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## PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 290 projects at a cost of $25 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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# Root Carbon Metabolism Associated with Bentgrass Tolerance to Heat Stress

Bingru Huang

## SUMMARY

Researchers at Rutgers University investigated root growth characteristics, carbon balance, and root respiration rate in relation to root thermotolerance for two bentgrass species contrasting in heat tolerance. The overall goal of the project was to identify physiological and metabolic mechanisms controlling heat tolerance in cool-season turfgrass species, specifically bentgrass. The study's findings included:

- Tolerance to high temperature was compared between thermal *A. scabra*, adapted to warm soils in geothermal areas in Yellowstone National Park, and creeping bentgrass (*A. stolonifera*) by exposing roots to optimum growth temperature 20°C or supraoptimal soil temperatures in water baths in growth chambers.
- Root viability, growth rate, and cell membrane stability data demonstrated that roots of thermal *A. scabra* were more thermotolerant than creeping bentgrass. Root respiration of thermal *A. scabra* was less responsive to increasing soil temperatures and significantly lower than creeping bentgrass at high soil temperatures.
- Lower root respiration may contribute to higher root thermotolerance in thermal grass. Both ion uptake and maintenance respiratory costs were significantly lower in thermal *A. scabra* than in creeping bentgrass at high temperature. Root thermotolerance could be related to the capacity of tighter control respiration by lowering maintenance and ion uptake costs.
- Carbon investment in growth of roots exposed to high soil temperatures was higher, while that invested in respiration was significantly lower, for thermal *A. scabra* than for creeping bentgrass.
- Heat tolerance in cool-season turfgrasses could be also related to efficient carbon use and adjustment of allocation patterns between growth and respiration.

High temperature is one of the most important environmental factors limiting growth and productivity of cool-season turfgrass species. Various studies suggest that high rootzone or soil temperature is more detrimental than high air temperature for the growth of shoots and roots, and roots play a critical role in regulating whole-plant responses to high air/soil temperature stress (14, 15, 16, 18). The significance of the root systems in plant survival of high temperatures is, in part, due to their high sensitivity to high temperatures and essential functions of hormone synthesis, water and nutrient uptake in plant growth. Therefore, protecting roots from high soil temperature stress is important in maintaining turfgrass growth during summer months.

Shoot responses to high-temperature stress have been studied extensively. However, roots have been investigated much less than above-ground parts, despite their importance to whole-plant responses to heat stress. The fundamental question remains of how roots can maintain viability and function under high soil temperature conditions. Insight into mechanisms controlling root growth and survival in high-temperature soils is critical for the improvement of turfgrass tolerance to heat stress.

*Agrostis scabra* collected near steam vents in Yellowstone National Park (shown above) exhibited root respiration less responsive to increasing soil temperatures and significantly lower than creeping bentgrass at high soil temperatures.

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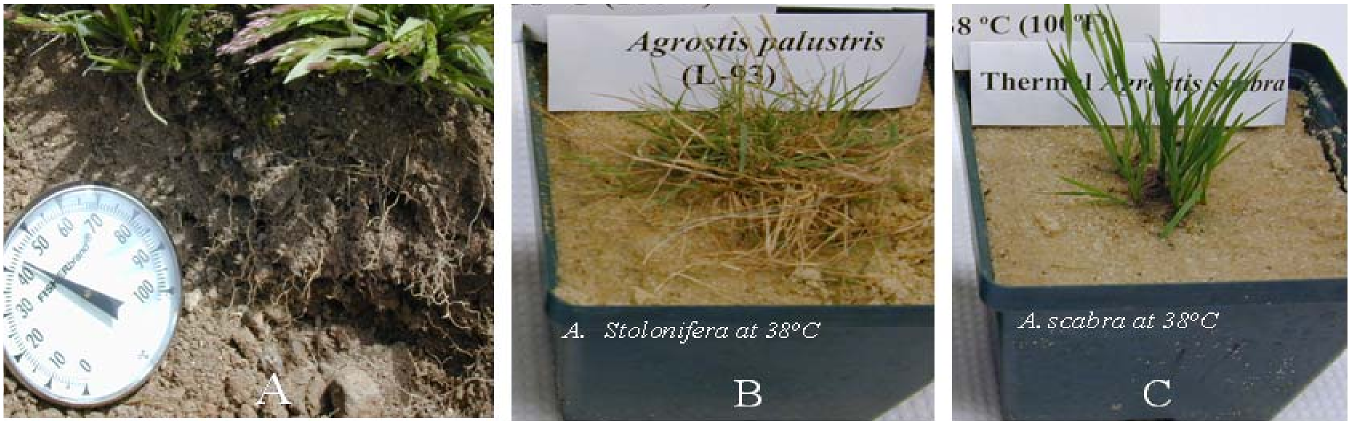

