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Heat-induced Leaf Senescence in Creeping Bentgrass Suppressed by Aminoethoxyvinylglycine Involving Regulation of Chlorophyll Metabolism

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Abstract. **Heat stress-induced or stress-accelerated leaf senescence is related to the accumulation of ethylene and loss of chlorophyll in cool-season grass species. The objective of this study was to determine whether foliar-spraying the ethylene inhibitor, aminoethoxyvinylglycine (AVG), may suppress heat-induced leaf senescence through effects on chlorophyll synthesis and degrading enzymes in creeping bentgrass (*Agrostis stolonifera*). Plants were maintained in environmentally controlled growth chambers under non-stress (22/17 °C day/night) or heat stress (35/30 °C day/night) temperature conditions for 25 days, and turf quality, electrolyte leakage, and chlorophyll content were measured to assess the extent of leaf senescence. Activities of chlorophyll-synthesizing and chlorophyll-degrading enzymes were quantified to determine whether AVG may regulate chlorophyll metabolism. Plants were foliar-sprayed with 25 μM AVG before and during heat stress at 7-day intervals. From 21 through 25 days of heat stress, AVG-treated plants had significantly higher turf quality and chlorophyll content, whereas electrolyte leakage was significantly lower in comparison with untreated controls. The activity of a chlorophyll-synthesizing enzyme, porphobilinogen deaminase, was significantly increased in AVG-treated plants at 21 days of heat stress. The activity of chlorophyll-degrading enzymes was significantly lower in plants treated with AVG from 14 through 25 days of heat stress for peroxidase, from 21 through 25 days of heat stress for pheophytinase, and at 25 days of heat stress for chlorophyllase. AVG may have suppressed heat-induced leaf senescence by regulating chlorophyll metabolic activities in cool-season grass species.**

Heat stress induces and accelerates leaf senescence, which is characterized by a loss of chlorophyll and cellular membrane deterioration, as well as oxidative damage (Jespersen et al. 2016; Liu and Huang 2000; Yu et al. 2021a). Leaf senescence can be exacerbated by the hormone ethylene, which acts as a signal to trigger cellular maturation and senescence (Bleecker and Kende 2000). Ethylene is produced at higher rates in plants exposed to heat stress, which induces premature leaf senescence (Balota et al. 2004; Hays et al. 2007; Morgan and Drew 1997). Inhibition of ethylene synthesis or action has been found to be effective for suppressing leaf senescence in different plant species subjected to abiotic stresses, including heat stress; therefore, the application of ethylene inhibitors is a viable approach to prolonging leaf growth to improve plant stress tolerance (Djanaguiraman and Prasad 2010; Djanaguiraman et al. 2011; Jespersen and Huang 2015; Yuan et al. 2015).

Aminoethoxyvinylglycine (AVG) impedes ethylene synthesis by inhibiting the catalytic reaction of 1-aminocyclopropane-1-carboxylate (ACC) synthase, which is the enzyme that catalyzes the transformation of S-adenosyl-methionine into ACC (Even-Chen et al. 1982). Previous studies have exhibited that exogenous application of AVG effectively delays leaf senescence in plants exposed to heat stress, as indicated by the higher levels of leaf chlorophyll in AVG-treated plants (Chen and Huang 2022; Jespersen and Huang 2015; Xu and Huang 2009; Zhang et al. 2019). The suppression of leaf senescence by AVG or an ethylene perception inhibitor, 1-methylcyclopropene, has been mainly attributed to their positive effects on enhancing antioxidant defense systems, including increases in antioxidant enzyme activities, as was reported for creeping bentgrass (*Agrostis stolonifera*) (Xu and Huang 2009) and soybean (*Glycine max*) (Djanaguiraman and Prasad 2010), rather than on their regulation of chlorophyll metabolism.

Leaves senescence caused by the loss of chlorophyll, which is the major pigment involved in photosynthesis, may occur as a result of enhanced degradation or suppression of synthesis (Papenbrock et al. 2000; Pruzinská et al. 2005; Rodriguez et al. 1987). Porphobilinogen deaminase (PBGD) is involved in chlorophyll synthesis and facilitates the formation of the ring structure of chlorophyll (Jones and Jordan 1994). It has been previously determined that the activity of this chlorophyll synthesis enzyme, as well as the activities of others, are inhibited during natural senescence in species such as poinsettia (*Euphorbia pulcherrima*) and pepper (*Capsicum anuum*) (Frydman and Frydman 1979). Additionally, a decline in PBGD activity has been exhibited in other species, such as cucumber (*Cucumis sativus*), under heat-induced leaf senescence (Tewari and Tripathy 1998), suggesting that it may have a key role in chlorophyll synthesis under heat stress. In the cool-season grass species, creeping bentgrass, a decline in chlorophyll content caused by heat stress was mainly attributed to increases in the activity of chlorophyll degradation enzymes, especially chlorophyllase (CHLASE), chlorophyll-degrading peroxidase (CHL-PRX), and pheophytinase (PPH) (Jespersen et al. 2016; Rossi et al. 2017, 2020, 2021). Chlorophyllase and PPH are responsible for excising the phytol chains from the rings of chlorophyll and pheophytin, respectively, and are rate-limiting steps during chlorophyll degradation (Matile et al. 1997; Schelbert et al. 2009). When hydrogen peroxide is concentrated in tissues along

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with phenolic compounds, CHL-PRX oxidizes chlorophyll, causing conformational changes (Huff 1982). Although it is well-known that these chlorophyll-synthesizing and chlorophyll-degrading enzymes are active in plants during senescence, whether AVG specifically mitigates heat-induced leaf senescence through the regulation of chlorophyll enzyme metabolism in cool-season grass species has yet to be established.

