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Scientitsts at Rutgers University conducted research to determine whether superior heat tolerance in *Agrostis scabra* (thermal bentgrass) is associated with metabolic factors regulating heat-induced leaf senescence, specifically changes in the three major senescence-related hormones (ethylene, abscissic acid, and cytokinins).

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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 350 projects at a cost of $29 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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Mechanisms Controlling Heat-induced Leaf Senescence and Heat Tolerance in Bentgrass

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SUMMARY

Heat stress is a primary factor causing summer bentgrass decline associated with thinning turf canopy and leaf yellowing or senescence. A growth-chamber study was conducted to examine whether heat-induced leaf senescence in bentgrass species were associated with changes in three major senescence-related hormones: ethylene, abscisic acid (ABA), and cytokinins. Plants of both species were exposed to 35ºC/30ºC (day/night; high temperature) or 20ºC/15ºC (control) for 35 days in growth chambers. The study found:

- Thermal bentgrass (*Agrostis scabra*) exhibited delayed and less severe leaf senescence, as demonstrated by lower decline in turf quality and levels of two pigments (chlorophyll and carotenoid) under high temperature compared to creeping bentgrass.
- Increases in ethylene and ABA, and decreases in cytokinins, were associated with heat-induced leaf senescence, and differences in heat tolerance were documented between the two bentgrass species.
- Ethylene accumulation was negative associated with turf quality, but cytokinin production was positively associated with turf quality.
- This study suggest that leaf senescence is an important factor accounting for genetic variations in heat tolerance of turfgrass species, and any approaches that can suppress endogenous ethylene or increase cytokinin levels may be used to delay foliar senescence and ultimately improve heat tolerance.

High temperature is a primary factor causing summer bentgrass decline. One of the typical symptoms of summer bentgrass decline is leaf senescence. Leaf senescence 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, which quickly lose color and suffer from a series of physiological injuries when exposed to high temperatures above 30ºC. Leaf senescence was observed after 20 days at 30ºC and only 8 days at 35ºC for 'Penncross' creeping bentgrass (8, 9).

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

Recently, a cool-season grass species, *Agrostis scabra* (thermal bentgrass), has been identified growing in geothermally heated areas in

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 bentgrass).

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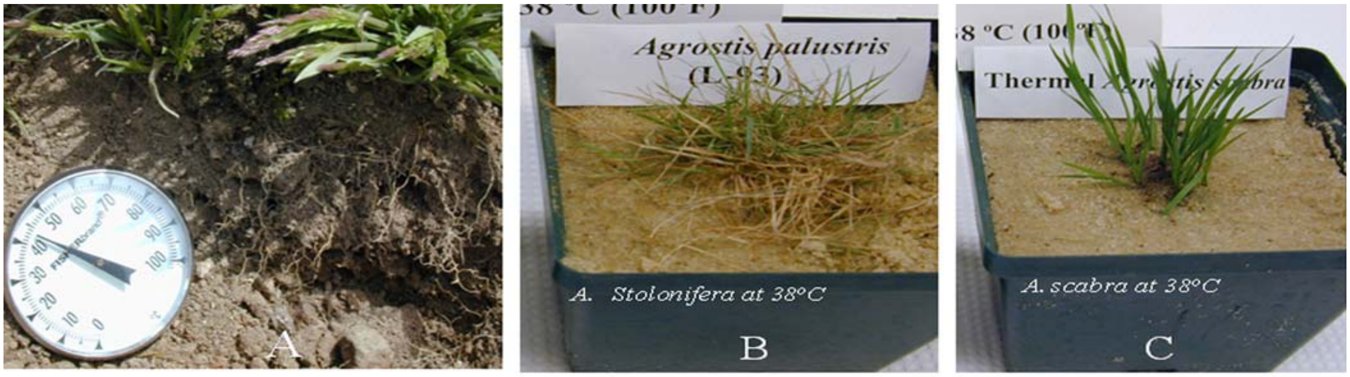

Soil temperature at a 5-cm depth was approximately 45º C at a thermal site in Yellowstone National Park (A) where thermal *Agrostis scabra* plants grow, showing healthy roots and leaves. Heat-sensitive creeping bentgrass (B) is compared to heat-tolerant thermal *A. scabra* (C) where both species were exposed to elevated air/soil temperatures in a growth chamber.

Yellowstone National Park (YNP) (20). It survives and even thrives in the chronically hot soils with temperatures up to 45ºC (23). Our studies demonstrated that when exposed to 35ºC, 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 (14, 17).

