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RESEARCH PAPER

Root respiratory characteristics associated with plant adaptation to high soil temperature for geothermal and turf-type *Agrostis* species

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Abstract

Respiration is a major avenue of carbohydrates loss. The objective of the present study was to examine root respiratory characteristics associated with root tolerance to high soil temperature for two *Agrostis* species: thermal *Agrostis scabra*, a species adapted to high-temperature soils in geothermal areas in Yellowstone National Park, and two cultivars ('L-93' and 'Penncross') of a cool-season turfgrass species, *A. stolonifera* (creeping bentgrass), that differ in their heat sensitivity. Roots of thermal *A. scabra* and both creeping bentgrass cultivars were exposed to high (37 °C) or low soil temperature (20 °C). Total root respiration rate and specific respiratory costs for maintenance and ion uptake increased with increasing soil temperatures in both species. The increases in root respiratory rate and costs for maintenance and ion uptake were less pronounced for *A. scabra* than for both creeping bentgrass cultivars (e.g. respiration rate increased by 50% for *A. scabra* upon exposure to high temperature for 28 d, as compared with 99% and 107% in 'L-93' and 'Penncross', respectively). Roots of *A. scabra* exhibited higher tolerance to high soil temperature than creeping bentgrass, as manifested by smaller decreases in relative growth rate, cell membrane stability, maximum root length, and nitrate uptake under high soil temperature. The results suggest that acclimation of respiratory carbon metabolism plays an important role in root survival of *Agrostis* species under high soil temperatures, particularly for the thermal grass adaptation to chronically high soil temperatures. The ability of roots to tolerate high soil temperatures could be related to the capacity to control respiratory rates and increase respiratory efficiency by lowering maintenance and ion uptake costs.

Key words: *Agrostis*, heat tolerance, root respiration, specific respiratory costs.

Introduction

High temperature is one of the most important environmental factors limiting growth and productivity for cool-season plant species. The optimum temperature for cool-season plant growth is generally between 10 °C and 24 °C; however, in many areas, soil temperatures often reach injuriously high levels during summer, which strongly influence shoot and root growth and survival of whole plants (Paulson, 1994). The biomass production for cool-season perennial grasses often declines with increasing temperatures, and plant death occurs at temperatures above 30 °C (DiPaola, 1992). Roots play critical roles in plant survival in environments with high soil temperatures, due, in part, to their lower optimum temperature range for growth, lower acclimation potential to extreme conditions, and higher sensitivity to fluctuations of environmental conditions (Nielsen, 1974). Moreover, the numerous functions of roots, including uptake of water and nutrients and synthesis and translocation of hormones such as cytokinin and ABA, are very sensitive to heat stress (McMichael and Burke, 1999). Shoot responses to high temperature stress

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have been studied extensively. However, roots have been investigated much less than above-ground parts, in spite of their importance to whole-plant responses to environmental stresses, largely due to limited accessibility in soil (McMichael and Taylor, 1987). The mechanisms controlling root growth and mortality at high soil temperatures are not well understood. Investigation into how roots survive in high-temperature soils is critical for the improvement of plant tolerance to heat stress.

One approach to understanding mechanisms of plant tolerance to stresses has been to examine plants adapted to extremely stressful environments. Several cool-season, C_3 perennial grass species have recently been identified growing in geothermally heated areas in Yellowstone National Park (YNP) (Stout and Al-Niemi, 2002). The predominant grass species in the thermal areas are *Dichanthelium lanuginosum* and *Agrostis scabra* ('thermal' rough bentgrass). These species grow well in the chronically hot soils that are permeated by hot steams. Little is known about why and how thermal plants survive and even thrive in hot soils. Delmer (1974) studied the thermostability of cytoplasmic proteins and shoot growth as affected by high temperature for another species (*Mimulus guttatus*) growing in the thermal areas and did not find specific heat-adaptation mechanisms in the above-ground parts. In their study of root responses to increasing soil temperatures for the thermal *Dichanthelium lanuginosum*, Stout *et al*. (1997) found that the grasses developed shorter and more highly branched roots at high soil temperatures than at low temperatures, but the nature of the alteration of root morphology has not yet been addressed. Recent research by Stout and Al-Niemi (2002) found that plants collected from the geothermal soils in YNP had strong expression of heat shock proteins (HSP101 and sHSP) in both roots and leaves when exposed to short-term heat shock (34 °C, 42 °C, and 48 °C), with higher levels of HSP induction in roots than in leaves of those plants.

Respiration is a major sink for carbohydrates. Carbon lost through respiration can account for up to 50% of the daily carbon gain by photosynthesis (Morgan and Austin, 1983; Poort *et al*., 1995). Respiration generally increases with temperatures with a short-term Q_{10} of about 2 (Lambers, 1985). Shortage of assimilates due to high respiratory losses has long been proposed to be a primary factor responsible for root growth inhibition and dysfunction (Youngner and Nudge, 1868). Heat-tolerant roots may be able to control their respiration rate or use more efficient respiratory pathways. Increasing temperature by 4 °C (from 16 °C to 20 °C) caused a 30% increase in root respiration rates in three species—two perennial grasses, *Dactylis glomerata*, and *Poa annua*, and *Bellis perennis*. However, *B. perennis*, that was better acclimated to high temperature conditions, maintained a slower respiration rate than the other two species (Gunn and Farrar, 1999). The critical question remains how plants adapted to high soil temperatures control root respiration rates to survive high temperatures for prolonged periods of time.

The rate of respiration depends on three major energy-requiring processes: maintenance of biomass, growth, and ion uptake transport and assimilation (Poorter *et al*., 1991). High respiratory carbon consumption has been found to be mainly due to high specific respiratory maintenance costs (Lawrence and Oechel, 1983; Scheurwater *et al*., 1998). Maintenance respiration increases with increasing temperatures (McCree, 1974; McCree and Amthor, 1982; Kase and Catsky, 1984), and its response is similar to the increased response of total respiration. The specific respiratory costs for growth, however, are usually thought to be relatively unaffected by temperature (Johnson and Thornley, 1885); i.e. as long as the substrate and product compositions do not change in response to temperature. In addition, even in a comparison of 24 species with a 3.5-fold variation in relative growth rate (RGR), growth respiration changed <20% (Poorter *et al*., 1991). While specific respiratory costs for maintenance and growth have frequently been determined (for reviews, see Amthor, 1984; Lambers, 1985), the specific respiratory costs for ion uptake have been little investigated (Scheurwater *et al*., 1998). Scheurwater *et al*. (1998) calculated costs of ~6 mmol O_2 g^{-1} DW for ion uptake by assuming costs for growth respiration similar to values found by Poorter *et al*. (1991). Their calculations were made for plants for which NO_3^- was the sole nitrogen source; therefore, the major costs of ion uptake were for NO_3^- uptake and assimilation. They concluded that the major cause for the relatively slow rates of root respiration in fast-growing grasses was lower specific respiratory costs for ion uptake.

