Responses of Cytokinins, Antioxidant Enzymes, and Lipid Peroxidation in Shoots of Creeping Bentgrass to High Root-zone Temperatures

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ABSTRACT. This study was designed to examine whether shoot injury induced by high root-zone temperature is associated with changes in shoot detoxifying metabolism and to determine the level and duration of high root-zone temperatures that would induce physiological changes in two cultivars of creeping bentgrass (*Agrostis stolonifera* var. *palustris* Huds) differing in heat tolerance. Plants of 'Penn A-4' (heat tolerant) and 'Putter' (heat susceptible) were grown in sand and exposed to root-zone temperatures of 20 (control), 21, 22, 23, 25, 27, 31, and 35 °C in water baths while air temperature was maintained at 20 °C in a growth chamber. Turf quality, leaf cytokinin content, and antioxidant enzyme activities declined at increased soil temperatures and the duration of treatment for both cultivars. A decline in turf quality occurred following 40 days of exposure to 35 °C for 'Penn A-4' and 26 days of exposure to 31 °C for 'Putter'. The root-zone temperature causing the decline of isopentenyl adenosine and zeatin cytokinins was 25 °C at 37 d for 'Putter' and 27 °C at 47 days for 'Penn A-4'. The temperature causing the decline of superoxide dismutase and catalase activities was 25 °C and 27 °C at 33 days for 'Putter' and 27 °C and 31 °C at 43 days for Penn A-4, respectively. Malondialdehyde content increased at 27 °C for 'Putter' and 31 °C for 'Penn A-4' at 43 days of treatment. The decline in cytokinin content and antioxidant enzyme activity occurred at a lower soil temperature and earlier during the treatment than the decline in turf quality, possibly contributing to turf quality decline. The root-zone temperatures causing the decline in turf quality, cytokinin content, and oxidative damage were higher in the heat-tolerant cultivar than heat-susceptible cultivar.

The optimal temperatures for the growth of cool-season grasses are between 10 and 24 °C (Beard, 1973). However, both air and soil temperatures often reach injuriously high levels during summer months, which strongly limits shoot and root growth and even survival of plants in many areas. Shoot injury in plants under environmental stresses has been associated with oxidative damage induced by the production of active oxygen species such as superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen. These species are highly reactive and can damage many important cellular components such as lipids, proteins, and nucleic acids in living cells (Foyer et al., 1994; Smirnoff, 1993). Plants normally develop antioxidant defense systems that scavenge active oxygen species and protect cells against oxidative stress injury (Bowler et al., 1992; Zhang and Kirkham, 1996a, 1996b). Superoxide dismutase (SOD) and catalase (CAT) are two key antioxidant enzymes, and their combined effect averts cellular damage. Changes in the levels of antioxidants may be indicative of the levels of oxidative stress and stress resistance. When plants are subjected to stresses such as high or low temperatures, drought and salinity, the activities of antioxidant enzymes are inhibited, which can result in lipid peroxidation of cell membranes and cause leaf senescence (Bowler et al., 1992; Price and Hendry, 1989; Zhang and Kirkham, 1994).

Shoot growth inhibition and leaf senescence could be related to the inhibition of growth and hormone synthesis in roots at high root-zone temperatures (Udomprasert et al., 1995; Xu and Huang 2000a). Cytokinins are essential plant hormones involved in shoot meristem and leaf formation, cell division, chloroplast biogenesis,

