 0 (the worst) to 9 (the best) (Turgeon, 1999). The minimum acceptable level was 6. Leaf relative water content (RWC) was determined according to the methods of Barrs and Weatherley (1962) based on the following calculation: $RWC = (FW-DW)/(SW-DW) \times 100$, where FW is leaf fresh weight, DW is dry weight of leaves after drying at 85°C for 3 d, and SW is the turgid weight of leaves after soaking in water for 4 h at room temperature (approximately 20°C).

Leaves were frozen and pressed with a hydraulic press to collect cell sap for the analysis of solute concentration and osmotic potential. The concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} were assayed on a 1:100 (v/v) dilution of cell sap using an inductively coupled plasma spectrophotometer (ICP) (Fisons Instruments Inc., Beverly, MA). Phosphorus was measured with a Technicon Autoanalyzer II according to the

manufacturer's instructions (Technicon, 1976). Chloride irons were determined by the method of Adriano and Doner (1982).

Leaf osmotic potentials of stressed (ψ_{π_o}) and fully rehydrated ($\psi_{\pi_{100}}$) leaves were measured using a vapor pressure osmometer (Wescor, Inc., Logan, UT). Osmotic adjustment was calculated as the difference in osmotic potential at full turgor ($\psi_{\pi_{100}}$) between control and stressed plants (ψ_{π_o}) (Blum and Sullivan, 1986; Blum, 1989).

For analysis of water soluble carbohydrate content (WSC), 20 to 30 mg of dry leaves or roots were extracted four times for 15 min in 10 mL of boiling water. After centrifugation at 3500 g for 10 min, supernatants were collected and pooled, and the final volume was adjusted to 50 mL. The WSC content was determined using the method of Dubois et al. (1956) modified by Buysse and Merckx (1993). Briefly, 1 mL of supernatant was put into glass tube, and 1 mL of 18 % phenol solution and 5 mL concentrated sulfuric acid were added. The mixture was shaken, and absorbance was read at 490 nm using a spectrophotometer (Spectronic Instruments, Inc., Rochester, NY).

Proline content was measured according to the method of Bates (1973). A 0.5 g sample of fresh leaves was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman #2 paper. Then 2 mL of filtrate was mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic and

heated at 100°C for 1 hr. The reaction was terminated in an ice bath; then 4 mL of toluene was added to the mixture, and contents of tubes were stirred for 15 to 20 s. The chromophore was aspirated from the aqueous phase, and the absorbance was read at 520 nm.

At the end of the experiment, roots were washed free of soil and separated from the 0-20 and 20-40 cm soil layers. Root dry weight was determined after samples were dried in an oven at 85°C for 3 d.

Experimental Design and Statistical Analysis

The experiment was a completely randomized design with four replicates. The heat stress treatment was repeated in four growth chambers. Drought-preconditioned and non-preconditioned plants were assigned randomly to each growth chamber. Analysis of variance was based on the general linear model procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC). Effects of the drought preconditioning treatment were analyzed by comparing it with the non-preconditioning control at a given measurement time. The least significance difference (LSD) at a 0.05 probability level was used to detect the differences between treatment means.

Turf Quality and Leaf Relative Water Content (RWC)

Drought-preconditioned plants had higher turf quality than non-preconditioned plants during the entire period of heat stress (Fig. 1A). By 21 d of heat stress, turf quality of non-preconditioned plants declined to 5.5, lower than the minimal acceptable level, whereas preconditioned plants still maintained a higher quality of 7.0. Leaves of drought-preconditioned plants were more turgid than non-preconditioned plants at 14 and 21 d of heat stress, whereas leave relative water content was 91 % and 88 % in drought-preconditioned plants and 86 % and 80 % in non-preconditioned plants, respectively (Fig. 1B).

Osmotic Adjustment and Solute Accumulation

Heat stress increased osmotic adjustment (OA) in both drought-preconditioned and non-preconditioned plants, however, OA was significantly higher in drought-preconditioned plants (0.23, 0.90, 0.42 MPa) than in non-preconditioned plants (0.13, 0.75, 0.22 MPa) at 7, 14, 21 days of heat stress, respectively. The difference in OA between the two groups of plants increased with the extended period of heat stress.

