Heat-induced Leaf Senescence and Hormonal Changes for Thermal Bentgrass and Turf-type Bentgrass Species Differing in Heat Tolerance

Yan Xu and Bingru Huang
Department of Plant Biology and Pathology, Rutgers University, 59 Dudley Road, New Brunswick, New Jersey 08901

ABSTRACT. Leaf senescence can be induced by many environmental stresses, including supraoptimal temperatures. The objectives of this study were to evaluate leaf senescence induced by heat stress for two Agrostis species contrasting in heat tolerance and to examine whether heat-induced leaf senescence in both species was associated with changes in three major senescence-related hormones: ethylene, abscisic acid (ABA), and cytokinins. Plants of heat-tolerant rough bentgrass (Agrostis scabra Willd.) and heat-sensitive creeping bentgrass (Agrostis stolonifera L.) were exposed to 35/30 °C (day/night) (high temperature) or 20/15 °C (control) for 35 d in growth chambers. Turf quality, photochemical efficiency (Fv/Fm), and the contents of two pigments (chlorophyll and carotenoid) for both species decreased under high temperature; however, heat-tolerant A. scabra exhibited delayed and less severe decline in all parameters compared with heat-sensitive A. stolonifera. Ethylene production rate increased in both species at 35 °C, but the increase was observed 21 days later in A. scabra compared with that in A. stolonifera. ABA content increased at the initiation of heat stress and then declined in both species after prolonged heat stress. However, the timing of the increase was delayed for 7 days and the highest level of ABA content was less in A. scabra (4.0 times that of the control) than that in A. stolonifera (5.9 times that of the control). Decreases in both forms of cytokinins (transzeatin/zeatin riboside and isopentenyl adenosine) were also delayed for 14 days and less pronounced in A. scabra. Correlation analysis revealed that leaf senescence induced by heat stress was negatively correlated to ethylene and ABA accumulation and positively correlated to cytokinin production. Delayed leaf senescence in A. scabra under heat stress could be related to slower and less magnitude of changes in ethylene, ABA, and cytokinins.

High temperature is a major factor limiting growth of cool-season plant species. One of the typical symptoms of heat injury for many plant species is leaf senescence (Thomas and Stoddart, 1980). Leaf senescence is characterized by loss of chlorophyll and photosynthetic activities in leaves (John et al., 1995). Heat-induced leaf senescence and associated changes in physiological activities have been reported in various plant species (Thomas and Stoddart, 1980; Xu et al., 1995; Yeh and Hsu, 2004). Xu et al. (1995) reported that increases in temperature during maturation of wheat (Triticum aestivum L.) enhanced leaf senescence, accentuated the loss of chloroplast integrity, and accelerated the decline of PSII-mediated electron transport. Cool-season turfgrass species such as Agrostis stolonifera are sensitive to heat stress and experience a series of physiological injuries when exposed to temperatures above 30 °C. Leaf senescence was observed after 20 d at 30 °C and only 8 d at 35 °C for A. stolonifera cv. Penncross (Huang and Gao, 2000; Huang et al., 1998).