and senescence (Binns, 1994). Cytokinins are produced mainly in roots and may regulate shoot responses to high root-zone temperatures. Cytokinin metabolism of roots is sensitive to heat stress. Two minutes of heat shock to the roots of tobacco (*Nicotiana rustica* L.) and bean (*Phaseolus vulgaris* L.) reduced cytokinin levels in both shoots and roots (Itai et al., 1973). Treatment with high air and soil temperatures (45/45 °C) for 5 h reduced the levels of zeatin riboside (ZR) and isopentenyl adenosine (iPA) in roots of both tepary bean (*Phaseolus acutifolius* A. Gray) and common bean (*P. vulgaris*) (Udomprasert et al., 1995). Heat stress also reduced ZR content in winter rape (*Brassica napus* L.) (Zhou and Leul, 1999). Leaves of transgenic plants with the isopentenyltransferase (*ipt*) gene inserted to enhance cytokinin synthesis maintained a green color under heat stress for a longer period than the non-transgenic control in Arabidopsis (*A. thaliana*) (Gan and Amasino, 1995). The positive effects of cytokinins on stress tolerance of plants are believed to be related to their protective effects from oxidative stress by preventing the formation of free radicals or by the regulation of antioxidant enzyme activities (Caers et al., 1985; Gunse and Eldtner, 1993; Leshem, 1984, 1988; Liu and Huang, 2002; Musgrave, 1994; Petit-Paly et al., 1999; Zhang and Schmidt, 2000).

Creeping bentgrass is a cool-season grass, widely used as turf on golf courses, and is sensitive to heat stress. Turf quality (color, uniformity, and density) often declines during summer months in many areas (Carrow, 1996). High root-zone temperature is more detrimental for plant growth than high air temperature, which can affect various physiological processes in shoots (Kuroyanagi and Paulsen, 1988; Huang and Xu, 2000; Paulsen, 1994; Ruter and Ingram, 1990, 1992; Udomprasert et al., 1995; Xu and Huang, 2000a, 2000b, 2001). Reducing root-zone temperature from 35 °C to 20 °C significantly improved shoot growth and photosynthetic

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activities (Xu et al., 2002). However, physiological factors involved in root-temperature effects on shoot growth of cool-season grasses are not well understood. Specifically, it is unclear whether shoot injury of cool-season grasses induced by high root-zone temperatures is associated with changes in cytokinin status and oxidative damage of leaves. Previous studies examined cytokinins and antioxidant responses to heat stress in creeping bentgrass focused on effects of air temperature and worked only with one temperature (35 °C) (Liu and Huang, 2002). The critical level of high root-zone temperature that induces changes in cytokinin status and antioxidant enzyme activities in creeping bentgrass has not been determined.

The objectives of this study were to 1) investigate cytokinin content, the activities of antioxidant enzymes, and lipid peroxidation in shoots as affected by high root-zone temperatures for two creeping bentgrass cultivars differing in heat tolerance, 'Penn A-4' and 'Putter'; 'Penn A-4' was relatively more heat tolerant than 'Putter' based on summer performance, evaluated by visual turf quality and shoot density, on golf green conditions (USGA/GCSAA/NTEP, 1998); and 2) to determine the level and duration of high root-zone temperatures that induce a decline in turf quality, cytokinin production, enzyme antioxidant activities, and lipid peroxidation in shoots.

Materials and Methods

PLANT MATERIALS AND GROWTH CONDITIONS. Two-year-old sod pieces of two creeping bentgrass cultivars, Putter and Penn A-4, were collected from field plots in Hort Farm II, Rutgers University, North Brunswick, N.J. Sod pieces were washed with water to remove the soil and then transplanted into clear polyethylene bags (5 cm in diameter and 40 cm in length, with eight holes drilled at the bottom for drainage), which were filled with washed sand (particle size of 0.2 to 0.5 mm) commonly used on golf greens. The polyethylene bags were placed in opaque polyvinylchloride (PVC) tubes of the same diameter and length, which were installed vertically in water baths with the lower open end exposed from the bottom of the water bath for drainage (Fig. 1) (Xu and Huang, 2001). The tubes were designed to enable plant growth to occur in well-drained sand in polyethylene tubes, while root-zone temperature was controlled at constant levels.