Under normal temperature condition (0 d), no differences in ion concentrations of cell sap were found between drought-preconditioned and non-preconditioned plants

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(Table 1). However, preconditioned plants had 18.9 %, 9.5 % and 7.9 % higher concentrations of K^+, and 31.7 %, 19.4 % and 29.8 % higher concentration of Ca^{2+} than non-preconditioned plants at 7, 14, and 21 d of heat stress, respectively. Significant higher (about 43 % at d 14 and 100 % at d 21) concentration of Na^+ was observed in drought-preconditioned plants than non-preconditioned plants. Preconditioned plants showed 20.6 % higher level of Cl^- and 21.1 % higher P than non-preconditioned plants at day 14, respectively. Drought reconditioning had no effect on the concentration of Mg^{2+} during heat stress.

The total concentration of the six ion solutes combined was about 16.2 %, 12.8 %, and 10.7 % higher in drought-preconditioned plants than in non-preconditioned plants at 7, 14 and 21 d of heat stress, respectively. The K^+ accounted for about 59 to 65 % of total ion solutes in both groups of plants during heat stress.

Heat stress increased shoot water soluble carbohydrate (WSC) (Fig. 2A). Drought-preconditioned plants had 21.3 % and 44 % higher leaf WSC content than non-preconditioned plants at 14 and 21 d of heat stress, respectively. About 23 % higher proline content of leave was found in preconditioned plants than non-preconditioned plants at 7 d of heat stress (Fig. 2B).

Root Growth

Heat stress reduced root dry weight in both the 0-20 and 20-40 cm soil layers for both drought-preconditioned and non-preconditioned plants (Table 2). Root dry weight in the 0-20 cm soil layer was not affected by drought preconditioning. However, drought-preconditioned plants had over 100 % higher root dry weight than non-preconditioned plants in the 20-40 cm soil layer. At 21 d of heat stress, root dry weight in the 20-40 cm soil layer accounted for 1.0 % of total root dry weight for non-preconditioned plants and 9.5 % of the total for preconditioned plants.

At both optimum temperature and 21 d of heat stress, drought preconditioning had no effect on WSC content of roots in the 0-20 cm soil layer, but increased over 100 % WSC of roots in the 20-40 cm soil layer (Table 2).

DISCUSSION

Enhanced turf quality and heat tolerance of Kentucky bluegrass by drought preconditioning could be associated with maintenance of leaf water status. Drought preconditioned plants had 5.8 % and 10 % higher leaf relative water content than non-preconditioned plants at 14 and 21 d of heat stress, respectively, suggesting that drought preconditioning could involve certain mechanisms contributing to water retention. The increased osmotic adjustment could be one of the mechanisms, which was observed about 90 % higher in drought-preconditioned than non-preconditioned plants at 21 d of heat stress. Other studies have also shown that osmotic adjustment through accumulation of organic or inorganic solutes facilitates maintenance of cell turgor and water retention (Ranney et al., 1991; Zhang and Archbold, 1993; Wang et al, 1995; Guicherd et al., 1997).

This increased osmotic adjustment was accompanied by the accumulation of the inorganic solutes such as K^+, Ca^{2+}, P and Cl^- and the organic solutes such as WSC and proline in this study. Among those ion solutes of cell sap, the predominant solute was K^+, which accounted for about 59 to 65 % of total ion concentration. Also drought-preconditioned plants showed about 8 to 19 % higher level of K^+ than non-preconditioned plants during heat stress. The accumulations of K^+ also has been found to be the major ion solute contributing to osmotic adjustment in other species under

