Research *You Can Use*

Some Like It Hot

Rutgers University scientists continue to unravel the mystery of creeping bentgrass heat tolerance in hopes of improving this vital turfgrass species.

BY BINGRU HUANG AND YAN XU

High temperature is a primary factor causing summer bentgrass decline. One of the typical symptoms of summer bentgrass decline is leaf senescence, which is characterized by loss of chlorophyll and photosynthetic activities in leaves. Cool-season turfgrass species, such as creeping bentgrass (*Agrostis stolonifera*), are sensitive to heat stress and quickly lose color and suffer from a series of physiological injuries when exposed to temperatures above 30°C (86°F). Leaf senescence was observed after 20 days at 30°C and only 8 days at 35°C (95°F) for Penncross creeping bentgrass.[1,2]

Phytohormones are major biochemical factors that regulate leaf senescence. Ethylene, abscisic acid (ABA), and cytokinins are three major phytohormones that mediate signaling events involved in leaf senescence, but the mechanisms of heat-induced leaf senescence in turfgrass are largely unknown. Identification of physiological or metabolic factors associated with leaf senescence has practical value for developing practices that promote healthy turf during the summer, and it is important for revealing basic mechanisms of turfgrass heat tolerance.

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. One of the two predominant grass species in thermal areas is *Agrostis scabra* (thermal rough bentgrass).

Recently, a cool-season grass species, *Agrostis scabra* (thermal rough bentgrass), has been identified growing in geothermally heated areas in Yellowstone National Park.[6] It survives and even thrives in chronically hot soils with temperatures up to 45°C (113°F).[23] Our studies demonstrated that when exposed to 35°C, thermal bentgrass exhibited much better heat tolerance than creeping bentgrass, exhibiting less leaf senescence, higher photosynthesis activity, more efficient carbon utilization, and better root growth.[4,5]

This study was designed to determine whether superior heat tolerance in the thermal bentgrass was associated with metabolic factors regulating heat-induced leaf senescence, specifically changes in the three major senescence-related hormones (ethylene, ABA, and cytokinins). Turf quality and the content of two pigments (chlorophyll and carotenoid) were measured to evaluate the degree of heat tolerance and leaf senescence. Quantitative changes in ethylene, ABA, and two major forms of cytokinins during heat stress were determined to examine their relationship with heat-induced leaf senescence.

EVALUATION OF HEAT-INDUCED LEAF SENESCENCE AND HORMONE PRODUCTION

Creeping bentgrass (cv. Penncross) plugs were collected from field plots at Hort Farm II, Rutgers University, N.J. Plants of *A. scabra*, originally collected from geothermally heated areas in Yellowstone National Park, Wyoming, were propagated in a greenhouse at Rutgers University. Both species were planted in plastic pots (15 cm diameter by 20 cm deep) filled with sterilized sand and fertilized weekly with full-strength Hoagland's solution. Plants of both species were exposed to 35°C/30°C (day/night, high temperature) or 20°C/15°C (68°F day/59°F night, optimum temperature) for 35 days in controlled-environment growth chambers.

Soil temperature at a 2-inch depth was approximately 113°F at a thermal site in Yellowstone National Park (A), where thermal *Agrostis scabra* plants grow and the plant still possesses healthy roots and leaves. Heat-sensitive creeping bentgrass (B) is compared to heat-tolerant thermal *A. scabra* (C), where both species were exposed to elevated air/soil temperatures in a growth chamber.

Turf quality was evaluated based on color, density, and uniformity of the grass canopy using a scale of 0 to 9, with 9 representing fully green, dense turf canopy and 0 representing completely dead plants. Leaf chlorophyll and carotenoid were extracted from fresh leaves. Ethylene production of leaves was determined using a gas chromatograph. ABA and two forms of cytokinin (trans-zeatin/zeatin riboside and isopentenyl adenosine) were quantified by an indirect competitive enzyme-linked immunosorbent assay.