This study was designed to determine whether superior heat tolerance in 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 [trans-zeatin/zeatin riboside (Z/ZR) and isopentenyl adenosine (IPA)] 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, NJ. Plants of *A. scabra*, originally collected from geothermally-heated areas in Yellowstone National Park (YNP), 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 35ºC/30ºC (day/night) (high temperature) or 20ºC/15ºC (day/night) (optimum temperature) for 35 days in controlled-environment growth chambers with 14-hour photoperiods, 50% relative humidity, and 400 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the canopy height.

Turf quality was evaluated based on color, density, and uniformity of the grass canopy using a 0 to 9 scale, 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 (14, 17).

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 bentgarss 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 less severe after 35 days of heat stress in the thermal bentgrass than in creeping bentgrass, suggesting 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 see 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 comparing tolerance to high temperature between thermal *A. scabra*, adapted to warm soils in geothermal areas in Yellowstone National Park and creeping bentgrass. The overall goal of the project was to identify physiological and metabolic mechanisms controlling heat tolerance in cool-season turfgrass species, specifically bentgrass.

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. (13) 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.

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^{o}C, and found that treated turf maintained greener and higher photosynthetic activity for a longer period of time compared to un-treated turf.

Our studies suggest that foliar application of cytokinins or ethylene inhibitors may be used 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 cytokinins and ethylene inhibitors as well as biostimulants in preventing summer bentgrass decline.

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References

1. Adedipe, N.O. 1971. Effects of benzyladenine on photosynthesis, growth, and senescence of bean plant. *Physiol. Plant.* 25:151.

2. Balota, M., S. Cristescu, W.A. Payne, S.T.L. Hekkert, L.J.J. Laarhoven, and F.J.M. Harren. 2004. Ethylene production of two wheat cultivars exposed to desiccation, heat, and paraquat-induced oxidation. *Crop Sci.* 44:812-818.

3. Becker, W.and K. Apel. 1993. Differences in gene expression between natural and artifically induced leaf senescence. *Planta* 189:74-79.

4. Chang, H.S., M.L. Jones, G.M. Banowetz, and D.G. Clark. 2003. Overproduction of cytokinins in petunia flowers transformed with P-SAG12-IPT delays corolla senescence and decreases sensitivity to ethylene. *Plant Physiol.* 132:2174-2183.

5. Farkhutdinov, R.G., S.U. Veselov, and G.R. Kudoyarova. 1997. Influence of temperature increase on evapotranspiration rate and cytokinin content in wheat seedlings. *Biol. Plant.* 39:289-291.

6. Fracheboud, Y., and J. Leipner. 2003. The application of chlorophyll fluorescence to study light, temperature and drought stress. Pages 125-150. *In* DeEll, J.R. and P.M.A. Toivonen (eds.) Practical Applications of Chlorophyll Fluorescence in Plant Biology. Kluwer Academic Publishers, Boston.

7. Grbic, V., and A.B. Bleecker. 1995. Ethylene regulates the timing of leaf senescence in *Arabidopsis*. *Plant J.* 8: 595-602.

8. Huang, B., and H. Gao. 2000. Growth and carbohydrate metabolism of creeping bentgrass cultivars in response to increasing temperatures. *Crop Sci.* 40:1115-1120. (TGIF Record 66973)

9. Huang, B., X. Liu, and J.D. Fry. 1998. Shoot physiological responses of two bentgrass cultivars to high temperature and poor soil aeration. *Crop Sci.* 38:1219-1224. (TGIF Record 54265)

10. John, I., R. Drake, A. Farrell, W. Cooper, P. Lee, P. Horton, and D. Grierson. 1995. Delayed leaf senescence in ethylene-deficient ACC-oxidase antisense tomato plants - molecular and physiological analysis. *Plant J.* 7:483-490.

11. Jordi, W., A. Schapendonk, E. Davelaar, G.M. Stoopen, C.S. Pot, R. De Visser, J.A. Van Rhijn, S. Gan, and R.M. Amasino. 2000. Increased cytokinin levels in transgenic P-SAG12-IPT tobacco plants have large direct and indirect effects on leaf senescence, photosynthesis and N partitioning. *Plant Cell Environ.* 23: 279-289.

