Light Intensity Affects Gibberellic Acid Content in Kentucky Bluegrass

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Abstract. Turfgrasses grown under low light conditions generally exhibit increased shoot elongation. Gibberellic acids (GAs), GA₁ in particular, promote stem elongation in grass species. GA₂₀ is the immediate precursor of GA₁. However, a direct quantitative measurement of GA₁ and GA₂₀ has not been reported for turfgrass under different light intensities. This study was conducted in a greenhouse to quantify the endogenous levels of GA₁ and GA₂₀ of 'KenblueTimes', 'Livingston', and 'NuGlade' Kentucky bluegrass (*Poa pratensis* L.) under two light intensities with and without trinexapac-ethyl (TE) application. GA₁ and GA₂₀ content in leaf tissue were analyzed using gas chromatography-mass spectrometry with deuterium-labeled GA₁ and GA₂₀ as internal standards. Light reduction of 73% under greenhouse conditions increased GA₁ by 44% to 47% and GA₂₀ by 16% to 50%. 'NuGlade' had a GA₁ content 20% lower than that of 'Kenblue', suggesting that the dwarf characteristics of 'NuGlade' may be related to its low GA₁ content. The application of TE (0.1 kg·ha⁻¹) reduced GA₁ concentration by 47%, but increased GA₂₀ concentration by 146%, supporting the contention that TE inhibited GA₁ biosynthesis by blocking the conversion of GA₂₀ to GA₁. Chemical names used: 4-(cyclopropyl-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester (trinexapac-ethyl); gibberellic acid (GA).

Gibberellins are a group of tetracyclic diterpenoids that are involved in various plant growth and development processes, such as stem growth, flower and seed development, and seed germination (Graebe, 1987). The involvement of gibberellic acid (GA) in regulating shoot elongation in higher plants is particularly interesting to the management of turfgrass. Gibberellic acids, GA₁, GA₁₉, GA₂₀, GA₄₄, and GA₅₃, have been identified from Kentucky bluegrass [*Poa pratensis* L. (KBG)], the most widely used cool-season turfgrass (Junttila et al., 1997). Junttila et al. (1997) found that long-day photoperiods stimulate gibberellic acid synthesis in KBG, and as a consequence, stimulate increased leaf elongation. Heide et al. (1985) suggested that GA₁, GA₃, and GA₄ cause shoot and leaf growth of Kentucky bluegrass. However, in grass species, GA₁ is primarily responsible for shoot and leaf elongation. GA₂₀ is the immediate precursor of GA₁ (Reid and Ross, 1991).

Much research about the influence of light and endogenous GAs on KBG shoot elongation has focused on photoperiod (Junttila et al., 1997; Heide et al., 1985). The effects of light intensity on shoot elongation and GAs levels are poorly understood. When KBG is grown under low light intensity, it first exhibits increased shoot and leaf elongation (Beard, 1965). Enhanced shoot elongation is a shade avoidance mechanism of plants. This is rather undesirable in turfgrass since the goal for turfgrass management

is to produce a dense grass sward with reduced upright growth. Rapid shoot elongation results in more shoot removal during regular mowing and accelerates energy depletion, which may result in reduced rooting and tillering, and a decline in overall turf quality (Qian et al., 1998). The increased shoot elongation in KBG under shade may, in part, result from increased GA₁ content, increased sensitivity of the plant to GAs, or both. However, no research has been done to quantify the effect of light intensity on endogenous GA₁ and GA₂₀ content for turfgrass. This information is essential in understanding the physiological response to low irradiance conditions and facilitate wise use of GA and GA-reducing compounds for the regulation of shoot elongation in turfgrass.

Trinexapac-ethyl (TE) is an anti-gibberellic acid type of plant growth regulator, which inhibits shoot elongation by blocking the conversion of GA₂₀ to GA₁, the last step of GA biosynthesis involving 3 β -hydroxylation of GA₂₀ (Adams et al., 1992). Research shows that TE restricts shoot elongation and improves Zoysiagrass and KBG quality under low light conditions (Qian and Engelke, 1999; Qian et al., 1998; Ervin et al., 1999; and Stier and Rogers, 2001).

The objectives of this study were: 1) to quantify the endogenous GA₁ and GA₂₀ content of three KBG cultivars under two light intensities, and 2) to determine the influence of TE on GA₁ and GA₂₀ content of KBG under low light intensity.