Many physiological and biochemical factors such as plant hormone levels can be related to leaf senescence. In many nonturf plant species, it has been found that both genetically and environmentally regulated senescence are associated with hormonal changes with either upregulating or downregulating effects (Nooden and Leopold, 1988). Ethylene, abscisic acid (ABA), and cytokinins are three major phytohormones that mediate signaling events involved in plant senescence (Chang et al., 2003). It has been reported that exogenous ethylene can accelerate processes characteristic of leaf senescence such as the decline of chlorophyll and increases in activity of hydrolytic enzymes (Grbic and Bleecker, 1995). In addition, delayed leaf senescence was observed in transgenic ethylene-deficient tomato (Lycopersicon esculentum Mill. cv. Ailsa Craig) plants (John et al., 1995). ABA is involved in many plant responses to biotic and abiotic stresses. Rapid ABA accumulation has been observed when plants are subjected to drought, salinity, and extreme temperatures (Xiong et al., 2002). Some studies also found that ABA accumulation may induce leaf senescence. Spraying ABA reduced leaf chlorophyll content in rice (Oryza sativa L.) (Yang et al., 2002). Becker and Apel (1993) reported that senescence-associated mRNAs were induced by ABA in detached leaves of barley (Hordeum vulgare L.). In contrast to the actions of ethylene and ABA, cytokinins retard senescence in vegetative and floral tissues (Nooden and Leopold, 1988). In transgenic P5SAG12–IPT tobacco (Nicotiana tabacum L.) plants, enhancement in cytokinin synthesis significantly delayed leaf senescence, reduced damage to chlorophyll and the photosynthetic system and nitrogen translocation to nonsenescing leaves (Jordi et al., 2000). Applying benzyladenine, a synthetic substance that is similar to cytokinin in structure and function, to the leaves of bean (Phaseolus vulgaris L.) plants helped maintain high chlorophyll content, thus delaying leaf senescence under normal environmental conditions (Adedipe, 1971).
The mechanisms of heat-induced leaf senescence in turfgrass species are largely unknown despite extensive research in other plant species as discussed previously. Identification of physiological or metabolic factors associated with senescence has practical value for turfgrass management and is important for revealing basic mechanisms of plant heat tolerance. Recently, a heat-tolerant C₃ perennial grass species, *Agrostis scabra,* has been identified growing in geothermally heated areas in Yellowstone National Park, Wyo. (Stout and Al-Niemi, 2002). It survives or even thrives in the chronically hot soils with temperatures up to 45 °C (Tercek et al., 2003). When exposed to 35 °C in controlled-environment chambers, these plants maintain green leaves longer than *A. stolonifera,* a widely cultivated cool-season grass whose optimal growth temperature is between 10 and 18 °C. Our objectives were to evaluate leaf senescence induced by heat stress for these two *Agrostis* species contrasting in heat tolerance and to determine whether heat-induced leaf senescence in both *Agrostis* species was associated with changes in the three major senescence-related hormones (ethylene, ABA, and cytokinins). Turf quality, photochemical efficiency, 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, transzeatin/zeatin riboside (Z/ZR) and isopentenyl adenosine (iPA), during heat stress were determined to examine their relationship with heat-induced leaf senescence.

**Materials and Methods**

**Plant materials.** *Agrostis stolonifera* cv. Penncross plugs were collected from field plots at Hort Farm II, Rutgers University, New Brunswick, N.J. Plants of *A. scabra,* originally collected from geothermally heated areas in Yellowstone National Park, Wyo., were propagated in a greenhouse at Rutgers University. Both species were planted in 24 well-drained plastic pots (15 cm diameter and 20 cm deep) filled with sterilized sand and fertilized weekly with 100 mL full-strength Hoagland’s solution. Plants were cut weekly to maintain a canopy height of ≈5 cm. After 1 month of establishment in the greenhouse, plants were transferred into controlled-environment growth chambers (Conviron, Winnipeg, Canada) with a temperature of 20/15 °C (day/night), 14-h photoperiod, 50% average relative humidity, and 400 μmol·m⁻²·s⁻¹ photosynthetic photon flux density at the canopy height. Plants were acclimated to growth chamber conditions for 1 week before exposure to different temperature treatments.

**Treatments and Experimental Design.** Plants of both species were exposed to 35/30 °C (day/night) (high temperature) or 20/15 °C (day/night) (control) for 35 d. A total of four growth chambers was used in this study with two chambers being used simultaneously for each temperature treatment. Two pots for each species were placed inside each chamber. Four pots of plants (four replicates) for each species were relocated every week to different chambers so that plants exposed to each temperature were treated in four different chambers during the treatment period. The experiment consisted of two factors (temperature and species), which were arranged as a completely randomized block design. Plants were watered twice daily until free drainage occurred from the bottom of a pot to prevent water deficit during the treatment period.