The objective of this study was to investigate whether root tolerance to high soil temperature is associated with a tighter control of respiration rate and with lower respiratory costs for maintenance and ion uptake in two *Agrostis* species differing in their heat tolerance: thermal *Agrostis scabra* adapted to geothermally heated soils in YNP, and two cultivars of creeping bentgrass (*A. stolonifera*), 'L-93' and 'Penncross'. Creeping bentgrass is a cool-season perennial grass and widely cultivated as turfgrass in cool-climate regions, and is sensitive to high temperatures (DiPaola, 1992). Root membrane permeability, RGR, maximum root length, and net NO_3^- uptake rate were determined to evaluate heat tolerance of roots. Total respiration rate (measured by both CO_2 evolution and O_2 consumption rates) and respiratory costs associated with maintenance, growth, and NO_3^- uptake were assessed to compare respiratory responses to high soil temperature of two *Agrostis* species.

Materials and methods

Plant materials

Plants (8–10 tillers) from thermal *Agrostis scabra*, and two cultivars, 'L-93' and 'Penncross' of creeping bentgrass (*A. stolonifera*) were

established in polyethylene bags filled with sand (5 cm in diameter and 40 cm in length) in a greenhouse. Holes were made at the bottom of the bags for drainage.

Plants were watered daily and fertilized twice a week with a nutrient solution containing 1 mM KNO_3, 0.2 mM $CaSO_4$, 0.35 mM KH_2PO_4, 2 mM $MgSO_4$, 0.2 g l^{-1} Fe-NaEDTA, and micronutrients (Epstein, 1972). After establishment for 6 weeks in the greenhouse, plants were transferred to a controlled-environment walk-in growth chamber (Conviron, Winnipeg, Canada) with a 14 h photoperiod, 20/15 °C day/night temperature, and a photosynthetic photon flux density of 350 mmol m^{-2} s^{-1} at canopy height. The plants were placed in tubes built in specially designed water baths. The tubes were designed to enable plant growth to occur in well-drained sand in polyethylene bags, while the root-zone temperature was constant. The detailed description of the system was presented in Wang *et al*. (2003). Plants were allowed to acclimate to growth chamber conditions for 1 week before temperature treatments were imposed.

Treatments and experimental design

Control plants were maintained at a constant air/soil temperature of 20 °C, in which roots were exposed to 20 °C in a water bath and shoots were exposed to ambient air of 20 °C in the growth chamber. For the high soil temperature treatment, shoots were maintained at 20 °C day/night air temperature, while roots were exposed to a constant day/night temperature of 37 °C in the water bath as described above. The temperature treatment was repeated in four water baths placed in the walk-in growth chamber. The entire root zone (a 40-cm-long sand column in a polyethylene bag) was kept in the water bath while the turf canopy was kept ~1.0 cm above the water level, with the water level maintained at the top edge of the water bath. The water-bath temperature was controlled with an immersion-circulating heater. The root-zone temperature was monitored daily using thermocouples located in the root zone at a depth of 10 cm. The root-zone temperature at 10 cm depth during the study was maintained at 37 °C for the high soil-temperature treatment and at 20 °C for the control plants.

Root respiration measurement

At weekly intervals, following the initiation of the heat treatment, roots were washed free of sand and transferred to 500 ml Erlenmeyer flasks containing the nutrient solution used to fertilize the plants, as described above. Shoots were sealed with a rubber stopper around the base into an Erlenmeyer flask, maintaining a 150 ml air space with 400 ml nutrient solution. The hydroponic solution in each flask was aerated via a circulating pump (Apollo Enterprises Inc., Oxnard, CA, USA) maintaining an open-flow system. Flasks were submerged in a water bath to maintain root temperature at either 20 °C or 37 °C, while shoots were exposed to the ambient air temperature (20 °C) of the growth chamber. After a 24 h period, a closed-flow system was created by connecting the exit air from the Erlenmeyer flask to a circulating pump. The rubber stopper was sealed with vacuum grease and Teflon tape in order to create air-tight conditions. The system was tested for leaks before each sampling. The air was sampled via a septum valve from each flask for the measurements of CO_2 evolution and O_2 consumption every 10 min for a total period of 60 min, using 1 ml air-tight syringes. Sampling started 3 h into the daily light period. Air samples were analysed via gas chromatography.

The concentrations of CO_2 and O_2 collected in the hydroponic system were measured using a gas chromatograph (model GC-8AIT, Shimadzu, Kyoto, Japan) with two injectors connected to two columns, one for CO_2 (model 8011/2; Alltech, Deerfield, IL, USA) and one for O_2 (model MR62827; Supelco, St Louis, MO, USA). The column temperature was set at 30 °C, and the detector has a thermal-conductivity set at 100 mA. Sampled air (0.5 ml) was injected into an injection port of either a CO_2 column or an O_2 column. CO_2 and O_2 concentrations were plotted versus time, and respiration rates were calculated from the regressions of either CO_2 evolution or O_2 consumption over time. In addition to the closed-flow system measurements of root respiration via gas chromatography, open-flow system measurements of CO_2 evolution were made using an infrared gas analyser (IRGA) (Li-Cor 6400, Li-Cor, Inc. Lincoln, NB, USA). The Erlenmeyer's exit air was connected to the sample inlet of the IRGA. The results were compared with results obtained using gas chromatography, and differences between the methods were found to be not significant (P >0.1). The data for respiration measurements using the closed-flow system are reported in this paper. The respiratory quotient (RQ) was calculated as CO_2 evolution/O_2 consumption rate.

Following CO_2 and O_2 sampling, roots were harvested and analysed for maximum length, dry weight, and membrane permeability. Roots were then dried in an oven at 80 °C for 72 h for dry weight determination.

Maximum root length and relative growth rate

Maximum root length was assessed as the length of the longest roots of a plant. Root RGR was determined as the slope of the natural logarithm of root dry mass versus time, and expressed as mg g^{-1} d^{-1} (Hunt, 1982).

Membrane permeability

Membrane permeability of fresh roots was assessed by measuring electrolyte leakage. Roots (0.2 g FW) were rinsed with deionized water, immersed in 20 ml deionized water, and shaken for 24 h on a shaker table (Lab-Line Instruments, Inc., Melrose Park, IL, USA). The initial conductivity of the solution was measured with a conductivity meter (YSI Model 32, Yellow Spring, OH, USA). The roots were then killed by autoclaving at 120 °C for 20 min, and the conductivity of the incubation solution with the killed tissues (maximum conductivity) was measured. Relative electrolyte leakage was calculated as the percentage of the initial conductivity over the maximum conductivity of the incubation solution with fresh and killed roots, respectively.