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drought stress (Jones et al., 1980; Morgan, 1992; Ranney et al., 1991; Premachandra et al., 1995). The pattern of the increased level of Ca^{2+} was similar to that of K^+, but its concentration was less than K^+ in both preconditioned and non-preconditioned plants. Both K^+ and Ca^{2+} regulate guard-cell turgor and stomatal aperture (Mansfield et al., 1990; Webb et al., 1996), and accumulation of K^+ and Ca^{2+} could contribute to the increased osmotic adjustment during heat stress following drought preconditioning observed in this study. Accumulation of cytosolic free Ca^{2+} also has been found during heat shock (Klein and Ferguson, 1987; Biyaseheva et al., 1993; Gong et al., 1998; Wang and Li, 1999), which may alleviate heat injury (Bamberg et al., 1998; Gong et al., 1998). Application of Ca^{2+} to leaves also increased heat tolerance in Kentucky bluegrass by regulating the Ca^{2+} concentration of cell sap (Jiang and Huang, 2000b). Concentrations of P and Cl^- also increased under heat stress, however, the accumulation of these two ions only showed at 14 d of heat stress. Drought-preconditioned plants had about 43 % and over 100 % higher level of Na^+ at 14 and 21 d of heat stress, respectively, but its contribution to total ion accumulation was less than 1 %. The results indicated that Na^+, P and Cl^- were less important to osmotic adjustment than K^+ and Ca^{2+} accumulation in Kentucky bluegrass.

Water-soluble carbohydrates have been found to be closely associated with osmotic adjustment in response to

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water stress in woody and herbaceous plants (Munns and Weir, 1981; Ranney et al., 1991; Tan et al., 1992; Zhang and Archbold, 1993). Premachandra et al. (1995) reported that soluble sugar was mainly responsible for solute accumulation and osmotic adjustment during the early period of drought stress. In this study, drought-preconditioned plants had about 21.3 % and 44 % higher leaf WSC content than non-preconditioned plants at 14 and 21 d of heat stress, respectively; when osmotic adjustment was observed higher in preconditioned plants. The indicated that the higher level of WSC in drought-preconditioned plants contributed to higher osmotic adjustment with the prolonged heat stress. The accumulation of WSC in shoots with increasing high temperature has been observed in a tolerant cultivar of Kentucky bluegrass (Aldous and Kaufmann, 1979).

Proline also accumulation in response to environmental stress in some species (Aspinall and Paleg, 1981; Mayer et al., 1990). In the present study, drought-preconditioned plants maintained 23 % higher level of proline at 7 d of heat stress. However, there was no difference in osmotic adjustment between preconditioned and non-preconditioned plants at day 7, suggesting that proline was not the major solute contributing to turgor maintenance. Similar results was found by others (Tan et al., 1992) in black spruce [*Picea mariana* (Mill.) B.S.P.]. Chu et al. (1974) found that proline accumulation showed no direct response to increased temperature in barley (*Hordeum vulgare* L.). Proline also was

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not detected in some other plant species in response to water stress (Zhang and Archbold, 1993). The responses of proline to drought or heat stress may vary with plant species and severity and duration of stress.

The maintenance of higher turf quality and leaf water content during heat stress following drought preconditioning also could be related to the development of a deeper root system and the accumulation of higher level of WSC. Drought-preconditioned plants had over 100 % higher root dry weight and WSC content than non-preconditioned plants in the lower soil layer (20 to 40 cm) before and after 21 d of heat stress. Deep rooting facilitates extraction of soil moisture from deeper soil profiles (Morgan and Condon, 1986; Tangpremsri et al., 1991). Higher WSC accumulation in roots could be beneficial for the maintenance of cell turgor and root penetration deeper into the soil profile (Sharp et al., 1990), and also associated with heat tolerance (Aldous and Kaufmann, 1979). Therefore, deeper root system and larger amount of WSC content could contribute to heat tolerance caused by drought preconditioning in Kentucky bluegrass.

In summary, drought preconditioning increased tolerance of Kentucky bluegrass to subsequent heat stress, confirming results of our previous study (Jiang and Huang, 2000a). The enhanced heat tolerance was related to increased osmotic adjustment and root growth deeper into the soil profile following drought preconditioning. The K^+, Ca^{2+}, and WSC were

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the major solutes contributing to osmotic adjustment in
Kentucky bluegrass during heat stress.

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Table 2-1. Ion concentrations in cell sap of drought-preconditioned (Pre) and non-preconditioned (Non) Kentucky bluegrass exposed to 7, 14, and 21 d of heat stress (HS).