**Measurements.** All the following measurements were made on plants in four pots (as four replicates) during the 14-h photoperiod. Turf quality was evaluated once a week based on color, density, and uniformity of the grass canopy using a 0 to 9 scale in which 9 represents fully green, dense turf canopy and 0 represents completely dead plants. Leaf photochemical efficiency of photosystem II (PSII) was also measured at a weekly interval with a fluorescence induction monitor (FIM 1500; Analytical Development Co. Ltd., Hoddesdon, U.K.) after a 30-min dark adaptation period. Photochemical efficiency was expressed as the ratio of Fv/Fm. [Fv/Fm = (Fm – Fo)/Fm. Fo is the ground state value of fluorescence, and Fm is the maximum value of fluorescence.] The Fv/Fm value is ≈0.8 in fully healthy plants. A lower value of Fv/Fm indicates that a proportion of PSII reaction centers are damaged (Fracheboud and Leipner, 2003).

In addition, leaf tissues were sampled weekly for pigment quantification. Chlorophyll and carotenoid were extracted using dimethyl sulfoxide (DMSO). A 0.2 g of fresh leaves was incubated in 10 mL DMSO for 48 h in darkness. Original leaf extracts were diluted eight times and then the absorbance of the diluted extracts was determined using a spectrophotometer (Spectronic Genesys2; Spectronic Instruments, Rochester, N.Y.). Chlorophyll content was calculated based on the absorbance at 663 and 645 nm, and carotenoid content was calculated based on the absorbance at 663, 645, and 470 nm using the formulas described by Amon (1949).

Ethylene production of leaves was determined using a gas chromatograph (GC-8A; Shimadzu Scientific Instruments, Columbia, Md.) (Watkins and Frenkel, 1987). An airtight system was designed to collect ethylene gas evolved from leaves. Five to seven attached leaves were grouped and sealed inside a 10-mL syringe (Becton, Dickinson and Co., Franklin Lakes, N.J.) with a rubber stopper around the leaf base. Vacuum grease and Teflon tape were used to prevent leaks. Four groups of leaves from each pot were randomly selected and sealed in syringes as four subsamples. A 0.5-mL gas sample from each syringe was taken through the rubber stopper every 2 h and injected into GC to determine ethylene concentration. Afterward, each group of leaves was excised and the fresh weight was measured. The average of hourly production of ethylene was calculated based on changes in ethylene concentration over time and a standard curve.

ABA and two forms of cytokinin (transZ/ZR and iPA) were quantified by an indirect competitive enzyme-linked immuno-sorbent assay. Extraction and quantification of hormones followed the method described by Setter et al. (2001) with some modifications (Wang and Huang, 2003). Briefly, samples were extracted in 80% v/v methanol and purified with reverse-phase C₁₅ columns. Hydrophilic contaminants were washed out with 200 μL of 20% solvent (20% methanol, 80% aqueous triethylamine (TEA; 10 mM, pH 3.5)). The cytokinin-containing fraction was eluted using 200 μL of 30% solvent (30% methanol, 70% aqueous TEA) and the ABA-containing fraction was eluted using 150 μL of 55% methanol.

**Statistical Analysis.** Analysis of variance and correlation were based on the general linear model procedure of SAS (SAS Institute, Cary, N.C.). Effects of species, temperature, and their interactions were tested separately for each sampling date for all the parameters. Differences between treatment means for each species were separated by Fisher’s protected least significant difference test at the *P* = 0.05 level.
Results

Changes in physiological parameters associated with leaf senescence and heat tolerance. Turf quality (TQ) of both species gradually declined over time at 35 °C (Fig. 1). Turf quality for *A. stolonifera* declined to below the control level beginning at 14 d of treatment, whereas a significant decline in *A. scabra* was not observed until 35 d. By 35 d of heat stress, turf quality decreased to 81% of the control for *A. scabra* and 56% of the control for *A. stolonifera*.