The objective of this study was to determine whether the ethylene inhibitor, AVG, may suppress heat-induced leaf senescence by affecting chlorophyll-synthesizing and chlorophyll-degrading enzymes in creeping bentgrass. To accomplish this, creeping bentgrass plants subjected to heat stress or non-stress temperature conditions were foliar-treated with AVG, and physiological properties were assessed along with the activities of chlorophyll synthesis and degradation enzymes. By studying the mechanisms by which AVG may mediate heat stress in creeping bentgrass, it may be possible to define how this ethylene inhibitor may be used to control heat-induced leaf senescence in cool-season plant species and enable the development of novel lines exhibiting traits associated with heat tolerance.

Materials and Methods

Plant materials and establishment conditions. A total of 20 creeping bentgrass (cultivar Penncross) sod plugs (diameter, 10.16 cm) were collected from the Rutgers University Horticultural Farm II research field site in North Brunswick, NJ, USA, and were immediately planted in plastic pots (length, 11.43 cm; width, 15.24 cm; height, 13.97 cm) filled with United States Golf Association-grade 310-grit sand that was screened for contaminants (Mitchell Products, Millville, NJ, USA). Plants were established to maturity for 28 d in a greenhouse at an average temperature of 24/20 °C day/night and a 14-h photoperiod at 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation at canopy height for 21 d. Plants were irrigated daily, fertilized every 3 d with three-quarter-strength Hoagland's nutrient solution (Hoagland and Arnon 1950), and trimmed to a height of 3.0 cm every 7 d. When plants were mature, they were immediately transferred into four controlled-climate growth chambers (Environmental Growth Chambers, Chagrin Falls, OH, USA) to acclimate to environmental conditions of 22/17 °C day/night, 60% relative humidity, and a 14-h photoperiod of 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation for 7 d.

Ethylene inhibitor treatment and experiment design. Plants were individually foliar-treated to canopy saturation with 40 mL of 25 μM AVG, an ethylene inhibitor, or water alone before initiation of heat stress and every subsequent 7 d. The concentration of AVG and application interval implemented during this experiment were effective for promoting the health of creeping bentgrass exposed to heat stress during our previous studies and preliminary screenings (Jespersen and Huang 2015; Jespersen et al. 2015). Plants were exposed to heat stress at 35/30 °C day/night or optimal temperature conditions of 22/17 °C day/night for a duration of 25 d. This experiment was arranged using a split-plot design, where the main plots were the temperature treatments and the subplots were the chemical treatments. There were five replicate pots consisting of one plant for each treatment, AVG or control, and replicates were distributed among four different growth chambers [two with optimal control conditions (22/17 °C day/night) and two with heat stress conditions (35/30 °C day/night)] and randomized every 3 d to diminish the impact of environmental variations among chambers.

Physiological measurements of leaf senescence. To measure overall plant health and the extent of leaf senescence, turf quality (TQ), chlorophyll content, and electrolyte leakage (EL) were evaluated before initiation of heat stress and every subsequent 7 d.

The TQ was visually rated using a scale of 1 (brown, desiccated, dead turf) to 9 (green, dense, uniform turf), with a rating of 6 defining minimally acceptable quality according to the methods of Beard (1972).

Using the methods of Hiscox and Israelstam (1979), with modifications, the leaf chlorophyll content was measured. Chlorophyll was extracted from ~100 mg of leaves from each sample using 10 mL dimethyl sulfoxide per sample and leaving samples in complete darkness for 72 h. Using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), the absorbance of chlorophyll extract was read at wavelengths of 663 and 645 nm. Leaf tissue was removed from the tubes and dried in a convection oven (Cole-Parmer, Vernon Hills, IL, USA) at 80 °C for 72 h; thereafter, dry weights of each sample were obtained. Using the formulas provided by Arnon (1949), the chlorophyll content was calculated.

The EL was quantified to measure the membrane stability of leaves. Approximately 200 mg of leaf tissue was excised from plants, cut into uniform pieces, and shaken in 35 mL of deionized water for ~8 h; thereafter, a conductance meter (model 32; YSI, Yellow Springs, OH, USA) was used to quantify the initial conductance (C_i). Samples were heated to a temperature of 121 °C