**Figure 1.** Soil temperature at a 5-cm depth was approximately 45$^{o}$ C at a thermal site in Yellowstone Naitonal Park (A) where thermal *Agrostis scabra* plants grow, showing healthy roots and leaves. Heat-sensitive creeping bentgrass (B) is compared to heat-tolerant thermal *A. scabra* (C) where both species were exposed to air/soil temperatures of 25/38 C for 24 hours in a growth chamber.

Among the physiological factors that form the basis of root growth and functions, carbohydrate metabolism including carbon and energy use efficiency is of fundamental importance because roots completely depend on shoots for the supply of assimilate. Carbon supply to roots typically decreases while root respiratory consumption increases with increasing temperatures. Excessive consumption of assimilates for maintenance of roots is a significant factor limiting plant productivity, particularly under stressful environments (8).

Maintaining low root respiration rate, particularly maintenance costs, has been associated with fast growth rate and with high temperature adaptation and root survival for various plant species (4, 10). Down-regulation of maintenance respiration can conserve carbon and energy, which may prolong root survival of high temperatures. Metabolic regulation of energy requirements for maintenance processes essential to root survival of heat stress is largely unexplored.

## Unique Plant Materials for the Study of Root Thermotolerance in Cool-season Grass Species

One approach to understand mechanisms of plant tolerance to stresses has been to examine plants adapted to extremely stressful environments. Several cool-season grass species have recently been identified growing in geothermally heated areas in Yellowstone National Park (YNP) (12, 13). The predominant grass species in thermal areas are *Dichanthelium lanuginosum* and *Agrostis scabra* ("thermal" bentgrass). Soil temperatures in the surface 5 cm near geothermal vents where thermal *A. scabra* are found range from 20 to 50$^{o}$ C, while air temperature range from 15 to 27$^{o}$ C from May to August in 2004 and 2005 (Huang, unpublished data). These geothermal grass species can survive and even grow at temperatures up to 45-50$^{o}$ C in soils that are permeated by steams (Fig. 1A). When exposed to 38$^{o}$ C soil temperature in a growth chamber, creeping bentgrass (cv. L-93) suffered severe injury, as indicated by leaf senescence (Fig. 1B); in contrast, thermal bentgrass plants maintained active growth (Fig. 1C).

Little is known about why and how roots of thermal bentgrass can grow in hot soils that are lethal to cool-season turfgrass species. The optimum temperature for commonly used cool-season turfgrasses is between 10-18$^{o}$ C for roots and 15-24$^{o}$ C for shoots. Physiological injury of roots in creeping bentgrass was observed occurred when soil temperature reached 23$^{o}$ C (9). A better understanding of underlying maintenance processes may provide crucial clues on how to manipulate plant characteristics to reduce the amount of assimilates consumed in these processes, ultimately resulting in improved heat tolerance.