Plants were grown in a growth chamber at 20 °C (day/night), 500 mmol·m⁻²·s⁻¹ photoflux density, and a 14-h photoperiod for 60 d before the treatments were imposed. Before and during the experiment, the turf was mowed daily at a 3-mm height with scissors, watered daily, and fertilized weekly with 50 mL of full strength Hoagland's nutrient solution (Hoagland and Arnon, 1950).

Shoots were maintained at 20 °C in the growth chamber. Root-zone temperatures were controlled at a constant day/night level of: 20 (control), 21, 22, 23, 25, 27, 31, and 35 °C by keeping the entire root-zone (40-cm-long sand column in a polyethylene bag) in different sub-compartments in a water bath while the turf canopy was kept approximately 1.0 cm above the water level in the water bath. The corresponding canopy temperatures were 20, 19, 19, 20, 20, 21, 22, and 23 °C. Figure 1 shows the temperature-controlled water bath.

Details of the root-zone temperature-controlling system were also described in Xu and Huang (2001). A gradient of root-zone temperatures were created in separate subcompartments in a water bath. A heater was installed at one end of the water bath to heat water in this compartment to 38 °C. At another end of the water bath, cool water (20 °C) was added to maintain root-zone temperature at 20 °C. Heating and cooling of water at the opposite ends of the water bath created different temperatures inside the water bath. Water levels were maintained at the top edge of the water bath and 0.5 cm below the top edge of PVC tubes during the experimental period. Root-zone temperatures were monitored daily using thermocouples placed into the root-zone at a depth of 10 cm.

Temperature and cultivars were arranged in a split-plot randomized design with temperature as the main plot and cultivar as the subplot. Each root-zone temperature treatment was replicated four times in four different water baths in a walk-in growth chamber. Effects of root-zone temperature, cultivar, duration of treatment, and their interactions were determined by the analysis of variance according to the general linear model procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.). Differences among treatments, cultivars, and durations of treatments were determined by the least significance difference (LSD) test at the 0.05 probability level.

MEASUREMENTS. After treatments were initiated, turf quality was visually rated weekly based on color, density, and uniformity on a scale of 1 to 9 (1 is the worst where all plants were dead, and 9 is the best where all plants were healthy). Grasses rated at 6 or above were considered to have acceptable quality. Leaf samples were randomly collected from each treatment at various times of treatment and immediately put into liquid nitrogen and stored at -80 °C for the assay of cytokinins and antioxidant enzymes. Cytokinin content and antioxidant activities were measured on different samples, because of the limited amount of leaf tissue for the analyses.

Fig. 1. Diagram of a water bath controlling root-zone temperatures. The water bath was divided into nine sub-compartments that were maintained at nine distinct temperatures. Nine PVC tubes were installed in each compartment (controlled at a specific temperature level), which held nine polyethylene bags with plants. A heater was installed at one end of the water bath to heat water in this compartment to 38 °C. At another end of the water bath, cool water (20 °C) was added to maintain a root-zone temperature at 20 °C. Heating and cooling of water at the opposite ends of the water bath created a temperature gradient inside the water bath.

To extract antioxidant enzymes, 0.5 g frozen leaves were ground using a tissue grinder in 8 mL of 50 mM ice-cold phosphate buffer (pH 7.0) containing 1 % (w/v) polyvinylpyrrolidone and 0.2 g of white quartz sand, which were placed in an ice bath. The homogenate was centrifuged at 15,000 g_n for 20 min at 4 °C. The supernatant was used for the assay of enzyme activity and the level of lipid peroxidation.

The activity of SOD was determined by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) following the method of Giannopolitis and Ries (1977). The reaction solution (3 mL) contained 50 μ M NBT, 1.3 μ M riboflavin, 13 mM methionine, 75 nM EDTA, 50 mM phosphate buffer (pH 7.8), and 20 μ L enzyme extract, with non-enzyme solution as the control. Test tubes containing the reaction solution were irradiated under a light bank at 78 μ mol·m⁻²·s⁻¹ for 15 min. The absorbance of the irradiated and nonirradiated solution at 560 nm was determined with a spectrophotometer (Spectronic Instruments, Inc., Rochester, N.Y.). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50% of the NBT photoreduction.