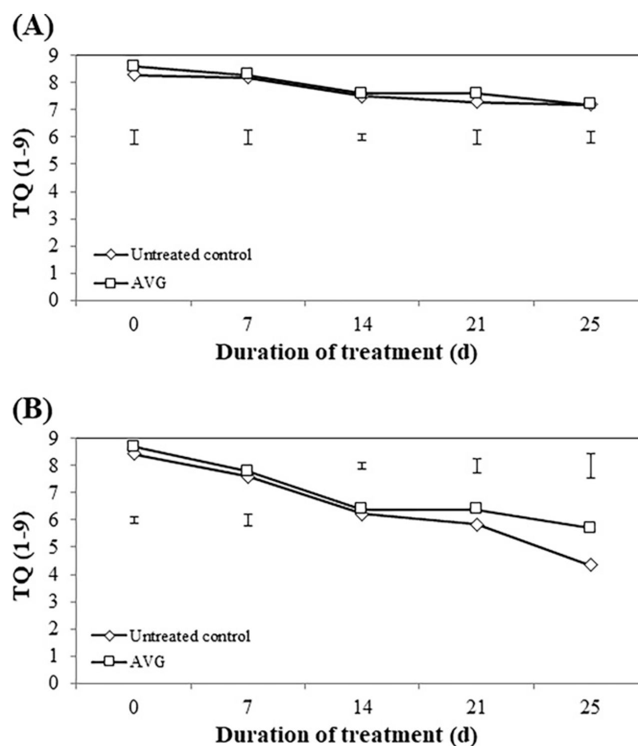

Fig. 1. Changes in turf quality (TQ) of creeping bentgrass foliar-treated with aminoethoxyvinylglycine (AVG) or water only (untreated control) over a duration of 25 d under non-stress control (**A**) or heat stress (**B**) temperature conditions. The TQ ratings were visually assigned using a scale of 1 to 9, with a value of 1 signifying turf that is brown, desiccated, and dead, and 9 defining turf that is completely healthy, turgid, and uniform green in color. Vertical bars signify least significant difference values between AVG and the untreated control treatments on the given day, with n defining the number of data points designated to each value (n = 5). Differences between treatment means were delineated according to Fisher's protected least significant difference test at $P = 0.05$.

for 30 min in an autoclave to kill leaf tissue and were then shaken for 8 h. After shaking, the maximum conductance (C_{max}) was measured. The EL was calculated as a percentage by dividing C_i by C_{max} and multiplying the result by 100, according to the formulas provided by Blum and Ebercon (1981).

Quantification of chlorophyll-synthesizing and chlorophyll-degrading enzyme activities. Activities of the chlorophyll synthesis enzyme, PBGD, and three chlorophyll degradation enzymes, CHLASE, CHL-PRX, and PPH, were measured using leaf tissue harvested every 7 d. Leaf tissue was frozen in liquid nitrogen and stored in a low-temperature freezer (Thermo Fisher Scientific) at −80 °C until enzyme activities were measured.

To obtain pure chlorophyll to be used in the series of enzymatic measurements, the methods of Iriyama et al. (1974) were used. Approximately 10 g of leaf tissue were harvested from healthy plants before initiation of heat stress. Leaves were frozen in liquid nitrogen and ground into a fine powder with a mortar and pestle; thereafter, 50 mL of cold acetone was added, and the slurry was ground and incubated in complete darkness at 4 °C for 2 h. The solution was centrifuged at 1000 g_n for 5 min, and 1,4-dioxane (1:7 volume/volume) was added to the supernatant. Deionized water was added one drop at a time until the solution became turbid, and then the solution was incubated under dark conditions for 1 h at 4 °C. After incubation, the solution was centrifuged at 10,000 g_n for 5 min, and the pellet was resolubilized in 1,4-dioxane (1:7 volume/volume). After adding deionized water drop-by-drop until the solution became turbid, the mixture was incubated again for 1 h at 4 °C under dark conditions, after which it was centrifuged for 5 min at 10,000 g_n. The supernatant was discarded, and the precipitate derived from the purification was dissolved by adding 50 mL of acetone to generate a concentration of 500 μg·mL^{-1} chlorophyll.

To extract chlorophyll enzymes from each sample, 400 mg of leaf tissue was frozen in liquid nitrogen and ground into a fine powder with a mortar and pestle. To each sample, 0.5 M KH_2PO_4 buffer (pH 7.0), 0.1 M phenylmethanesulfonyl fluoride, 1.0% nonionic surfactant {2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy] ethanol (Triton X-100; Union Carbide Co., Inc., Houston, TX, USA)}, and deionized water were mixed. The sample solution was ground again with 3.0% polyvinylpyrrolidone and centrifuged at 4 °C for 20 min at 9000 g_n, and the resulting solution was stored at −80 °C for future use in enzyme activity assays.

Activity of PBGD was quantified according to Jones and Jordan (1994), with slight modifications to their procedure. A 0.1 M Tris-HCl buffer (pH 7.5) comprising 2.5 mM ethylenediaminetetraacetic acid, 2 mM porphobilinogen, 15 mM $MgCl_2$, and 0.1% bovine serum albumin (BSA) was mixed, and 100 μL of enzyme extract was added to 500 μL of the buffer. The reaction mixture was heated at 37 °C for 1 h in a water bath; thereafter, 5 M HCl and 0.1% *p*-benzoquinone were added to halt the reaction. The absorbance of each sample was determined at 405 nm using a spectrophotometer.