Under high temperature, plants of both species exhibited lower photochemical efficiency (Fv/Fm) than the control beginning at 14 d of treatment (Fig. 2). The decline in Fv/Fm became more dramatic with prolonging stress duration and more pronounced for *A. stolonifera* than *A. scabra* at 35 °C.

Leaf chlorophyll content for *A. stolonifera* decreased below the control level at 7 d of high temperature treatment, whereas that for *A. scabra* was maintained at the control level until 21 d of treatment (Fig. 3). Chlorophyll content in *A. stolonifera* exhibited a greater extent of decline than that in *A. scabra* with prolonging duration of heat stress. Species variation and the

change in carotenoid content with stress duration followed a similar pattern to that of chlorophyll content (Fig. 4). In *A. scabra* plants exposed to high temperature, carotenoid content was maintained at the control level for 28 d before a significant decrease occurred. For *A. stolonifera* plants, the decrease started at 7 d and became significantly lower than the control after 14 d. The decline in carotenoid contents for *A. stolonifera* was more pronounced than for *A. scabra* during the entire experimental period of the high temperature treatment.

Changes in ethylene, abscisic acid, and cytokinin content. Ethylene production rate increased with treatment duration in both species under high temperature except for *A. scabra* at 14 d of heat stress and was two to three times the initial level for both species at 35 d of treatment (Fig. 5). The timing of ethylene increase varied with species. In *A. scabra*, the rate of ethylene production at 35 °C did not increase significantly above the control level until 35 d, whereas a significant increase occurred at 14 through 35 d of heat treatment for *A. stolonifera*.

At 35 °C, ABA content increased to the highest level at 14 d for *A. stolonifera* and at 21 d for *A. scabra* and declined


187
thereafter. However, ABA content was significantly higher than the control level after only 7 d of treatment for *Agrostis stolonifera* and after 21 d for *A. scabra* (Fig. 6). In addition, the peak values of ABA varied with species, which was 77.4 pmol g\(^{-1}\) fresh weight (FW) for *A. stolonifera* (5.9 times that of the control) and 64.6 pmol g\(^{-1}\) FW for *A. scabra*, which was equal to 4.0 times that of the control value.

The content of both forms of cytokinins declined with the progression of heat stress for both species (Figs. 7 and 8). For iPAt content, the decrease in *A. scabra* became significant after 14 d, whereas in *A. stolonifera*, it became significant after only 7 d (Fig. 7). At 35 d of heat stress, the content of iPAt for *A. scabra* was reduced to 30% of the initial level, whereas that in *A. stolonifera* was reduced to 22%. The decrease in Z/ZR content became significant after 14 d of heat stress for *A. scabra* and 7 d for *A. stolonifera*. At the end of the treatment period, Z/ZR cytokinin declined by 65% for *A. stolonifera* and by 50% for *A. scabra* (Fig. 8).

**Correlation between hormones and senescence parameters.** In general, ethylene and ABA contents were negatively correlated to TQ, leaf Fv/Fm, chlorophyll, and carotenoid contents, whereas both forms of cytokinin contents had positive correlations with these parameters for both species (Tables 1 and 2). For *A. stolonifera*, there were significant correlations of ethylene and cytokinins with all senescence parameters with higher correlation coefficients for ethylene (ranging from –0.90 to –0.96) than cytokinins (ranging from 0.72 to 0.94) (Table 1). No significant correlations were detected between ABA and physiological parameters. For *A. scabra*, the correlation coefficients between all three hormones and senescence parameters were highly significant, which ranged from –0.51 (Fv/Fm) to –0.75 (chlorophyll) for ABA, 0.60 (TQ) to 0.79 (Fv/Fm) for iPAt and 0.63 (TQ) to 0.89 (Fv/Fm) for Z/ZR (Table 2).