Activity of CAT was measured using the method of Chance and Maehly (1955). The reaction solution (3 mL) contained 50 mM phosphate buffer (pH 7.0), 15 mM H₂O₂, and 0.1 mL enzyme extract. The reaction was initiated by adding the enzyme extract. Because of the linear decline of absorbance at 240 nm within the first 3 min, changes in absorbance were read every min. One unit CAT activity was defined as an absorbance change of 0.01 units/min.

The lipid peroxidation level was determined in terms of malondialdehyde (MDA) content using the method described by Dhindsa et al. (1981). A 0.5-mL aliquot of extract was added to a tube containing 1 mL 20 % (v/v) trichloroacetic acid and 0.5 (v/v) thiobarbituric acid. The mixture was heated in a water bath at 95 °C for 30 min. After cooling to room temperature and centrifuging at 10,000 g_n for 10 min, the absorbance of the supernatant was read at 532 and 600 nm. The MDA content was calculated using the extinction coefficient of 155 mm·cm⁻¹ (Heath and Packer, 1968).

The procedure for extraction and quantification of cytokinins in leaves followed the methods described by Setter et al. (2001) with modifications. Briefly, plant samples were extracted in 80% (by volume) methanol and then partially purified with reverse-phase chromatography on columns packed with 25 mg of 40- μ m-diameter C₁₈-silica material (SPE-96, Supelco, Bellefonte, Pa.) (instead of self packed column in the original method). Samples were loaded in 100 μ L of solvent A (10 mM tri-ethylamine-acetate, pH 3.4). Hydrophilic contaminants were washed out with 200 μ L solvent B (85% solvent A, 15% methanol, v/v) (instead of 20% methanol in the original method), and then the cytokinin-containing fraction was eluted with 200 μ L solvent C (65% solvent A, 55% methanol, v/v). Corresponding radioactive chemicals (Amersham Co., Arlington Heights, Ill.) were added to each sample to monitor the loss of radioactivity during the purification step. Recovery of each cytokinin compound averaged >90% on the basis of analysis of radioactivity in non-cytokinin fractions.

An indirect competitive enzyme linked immunosorbent assay (ELISA) was used to quantify three kinds of cytokinins, trans-zeatin/zeatin riboside (Z/ZR), dihydrozeatin/dihydrozeatin riboside (DHZ/

DHZR), and isopentenyl adenosine (iPA) as previously described by Setter et al. (2001). Partially purified cytokinin samples from C₁₈ chromatography were dried in vacuo at <24 °C, then were dissolved in 100 μ L of TBST (Tris-buffered saline, containing 1 g·L⁻¹ Tween-20, pH 7.5). ELISA plates (Costar High Binding 3366, Corning, N.Y.) were coated overnight at 4 °C with 4 ng corresponding cytokinin-bovine serum albumin conjugate (MacDonald and Morris, 1985) in 200 μ L of 50 mM NaHCO₃ buffer, pH 9.6, containing 0.2 g·L⁻¹ NaN₃. Plates were washed four times with TBST, and the following were added to each well: 10 to 30 μ L of sample, sufficient additional TBST (Tris-buffered saline, containing 1 g·kg⁻¹ bovine serum albumin, pH 7.5) to bring to 100 μ L, and 100 μ L of TBST containing 1.4 μ g of monoclonal antibody. Monoclonal antibody for Z/ZR, DHZ/DHZR, and iPA were from clone t-ZR-J3-I-B3, clone DHZR-J23-II-B1, clone iPA-J40-IV-C4, respectively (Eberle et al., 1986; currently available from Agdia Inc., Elkhart, Ind.). On each plate, the C and F rows were set for zeatin-riboside, dihydrozeatin-riboside, or isopentenyladenosine (Sigma Chem. Co., St. Louis, Mo.) standards containing a 1:2 dilution series; 12 values from 20 to 0.01 pmol/well served as a calibration curve in each assay. After incubation overnight at 4 °C and four washes by TBST, 200 μ L secondary antibody solution containing 10 nL of antimouse IgG-alkaline phosphatase conjugate (A-3562, Sigma Chemical Co., St. Louis, Mo.) in TBST was added per well. After incubation overnight at 4 °C and four washes of TBST, 200 μ L of *p*-nitrophenyl phosphate (PNPP) solution (1 g·L⁻¹ PNPP, 0.9 M diethanolamine, 3 mM MgCl₂, pH 9.8) was added for color development, and plates were incubated for about 1 h at 24 °C. The absorbance at 405 nm was read with a plate reader (model EL 800, Bio-Tek Instruments, Inc., Winooski, Vt.). Z/ZR, DHZ/DHZR, and iPA content were determined based on their calibration standards and a logit transformation of data (Wang et al., 2002). Assays were validated for absence