The methods of Fang et al. (1998) were used to determine CHLASE activity. A solution containing 700 μL Tris-HCl buffer (pH 7), 300 μL acetone, 200 μL purified chlorophyll, 100 μL

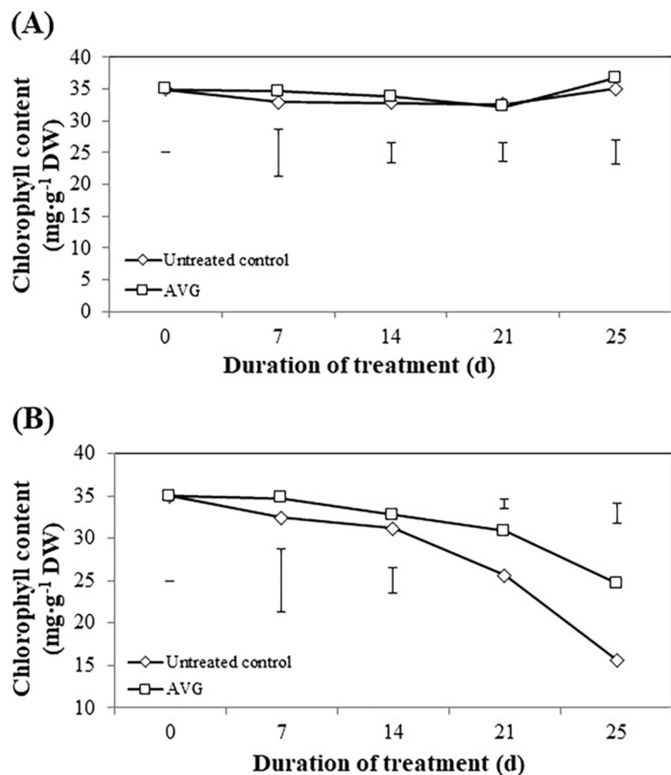

Fig. 2. Leaf chlorophyll content of creeping bentgrass foliar-treated with aminoethoxyvinylglycine (AVG) or water only (untreated control) over a duration of 25 d under non-stress control (**A**) or heat stress (**B**) temperature conditions. Vertical bars signify least significant difference values between AVG and the untreated control treatments on the given day, with n defining the number of data points designated to each value (n = 5). Differences between treatment means were delineated according to Fisher's protected least significant difference test at $P = 0.05$.

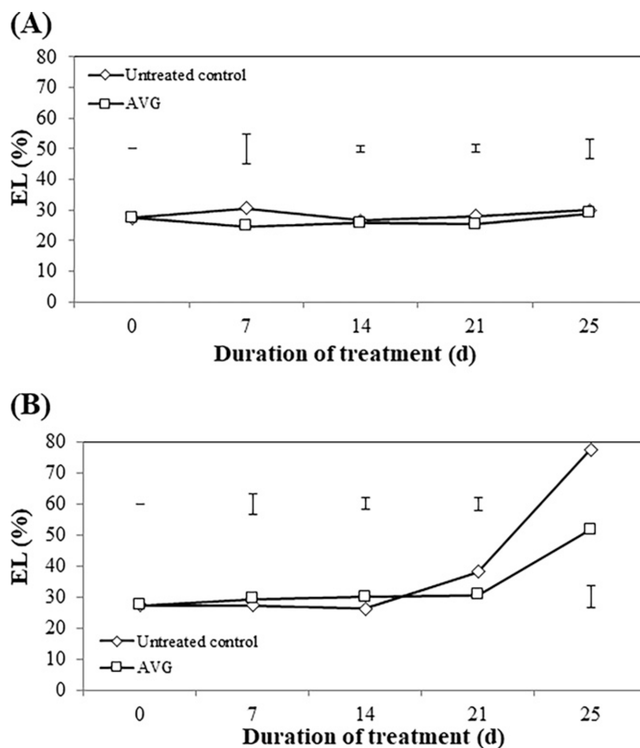

Fig. 3. Leaf electrolyte leakage (EL) of creeping bentgrass foliar-treated with aminoethoxyvinylglycine (AVG) or water only (untreated control) over a duration of 25 d under non-stress control (**A**) or heat stress (**B**) temperature conditions. Vertical bars signify least significant difference values between AVG and the untreated control treatments on the given day, with n defining the number of data points designated to each value (n = 5). Differences between treatment means were delineated according to Fisher's protected least significant difference test at $P = 0.05$.

enzyme extract, and 20 μL 0.1 M ascorbate was mixed and incubated under dark conditions in a water bath for 1 h at 35 °C. After the incubation period, 1 mL 0.5 M Tris-HCl buffer (pH 9), 2 mL hexane, and 1 mL acetone were added to each reaction vial, and samples were vortexed to move chlorophyll to the upper phase of hexane and chlorophyllide to the lower aqueous phase. To better-delineate the phases, samples were centrifuged at 10,000 g_n for 15 s, and the absorbance of chlorophyllide was determined using a spectrophotometer set to a wavelength of 665 nm.