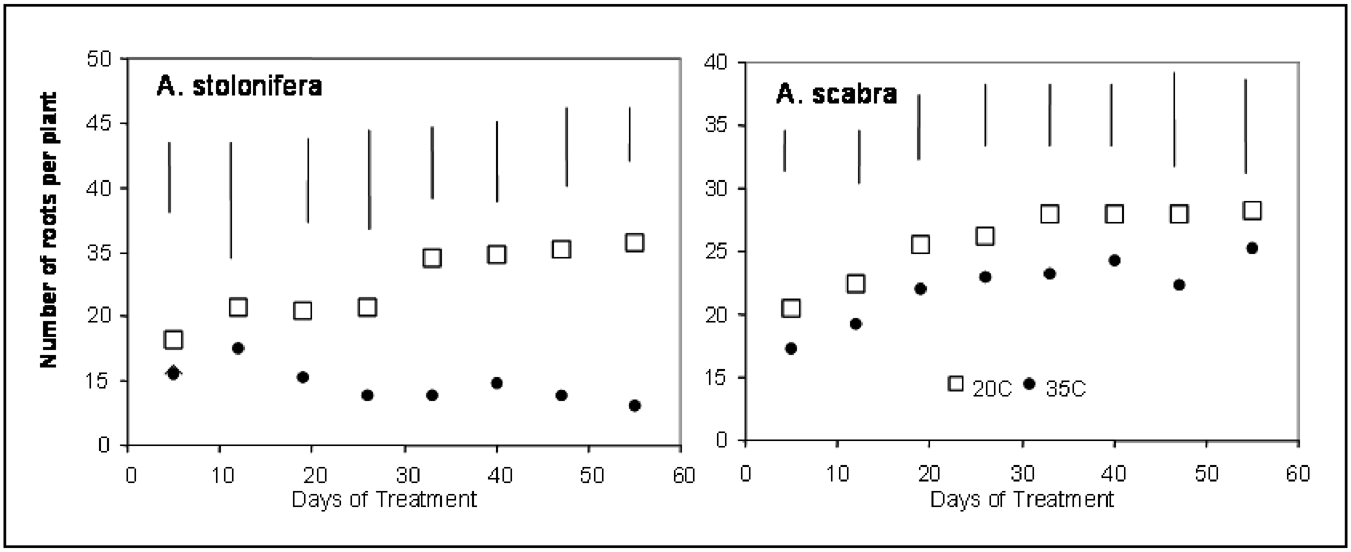

**Figure 2.** Root production of creeping bentgrass (*Agrostis stononifera*) and thermal *A. scabra* exposed to 20° or 35° C soil temperatures. Creeping bentgrass exhibited root decline, whereas *A. scabra* had increased root production during heat treatments. Vertical bars indicate valuses of least significant differences (p = 0.05)..

## Respiratory Carbon Metabolism in Relation to Heat Tolerance

Physiological studies conducted recently in our lab indicated that root thermotolerance is critical for cool-season grass tolerance to high soil temperatures (5, 14, 15, 16, 17, 18, 19). We also compared gene expression and protein levels in roots between thermal *A. scabra* and creeping bentgrass under heat-stress conditions. Our study found differential expression of genes and proteins in roots between the two species contrasting in heat tolerance (Xu and Huang, unpublished data). Several other studies from our lab found that thermal *A. scabra* was able to maintain high root viability and continue to produce new roots in high-temperature (35° C) soils that are detrimental to creeping bentgrass (Figure 2; 7, 10).

In a study of root responses to increasing soil temperatures for thermal *D. lanuginosum*, Stout et al. (12) found that thermal grasses developed shorter and more highly branched roots at high soil temperatures than at low temperatures. Limited studies have examined mechanisms of root tolerance to high soil temperatures for temperate plant species, but the few available studies suggest that roots play a critical role in plant adaptation to high temperatures. How perennial grass roots proliferate and survive at high soil temperatures is not well understood. Understanding mechanisms of root thermotolerance is essential for improving plant tolerance to high temperatures, especially for warm climatic areas where grass quality and productivity are limited due to high temperatures during summer.

Our studies suggested that root survival in high-temperature soils in thermal bentgrass was associated with more efficient carbon utilization and allocation, including tighter control of respiratory costs or down-regulation of root respiration, when compared with heat-sensitive creeping bentgrass (Figures 3 and 4; 10, 11). We also examined the potential role of maintenance respiration and nonphosphorylating pathways such as alternative respiration in controlling total root respiration sensitivity in thermal bentgrass in comparison to creeping bentgrass. Our results indicated that the control of root respiration increases in response to increasing temperature in thermal bentgrass was mainly associated with lower maintenance respiration.