**Discussion**

The decline of turfgrass quality under heat stress was observed 3 weeks earlier in *A. stolonifera* compared with *A. scabra*. Changes in chlorophyll and carotenoid exhibited consistent differences in the timing and severity of leaf senescence induced by heat treatment between two species. Both chlorophyll and carotenoid contents were maintained at
the control level for $\approx 14$ d in *A. scabra* without any significant decrease until 21 and 28 d, respectively. The decline in TQ, chlorophyll, and carotenoid content was less severe for *A. scabra* than *A. stolonifera*. These results demonstrated that heat-tolerant *A. scabra* 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 *A. scabra* exhibited higher tolerance to high soil temperature than *A. stolonifera* with smaller decreases in root growth rate, cell membrane stability, maximum root length, and nitrate uptake (Lyons et al., 2006; Rachmilevitch et al., 2006).

Ethylene production has been considered an important factor contributing to natural senescence or abscission of leaves and reproductive organs (Yang and Hoffman, 1984). Enhanced ethylene production has also been associated with reduced growth and accelerated senescence in response to environmental stresses (Balota et al., 2004; Huberman et al., 2004). Limited literature is available in the association of ethylene production with heat tolerance. In our studies, ethylene production rate of both bentgrass species increased significantly under heat stress when there was a 20% decline in chlorophyll content (Fig. 3). One study in winter wheat (*T. aestivum*) reported that the stress-resistant ‘Dropia’ produced significantly greater ethylene than stress-susceptible ‘Delia’ under high temperature (38 °C) (Balota et al., 2004). We did not observe significant differences in ethylene accumulation between the two bentgrass species tested. Ethylene production increased to a similar extent for both species after 35 d of heat treatment. However, the initiation of the increase was 14 d later in *A. scabra* than in *A. stolonifera* under stress conditions. In addition, a significant decrease in ethylene content was observed for *A. scabra* at 14 d of heat stress, which may be part of the defense mechanisms of this species against heat stress. The delay of ethylene accumulation in *A. scabra* was consistent with the delay of leaf senescence as manifested by TQ and chlorophyll and carotenoid contents.

The accumulation of ABA in response to heat shock and other stresses has been reported in several plant species, including maize (*Zea mays* L.), durum wheat (*Triticum durum* Desf.), and pea (*Pisum sativum* L.) (Musatenko et al., 2003; Shakirova et al., 1995; Veselov et al., 1998). In our study, ABA accumulation occurred at 35 °C for both species, exhibiting a peak at 14 and 21 d for *A. stolonifera* and *A. scabra*, respectively. Similar results have recently been reported for

![Figure 5. Changes in leaf ethylene production rate [nL g⁻¹ h⁻¹ (FW basis)] over time at 20 °C (dotted lines and open symbols) or 35 °C (solid lines and filled symbols) for (A) *Agrostis stolonifera* and (B) *Agrostis scabra*. Vertical bars on the bottom (A) or top (B) indicate least significant differences ($P \leq 0.05$) for temperature treatment comparison on a given day of treatment.](image)

![Figure 6. Changes in leaf abscisic acid content (pmol g⁻¹ FW) over time at 20 °C (dotted lines and open symbols) or 35 °C (solid lines and filled symbols) for (A) *Agrostis stolonifera* and (B) *Agrostis scabra*. Vertical bars on the top indicate least significant differences ($P \leq 0.05$) for temperature treatment comparison on a given day of treatment.](image)
grape (*Vitis vinifera* L. cv. Jinoxiu), in which ABA content drastically increased within 1 h after heat treatments (38°C) (Wang et al., 2005). However, the increase in *A. scabra* was delayed for 14 d and the maximum accumulation at the peak was smaller compared with *A. stolonifera*. These results suggested that *A. scabra* could maintain its ABA content for a longer period of time and exhibit a less pronounced increase when exposed to prolonged heat treatment, which could possibly be associated with less stress injury and better heat tolerance.