Net nitrate uptake rate (NNUR)

Dry root samples were used to determine total N content with a C-H-N elemental analyser (Model EA 1108, Fisons Instruments, Beverly, MA, USA). The final NNUR per unit weight was calculated from RGR, total nitrogen content per plant, and the fraction of total biomass invested in roots (Garnier, 1991). NNUR gives a good approximation of total anion uptake (Veen, 1981).

Specific respiratory costs

Using a linear regression approach described by Scheurwater *et al*. (1998), specific respiratory costs for maintenance of biomass and specific respiratory costs for growth and ion uptake of roots were determined using the following equation:

$$R_t = R_m + C(g+u) \times RGR$$

The slope of the regression line of the total rate of root respiration (R_t, mmol O_2 g^{-1} DW d^{-1}) versus RGR gives the specific respiratory costs for growth including ion uptake [$C(g+u)$, mmol O_2 g^{-1} DW]. The y intercept of the regression line gives specific respiratory costs for maintenance of biomass (R_m, mmol O_2 g^{-1} DW d^{-1}). The costs for root growth and the costs for ion uptake were separated using three different values for growth costs as described by Scheurwater *et al*. (1998). The commonly used value for respiratory cost of growth is 6.5 mmol O_2 g^{-1} DW, and two alternative values were used with the assumption that respiratory costs for growth could either be 25%

higher (8.13 mmol O_2 g^{-1} DW) or 25% lower (4.88 mmol O_2 g^{-1} DW) (Poorter *et al*., 1991; Scheurwater *et al*., 1998). The use of the above equation is based on the following assumptions: (i) plants with similar RGR have similar respiratory costs for growth; (ii) respiratory costs for growth are relatively constant, and independent of growth temperature or RGR; within a 4-fold change in RGR, the respiratory costs for growth changes only within a range <25% (Poorter *et al*., 1991); (iii) given that nitrogen is the major mineral nutrient needed for plant biomass, the major costs of anion uptake in NO_3^--fed plants are associated with NO_3^- uptake, transport, and assimilation; (iv) no diurnal variation in shoot and root respiration and an average constant rate of net photosynthesis under constant day/night temperatures. Multiplying these costs for growth by the RGR values resulted in the rate of root respiration necessary for growth. By subtracting the portion of root respiration associated with growth from total root respiration, the rate of respiration for maintenance and ion uptake was obtained. The slope of the regression of root respiration rates for maintenance and ion uptake against the rates of net nitrate uptake gives specific respiratory costs for ion uptake expressed per mole of nitrate.

Statistical analyses

The experiment was a completely randomized design with measurements made at weekly intervals. An analysis of variance was conducted [PROC GLM using repeated measures (using sum of squares type III) in SAS 8.02, SAS Institute, Cary, NC, USA] to determine the effects of temperature and time and their interaction on RGR, maximum root length, membrane permeability, NNUR, CO_2 evolution, O_2 consumption, and RQ. Treatment means were separated using the least significance test at P=0.05. Relationships between RGR and respiration were determined with linear regression equations. Significant differences between regression lines were tested using analysis of variance. Probabilities of <5% were deemed significant.

Results

Root tolerance to high soil temperature

Root membrane permeability increased significantly (P <0.05) at high temperatures (37 °C) in all species/genotypes compared with their respective controls (20 °C) (Fig. 1). The smallest increase was found in thermal *A. scabra* (83%) after roots had been subjected to 7 d at 37 °C (Fig. 1), as compared with 225% and 208% increases in 'L-93' and 'Penncross', respectively. The highest increase (420%) was detected in 'Penncross' after 14 d at 37 °C, as compared with 177% and 269% increases in *A. scabra* and 'L-93', respectively, after 14 d. The increase in root membrane permeability was similar for 'L-93' and 'Penncross' after 21 d at 37 °C. However, after 28 d, root membrane permeability was significantly lower (P <0.05) in 'L-93' than in 'Penncross' (Fig. 1).

Maximum root length decreased significantly (P <0.05) in 'L-93' and 'Penncross' after 7 d at high soil temperature (Fig. 2b, c), but the decline in thermal *A. scabra* was not observed until 21 d (Fig. 2a). *Agrostis scabra* exhibited a less severe decrease in maximum root length than 'L-93' and 'Penncross' throughout the entire experimental period. By 28 d at 37 °C, maximum root length had decreased by 64% in both 'L-93' and 'Penncross' (Fig. 2b, c), and by 51% in *A. scabra* (Fig. 2a).

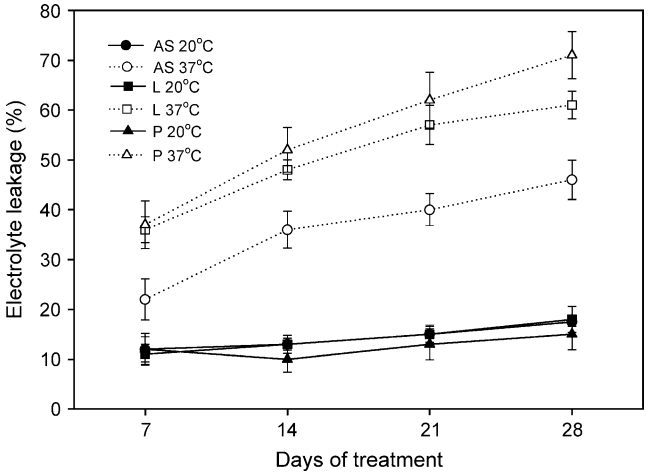

Fig. 1. Root membrane permeability expressed as electrolyte leakage of two *Agrostis* species: *Agrostis scabra* (circles) and two creeping bentgrass (*A. stolonifera*) cultivars, 'L-93' (squares) and 'Penncross' (triangles), in response to soil temperature: 20 °C (continuous lines and filled symbols) and 37 °C (dotted lines and open symbols). Error bars represent standard errors (n=5 or 6).

The RGR of roots decreased over time in all species/genotypes, irrespective of root temperature, 20 °C or 37 °C (Fig. 3). Effects of high temperature on RGR were similar to effects on maximum root length. Thermal *A. scabra* had a significantly (P <0.05) higher RGR at 37 °C than 'Penncross' and 'L-93' throughout the entire experimental period. In both 'Penncross' and 'L-93', RGR decreased by >80% at 37 °C compared with roots grown at 20 °C, whereas RGR in thermal *A. scabra* decreased by 65%.

NNUR decreased significantly (P <0.05) in all species at 37 °C compared with NNUR at 20 °C, except at 28 d for thermal *A. scabra*. The largest decrease was a 50% reduction in 'Penncross' at 7 d, as compared with 19% and 36% in thermal *A. scabra* and 'L-93', respectively, at 7 d (Fig. 4).