Activity of CHL-PRX was determined using the methods of Aiamla-or et al. (2010), with some alterations. A mixture of 1 mL 0.1 M potassium phosphate buffer (pH 7), 100 μL 25 mM *p*-coumaric acid, 100 μL 1% nonionic surfactant, 50 μL enzyme extract, and 100 μL purified chlorophyll was made. To initiate the rate reaction, 100 μL 1% hydrogen peroxide was added to the mixture, and the rate of chlorophyll degradation by CHL-PRX was read using a spectrophotometer at a wavelength of 668 nm at 20-s intervals for a duration of 10 min. A single unit of CHL-PRX activity was expressed as a change in absorbance of 0.1 $mg^{-1} \cdot min^{-1}$ protein.

The PPH activity was determined by following the protocol of Kaewsuksaeng et al. (2011), with modifications. Pheophytin, the substrate on which PPH acts, was derived by combining 1 mL purified chlorophyll with 60 μL 0.1 M HCl and letting the mixture react for 5 min. To 100 μL pheophytin, 100 μL 1% nonionic surfactant and 600 μL 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.75) were added, and this mixture was reacted with 200 μL sample enzyme extract. The reaction was performed at 30 °C in complete darkness for 30 min. To cease the reaction, 100 μL 0.5 M Tris-HCl buffer (pH 9), 1 mL deionized water, 1 mL acetone, and 2 mL hexane were added to each reaction vial, and samples were vortexed to move pheophytin to the upper phase and pheophorbide to the lower phase. The absorbance of pheophorbide was read at 665 nm using a spectrophotometer.

To quantify protein content, the enzyme extract from each sample was combined with 20% trichloroacetic acid and incubated for 1 h at 4 °C so that the protein would precipitate onto the bottom of the vial. Samples were centrifuged for 5 min at 11,500 g_n, and the supernatant was discarded. After the protein pellet was air-dried, it was resuspended with 1 M sodium hydroxide. To determine the protein content of each sample, the Bradford Assay was used, whereby a series of concentrations of the standard, BSA, was created by serially diluting the BSA with Coomassie Brilliant Blue G-250 Dye (Bio-Rad Laboratories, Hercules, CA, USA) so that a standard curve could be generated (Bradford 1976). Using a spectrophotometer, the absorbance of each sample was measured at a wavelength of 595 nm, and these absorbance values were individually substituted into the standard curve equation to determine protein content.

Statistical analysis. To determine the effects of temperature and chemical treatments on the physiological and enzymatic parameters measured, a two-way analysis of variance was implemented using the general linear model procedure

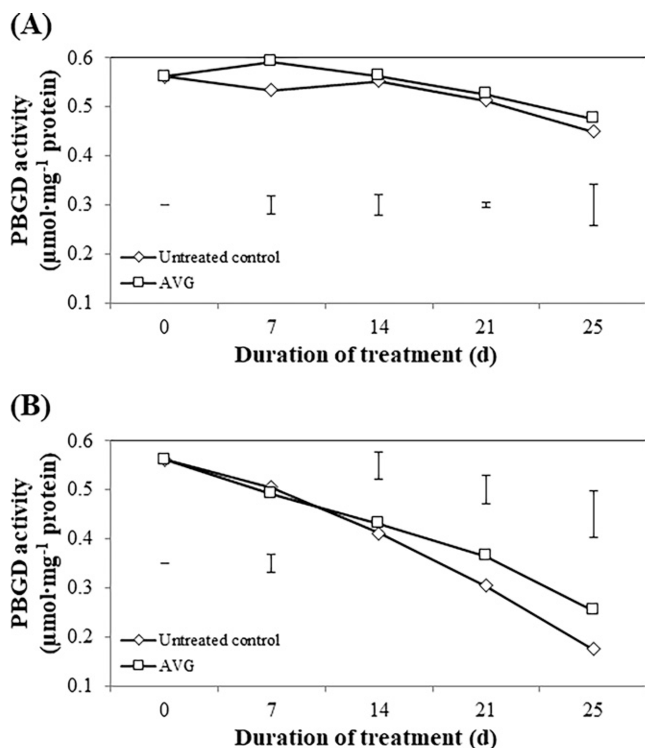

Fig. 4. Porphobilinogen deaminase (PBGD) activity of creeping bentgrass foliar-treated with aminoethoxyvinylglycine (AVG) or water only (untreated control) over a duration of 25 d under non-stress control (**A**) or heat stress (**B**) temperature conditions. Vertical bars signify least significant difference values between AVG and the untreated control treatments on the given day, with n defining the number of data points designated to each value (n = 5). Differences between treatment means were delineated according to Fisher's protected least significant difference test at $P = 0.05$.