Fig. 2. Turf quality of 'Penn A-4' (heat tolerant) and 'Putter' (heat susceptible) creeping bentgrasses as affected by root-zone temperatures. Vertical bars at the top of the graphs indicate LSD values ($p = 0.05$) for cultivar comparisons within a given soil temperature treatment. Vertical bars on the right indicate LSD values ($p = 0.05$) for comparisons of different soil temperature treatments within a given cultivar: (A) 19 d after treatment; (B) 26 d after treatment; (C) 33 d after treatment; (D) 40 d after treatment.

Fig. 3. Responses of shoot superoxide dismutase (SOD) activity of 'Penn A-4' (heat tolerant) and 'Putter' (heat susceptible) creeping bentgrasses to high soil temperatures. Vertical bars at the top of the graphs indicate LSD values ($p = 0.05$) for cultivar comparisons within a given soil temperature treatment. Vertical bars on the right indicate LSD values ($p = 0.05$) for comparisons of different soil temperature treatments within a given cultivar: (A) 16 d after treatment; (B) 23 d after treatment; (C) 33 d after treatment; (D) 43 d after treatment.

of interfering substances after chromatography as described by Pengelly (1986). Such tests indicated that a) when an equal quantity of hormone standard was added to various quantities of samples, prepared as described above, parallel curves were obtained, indicating a linearly additive response throughout the range, and b) when extracts were diluted, ELISA estimates were consistent with the dilution.

Results

TURF QUALITY. High root-zone temperatures had no effect on turf quality of either cultivar at 19 d of treatment (Fig. 2A). Turf quality of 'Putter' declined significantly at root-zone temperatures of 31 °C at 26 d, while turf quality of 'Penn A-4' declined under the 35 °C temperature regime only at 40 d of treatment (Fig. 2B, D). Turf quality of 'Penn A-4' was significantly higher than 'Putter' at 31 and 35 °C at 26, 33, and 40 d of treatment.

ANTIOXIDANT ENZYME ACTIVITY. SOD activity of 'Putter' declined below the control level (20 °C) when root-zone temperature was maintained at 25 °C or above for 33 d (Fig. 3C); this decline occurred at 27 °C at 16 d of treatment (Fig. 3A). SOD activity of 'Penn A-4' did not change under any temperature at 16 d. The decline of SOD activity of 'Penn A-4' at 27 °C occurred only at 43 d of treatment (Fig. 3D).

The severity of the decline in SOD activity increased with temperature and the duration

of treatment for both cultivars. After 16 d of treatment (Fig. 3A) SOD activity for Penn A-4 was maintained at a higher level than 'Putter' at 27 °C. 'Penn A-4' had higher SOD activity than 'Putter' maintained at 31 °C for 23 d (Fig. 3B), at 27 °C for 33 d (Fig. 3C), and at 27 and 31 °C for 43 d (Fig. 3D). The cultivar difference diminished when root-zone temperatures were maintained at 35 °C.