Root respiration rate in response to high soil temperature

Root respiration measured either as O_2 consumption or as CO_2 evolution increased significantly (P <0.05) at high temperature in all species/genotypes, but was affected by the time of exposure to high temperature and varied among species/genotypes (Figs 5, 6). O_2 consumption rate at 37 °C increased above that at 20 °C, beginning at 7 d after exposure for both 'L-93' and 'Penncross', but not until 21 d after exposure to high soil temperature for thermal *A. scabra* (Fig. 5). The largest increase (108%) was found in 'Penncross' after 28 d at 37 °C, as compared with a 51% and 99% increase in *A. scabra* and 'L-93', respectively, after 28 d. The effects of high temperatures on CO_2 evolution (Fig. 6) were similar to the effects on O_2 consumption (Fig. 5) in both species at both 20 °C and 37 °C.

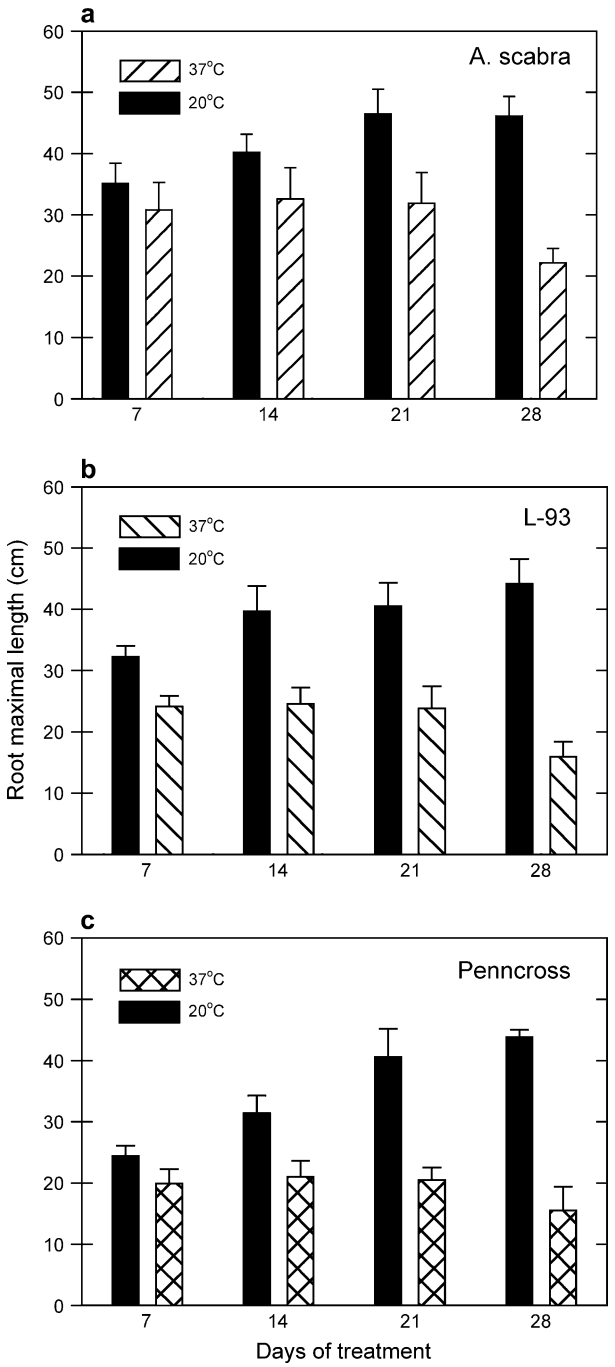

Fig. 2. Maximum root length of two *Agrostis* species: *Agrostis scabra* (a) and two creeping bentgrass (*A. stolonifera*) cultivars, 'L-93' (b) and 'Penncross' (c), in response to soil temperature: 20 °C (filled columns) and 37 °C (hatched columns). Error bars represent standard errors (n=5 or 6).

The respiratory quotient (RQ), the ratio of moles of CO_2 evolved per mole of O_2 consumed, decreased significantly (P <0.05) at 37 °C compared with that at 20 °C in all species (Fig. 7). RQ was not significantly different (P >0.05) between 'Penncross' and 'L-93' at 37 °C. However, RQ values in the thermal *A. scabra* were significantly (P <0.05) higher than those in both 'Penncross' and 'L-93' at 37 °C.

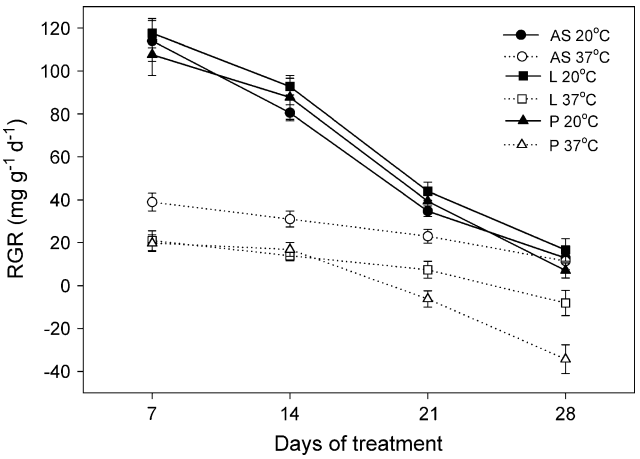

Fig. 3. Relative growth rate (RGR) of two *Agrostis* species: *Agrostis scabra* (circles) and two creeping bentgrass (*A. stolonifera*) cultivars, 'L-93' (squares) and 'Penncross' (triangles), in response to soil temperature: 20 °C (continuous lines and filled symbols) and 37 °C (dotted lines and open symbols). Error bars represent standard errors (n=5 or 6).

At 20 °C, RQ values did not differ significantly among species/genotypes (P >0.05) (Fig. 7).

Specific respiratory costs for maintenance and ion uptake

The correlation between the O_2-consumption rate and RGR was used to calculate specific respiratory costs for maintenance. The y intercept of the regression line is an estimate of maintenance respiration costs. Specific respiratory costs for maintenance were higher at 37 °C (1.8, 2.7, and 3.0 mmol O_2 g^{-1} DW d^{-1} in *A. scabra*, 'L-93', and 'Penncross', respectively) than those at 20 °C (1.2, 1.1, and 1.2 mmol O_2 g^{-1} d^{-1} in *A. scabra*, 'L-93', and 'Penncross', respectively) (Fig. 8). The smallest increase at 37 °C was 53% in thermal *A. scabra*, and the largest increase was 152% in 'Penncross'. The maintenance costs for 'L-93' increased by 134% at 37 °C, and were not significantly different from the costs for 'Penncross' (P >0.05), whereas the maintenance costs of thermal *A. scabra* at 37 °C were significantly lower (P <0.05) than those for both 'L-93' and 'Penncross' (Fig. 8).