## Respiratory Acclimation in Relation to Heat Stress Adaptation

Temperature is one of the important fac-

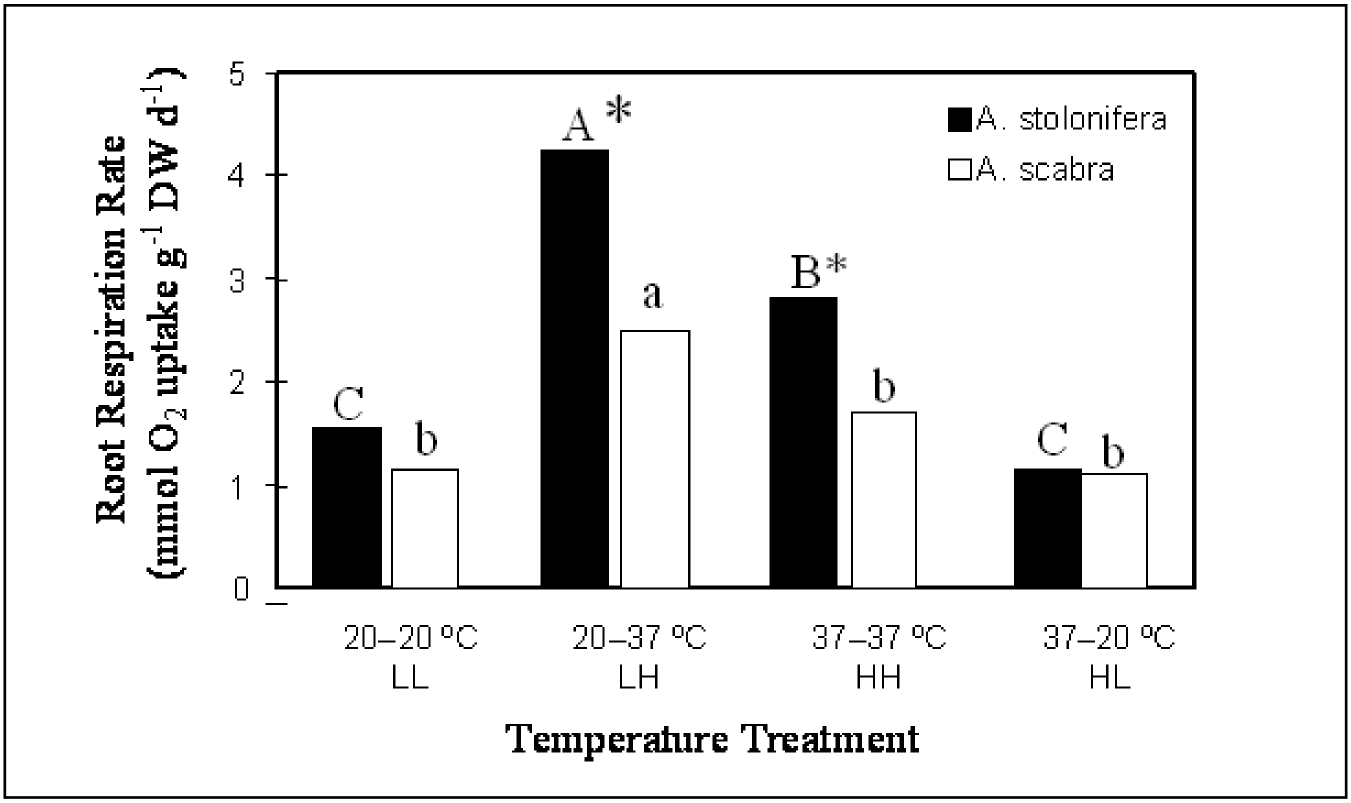

**Figure 3.** Root respiration of creeping bentgrass (*Agrostis stolonifera*) and *A. scabra* grown at 20 or 37° C for 17 days and measure at 20° or 37° C. LL represents grown and measured at 20°C. LH represents gown at 20°C and measure at 20°C. Different uppercase (*A. stolonifera*) and lowercase (*A. scabra*) letters indicate differences in root respiration among temperature treatments (P=0.05). Asterisks (*) represent sigificant differences between species at a given temperature.

tors affecting respiratory rates of plants. Long-term temperature exposure to certain temperatures can result in respiratory acclimation, the adjustment of respiration rates to compensate for a change in temperature (1). Acclimation may result in respiratory homeostasis, the maintenance of identical rates of respiration in plants grown at different temperatures. Thermal acclimation of root respiration has received far less attention than acclimation of above-ground processes.