CAT activity declined below the control level (20 °C) when root-zone temperature was maintained at 27 °C and above for 23 d for 'Putter' and at 31 °C and above at 23 d for 'Penn A-4' (Fig. 4B). At 16 d of treatment at 31 °C, 'Putter' had a lower CAT activity than 'Penn A-4' (Fig. 4A). The decline of CAT activity for 'Penn A-4' occurred at higher temperatures and later during the treatment than 'Putter'. CAT activity for 'Penn A-4' was higher than 'Putter' at 16 d of 27 and 31 °C (Fig. 4A). The difference also occurred at 23 d at 27, 31, and 35 °C (Fig. 4B), and 43 d at 25 °C and higher levels of temperatures (Fig. 4D).

LIPID PEROXIDATION. No cultivar differences in MDA content were detected under any temperature regime at 16 d (Fig. 5A) and 23 d (Fig. 5B). At 33 d, 'Putter' had a significantly higher MDA content than 'Penn A-4' at 25 and 27 °C (Fig. 5C). At 43 d, MDA content in 'Putter' was significantly higher than that in 'Penn A-4' at 27 °C and higher temperatures (Fig. 5D).

MDA content was higher at higher root-zone temperature than at lower temperature for both cultivars (Fig. 5). Increases in MDA content occurred at 43 d at 27 °C for 'Putter' and 35 °C for 'Penn A-4'.

Fig. 4. Responses of shoot catalase (CAT) activity of 'Penn A-4' (heat tolerant) and 'Putter' (heat susceptible) creeping bentgrasses to high soil temperatures. Vertical bars at the top of the graphs indicate LSD values ($p = 0.05$) for cultivar comparisons within a given soil temperature treatment. Vertical bars on the right indicate LSD values ($p = 0.05$) for comparisons of different soil temperature treatments within a given cultivar: (A) 16 d after treatment; (B) 23 d after treatment; (C) 33 d after treatment; (D) 43 d after treatment.

Fig. 5. Responses of shoot malondialdehyde content of 'Penn A-4' and 'Putter' creeping bentgrasses to high soil temperatures. Vertical bars at the bottom of the graphs indicate LSD values ($p = 0.05$) for cultivar comparisons within a given soil temperature treatment. Vertical bars on the right indicate LSD values ($p = 0.05$) for comparisons of different soil temperature treatments within a given cultivar: (A) 16 d after treatment; (B) 23 d after treatment; (C) 33 d after treatment; (D) 43 d after treatment.

CYTOKININS. Z/ZR content of 'Putter' declined at increased root-zone temperatures; the decline occurred at 20 d at 27 °C (Fig. 6A) and 37 d at 25 °C (Fig. 6C). The decline of Z/ZR content in 'Penn A-4' at the 27 °C root-zone temperature was found at 37 d of treatment (Fig. 6C). 'Penn A-4' had a higher Z/ZR content than 'Putter' at 37 (Fig. 6C) and 47 d (Fig. 6D) at temperatures between 25 and 35 °C.

The decline of iPA content for 'Putter' started at 20 d at 27 °C (Fig. 7A) and 37 d at 25 °C (Fig. 7C). For 'Penn A-4', no iPA decline was observed at 20 d at any temperature. The decrease in iPA content at 27 °C for 'Penn A-4' was noted at 37 d (Fig. 7C). 'Penn A-4' had a higher iPA content than 'Putter' at all temperatures at or above 27 °C at 37 and 47 d of treatment (Fig. 7C and D).