12. Larkindale, J., and M.R. Knight. 2002. Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiol.* 128: 682-695.

13 Liu, X., B. Huang, and G. Banowetz. 2002. Cytokinin effects on creeping bentgrass responses to heat stress: I. Shoot and root growth. *Crop Sci.* 42: 457-465. (TGIF Record 79200)

14. Lyons, E., J. Pote, M. DaCosta, and B. Huang. 2007. Whole-plant carbon relations and root respiration associated with root tolerance to high soil tempertures for *Agrostis* grasses. *Environ. Exp. Bot*. 59(30):307-313 (TGIF Record 124345)

15. Musatenko, L.I., N.P. Vedenicheva, V.A. Vasyuk, and V.N. Generalova. 2003. Phytohormones in seedlings of maize hybrids differing in their tolerance to high temperature. *Russian J. Plant Physiol.* 50: 499-504.

16. Nooden, L.D. and A.C. Leopoid. 1988. Senescence and aging in plants. Academic Press, San Diego, CA.

17. Rachmilevitch, S., H. Lambers, and B.R. Huang. 2006. Root respiratory characteristics associated with plant adaptation to high soil temperature for geothermal and turf-type *Agrostis* species. *J. Exp. Bot.* 57:623-631. (TGIF Record 124348)

18. Setter, T.L., B.A. Flannigan, and J. Melkonian. 2001. Loss of kernel set due to water deficit and shade in maize: carbohydrate supplies, abscisic acid, and cytokinins. *Crop Sci.* 41: 1530-1540.

19. Shakirova, F.M., M.V. Bezrukova, and I.F. Shayakhmetov. 1995. The effect of heat-shock on the accumulation of ABA and lectin in wheat callus cells. *Russian J. Plant Physiol.* 42:621-623.

20. Stout, R.G., and T.S. Al-Niemi. 2002. Heat-tolerant flowering plants of active geothermal areas in Yellowstone National Park. *Ann. Bot.* 90:259-267.

21. Suzuki, N., L. Rizhsky, H.J. Liang, J. Shuman, V. Shulaev, and R. Mittler. 2005. Enhanced tolerance to environmental stress in transgenic plants expressing the transcriptional coactivator multiprotein bridging factor 1c. *Plant Physiol.* 139: 1313-1322.

22. Teplova, I.R., R.G. Farkhutdinov, A.N. Mitrichenko, I.I. Ivanov, S.Y. Veselov, R.L. Valcke, and G.R. Kudoyarova. 2000. Response of tobacco plants transformed with the ipt gene to elevated temperature. *Russian J. Plant Physiol.* 47: 367-369.

23. Tercek, M.T., D.P. Hauber, and S.P. Darwin. 2003. Genetic and historical relationships among geothermally adapted *Agrostis* (bentgrass) of North America and Kamchatka: evidence for a previously unrecognized, thermally adapted taxon. *Amer. J. Bot.* 90: 1306-1312. (TGIF Record 124350)

24. Thomas, H., and J.L. Stoddart. 1980. Leaf senescence. *Ann. Rev. Plant Physiol.* 31:83-111.

25. Veselov, A.P., V.P. Lobov, and L.N. Olyunina. 1998. Phytohormones during heat shock and recovery. *Russian J. Plant Physiol.* 45:611-616.

26. Wang, L.J., W.D. Huang, Y.P. Liu, and J.C. Zhan. 2005. Changes in salicylic and abscisic acid contents during heat treatment and their effect on thermotolerance of grape plants. *Russian J. Plant Physiol.* 52:516-520.

27. Wang, Z. and B. Huang. 2003. Genotypic variation in abscisic acid accumulation, water relations, and gas exchange for Kentucky bluegrass exposed to drought stress. *J. Amer. Soc. Hort. Sci.*. 128:349-355. (TGIF Record 86218)

28. Xiong, L.M., K.S. Schumaker, and J.K. Zhu. 2002. Cell signaling during cold, drought, and salt stress. *Plant Cell* 14: S165-S183.

29. Xu, Q.A., A.Q. Paulsen, J.A. Guikema, and G.M. Paulsen. 1995. Functional and ultrastructural injury to photosynthesis in wheat by high temperature during maturation. *Environ. Exp. Bot.* 35: 43-54.

30. Yang, J.C., J.H. Zhang, Z.Q. Wang, Q.S. Zhu, and L.J. Liu. 2002. Abscisic acid and cytokinins in the root exudates and leaves and their relationship to senescence and remobilization of carbon reserves in rice subjected to water stress during grain filling. *Planta* 215:645-652.

31. Yeh, D.M., and P.Y. Hsu. 2004. Heat tolerance in English ivy as measured by an electrolyte leakage technique. *J. Hort. Sci. Biotech.* 79:298-302.