Limited data on root acclimation to temperatures suggest that the ability for root respiration acclimation varies with plants species. We compared total root respiration responses to short-term (24 h) exposure to low temperature (20° C) or high soil temperature (37° C) for thermal *A. scabra* and heat-sensitive creeping bentgrass grown at 20° C or 37° C (Figure 3). We found that when plants previously grown at 20° C were transferred to 37° C (LH), root respiration rate increased dramatically for creeping bentgrass. Root respiration rate for thermal *A. scabra* was less responsive to temperature changes than that for creeping bentgrass. Thermal *A. scabra* maintained lower root respiration rates than creeping bentgrass when previously grown at 20° C and transferred to 37° C (LH) or grown and measured at 37° C (HH).

Other researchers reported that some species such as *Poa costiniana* (6) and *Dactylis glomerata* (3) show no ability for acclimation of root respiration, and other species such as *Festuca ovina* and *Poa annua* exhibit full acclimation or homeostasis (3). Plants with a large degree of acclimation exhibit less variation in relative growth rate with temperature than those with a low degree of acclimation, showing that acclimation in respiration coincided with acclimation in

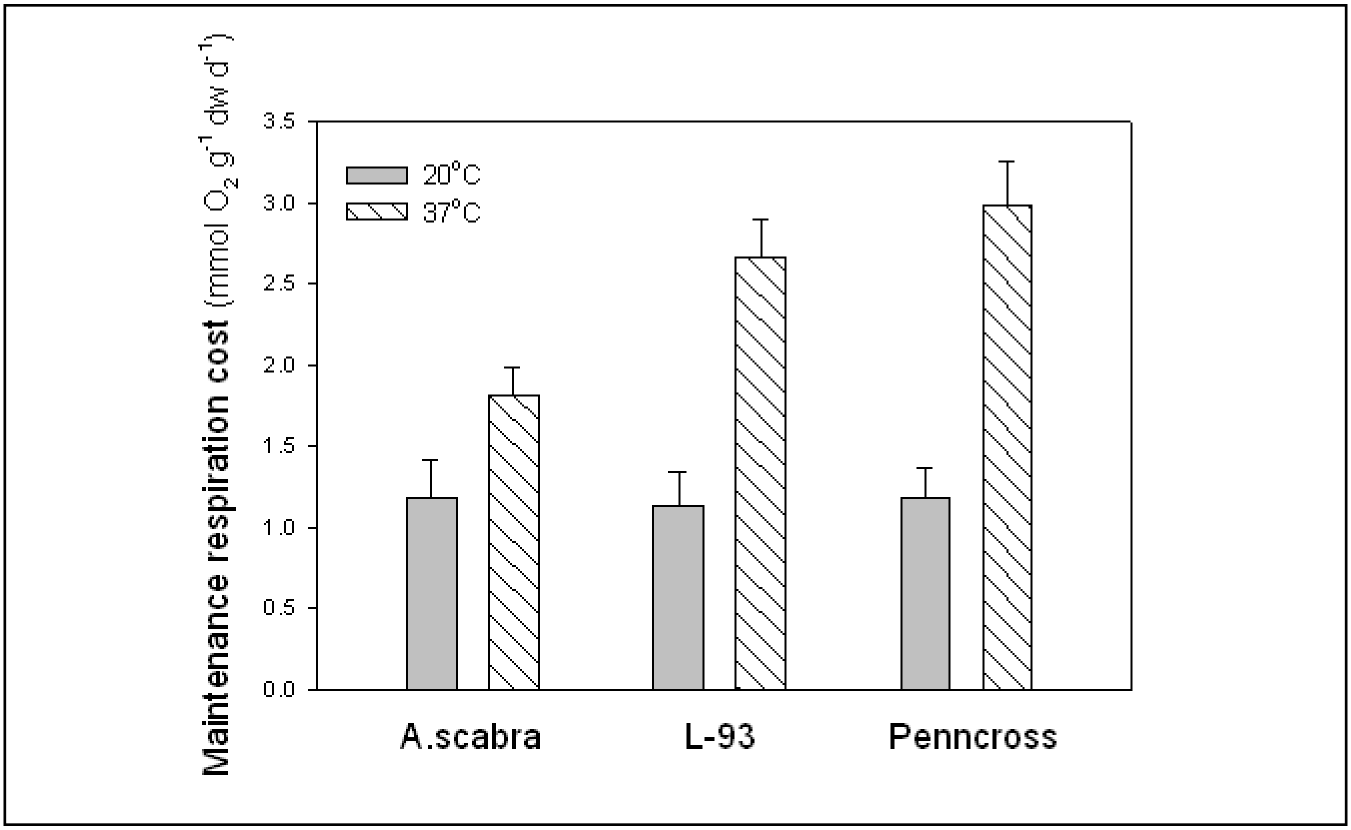

**Figure 4.** Specific respiratory costs of maintenance respiration (mmol O2 g-1 dry weight day-1) of A. scabra and two creeping bengrass cultivars ('L-93' and 'Penncross') in response to increasing soil tempertures: 20$^{o}$C (solid columns) and 37$^{o}$C (cross-hatched columns). Error bars represent standard errors.

growth rate. Plants showing homeostasis in root respiration have a greater ability to maintain growth rate at stressful temperatures, and those plants appear to use more efficient respiratory pathways under stress. Therefore, respiration acclimation or homeostasis may be important for plant adaptation to long-term exposure to high temperatures in terms of carbon economy.

## Maintenance Respiratory Costs Associated with Heat Tolerance

Carbon lost through respiration can account for up to 50% of the daily carbon gain by photosynthesis. Shortage of assimilates due to high respiratory losses has long been considered to be a primary factor responsible for root growth inhibition and dysfunction at high temperatures (20). Thermotolerant roots may be able to control respiration rates or use more efficient respiratory pathways.