Days of HS	Treatment	Ion concentrations						
		K	Ca	Na	Mg	Cl	P	Total ions
		($mmol\,l^{-1}$)						
0	Non	108.6a*	8.7a	0.88a	9.1a	13.9a	14.4a	155.6a
	Pre	108.4a	7.5a	0.99a	7.6a	14.0a	12.6a	155.1a
7	Non	98.4b	10.1b	0.76a	10.6a	15.5a	18.5a	153.9b
	Pre	117.0a	13.3a	0.75a	11.2a	17.3a	19.4a	178.9a
14	Non	115.3b	17.5b	1.00b	13.8a	17.0b	21.8b	186.4b
	Pre	126.3a	21.9a	1.43a	13.8a	20.5a	26.4a	210.3a
21	Non	95.8b	14.1b	0.87a	11.4a	12.0a	23.6a	159.8b
	Pre	103.4a	18.3a	1.78b	12.5a	14.2a	26.5a	176.9a

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

Table 2-2. Root dry weight (DW) and root water soluble carbohydrate (WSC) content in 0-20 and 20-40 cm soil layers for drought-preconditioned (Pre) and non-preconditioned (Non) Kentucky bluegrass under optimum temperature (control) and at 21 d of heat stress.

Treatment		0-20 cm		20-40 cm	
		DW	WSC	DW	WSC
		(g)	(mg/gDW)	(g)	(mg/gDW)
Control	Non	0.46a*	18.1a	0.07b	13.5b
	Pre	0.55a	21.5a	0.20a	36.1a
Heat stress	Non	0.32a	25.5a	0.003b	0.004b
(21 d)	Pre	0.38a	25.2a	0.04a	20.3a