Materials and Methods

Plant materials. The experiments were conducted in a fiberglass-reinforced plastic greenhouse from Nov. 1999 to Jul. 2000. Average air temperature in the greenhouse was $\approx$ 20

°C at 0800 HR, 24 °C at 1400 HR, and 18 °C at 2000 HR. Three cultivars of KBG were selected for the experiment based on their morphological characteristics: 'NuGlade' KBG exhibits dwarf, compact shoot growth; 'Livingston' KBG produces medium shoot growth; and 'Kenblue' KBG has a fast shoot growth rate and leaf elongation. Sod pieces 30 cm in diam. were excised from field plots. They were hand-washed to remove soil, and planted in a total of 48 plastic containers (30 cm in diameter and 40 cm deep). The containers were filled with a mix of 85 sand :15 compost (by volume). Initially, plants were grown under natural light in the greenhouse, with photosynthetically active radiation ranged from $\approx$ 150 μ mol·m⁻²·s⁻¹ on cloudy days to 1150 μ mol·m⁻²·s⁻¹ on sunny days. Turf was clipped weekly at a 3.5-cm height and clippings were removed. This experiment was conducted as a three-way factorial design with four replications. The three treatment factors were cultivar, shade, and TE.

Five months after sodding, light treatments were randomly applied to the 48 containers. The light treatments consisted of 13% sunlight (low irradiance from shade cloth) and 52% sunlight (high irradiance from natural greenhouse conditions). Based on measurements on photon flux densities (μ mol·m⁻²·s⁻¹) with a quantum radiometer (model LI-170, LI-COR, Lincoln, Nebr.), the fiberglass roof provided $\approx$ 52% sunlight transmission. The low irradiance treatment was achieved by mounting a saran shade cloth with 73% light filtering property on a PVC frame and supported 50 cm above the turf containers. The shade cloth reduces light intensity but does not affect quality (Allard et al., 1991). The shade cloth was draped on all sides to prevent the effect of incline light. The shade cloth plus fiberglass roof allowed only 13% sunlight transmission.

Trinexapac-ethyl was applied to 24 pots at 0.048 kg·ha⁻¹ a.i. 2 d after the application of irradiance treatments with a CO₂-pressurized sprayer that delivered 550 L·ha⁻¹ at a pressure of 1.5 kg·cm⁻². Trinexapac-ethyl was repeatedly applied at the same rate at monthly intervals. The remaining 24 pots that subjected to no TE application were considered the controls.

During week 1 to week 6 after the initiation of shade treatment, data on canopy height and clipping yield were determined weekly. Canopy height was measured prior to each clipping event. A ruler was set on end on the soil surface, and a thin 10-cm diameter cardboard disc with a hole in its center was dropped freely over the ruler onto the turf canopy. Canopy height was read as the distance between the soil surface and the disk. Clippings were collected at each clipping event, and dried at 70 °C for 48 h for dry weight determination.

Gibberellic acid measurement. About 6 weeks after the initial shading treatments and 2 weeks after the second TE treatment, leaf tissues were sampled to determine GA₁ and GA₂₀ content. Samples were collected from each container, immediately freeze-dried (Genesis 25LL Lyophilizer, Virtis, Gardiner, N.Y.), and subsequently stored in air-tight bottles at -20 °C until homogenization in 250 mL of cold 80% methanol. During homogenization,

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deuterium-labeled (17,17-2H₂) GA₁ and (17,17-2H₂) GA₂₀ were added to the extracts as internal standards. Deuterated GAs were obtained from Prof. Mander, Research School of Chemistry, Australian National Univ., Canberra, Australia. After overnight extraction, the filtered extracts were partitioned at least three times against hexane and diethyl ether, and further purified by column chromatography with charcoal : Celite, silicic acid : Celite, and QEA-E-Sephadex-25. Detailed procedures used on GA purification were described by Talon and Zeevaart (1990) and Zeevaart (1971).

The extracted and purified GAs were methylated with ethereal diazomethane and then trimethylsilylated. Samples were analyzed by a gas chromatograph-mass spectrometer (GC-MS) (SATURN 2000, Varian Associates, Calif.). After injection, the oven temperature was maintained at 80 °C for 0.5 min, and then increased to 200 °C at 25 °C/min, followed by a further increase to 300 °C at 5 °C/min. The He inlet pressure was 100 kPa and the injector, interface, and source temperatures were 250 °C, 250 °C, and 190 °C, respectively. The prominent ion pairs of 506/508 and 418/420 (endogenous/di-deuterated) of GA₁ and GA₂₀ were monitored. Since the amounts of deuterium-labeled (17,17-2H₂) GA₁ and (17,17-2H₂) GA₂₀ (internal standards) added to the extracts were known, endogenous GA₁ and GA₂₀ content was calculated from the peak area ratios of m/z (mass to charge signals) 506/508 and 418/420, respectively (Watson, 1997).

Data analysis. There were variations in canopy height and clipping yield among different measurement dates. However, the rank of treatments for clipping yield and canopy height was largely orderly, therefore, the averages over six consecutive weekly measurements for clipping yield and canopy height were subjected to analysis of variance (ANOVA) test. The ANOVA was conducted for data on canopy height (average over six measurements), clipping yield (average over six measurements), GA₁ and GA₂₀ content (week 6 sampling only) using the GLM procedure (Table 1). Means were separated using Fisher's protected least significant difference (LSD) (SAS Institute, 1989). Correlation analysis was performed to determine the relationship between the canopy height and GA content at 6 weeks after shading treatment.