The DHZ/DHZR content of both cultivars did not change for plants maintained at different root-zone temperatures within the first 20 d of treatment (Fig. 8A). For 'Putter', the decrease in DHZ/DHZR content at a 25 °C root-zone temperature started at 47 d (Fig. 8D), and it occurred at 27 °C at 37 d of treatment (Fig. 8C). For 'Penn A-4', the decrease in DHZ/DHZR content was only observed at 27 °C and higher temperatures at 37 and 47 d (Fig. 8C, D); no decrease in DHZ/DHZR content was observed for 25 °C and lower temperatures even at 47 d. The decrease of DHZ/DHZR content with higher root-zone temperatures was greater for 'Putter' than for 'Penn A-4' at 37 d of treatment (Fig. 8C).

The decline of total cytokinin content at 25 °C for 'Putter' started at 37 d (Fig. 9C). For 'Penn A-4', the decline of total cytokinin content at 27 °C started at 37 d (Fig. 9C). However, total cytokinin content in 'Penn A-4' did not show a decline at root-zone temperatures

under 25 °C by the end of this experiment (47 d) (Fig. 9D).

Discussion

High temperatures are known to induce oxidative injury in plants by inhibiting the antioxidant protection system (Burke and Oliver, 1992; Foyer et al., 1994; Gong et al., 1997; Jagtap and Bhargava, 1995). Liu and Huang (2000) reported the suppression of activity of antioxidant enzymes and the induction of lipid peroxidation when both shoots and roots of creeping bentgrass were exposed to heat stress (35 °C) in creeping bentgrass. However, limited information is available on how increasing only root-zone temperature influences the antioxidant protection system in shoots, even though root-zone temperature is found to be more critical than air temperature in the regulation of plant growth (Kuroyanagi and Paulsen, 1988; Xu and Huang, 2000a, 2000b, 2001). In the present study, the activity of SOD and CAT enzymes for both cultivars decreased at higher root-zone temperatures, even though shoots were maintained at the optimal air temperature. Our results suggested that heating roots alone decreased SOD and CAT activity, which could cause accumulation of free radical species and severe oxidative damage in shoots. MDA is a final product of peroxidation of unsaturated fatty acids in phospholipids, and it often is used as a measure of level of lipid peroxidation (Gutteridge and Halliwell, 1990). MDA content of leaves increased at higher root-zone temperatures, and this was accompanied by a decline in antioxidant enzyme activities (Figs. 3, 4, and 5).

Fig. 6. Responses of shoot zeatin and zeatin riboside content of 'Penn A-4' and 'Putter' creeping bentgrasses to high soil temperatures. Vertical bars at the top of the graphs indicate LSD values ($p = 0.05$) for cultivar comparisons within a given soil temperature treatment. Vertical bars on the right indicate LSD values ($p = 0.05$) for comparisons of different soil temperature treatments within a given cultivar: (A) 20 d after treatment; (B) 30 d after treatment; (C) 37 d after treatment; (D) 47 d after treatment.

Fig. 7. Responses of shoot isopentenyl adenosine content of 'Penn A-4' and 'Putter' creeping bentgrasses to high soil temperatures. Vertical bars at the top of the graphs indicate LSD values ($p = 0.05$) for cultivar comparisons within a given soil temperature treatment. Vertical bars on the right indicate LSD values ($p = 0.05$) for comparisons of different soil temperature treatments within a given cultivar: (A) 20 d after treatment; (B) 30 d after treatment; (C) 37 d after treatment; (D) 47 d after treatment.

The effects of root-zone temperature on antioxidant activities and lipid peroxidation varied with the level and duration of the stress, and with cultivars differing in heat tolerance. SOD activity decreased at 25 °C at 33 d for 'Putter' (Fig. 3C) and at 27 °C at 43 d for 'Penn A-4' (Fig. 3D). The decrease in CAT activity was observed at 27 °C after 23 d for 'Putter' and 31 °C at 23 d for 'Penn A-4' (Fig. 4B). For MDA, the increase started when roots were exposed to 27 °C for 43 d for 'Putter', and to 31 °C for 43 d for 'Penn A-4' (Fig. 5D). The changes in both SOD and CAT activities occurred at a lower temperature or earlier during the treatment than MDA, suggesting that the decline in activity of both enzymes led to lipid peroxidation. The cultivar differences in the responses of SOD, CAT, and MDA to higher root-zone temperatures indicate that maintenance of antioxidant activities at low levels of lipid peroxidation were related to an increased tolerance of creeping bentgrass to high root-zone temperatures.