* Means followed by the same letters within a column were not significantly different based on LSD test (P=0.05).
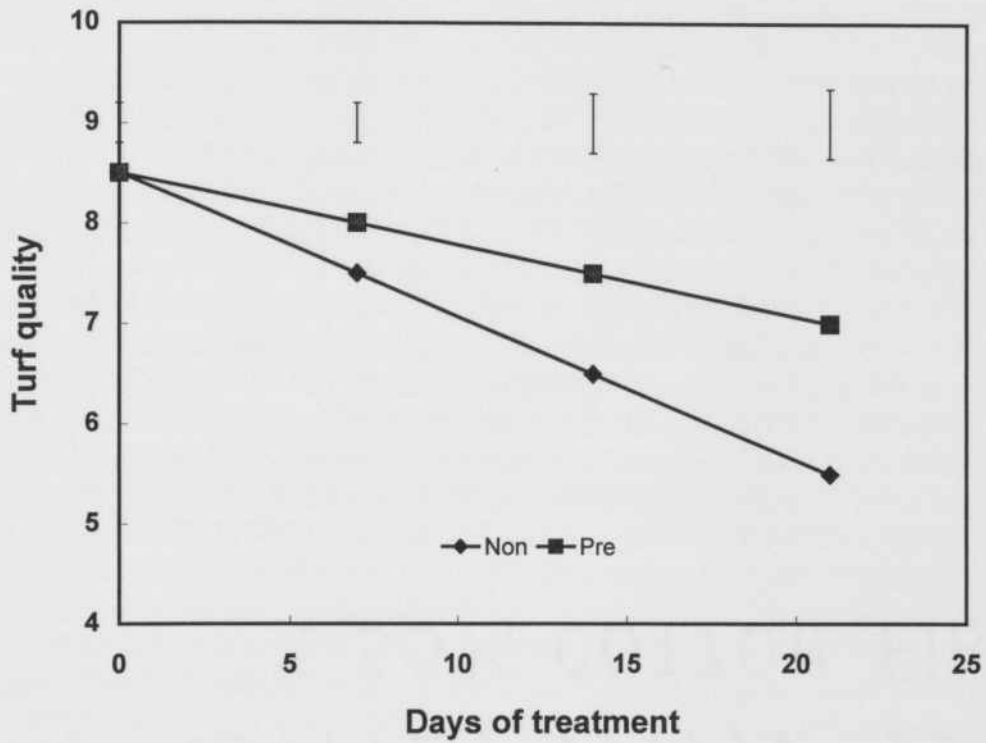
Fig. 2-1. Turf quality as affected by heat stress in drought-preconditioned (Pre) and non-preconditioned (Non) Kentucky bluegrass. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.
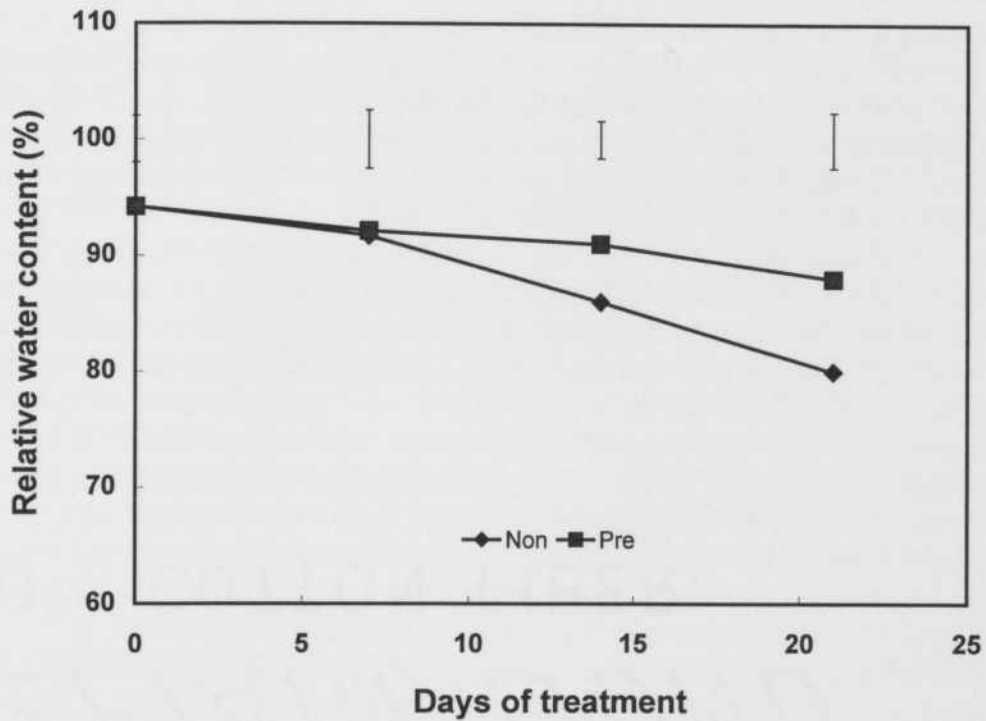
Fig. 2-2. Leaf relative water content as affected by heat stress in drought-preconditioned (Pre) and non-preconditioned (Non) Kentucky bluegrass. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.
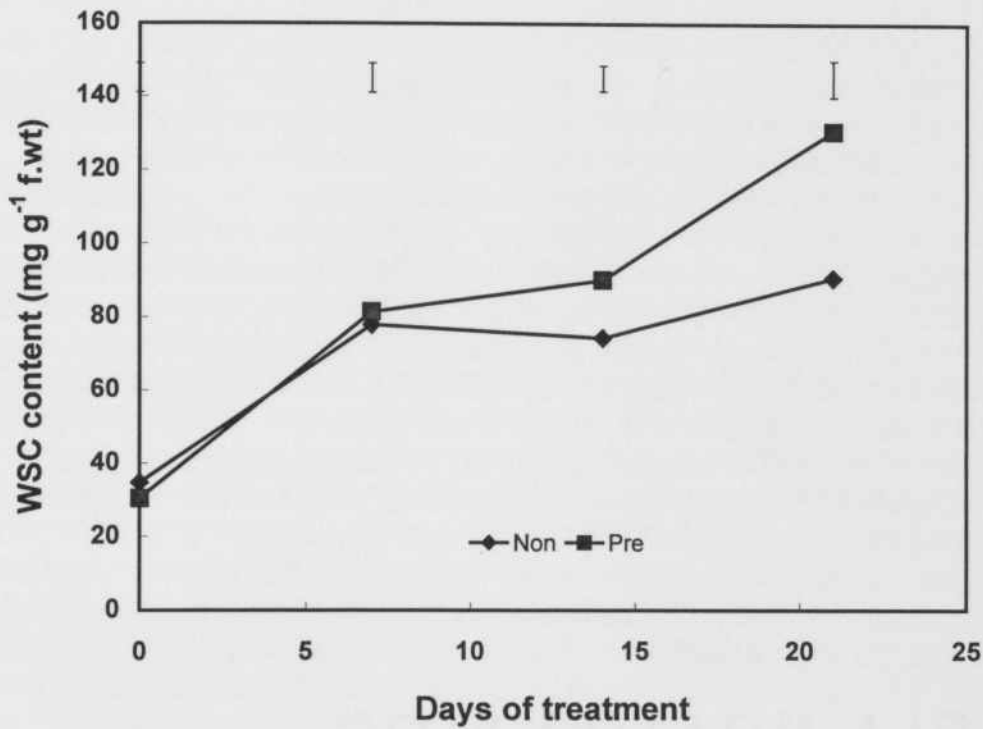