Specific respiratory costs for ion uptake increased significantly (P <0.05) in roots grown at 37 °C in both species, under the assumption that growth costs were either 6.50 or 8.13 mmol O_2 g^{-1} DW (Table 1). When the growth cost was assumed to be 4.88 mmol O_2 g^{-1} DW, costs for ion uptake at 37 °C increased only in 'L-93' and 'Penncross', but did not change significantly (P >0.05) for thermal *A. scabra*. The largest increases in specific respiratory costs for ion uptake in all species/genotypes were found under the assumption that the cost for growth was 8.13 mmol O_2 g^{-1} DW. 'Penncross' and thermal *A. scabra*, respectively, exhibited the largest and smallest increases in costs for ion uptake, irrespective of what assumption was made with respect to growth cost.

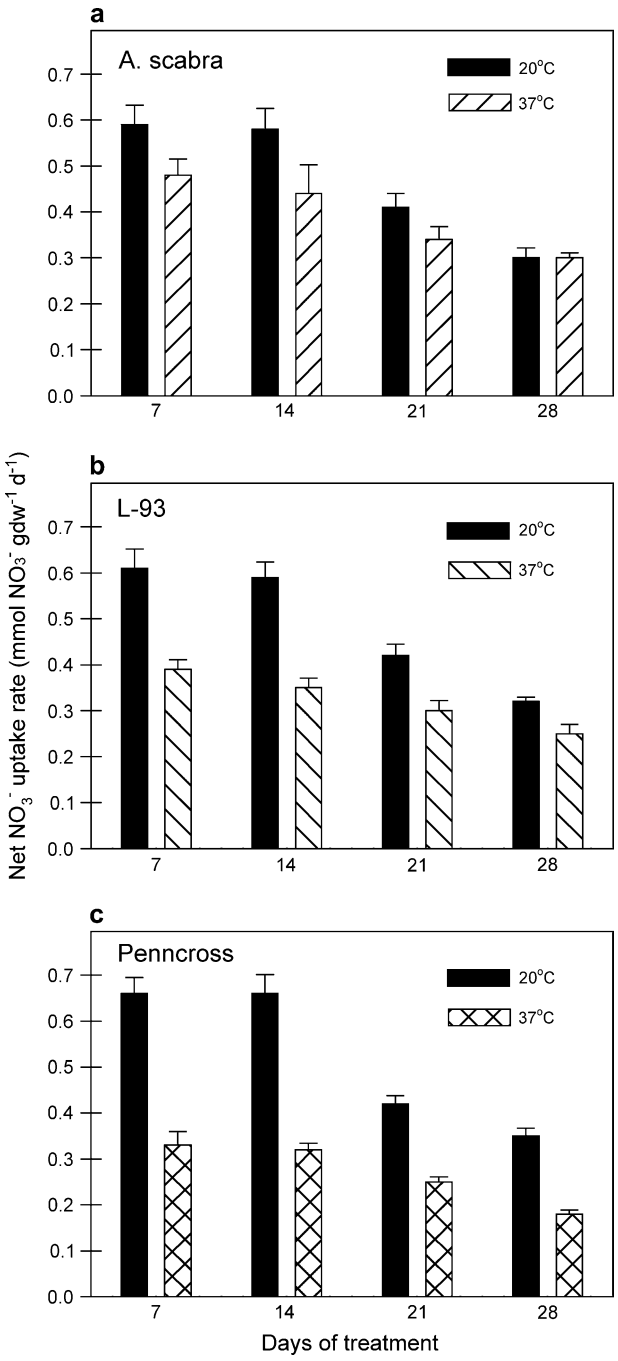

Fig. 4. Net nitrate uptake (mmol NO_3^- g^{-1} DW d^{-1}) of two *Agrostis* species: *Agrostis scabra* (a) and two creeping bentgrass (*A. stolonifera*) cultivars, 'L-93' (b) and 'Penncross' (c), in response to soil temperature: 20 °C (filled columns) and 37 °C (hatched columns). Error bars represent standard errors (n=5 or 6).

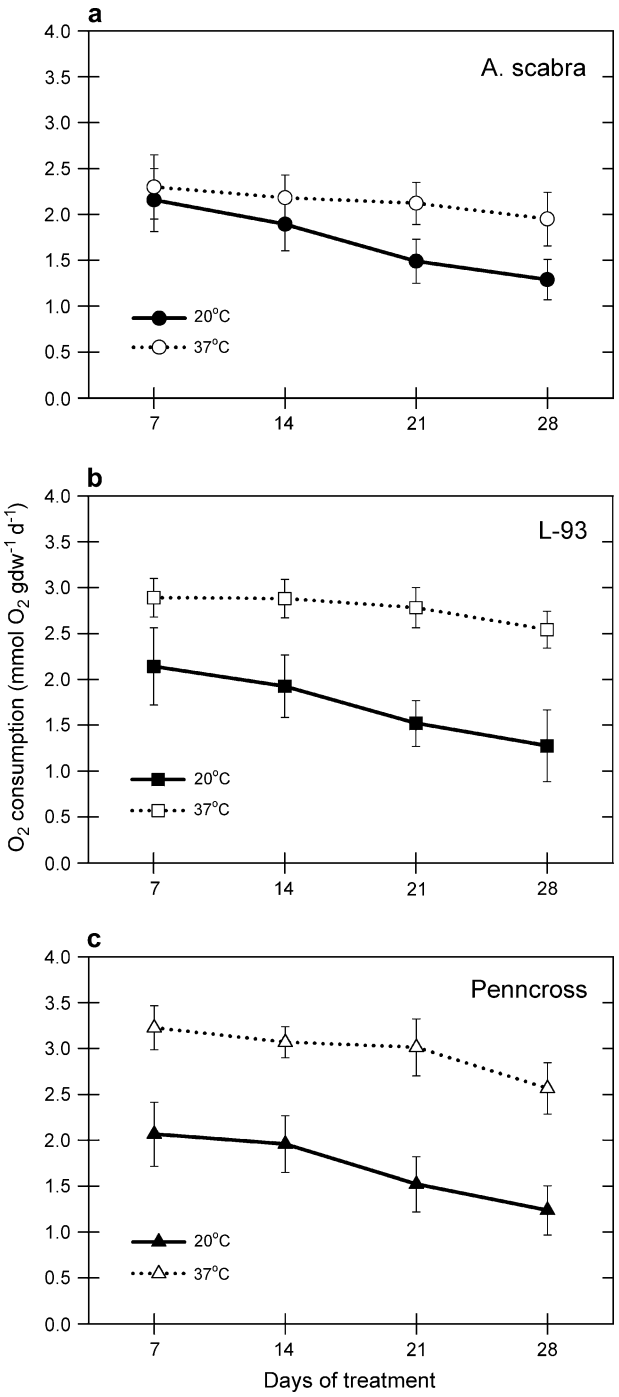

Fig. 5. Root respiration expressed rate as O_2 consumption rate (mmol O_2 g^{-1} DW d^{-1}) of two *Agrostis* species: *Agrostis scabra* (a) and two creeping bentgrass (*A. stolonifera*) cultivars, 'L-93' (b) and 'Penncross' (c) in response to soil temperature: 20 °C (continuous lines and filled symbols) and 37 °C (dotted lines and open symbols). Error bars represent standard errors (n=5 or 6).

