RESEARCH PAPER

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

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Received 14 June 2005; Accepted 7 November 2005

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...
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, C₃ 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 Q₁₀ 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 O₂ 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 NO₃⁻ was the sole nitrogen source; therefore, the major costs of ion uptake were for NO₃⁻ 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 NO₃⁻ uptake rate were determined to evaluate heat tolerance of roots. Total respiration rate (measured by both CO₂ evolution and O₂ consumption rates) and respiratory costs associated with maintenance, growth, and NO₃⁻ 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 KNO₃, 0.2 mM CaSO₄, 0.35 mM KH₂PO₄, 2 mM MgSO₄, 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 growth chamber. After a 24 h period, a closed-flow system was imposed. Shoots were exposed to the ambient air temperature (20 °C, in which roots were exposed to 20 °C in the water bath as described above). 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 found to be not significant (P > 0.1). The data for respiration measurements using the closed-flow system are reported in this paper. The respiralmost quotient (RQ) was calculated as CO₂ evolution/O₂ consumption rate.

Following CO₂ and O₂ 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:

\[ R_i = R_m + C(g + u) \times RGR \]

The slope of the regression line of the total rate of root respiration (\( R_i \)), mmol O₂ g⁻¹ DW d⁻¹) versus RGR gives the specific respiratory costs for growth including ion uptake [C(g + u), mmol O₂ g⁻¹ DW]. The y intercept of the regression line gives specific respiratory costs for maintenance of biomass (\( R_m \), mmol O₂ 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 O₂ 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 O₂ g⁻¹ DW) or 25% lower (4.88 mmol O₂ 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 NO₃⁻/C255 was not detected in ‘Penncross’ after 14 d at 37 °C, as compared with 225% and 208% increases in ‘L-93’ and ‘Penncross’, respectively. The highest increase (420%) was found in ‘Penncross’ after 28 d at 37 °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 O₂ consumption or as CO₂ 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). O₂ 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 CO₂ evolution (Fig. 6) were similar to the effects on O₂ consumption (Fig. 5) in both species at both 20 °C and 37 °C.
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.

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.
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 *Agrostis*. 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...
“Penncross” were the most heat sensitive and roots of thermal Agrostis 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 Agrostis stolonifera cultivars, beginning at 7 d of exposure to 37°C. In the thermal Agrostis 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
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 O₂ consumption or CO₂ 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 CO₂ and O₂ fluxes permitted calculation of the respiratory quotient (RQ, ratio of CO₂ evolved and O₂ consumed). The RQ is associated with NO₃ assimilation and strongly depends on NO₃ reduction, because reducing equivalent generated in the catabolism of carbohydrates to CO₂ is transferred to NO₃ and NO₂, rather than O₂ (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.
Acknowledgements

This research was supported by a USDA grant (2003-00759). We thank Dr H Frenkel for his help with the gas chromatography. Thanks also go to Michelle DaCosta and Yan Xu for critically reviewing of the manuscript.

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