Ultraviolet-B Radiation Damage on Kentucky Bluegrass II: Hormone Supplement Effects

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Abstract. High ultraviolet-B (UV-B; 290-320 nm wavelength) radiation may significantly contribute to the quality decline and death of kentucky bluegrass (Poa pratensis L.) sod during summer transplanting. Antioxidants and protective pigments may be involved in plant defense against oxidative stress caused by UV-B. Selected exogenous hormones may alleviate UV-B damage by upregulating plant defense systems. The objectives of this study were to determine if exogenous hormone or hormone-like substances could alleviate UV-B damage to 'Georgetown' kentucky bluegrass (Poa pratensis L.) under greenhouse conditions. The hormone salicylic acid at 150 mg·m⁻² and the hormone-containing substances, humic acid (HA) at 150 mg·m⁻² and seaweed extract (SWE) at 50 mg·m⁻², were applied to plugs of kentucky bluegrass and then subjected to UV-B radiation (70 μmol·m⁻²·s⁻¹). The UV-B irradiation stress reduced turf quality by 51% to 66% and photochemical efficiency by 63% to 68% when measured 10 or 12 days after initiation of UV-B. Endogenous alpha-tocopherol (AT) and antioxidant enzymes (superoxide dismutase (SOD) and catalase) were reduced by UV-B stress. Anthocyanin content was increased from day 1 to 5 and then decreased from day 5 to 10 of continuous UV-B irradiation. Application of SA and HA + SWE enhanced photochemical efficiency by 86% and 82%, respectively, when measured 10 or 12 days after UV-B initiation. In addition, application of the hormonal supplements increased AT concentration, SOD, catalase activity, and anthocyanin content when compared to the control at 10 days after UV-B initiation. Bluegrass with greater AT concentration and SOD and catalase activity exhibited better visual quality under UV-B stress. The results of this study suggest that foliar application of SA and HA + SWE may alleviate decline of photochemical efficiency and turf quality associated with increased UV-B light levels during summer.

The vagaries of the turfgrass and landscaping industry often dictate that kentucky bluegrass sod be harvested, transported, and transplanted during the summer. Frequently, increased respiratory heating during storage, transport, and subsequent exposure to high UV-B during transplanting can cause shock (Giese et al., 1997). The shock often results in bleached, inactive turfgrass leaves. Plants better adapted to resist UV-B induced photo-bleaching usually contain more robust screening (pigment) and scavenging (antioxidant) protection systems (Mackerness, 2000). Resistance to UV-B damage most likely involves both avoidance and tolerance mechanisms.

Day et al. (1992) noted conifers are efficient avoiders of UV-B damage due to higher needle levels of flavonoid and related phenolic pigments (anthocyanins) that strongly absorb UV and transmit nondamaging longer wavelengths. Most herbaceous species do not appear to have such robust screening systems. However, it has been established that exposure of herbaceous plants to UV radiation will up-regulate the formation of flavonoids (Salisbury and Ross, 1991). Additionally, application of a green-pigmented colorant that strongly absorbs in the UV range (250 to 400 nm) has been shown to improve avoidance of UV-B damage in kentucky bluegrass (Ervin et al., 2004) and creeping bentgrass (Agrostis stolonifera L.; Schmidt and Zhang, 2001). Further, by exogenously applying ascorbic acid and alpha-tocopherol, endogenous alpha-tocopherol and antioxidant enzyme activities increased and directly correlated with greater tolerance of UV-B by kentucky bluegrass (Ervin et al., 2004).

Greater tolerance to oxidative stress caused by high UV-B may involve hormonal signals to up-regulate antioxidant enzyme activity. One of the implicated hormones is the phenolic compound, salicylic acid (SA). Numerous studies have shown that SA is a key signaling compound involved in the activation of certain plant defense mechanisms including induction of pathogenesis-related genes and antioxidant enzymes (Mackerness, 2000; Jordan, 1996). Recently, Clarke et al. (2002) noted that SA reduced heat-induced oxidative damage and increased catalase, glutathione reductase and peroxidase activity in Phaseolus vulgaris. Sanjay et al. (2001) indicated SA increased

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Materials and Methods

The detailed experimental procedures, including field growth conditions of harvested plant material, subsequent greenhouse growing conditions, UV-B treatments, data collection, measurement, and statistical protocols were described in a companion paper (Ervin et al., 2004).

Experiment 1. Treatments for this study were 1) SA at 150 mg·m⁻²; 2) HA at 150 mg·m⁻² + SWE at 50 mg·m⁻²; and 3) control. Three replications were arranged in randomized complete blocks on the greenhouse bench. Salicylic acid (Research Organics; Cleveland, Ohio), seaweed extract (Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada) and HA (Plant Wise Biostimulants, Inc. Louisville, Ky.) are each available as dry powders. Solutions were made by mixing the products with water. Salicylic acid solution required addition of a surfactant (Aqua-Gro, Aquatrols Corporation, Cherry Hill, N.J.) at 0.05% to encourage uniform coverage.