Discussion

Roots are sensitive to high soil temperatures (Cooper, 1973). Pote *et al*. (2006) reported that soil temperatures of 23 °C or above are detrimental to root growth and activities of *A. stolonifera*. In the present study, maximum root length, root RGR, and nitrate uptake decreased for thermal *A. scabra* and both cultivars of *A. stolonifera* following a prolonged exposure to 37 °C soil temperature. Comparing root responses to high soil temperature between thermal *A. scabra* and two cultivars of *A. stolonifera*, roots of

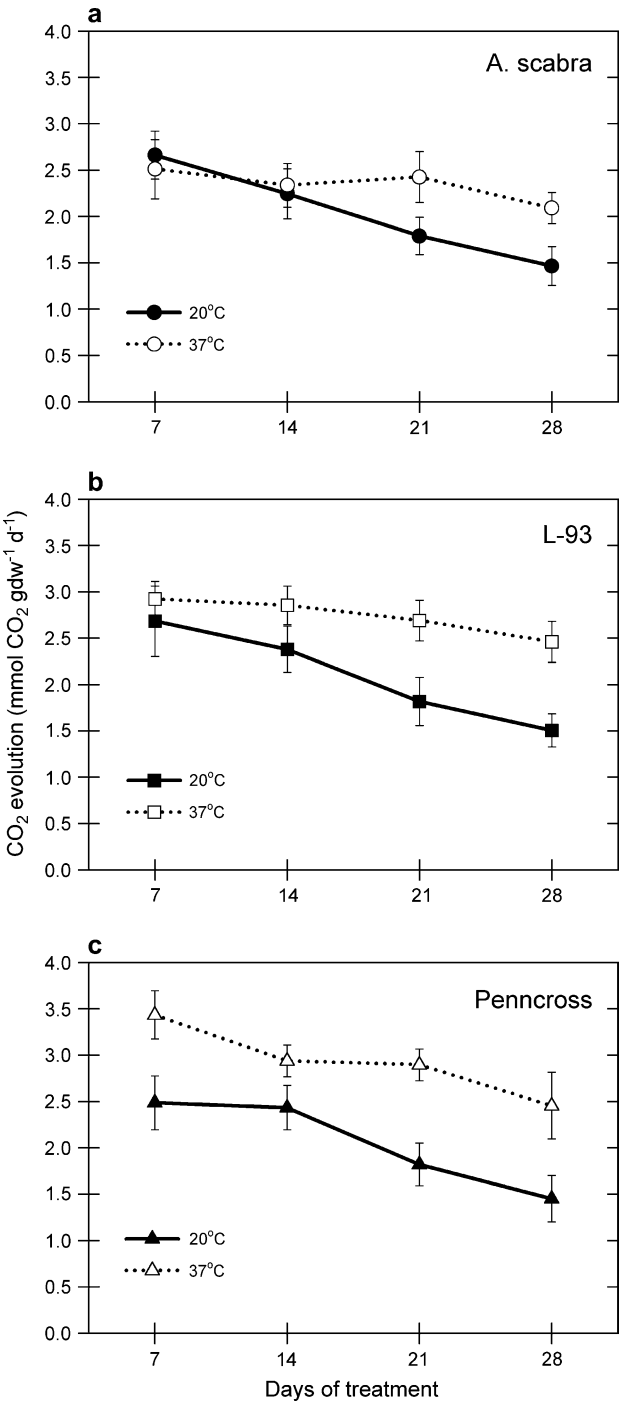

Fig. 6. Root respiration expressed rate as CO_2 evolution rate (mmol CO_2 g^{-1} DW d^{-1}) of two *Agrostis* species: *Agrostis scabra* (a) and two creeping bentgrass (*A. stolonifera*) cultivars, 'L-93' (b) and 'Penncross' (c) in response to soil temperature: 20 °C (continuous lines and filled symbols) and 37 °C (dotted lines and open symbols). Error bars represent standard errors (n=5 or 6).

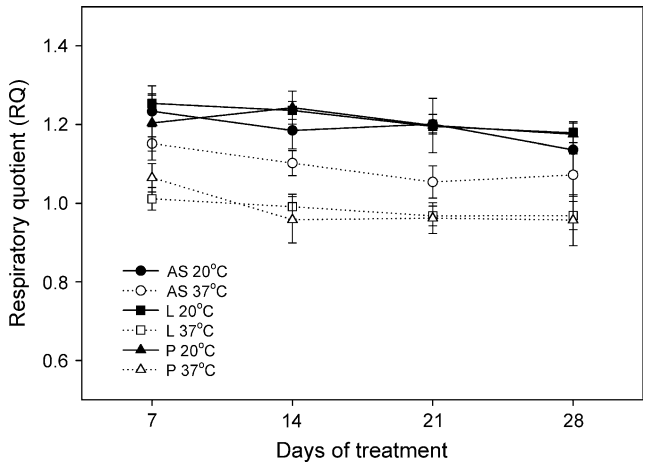

Fig. 7. Respiratory quotient of root respiration (moles of CO_2 evolved per mole of O_2 consumed) of two *Agrostis* species: *Agrostis scabra* (circles) and two creeping bentgrass (*A. stolonifera*) cultivars, 'L-93' (squares) and 'Penncross' (triangles), in response to soil temperature: 20 °C (continuous lines and filled symbols) and 37 °C (dotted lines and open symbols). Error bars represent standard errors (n=5 or 6).

'Penncross' were the most heat sensitive and roots of thermal *A. scabra* exhibited the highest tolerance to high soil temperature.

High soil temperature affects both shoot and root physiology and growth (Xu and Huang, 2000; Huang and Fu, 2001). The adverse effects of high soil temperature on plant growth and physiological activities have been attributed to direct inhibition of root growth and activity, and to limitation of nutrient and water uptake (Itai and Benzioni, 1974). Most terrestrial plants absorb nutrients required for growth via their roots. Nitrogen is the major nutrient required for plant growth, and the major costs for anion uptake in plants are towards NO_3^- uptake, transport, and assimilation. NO_3^- reduction is a highly carbohydrate-intensive process (Noctor and Foyer, 1998). The present results indicate that changes in NNUR exhibited the same trend as that for RGR, maximum root length, and membrane stability in response to heat stress. Thermal *Agrostis scabra* had the lowest decreases in NNUR, whereas decreases in NNUR in 'Penncross' were the highest at 37 °C. The thermal species generally maintained a higher RGR, maximum root length, cell membrane stability, and nitrate uptake under prolonged periods of high soil temperature stress, which could account for its long-term adaptation to geothermally heated soils at YNP.