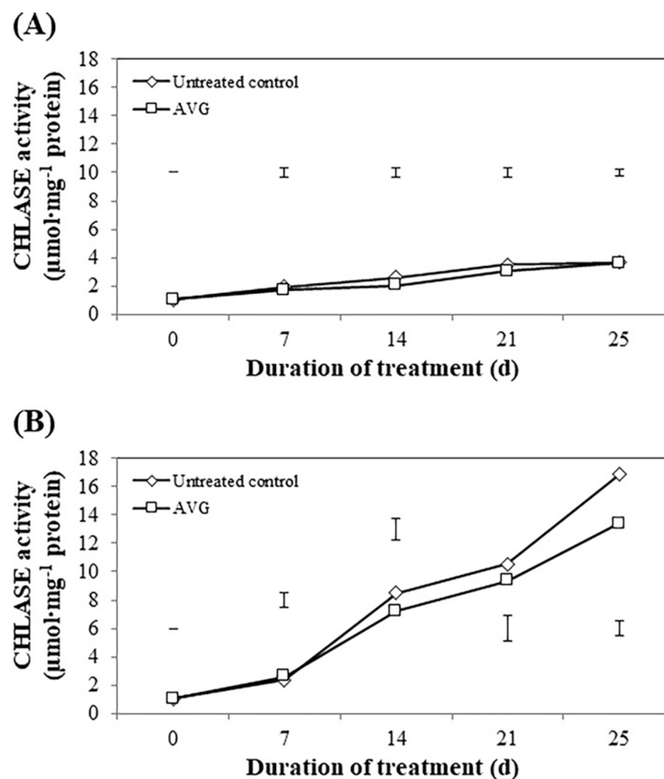

Fig. 5. Chlorophyllase (CHLASE) activity of creeping bentgrass foliar-treated with aminoethoxyvinylglycine (AVG) or water only (untreated control) over a duration of 25 d under non-stress control (**A**) or heat stress (**B**) temperature conditions. Vertical bars signify least significant difference values between AVG and the untreated control treatments on the given day, with n defining the number of data points designated to each value (n = 5). Differences between treatment means were delineated according to Fisher's protected least significant difference test at $P = 0.05$.

(PROC GLM) in SAS (version 9.2; SAS Institute, Cary, NC, USA). Fisher's least significant difference test was implemented at the $P = 0.05$ level to separate the differences between temperature and chemical treatments.

Results

Physiological responses of AVG-treated creeping bentgrass under heat stress. Under non-stress conditions, the TQ, chlorophyll content, and EL were not altered in response to AVG application for a majority of treatment dates (Figs. 1A, 2A, 3A). There was a steady decline in TQ and chlorophyll content through the duration of heat stress, whereas EL increased; however, TQ, chlorophyll content, and EL did not respond to heat stress as dramatically in AVG-treated plants when compared with the untreated control plants (Figs. 1B, 2B, 3B). The TQ was significantly higher in plants treated with AVG at 0, 21, and 25 d of heat stress (by 3.03%, 9.71%, and 31.54%, respectively) in comparison with the untreated controls. Chlorophyll content was significantly increased in AVG-treated plants at 21 and 25 d of heat stress (by 20.3% and 58.03%, respectively), whereas EL was significantly lower in plants treated with AVG at 21 and 25 d of heat stress (by 19.99% and 33.35%, respectively).

Effects of the exogenous application of AVG on chlorophyll-synthesizing and chlorophyll-degrading enzymes. The activities of chlorophyll synthesis (PBGD) and degradation (CHLASE, CHL-PRX, and PPH) enzymes were measured to understand whether AVG may suppress heat-induced leaf senescence by promoting chlorophyll production or inhibiting chlorophyll breakdown.

Under non-stress control conditions, enzymatic activities for chlorophyll-synthesizing and chlorophyll-degrading enzymes were not significantly altered by AVG on a majority of treatment dates (Figs. 4A, 5A, 6A, 7A). Through the duration of heat stress, the activity of the chlorophyll-synthesizing enzyme, PBGD, exhibited a decreasing trend overall, but the decline was not as pronounced in AVG-treated plants (Fig. 4B). Activity of PBGD was significantly higher in AVG-treated plants at 21 d of heat stress (by 19.7%).

Activities of the chlorophyll-degrading enzymes increased over the course of heat stress, but the inclining trend was not as severe in AVG-treated plants when compared with the untreated control plants (Figs. 5B, 6B, 7B). The activity of CHLASE was significantly lower in AVG-treated plants at 25 d of heat stress (by 20.58%). Treating plants with AVG led to a significant decline in CHL-PRX activity at 14, 21, and 25 d of heat stress (by 8.04%, 10.05%, and 12.4%, respectively). At 21 and 25 d of heat stress, AVG significantly reduced the activity of PPH (by 21.92% and 27.84%, respectively).