Light absorbance of the two treatment solutions was measured across the UV spectral range (250 to 400 nm) and results are presented as percent absorbance at each wavelength (Fig. 1). The SA-treatment solution had 20%
to 30% absorbance across the UV-B spectra, while maximum absorbance of 20% occurred at 290 to 300 nm for the HA + SWE solution. The treatments were sprayed onto Kentucky bluegrass foliage using 60-mL syringes with needles on 15 Mar. 2001. The treated turfgrass was not irrigated for 24 h.

Twenty-four hours after treatment the plugs were placed under artificial UV-B radiation (70 µmol·m⁻²·s⁻¹) provided by three 40-W UV-B fluorescent lamps (UVB-313, Cleveland, Ohio). The plugs were spaced evenly (2.5 cm), kept 0.5 m below the UV-B source, and grown under continuous UV-B in a greenhouse maintained at 22 ± 2°C. Daylength averaged 11 h. Plugs were subjected to UV-B from 16 to 26 Mar. and irrigated three times a week to prevent moisture stress. On 27 Mar., the plugs were removed from UV-B treatment and placed under a mist system for recovery. Photochemical efficiency and turf quality (based on a visual scale of 1 to 9, with 9 indicating the best quality) scores (as an indication of the degree of visual injury or lack thereof) were determined as in previous trials.

Experiment 2. Treatments remained the same as in Expt. 1. The plugs were treated on 31 May and then placed under continuous UV-B irradiation from 1 through 12 June 2001. On 13 June, UV-B treatment ceased and the plugs were set under a mist system for recovery. Photochemical efficiency and turf quality scores were determined as in previous trials.

Experiment 3. Treatments remained the same as those in Expts. 1 and 2. However, there were four replications instead of three. The plugs were treated on 24 Jan. 2002 and placed under continuous UV-B from 25 Jan. through 4 Feb. 2002. On 5 Feb., UV-B irradiation stress was discontinued and the plugs were placed under a mist system for recovery. Photochemical efficiency and turf quality scores were determined as in previous trials.

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Table 2. Kentucky bluegrass antioxidant and pigment responses to hormone supplement treatments and UV-B irradiance over 10 d; Expt. 3

<table>
<thead>
<tr>
<th>Supplement treatment</th>
<th>Rate (mg·m⁻²)</th>
<th>Days of UV-B exposure</th>
<th>Alpha-tocopherol (µg·g⁻¹ FW)</th>
<th>SOD activity (unit/mg protein)</th>
<th>Catalase activity (unit/mg protein)</th>
<th>APX activity (unit/mg protein)</th>
<th>Total Chlorophyll (µg·mL⁻¹)</th>
<th>Total Carotenoids (µg·mL⁻¹)</th>
<th>Anthocyanin (A530 – (0.25 × A657))</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1</td>
<td>6.4 a</td>
<td>4.0 b</td>
<td>4.2 b</td>
<td>85.5 c</td>
<td>6.9 a</td>
<td>55.8 b</td>
<td>2.6 a</td>
</tr>
<tr>
<td>HA + SWE</td>
<td>150</td>
<td>1</td>
<td>6.9 a</td>
<td>5.9 a</td>
<td>4.0 a</td>
<td>85.5 b</td>
<td>77.1 a</td>
<td>45.3 a</td>
<td>1.7 a</td>
</tr>
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<td>4.6 ab</td>
<td>4.2 a</td>
<td>84.5 b</td>
<td>44.5 c</td>
<td>45.6 a</td>
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<tr>
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<td>15.1 b</td>
<td>18.8 b</td>
<td>117.5 a</td>
<td>0.5 a</td>
<td>0.6 a</td>
<td>0.7 a</td>
</tr>
<tr>
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<td>17.5 a</td>
<td>18.2 a</td>
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<td>21.6 a</td>
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<td>0.7 a</td>
<td>0.9 a</td>
<td>1.0 a</td>
</tr>
<tr>
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<td>84.5 b</td>
<td>44.5 c</td>
<td>45.6 a</td>
<td>85.5 b</td>
<td>0.5 a</td>
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<td>1.3 a</td>
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<td>1.3 a</td>
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<td>1.5 a</td>
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<td>2.9 a</td>
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<tr>
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<tr>
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<td>0.34 b</td>
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<td>0.56 a</td>
<td>0.61 a</td>
<td>0.36 a</td>
<td>0.36 a</td>
</tr>
</tbody>
</table>

*HA = humic acid; SWE = seaweed extract.

Values within the same column marked with same letters are not different significantly at α = 0.05.

*SOD = superoxide dismutase; APX = ascorbate peroxidase.