Fig. 2-3. Leaf water soluble carbohydrate content as affected by heat stress in drought-preconditioned (Pre) and non-preconditioned (Non) Kentucky bluegrass. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.
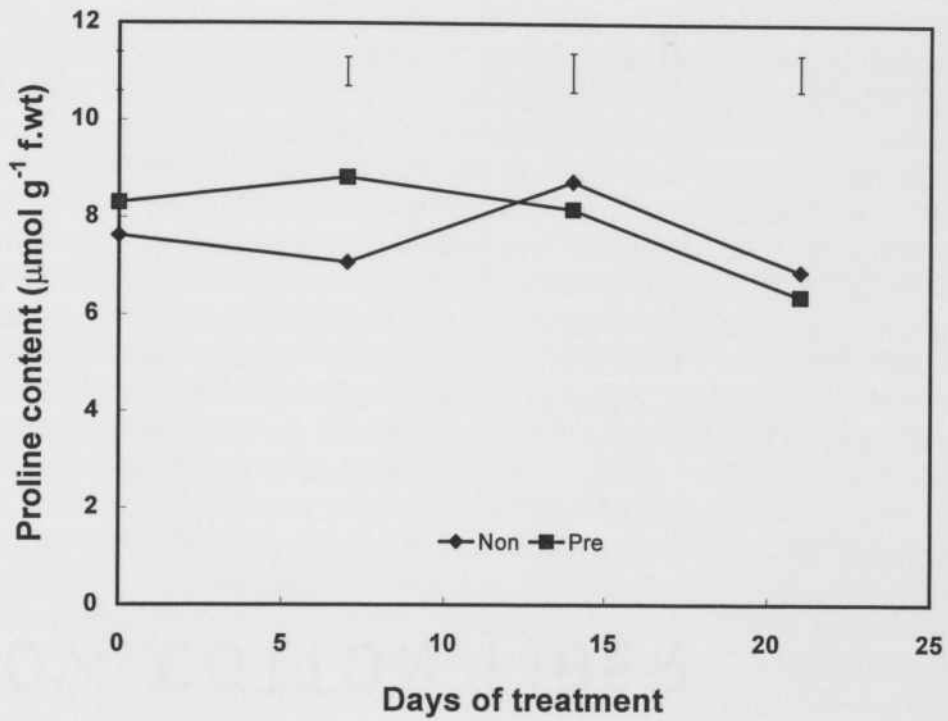
Fig. 2-4. Proline content (B) as affected by heat stress in drought-preconditioned (Pre) and non-preconditioned (Non) Kentucky bluegrass. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

CHAPTER III*

DROUGHT AND HEAT STRESS INJURY TO TWO COOL-SEASON

TURFGRASSES IN RELATION TO ANTIOXIDATION METABOLISM AND

LIPID PEROXIDATION