RELATIONSHIP BETWEEN HORMONE ACCUMULATION AND HEAT-INDUCED LEAF SENESCENCE

Heat stress caused decline in turf quality in both bentgrass species, but the decline occurred three weeks later in the thermal bentgrass than creeping bentgrass. Chlorophyll and carotenoid content of the thermal bentgrass exposed to heat stress were maintained at the optimum temperature level for approximately 14 days without any significant decrease until 21 and 28 days, respectively. The decline in turf quality, chlorophyll, and carotenoid content was less severe for the thermal bentgrass than creeping bentgrass. The thermal bentgrass exhibited delayed and less severe leaf senescence under heat stress. Previous studies on root response to high temperatures for these two species also found that the thermal bentgrass exhibited higher tolerance to high soil temperature than creeping bentgrass, with smaller decreases in root growth rate, cell membrane stability, maximum root length, and nitrate uptake.[4,5]

The ethylene production rate of both bentgrass species increased significantly under heat stress, when there was a 20% decline in chlorophyll content. Leaf ABA content also increased under heat stress for both species. However, the increased production of ethylene and ABA in the thermal bentgrass occurred 14 days later than that in creeping bentgrass. This delay of ethylene or ABA accumulation in the thermal bentgrass was consistent with the delay of leaf senescence as manifested by decline in turf quality and chlorophyll and carotenoid contents.

The production of both forms of cytokinins (Z/ZR and IPA) consistently decreased under heat stress in both bentgrass species. In terms of species variation, the decreases of both forms of cytokinins were delayed for 7 days and were less severe after 35 days of heat stress in the thermal bentgrass than in creeping bentgrass. This suggests that maintenance of a higher level of endogenous cytokinin for a longer period of time may contribute to better heat tolerance.

We performed a correlation analysis between hormone accumulation and leaf senescence to determine whether changes in hormone production during heat stress are associated with heat-induced leaf senescence, and to determine which hormone is more important in controlling leaf senescence. The results suggested that endogenous ethylene and ABA production was negatively correlated and cytokinin production was positively correlated with turf performance under heat stress.

Researchers at Rutgers University are using thermal rough bentgrass (*Agrostis scabra*) plants collected from geothermal sites at Yellowstone National Park (left) to identify high-temperature tolerance genes. The goal is to identify the mechanisms in an effort to improve heat tolerance of other creeping bentgrass varieties.

PRACTICAL IMPLICATIONS

The results in this study suggest that approaches that can increase endogenous cytokinin levels or suppress ethylene production may lead to improved heat tolerance and delayed foliar senescence. Exogenous spray of cytokinin, or its derivatives, may be one possible method. Liu et al.[3] reported that applications of 1 and 10 mM zeatin riboside to the rootzone of creeping bentgrass increased cytokinin content in leaves and roots and mitigated heat stress injury in both shoots and roots.

Endogenous cytokinin levels may also be increased by transgenic approaches, introducing favorable genes. In another study, we transformed creeping bentgrass plants with a gene controlling cytokinin synthesis and found that transgenic plants exhibited superior heat tolerance compared to non-transgenic plants. This demonstrated that heat tolerance was associated with the maintenance of cytokinin production and leaf chlorophyll content during heat stress (unpublished data).

Conversely, since ethylene production was negatively correlated with heat-induced senescence, delayed leaf senescence may also be achieved by transgenic approaches or using ethylene inhibitors. In a recent study, we sprayed an ethylene inhibitor to the canopy of creeping bentgrass exposed to 35°C and found that treated turf maintained greener and higher photosynthetic activity for a longer period of time compared to untreated turf.

Our studies suggest that foliar application of cytokinins or ethylene inhibitors may be useful to suppress or delay leaf senescence and ultimately improve turfgrass performance during summer months. A field study is in progress at Rutgers University to test the effectiveness of exogenous application of cytokinins and ethylene inhibitors as well as biostimulants in preventing summer bentgrass decline.

