Scientists 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).
PURPOSE

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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 35°C/30°C (day/night; high temperature) or 20°C/15°C (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 negatively associated with turf quality, but cytokinin production was positively associated with turf quality.
- This study suggests 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 30°C. Leaf senescence was observed after 20 days at 30°C and only 8 days at 35°C 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 Yellowstone National Park. One of the two predominant grass species in thermal areas is *Agrostis scabra* (thermal bentgrass).
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 tempera-
ture 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 bentgrass was consistent with the delay of leaf senescence as manifest-
ed 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°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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