Superoxide dismutase activity was equivalent across treatments at day 1 (Table 2). At day 5, SA- and HA + SWE-treated plants had greater SOD activity. From day 1 to 10, SOD activity decreased by 38% in control plants, while SA- and HA + SWE-treated plants only lost 16% and 9% SOD activity, respectively. Correlation between turf quality and SOD activity was not significant over the 10-d period.

Catalase activity declined sharply following 5 d of UV-B irradiation (Table 2). However, relative to the control, bluegrass pretreated with HA + SWE or SA maintained 25% and 73% higher catalase activity, respectively, at 5 d after UV-B initiation. When measured at 10 d, HA + SWE or SA resulted in 2.4-fold greater catalase activity. Accordingly, HA + SWE (r = 0.97”) and SA (r = 0.78”) were correlated with greater quality ratings over the 10-d UV-B exposure period. Ascorbate peroxidase activity was unaffected by UV-B irradiation and supplement pretreatment (Table 2).

A greater total chlorophyll content (i.e., chl a + b) remained following 10 d of UV-B due to HA + SWE and SA (Table 2); both treatments also resulted in greater carotenoids at day 10. Anthocyanin content for all three treatments increased under continuous UV-B from day 1 to 5, and decreased from day 5 to day 10 (Table 2). Relative to the control, SA-treated bluegrass had greater anthocyanin concentrations at all sampling dates, while HA + SWE plants had greater anthocyanin only at day 10. Patterns of anthocyanin concentration suggest control plants had less ability to maintain this defense with prolonged exposure to UV-B radiation.

Discussion

Similar to results reported in the companion paper (Ervin et al., 2004), pretreatment with SA or SWE + HA before continuous UV-B exposure alleviated decline of kentucky bluegrass photochemical efficiency and visual quality. In general, the magnitude of protection was similar for SA and HA + SWE treatments across all three experiments. Schmidt and Zhang (2001) reported similar protection of Kentucky bluegrass photosynthetic function under UV-B stress due to SA and SWE + HA treatments. However, our data expand these findings by indicating that damage mitigation was associated with higher AT concentration, higher SOD and catalase activity, and greater leaf pigment concentrations.

Increased SOD activity with exogenous application of SA has been shown with Nicotiana plumbaginifolia (Bowler et al., 1989). Mackerness (2000) pointed out that while UV-B exposure leads primarily to oxidative damage as leaves senesce (Field et al., 2001) and many species have been noted to up-regulate anthocyanin production in response to increased UV radiation (Holton and Cornish, 1995). In general, hormone-supplement treated plants maintained greater overall pigment concentrations over the 10-d radiation period. Less pigment destruction was most likely associated with the more robust antioxidative protection systems reported herein due to HA + SWE and SA treatments.

The hormone-like activities of seaweed extract and humic acid have been identified with bioassay and GC–MS techniques (Cacco and Dell’Agnola, 1984; Sanderson et al., 1987; Yan, 1993). Indirect ELISA indicates that the SWE used in these experiments contained 70 mg kg⁻¹ of the cytokinin, zeatin riboside (Zhang and Ervin, 2004). Applications of a combination of SWE and HA have been previously shown to increase antioxidant concentrations and activities of drought-stressed kentucky bluegrass (Zhang and Schmidt, 1999). Our results also indicate that antioxidant increases are correlated with reduced UV-B irradiation damage. Based on our reading of the literature, it is possible that the SWE + HA treatment affected cell membrane integrity either directly, by altering membrane components, or indirectly, by increasing antioxidative protective mechanisms. Yan et al. (1997) reported that perennial ryegrass (Lolium perenne L.), pretreated with SWE fortified with HA and Fe had increased drought tolerance associated with increased membrane fluidity (i.e., greater levels of unsaturated fatty acids). It is also clear that cytokinins directly function to scavenge free radicals and prevent their formation (Leshem, 1981; Musgrave, 1994). A further role of cytokinins in antioxidative protection has recently been shown by Liu and Huang (2002) who reported that treatment of creeping bentgrass roots with zeatin riboside reduced lipid peroxidation and senescence of leaves exposed to high temperature stress by suppressing loss of SOD and catalase activity.

In summary, our data indicate that increasing the robustness of the antioxidative protection system in Kentucky bluegrass through the application of phytohormone supplements prior to UV-B stress served to mitigate pigment destruction, alleviate decline of photochemical efficiency, delay senescence, and improve the rate of visual quality recovery. As terrestrial UV-B levels continue to increase, our approach of boosting plant defense systems through direct application of antioxidants (Ervin et al., 2004) or application of hormone supplements may prove of high utility for improving the abiotic stress resistance of cool-season turfgrasses. However, further research is required to confirm our results in field environments and fine-tune application protocols.

Literature Cited