CONNECTING THE DOTS

An interview with Dr. Bingru Huang, *Rutgers University, regarding heat-induced leaf senescence of creeping bentgrass.*

Q: *You stated that the mechanisms of heat-induced leaf senescence in turfgrasses are largely unknown. Are there other plant species for which the mechanisms of heat-induced leaf senescence have been established that can serve as a working model for turfgrasses?*

A: Winter wheat has been the most studied plant species in terms of heat-induced leaf senescence. It is also a cool-season plant species and can be used as a working model for cool-season turfgrasses.

Q: *Your work involves investigations with* Agrostis scabra, *which is adapted to the high air and soil temperatures surrounding geothermally heated areas of Yellowstone National Park. How did you become aware of this remarkably adapted grass species?*

A: While searching for literature on heat tolerance mechanisms of grass species, we found an article published by Richard Stout and his associate (Montana State University) on *Dichanthelium lanuginosum*, a predominant flowering plant in geothermal areas with soil temperature >40°C at 2-5 cm depth in Yellowstone National Park. This species has wide leaf blades, which is not suitable for turf use. We inquired about the existence of *Agrostis* species in geothermal areas, and luckily *Agrostis scabra* is found to grow in different geothermal sites in the park. We now have a collection of different ecotypes at Rutgers.

Q: *Is it your long-range goal to identify "heat tolerance genes" in* A. scabra *and eventually incorporate them into creeping bentgrass? If so, how long of a process is this? When might golf course superintendents see new creeping bentgrass cultivars with these "heat tolerance genes" from* A. scabra*?*

A: Our long-term goal is to develop better heat-tolerant creeping bentgrass, utilizing the genes identified in the thermal grass species either through molecular-marker associated breeding or genetic engineering. Currently, we focus on identification and development of molecular markers of heat tolerance that may be used in breeding to select for heat-tolerant germplasm in creeping bentgrass and other cool-season turfgrass species. We have already found several genes that are highly up-regulated in this grass species when exposed to heat stress, and they may be used as molecular markers. We are in the process of using these markers to screen creeping bentgrass cultivars that differ in heat tolerance in the lab. Field screening trials may be conducted in the next few years. At this point, we are not certain about the future of transgenic plants on golf courses, and therefore we may work on gene transformation in the near future.

Q: *Although* A. scabra *will grow and thrive at elevated air and soil temperatures, will it also perform well at temperatures that are typically found associated with creeping bentgrass? How feasible is it that cultivars of* A. scabra *could be developed for heat-prone areas such as the southern United States?*

A: *A. scabra* plants are able to grow actively at the temperature requirement range for creeping bentgrass, except it has a higher upper temperature limit. Developing cultivars of *A. scabra* may not be feasible, at least in the near future, due to National Park regulation of plant conservation and other issues. We are exploring the possibilities.

Q: *Your paper seemed to suggest that significant ethylene production occurred only after a 20% decline in chlorophyll content. In light of that, do you think ethylene inhibitors would be an effective way to limit heat-induced leaf senescence?*

A: Ethylene inhibitors may be able to suppress heat-induced leaf senescence, but not eliminate the problem.

Q: *Your work with exogenous applications of cytokinins was very interesting in that such applications mitigated heat-stress injury in both shoots and roots. Do you think it is feasible that such applications (e.g., seaweed extract) will become an accepted practice for limiting high-temperature injury to turfgrasses?*

A: Most of our research and others' research on cytokinins effects on heat tolerance were conducted in controlled environmental conditions. Most studies used pure cytokinins. The feasibility of using cytokinin-containing products such as seaweed extract for limiting summer heat injury in creeping bentgrass under natural field conditions needs to be further investigated.

Q: *What's the next step in this research, and what can golf course superintendents expect to come out of this work?*

A: We will explore practical means of preventing or controlling summer bentgrass decline based on the physiological and molecular information. Field studies will be conducted to further confirm our findings from the controlled-environment studies.

Jeff Nus, Ph.D., *manager, Green Section research.*

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