Respiration is sensitive to temperatures within the plant's physiological range (Amthor, 1989) and, in most cases, has a short-term Q_{10} of around 2 (Lambers, 1985). In the present study, exposure of roots to 37 °C resulted in an increase in root respiration rates, assessed as O_2 consumption, in both *A. stolonifera* cultivars, beginning at 7 d of exposure to 37 °C. In the thermal *A. scabra*, a significant increase in root respiration rate was not observed until 21 d of exposure to heat stress. The present results on root respiration rate, assessed as CO_2 evolution, are consistent with those on O_2 consumption, except for 'L-93' in which respiration did not increase until 14 d at 37 °C. Respiration is a major sink for carbohydrates. Fast respiratory rates have

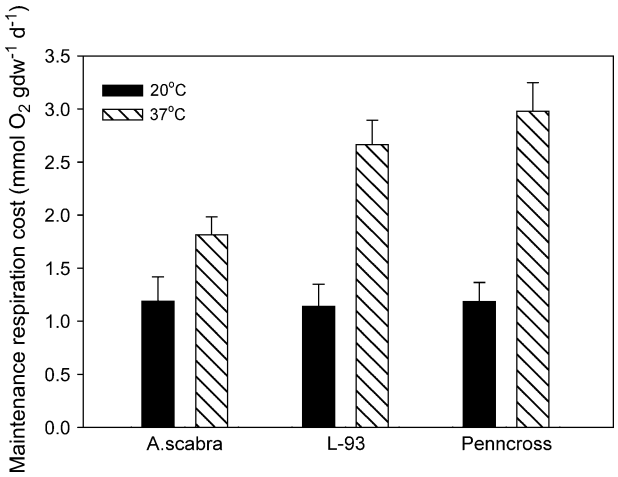

Fig. 8. Specific respiratory costs of maintenance respiration (mmol O_2 g^{-1} DW d^{-1}) of two *Agrostis* species: *Agrostis scabra* and two creeping bentgrass (*A. stolonifera*) cultivars, 'L-93' and 'Penncross', in response to soil temperature: 20 °C (filled columns) and 37 °C (hatched columns). Error bars represent standard errors (n=5 or 6).

Table 1. *Change in specific respiratory costs for ion uptake [C_w, mol O_2 (mol NO_3^-)$^{-1}$] estimated assuming three different values for specific respiratory costs for growth (C_g, mmol O_2 g^{-1} DW) for* Agrostis scabra *and two cultivars, 'L-93' and 'Penncross', of* Agrostis stolonifera *in response to two different soil temperatures (20 °C and 37 °C)*

Values are mean ±standard error. Different letters indicate significant differences (P <0.05) between species and temperatures at each specific growth respiratory cost (n=5 or 6).

Root temperature (°C)	Estimate of C_g (mmol O_2 g^{-1} DW)	*A. scabra*	L-93	Penncross
20	6.50	0.67±0.04 a	0.68±0.03 a	0.56±0.04 b
37	6.50	0.83±0.05 c	1.19±0.11 d	1.50±0.14 e
20	8.13 (+25%)	0.17±0.03 a	0.13±0.04 a	0.11±0.07 a
37	8.13 (+25%)	0.63±0.04 a	0.86±0.07 b	0.91±0.08 b
20	4.88 (–25%)	1.18±0.18 a	1.23±0.07 a	1.01±0.11 a
37	4.88 (–25%)	1.03±0.16 a	1.51±0.04 b	2.08±0.12 c

long been proposed to be a primary factor responsible for root-growth inhibition (Heichel, 1971; Barneix *et al*., 1984; Lambers *et al*., 1996). The present results show that the effect of high temperature on respiration rate is positively associated with the increases in cell membrane damage and negatively related to RGR and maximum root length. Comparing the differences in respiration rates, expressed either as O_2 consumption or CO_2 evolution, to root growth and membrane stability between thermal *A. scabra* and *A. stolonifera* revealed that greater tolerance of roots to high soil temperature of thermal *A. scabra* was correlated with its greater control of respiration. Therefore, slower respiration rates in the thermal *A. scabra* are largely accounted for by its greater capacity to maintain membrane stability at high temperatures.

Monitoring both net CO_2 and O_2 fluxes permitted calculation of the respiratory quotient (RQ, ratio of CO_2 evolved and O_2 consumed). The RQ is associated with NO_3^- assimilation and strongly depends on NO_3^- reduction, because reducing equivalent generated in the catabolism of carbohydrates to CO_2 is transferred to NO_3^- and NO_2^-, rather than O_2 (Lambers *et al*., 1998). The present results confirmed the correlation between RQ and NNUR. RQ values decreased in both species at 37 °C; however, the decrease in the two *A. stolonifera* cultivars was significantly greater than the decrease in thermal *A. scabra*, in accordance with greater maintenance of membrane stability and root growth.

Specific respiratory costs for maintenance for both *Agrostis* species were similar to the specific respiratory cost for maintenance of barley genotypes (Bloom *et al*., 1992) and of *Dactylis glomerata* and *Festuca ovina* grasses (Scheurwater *et al*., 1998) estimated in other studies. In the present study, maintenance respiration increased at high soil temperatures. These results are similar to other reports of increasing maintenance respiration as a response to increasing temperature (McCree, 1974; McCree and Amthor, 1982; Kase and Catsky, 1984). Scheurwater *et al*. (1998) found that the major difference in specific respiratory costs between slow- and fast-growing grasses was the cost of ion uptake, whereas changes in maintenance costs were small. In the present study, both ion uptake (calculated assuming three different growth costs) and maintenance costs were significantly lower in thermal *A. scabra* than in both cultivars of *A. stolonifera* under high soil temperature conditions, suggesting that in the heat-adapted species, heat tolerance of roots was related to the ability to maintain membrane stability and control specific respiratory costs and respiratory efficiency by maintaining lower maintenance and ion uptake costs.

In summary, a soil temperature of 37 °C inhibited root growth and nitrogen uptake, but increased cell permeability and root respiration rate for both *Agrostis* species; however, roots of the thermal *A. scabra* exhibited higher tolerance to high soil temperature than both cultivars of *A. stolonifera*. Thermotolerance of *A. scabra* roots was related to lower specific respiratory costs for maintenance and ion uptake, and the maintenance of higher respiratory and ion-acquisition efficiencies as indicated by smaller changes in respiratory costs, NNUR, and RQ. Therefore, it is inferred that, in order to survive at chronically high-soil temperatures, heat-adapted roots of thermal *A. scabra* could control respiratory costs and increase their respiratory efficiency by lowering their maintenance and ion acquisition costs. The present study elucidated the importance of root respiration control in root thermotolerance, particularly for the unique thermal *Agrostis* species from YNP. Such mechanisms could be incorporated in breeding or genetic engineering programmes to improve heat tolerance of cultivated cool-season grass species.