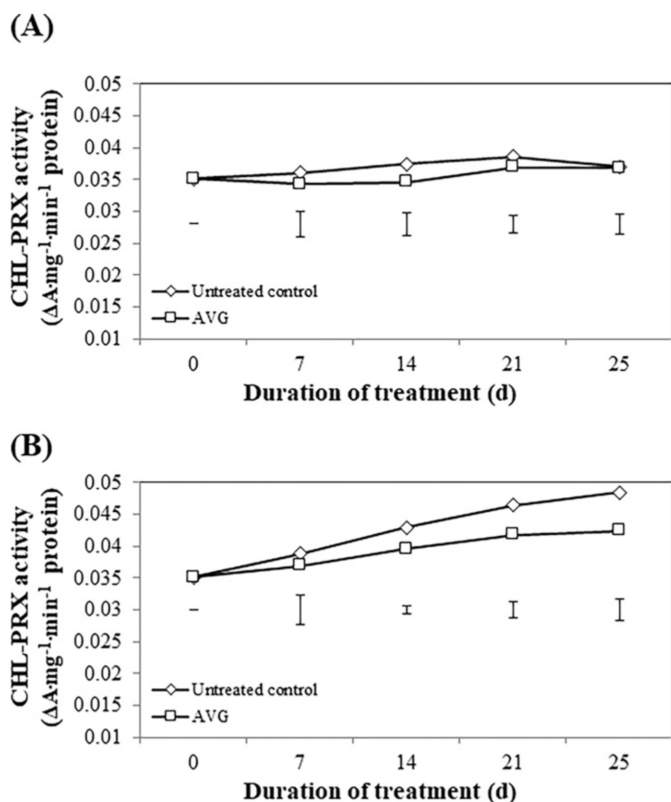

Fig. 6. Chlorophyll-degrading peroxidase (CHL-PRX) activity of creeping bentgrass foliar-treated with aminoethoxyvinylglycine (AVG) or water only (untreated control) over a duration of 25 d under non-stress control (**A**) or heat stress (**B**) temperature conditions (ΔA is defined as the change in absorbance). Vertical bars signify least significant difference values between AVG and untreated control treatments on the given day, with n defining the number of data points designated to each value (n = 5). Differences between treatment means were delineated according to Fisher's protected least significant difference test at $P = 0.05$.

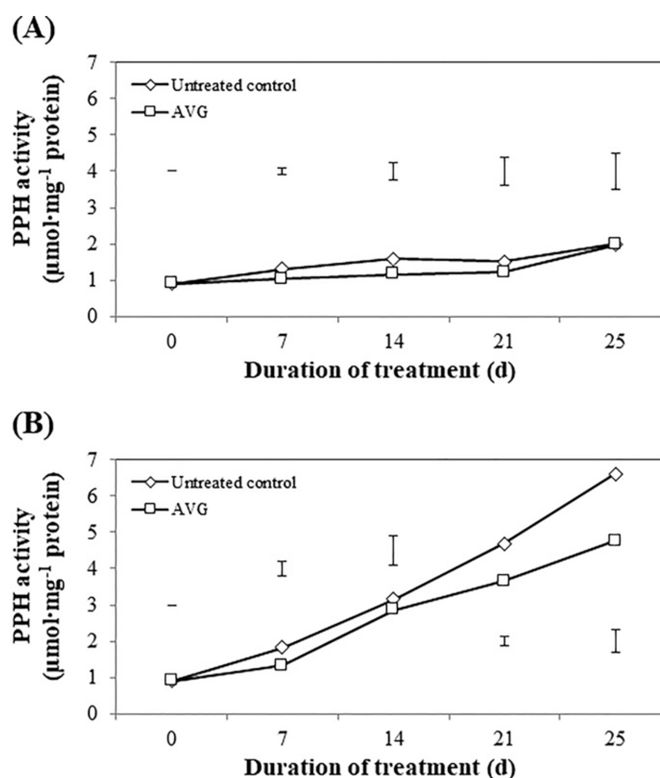

Fig. 7. Pheophytinase (PPH) activity of creeping bentgrass foliar-treated with aminoethoxyvinylglycine (AVG) or water only (untreated control) over a duration of 25 d under non-stress control (**A**) or heat stress (**B**) temperature conditions. Vertical bars signify least significant difference values between AVG and the untreated control treatments on the given day, with n defining the number of data points designated to each value (n = 5). Differences between treatment means were delineated according to Fisher's protected least significant difference test at $P = 0.05$.

Discussion

Stay-green or delayed leaf senescence is a highly desirable trait for turfgrass because of its benefits on maintaining turf aesthetic quality and photosynthetic functionality (Xu et al. 2019; Yu et al. 2021b; Zhang et al. 2022). Although heat stress negatively impacted chlorophyll synthesis and accelerated chlorophyll degradation in creeping bentgrass, foliar application of the ethylene inhibitor, AVG, effectively alleviated heat-induced leaf senescence, as indicated by an increase in TQ and chlorophyll content, as well as a reduction in EL. These results suggest that AVG could be used as a biostimulant component to extend the stay-green period during the summer months in warm climatic regions.