Under prolonged heat stress conditions, leaf senescence is often accompanied by decline of endogenous cytokinin content in leaves (Cheikh and Jones, 1994). Liu et al. (2002) reported that cytokinin content in both leaves and roots decreased when both roots and shoots of creeping bentgrass were exposed to heat stress (35 °C). However, the critical root-zone temperature inducing cytokinin decline in cool-season grasses has not been reported. The root-zone temperature range that

caused a decrease in the content of all three types of cytokinins was 25 to 27 °C for 'Putter' and 27 to 31 °C for 'Penn A-4' at 37 d of treatment (Figs. 6, 7, and 8).

The cytokinin decline with higher root-zone temperatures corresponded with the changes in SOD and CAT in both cultivars. Cytokinins may suppress the production of free radicals by preventing their formation or by direct scavenging (Caers et al., 1985; Gunse and Eldtner, 1993; Leshem, 1984, 1988; Musgrave, 1994; Petit-Paly et al., 1999). Exogenous application of cytokinins has been found to increase the activities of SOD and CAT and prevent heat-induced lipid peroxidation and membrane damage in creeping bentgrass (Liu and Huang, 2002). Similar results were reported in rape (*Brassica napus* L.) (Zhou and Leul, 1999). Zhang and Schmidt (2000) reported that application of seaweed extracts containing cytokinin-like compounds increased the concentration of antioxidants in creeping bentgrass exposed to drought stress. The relative sensitivity of cytokinin and antioxidant enzymes to higher root-zone temperatures, however, could not be evaluated in our study. Cytokinin content and antioxidant assays were analyzed at different sampling times due to the limited amount of leaf samples.

Both cytokinin content and antioxidant enzyme activities were affected at lower root-zone temperatures and earlier during the treatment than turf quality in both cultivars. Turf quality started to decline at 31 °C at 26 d for 'Putter' and at 35 °C after 40 d for 'Penn A-4' (Fig. 2). Huang and Gao (2000) reported the decline in turf quality of 'L-93', 'Crenshaw' and 'Penncross' started when both air and soil temperatures increased to 30 °C for 20 d.

Fig. 8. Responses of shoot dihydrozeatin and dihydrozeatin riboside content of 'Penn A-4' and 'Putter' creeping bentgrasses to high soil temperatures. Vertical bars at the top of the graphs indicate LSD values ($p = 0.05$) for cultivar comparisons within a given soil temperature treatment. Vertical bars on the right indicate LSD values ($p = 0.05$) for comparisons of different soil temperature treatments within a given cultivar: (A) 20 d after treatment; (B) 30 d after treatment; (C) 37 d after treatment; (D) 47 d after treatment.

Fig. 9 Responses of total cytokinin content of 'Penn A-4' and 'Putter' creeping bentgrasses to high soil temperatures. Vertical bars at the top of the graphs indicate LSD values ($p = 0.05$) for cultivar comparisons within a given soil temperature treatment. Vertical bars on the right indicate LSD values ($p = 0.05$) for comparisons of different soil temperature treatments within a given cultivar: (A) 20 d after treatment; (B) 30 d after treatment; (C) 37 d after treatment; (D) 47 d after treatment.

These results suggest that shoot injury induced by high root-zone temperatures was associated with the inhibition of cytokinin production and the induction of oxidative stress. Both physiological parameters could be used as an early warning signal of heat injury in creeping bentgrass.

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