Both Z/ZR and iPA production consistently decreased under heat stress as demonstrated in both bentgrass species in our study and many nonturf plant species in various other studies. One potential explanation for cytokinin reduction in shoots is the result of inhibition of cytokinin transport from roots to shoots under elevated temperatures, because cytokinins are primarily produced in roots. This perspective is supported by a study imposed in wheat, which reported that the content of cytokinins decreased in shoots and accumulated in roots simultaneously as a result of temperature increase (Farkhutdinov et al., 1997). Additionally, the decline of endogenous cytokinins in senescing tissues may be the result of a decrease in biosynthesis or an increase in catabolism (Nooden and Leopold, 1988). It would be necessary to examine specific changes in cytokinin synthesis, degradation, and mobilization to reveal the mode of actions of cytokinin in heat tolerance of plants, including turfgrass. In terms of species variation, the decreases of both forms of cytokinins were delayed for 7 d and less severe after 35 d of heat stress in *A. scabra* than in *A. stolonifera*, suggesting that maintenance of a higher level of endogenous

| Table 1. Correlations of ethylene, abscisic acid (ABA), isopentenyl adenosine (iPA), and transzeatin/zeatin riboside (Z/ZR) with senescence parameters (turf quality, Fv/Fm, chlorophyll, and carotenoid contents) under heat stress (35°C) for *Agrostis stolonifera*. |
|-----------------|----------------|----------------|----------------|
| Ethylene | ABA | iPA | Z/ZR |
| Turf quality | -0.90** | -0.25 | 0.72** | 0.79** |
| Fv/Fm | -0.92** | -0.29 | 0.73** | 0.74** |
| Chlorophyll | -0.96** | -0.42 | 0.88** | 0.94** |
| Carotenoid | -0.90** | -0.45 | 0.87** | 0.93** |

The significance of correlation coefficients was tested at \(P \leq 0.05\) (*) and \(P \leq 0.01\) (**).
cytokinin for a longer period of time may contribute to better heat tolerance.

Data from correlation analysis demonstrated that endogenous ethylene and ABA production were negatively correlated and cytokinin production was positively correlated with heat-induced leaf senescence; however, the correlation with ABA was not significant for *A. stolonifera*. Thus, approaches that can increase endogenous cytokinin levels or suppress ethylene production may lead to improved heat tolerance and delayed foliar senescence. Exogenous application of cytokinin, or its derivatives, may be one possible method. Liu et al. (2002) reported that applications of 1 and 10 mM ZR to the root zone 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. Teplova et al. (2000) transformed tobacco plants with the *ipt* gene that codes for isopentenyltransferase, which was subsequently expressed after a heat shock treatment. Elevated temperature resulted in a decrease in the transpiration of wild-type plants, whereas the transpiration rate was maintained at high levels in transgenic plants. Conversely, because ethylene production was negatively correlated with heat-induced senescence, delayed leaf senescence may also be achieved by transgenic approaches that can perturb endogenous ethylene-response signal transduction pathways (Suzuki et al., 2005). However, blocking ethylene production may be detrimental to heat tolerance. Larkindale and Knight (2002) reported that ethylene was necessary in protection against heat-induced oxidative damage in *Arabidopsis thaliana* (L.) Heyn. because the ethylene-insensitive mutant *etr-1* showed increased susceptibility to heat. These studies indicate the complex interaction of hormones and stress tolerance.

In summary, heat-tolerant *A. scabra* exhibited delayed and less severe leaf senescence during heat stress compared with heat-sensitive *A. stolonifera*. The increases in ethylene and ABA, and decreases in cytokinins, could contribute to heat-induced leaf senescence and differences in heat tolerance between the two bentgrass species. Ethylene and cytokinins were more closely correlated to physiological parameters associated with leaf senescence and heat tolerance than ABA, especially for heat-sensitive *A. stolonifera*. This suggested that approaches that can suppress endogenous ethylene, or increase cytokinin levels, may be used to delay foliar senescence and ultimately improve heat tolerance.

**Literature Cited**