It has been indicated that accumulation of ethylene is detrimental to heat tolerance in several plant species, including soybean, winter wheat (*Triticum aestivum*), and pea (*Pisum sativum*) (Djanaguiraman and Prasad 2010; Hays et al. 2007; Kaur et al. 2021). The effects of AVG on improving heat tolerance have been associated with an increase in activities of antioxidant enzymes and reduction in lipid peroxidation through exogenous application of this compound (Xu and Huang 2009) and enhanced content of metabolites, including organic acids, sugar alcohols, and sucrose during heat stress after AVG treatment (Jespersen and Huang 2015). Previous studies suggested that AVG may enhance antioxidant metabolism and osmoregulation for plant defense against heat stress. Although the effects of AVG on suppressing leaf senescence have been reported, very few studies have focused on how AVG may specifically regulate chlorophyll metabolism in plants exposed to heat stress. In a recent study that examined the effects of AVG on heat tolerance in perennial ryegrass (*Lolium perenne*), it was exhibited that applying this compound enhanced the activity of PBGD and suppressed the activity of CHLASE and PPH; however, the effects of AVG on CHL-PRX activity were not significant (Chen and Huang 2022). In this study, creeping bentgrass treated with AVG exhibited increased activity of the chlorophyll-synthesizing enzyme, PBGD, and reduced activity of the three chlorophyll-degrading enzymes, CHLASE, PPH, and CHL-PRX, under heat stress with regard to the untreated controls; however, heat stress inhibited chlorophyll-synthesizing enzyme activity and enhanced the activity of the three chlorophyll-degrading enzymes (Fig. 8). The increase in CHLASE, PPH, and CHL-PRX by heat stress was also reported in creeping bentgrass in our previous study (Rossi et al. 2017). The application of AVG to heat-stressed perennial ryegrass was found to alleviate leaf senescence by downregulating expression of the *LpPPH* gene, which encodes the PPH enzyme (Zhang et al. 2019). A recent study found that ethylene accelerated leaf senescence under heat stress through the regulation of the transcription factor, WRKY, in perennial ryegrass (Chen and Huang 2022); however, whether AVG may regulate PBGD, CHLASE, PPH, and CHL-PRX at the gene transcript level in creeping bentgrass under heat stress conditions deserves further investigation.

The findings of the current study in tandem with these previous studies suggest that AVG could suppress heat-induced leaf senescence by maintaining chlorophyll synthesis and alleviate chlorophyll degradation in creeping bentgrass exposed to heat stress through action on enzymatic activities, in addition to the other mechanisms previously reported. Understanding how AVG may regulate chlorophyll metabolism at the molecular level may

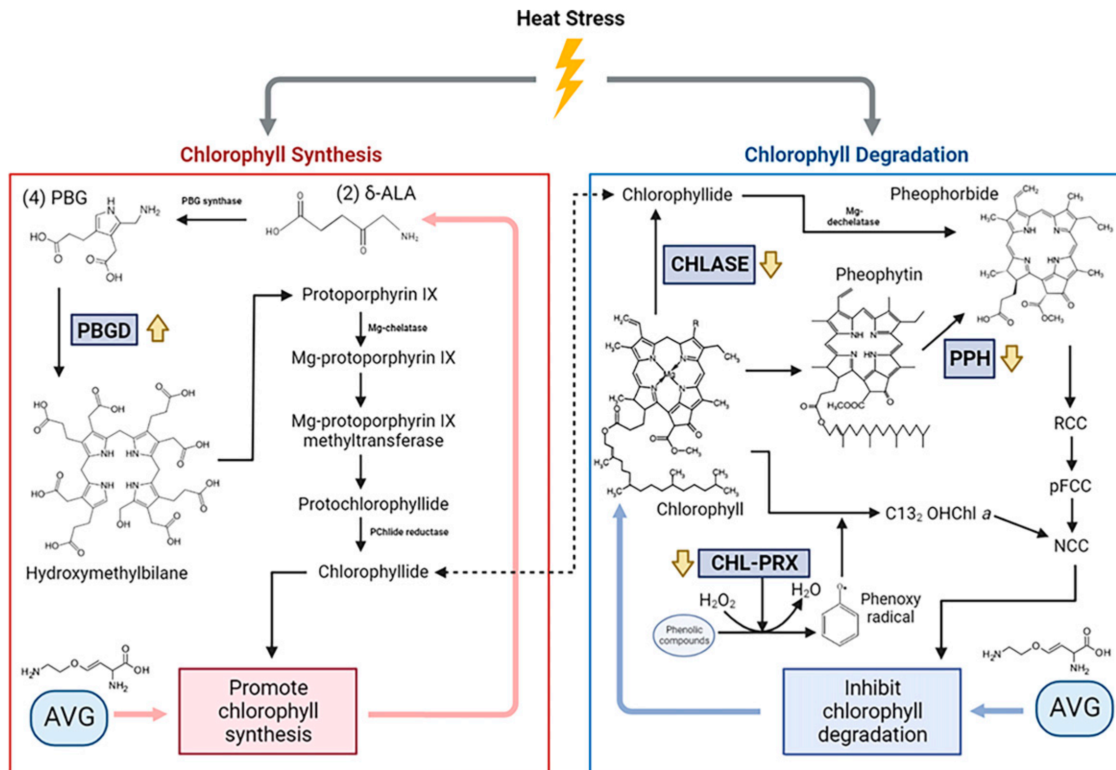

Fig. 8. Pathway map for chlorophyll synthesis and degradation in creeping bentgrass foliar-treated with aminoethoxyvinylglycine (AVG) under heat stress showing chlorophyll enzymes promoted or suppressed by AVG treatment: δ-ALA = δ-aminolevulinic acid; $C13_2$ OHChl *a* = $C13_2$ hydroxychlorophyll a; CHL-PRX = chlorophyll-degrading peroxidase; CHLASE = chlorophyllase; NCC = nonfluorescent chlorophyll catabolites; pFCC = primary fluorescent chlorophyll catabolite; PBG = porphobilinogen; PBGD = porphobilinogen deaminase; PPH = pheophytinase; RCC = red chlorophyll catabolite.

facilitate the development of novel lines of cool-season grass species with stay-green traits to maintain high-quality turfgrass during summer months.

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