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References

Amthor JS. 1984. The role of maintenance respiration in plant growth. *Plant, Cell and Environment* **7,** 561–569.

Amthor JS. 1989. *Respiration and crop productivity.* New York: Springer-Verlag.

Barneix AJ, James DM, Watson EF, Hewitt EJ. 1984. Some effects of nitrate abundance and starvation on metabolism and accumulation of nitrogen in barley (*Hordeum vulgare* L. cv Sonja). *Planta* **162,** 469–476.

Bloom AJ, Sukrapanna SS, Warner RL. 1992. Root respiration associated with ammonium and nitrate absorption and assimilation by barley. *Plant Physiology* **99,** 1294–1301.

Cooper AJ. 1973. Root temperature and plant growth: a review. *Commonwealth Bureau of Horticulture and Plantation Crops* **4,** 73.

Delmer DP. 1974. Studies on the nature of adaptation of the monkey flower, *Mimulus gutattus*, to a thermophilic environment. *Candian Journal of Botany* **52,** 1509–1514.

DiPaola J. 1992. Physiological responses of turfgrasses to heat stress. In: Waddington DV, Carrow RN, Shearman RC, eds. *Turfgrass.* Madison, WI: Agronomy Society of America.

Epstein E. 1972. *Mineral nutrition of plants: principles and perspectives.* New York, NY: John Wiley & Sons.

Garnier E. 1991. Above- and below-ground resource capture in herbaceous plants: relationships with growth and biomass allocation. *Trends in Ecology and Evolution* **6,** 126–131.

Gunn S, Farrar JF. 1999. Effects of a 4 °C increase in temperature on partitioning of leaf area and dry mass, root respiration and carbohydrates. *Functional Ecology* **13,** 12–20.

Heichel GH. 1971. Confirming measurements of respiration and photosynthesis with dry matter accumulation. *Photosynthetica* **5,** 93–98.

Huang B, Fu J. 2001. Growth and physiological responses of tall fescue to surface soil drying. *International Turfgrass Society Research Journal* **9,** 291–296.

Hunt R. 1982. *Plant growth curves: the functional approach to plant growth analysis.* Baltimore, MD: University Park Press.

Itai C, Benzioni A. 1974. Regulation of plant response to high temperature. *Royal Society of New Zealand Bulletin* **12,** 477–482.

Johnson IR, Thornley JHM. 1985. Temperature dependence of plant and crop processes. *Annals of Botany* **55,** 1–24.

Kase M, Catsky J. 1984. Maintenance and growth componenets of dark respiration rate in leaves of C_3 and C_4 plants as affected by leaf temperature. *Biologia Plantarum* **26,** 461–470.

Lambers H. 1985. Respiration in intact plants and tissues: its regulation and dependence on environmental factors, metabolism and invaded organisms. In: Douce R, Day DA, eds. *Encyclopedia of plant physiology.* Berlin: Springer-Verlag.

Lambers H, Stulen I, Van Der Werf A. 1996. Carbon use in root respiration as affected by elevated atmospheric O_2. *Plant and Soil* **187,** 251–263.

Lawrence WT, Oechel WC. 1983. Effects of soil temperature on the carbon exchange of taiga seedlings. *Canadian Journal of Forestry Research* **13,** 840–849.

McCree KJ. 1974. Equations for the rate of dark respiration of white clover and grain sorghum, as functions of dry weight, photosynthetic rate, and temperature. *Crop Science* **14,** 509–514.

McCree KJ, Amthor ME. 1982. Effects of diurnal variation in temperature on the carbon balances of white clover plants. *Crop Science* **22,** 822–827.

McMichael BL, Taylor HM. 1987. Applications and limitations of rhizotrons and minirhizotrons. In: Taylor HM, ed. *Minirhizotron observation tubes: methods and applications for measuring rhizosphere dynamics.* Madison, WI: ASA, CSSA, SSA, 1–14.

McMichael BL, Burke JJ. 1999. Temperature effects on root growth. In: Eshel Y, Kafkafi U, Waisel Y, eds. *Plant roots: the hidden half.* New York, NY: Marcel Dekker.

Morgan CL, Austin RB. 1983. Respiratory loss of recently assimilated carbon in wheat. *Annals of Botany* **51,** 85–95.

Nielsen KF. 1974. Roots and root temperatures. In: Carson EW, ed. *The plant root and its environment.* Charlottesville, VA: University Press Virginia, 293–333.

Noctor G, Foyer CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49,** 249–279.

Paulsen GM. 1994. High temperature responses of crop plants. In: Boote KJ, Bennett JM, Sinclair TR, Paulsen GM, eds. *Physiology and determination of crop yield.* Madison, WI: ASA, CSSA, SSA, 365–389.

Poorter H, Van Der Werf A, Atkin OK, Lambers H. 1991. Respiratory energy requirements of roots vary with the potential growth rate of plant species. *Physiologia Plantarum* **83,** 469–475.

Poorter H, Vandevijver C, Boot RGA, Lambers H. 1995. Growth and carbon economy of a fast-growing and a slow-growing grass species as dependent on nitrate supply. *Plant and Soil* **171,** 217–227.

Pote J, Wang Z, Huang B. 2006. Timing and temperature of physiological decline in creeping bentgrass. *Journal of The American Society for Horticulture Science* (in press).

Scheurwater I, Cornelissen C, Dictus F, Welschen R, Lambers H. 1998. Why do fast- and slow-growing grass species differ so little in their rate of root respiration, considering the large differences in rate of growth and ion uptake? *Plant, Cell and Environment* **21,** 995–1005.

Stout RG, Al-Niemi TS. 2002. Heat-tolerant flowering plants of active geothermal areas in Yellowstone National Park. *Annals of Botany* **90,** 259–267.

Stout RG, Summers ML, Kerstetter T, McDermott TR. 1997. Heat- and acid-tolerance of a grass commonly found in geothermal areas within Yellowstone National Park. *Plant Science* **130,** 1–9.

Veen BW. 1981. Relation between root respiration and root activity. *Plant and Soil* **63,** 73–76.

Wang Z, Huang B, Xu Q. 2003. Effects of abscisic acid on drought responses of Kentucky bluegrass. *Journal of the American Society for Horticultural Science* **128,** 36–41.

Xu Q, Huang B. 2000. Growth and physiological responses of creeping bentgrass to changes in air and soil temperatures. *Crop Science* **40,** 1363–1368.

Youngner VB, Nudge FG. 1968. Growth and carbohydrate storage of three *Poa pratensis* L. strains as influenced by temperatures. *Crop Science* **8,** 455–456.