Respiration involves three major energy-requiring processes: maintenance, growth, and ion transport. The term "maintenance" includes the processes that maintain cellular structures and gradients of ions and metabolites, and the process of physiological adaptation that maintain cells as active units in a changing environment. High respiratory carbon consumption is mainly due to high maintenance costs. Respiratory maintenance costs account for 20 to 60% of daily assimilates in herbaceous plant species (2). Maintenance respiration typically increases with increasing temperatures. Plant that are adapted to high temperatures may reduce their energy requirements for maintenance to conserve energy and/or direct the limited amount of energy produced to those energy-consuming processes that are critical to survival when energy supply becomes insufficient during stress.

We found that thermal *A. scabra* was able to control root maintenance respiration rate at a significantly lower level under high soil temperatures than creeping bentgrass cultivars ('L-93' and 'Penncross') (Figure 4; 11). Specific respiratory costs for maintenance were higher at $37^{o}$ C (1.8, 2.7, 3.0 mmol $O_2$ $g^{-1}$ dm $day^{-1}$ in *A. scabra*, 'L-93', and 'Penncross', respectively) than those at $20^{o}$C (1.2, 1.1 and 1.2 mmol $O_2$ $g^{-1}$ $d^{-1}$ in *A. scabra*, 'L-93', and 'Penncross', respectively). Root maintenance costs increased only by 53% in thermal *A. scabra*, but by 150% in heat-sensitive 'Penncross'.

Our results suggest that higher root thermotolerance of *A. scabra* was related to lower respiratory costs for maintenance, and therefore, we infer that in order to survive at chronically high soil temperatures, thermotolerant roots control respiratory costs and increase their respiratory efficiency by lowering their maintenance costs. Thermotolerant roots may exhibit high respiratory acclimation potential, which enables the adjustment of their maintenance respiration in response to increasing temperatures in order to reduce carbon expenditure for long-term survival.

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