Results and Discussion

Canopy height and clipping yield. Shoot elongation, expressed as canopy height, was different among the three cultivars. At high irradiance, 'Kenblue' and 'Livingston' exhibited 23% and 19% higher shoot elongation than 'NuGlade', respectively, over six measurement dates (Fig. 1). Reducing light intensity from 52% to 13% sunlight promoted control (pots that were not subjected to TE treatments) shoot elongation by 33% for 'Kenblue', 32% for 'Livingston', and 26% for 'NuGlade'. Application of TE reduced shoot elongation of all KBG cultivars at both high and low irradiance conditions.

Table 1. Analysis of variances with mean square and treatment significance of canopy height, clipping yield, GA₁, and GA₂₀ content.

Source	Canopy height (cm)	Clipping yield (g/pot)	GA ₁ (ng/g)	GA ₂₀ (ng/g)
Shade (S)	50.5****	14.6****	121.4****	143****
TE	25.2****	8.2****	313.0****	1677****
S × TE	0.76 ^{ns}	1.9***	7.4	10.7
Cultivar (C)	11.4****	0.15**	10.0**	5.6 ^{ns}
S × C	2.9**	0.18**	0.5 ^{ns}	1.7 ^{ns}
TE × C	1.0 ^{ns}	0.14*	1.4 ^{ns}	2.4 ^{ns}
S × TE × C	0.3 ^{ns}	0.1 ^{ns}	0.5 ^{ns}	3.5 ^{ns}

^{ns}, *, **, **** Nonsignificant or significant at 0.05, 0.01, 0.001, and 0.0001 levels, respectively.

Fig. 1. Effects of irradiance and trinexapac-ethyl treatment on mean canopy height of 'Kenblue', 'Livingston', and 'NuGlade' Kentucky bluegrass. Canopy height was measured weekly prior to each clipping event and data were averaged 1 to 6 weeks after shading treatment. Vertical line represents Fisher's protected LSD.

Fig. 2. Mean weekly clipping yield of 'Kenblue', 'Livingston', and 'NuGlade' Kentucky bluegrass as influenced by irradiance and trinexapac-ethyl treatment. Clipping yield was measured weekly and data were averaged 1 to 6 weeks after shading treatment. Vertical line represents Fisher's protected LSD.

In contrast to height, clipping yield decreased 59% across all three cultivars as the level of irradiance decreased from 52% to 13% sunlight (Fig. 2). At high irradiance, 'Kenblue' and 'Livingston' control plants produced higher clipping yield than 'NuGlade'. At low irradiance, however, cultivars did not differ in their clipping yields. This result suggested that 'NuGlade' had less reduction in clipping yield than 'Kenblue' and 'Livingston' as light intensity was reduced. Application of TE reduced clipping yield at both high and low irradiance through out the study (Fig. 2).

GA₁ content. The shoot content of GA₁, the biological active GA, was different between the two light intensities and among the three cultivars (Fig. 3).

At high irradiance, GA₁ content ranged from 8 to 10 ng/g. Reducing light intensity from 52% to 12% sunlight increased GA₁ by 47% in all control plants. This increase in GA₁ was moderate when compared with *Brassica* (*Brassica napus* L.) and pea (*Pisum sativum* L.) (Gawronska et al., 1995; Potter et al., 1999). In *Brassica*, GA₁ concentration increased 7- to 25-fold and GA₃ concentration increased 8- to 25-fold when light intensity decreased from 500 to 25 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Potter et al., 1999). The increase was 2- to 3-fold when light intensity decreased from 500 to 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In pea, GA₁ level increased 2-fold as light level decreased from 386 to 193 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Gawronska et al., 1995). The authors found that increased biosynthesis of GA₁ partially contributed to excessive shoot growth of pea in response to low light intensity.

'NuGlade' exhibited 20% lower GA₁ content than 'Kenblue'. GA₁ content in 'Livingston' was not different from either 'Kenblue' or 'NuGlade'. This result suggested that the dwarf characteristics of 'NuGlade' were, at least in part, associated with low GA₁ content (Fig. 3).

Across cultivars, application of TE reduced GA₁ content by 49% under 13% sunlight and by 47% for KBG grown under 52% sunlight, indicating that the efficiency of TE was similar between two irradiance levels in KBG. However, in zoysiagrass (*Zoysia japonica* Steud.), Qian et al. (1998) found that the effectiveness of TE in reducing vertical shoot growth generally increased with increasing shade level.

Correlation analysis revealed that shoot elongation was correlated with GA₁ content with a correlation coefficient of 0.71 for the control and 0.53 for TE-treated plants (Fig. 5), indicating GA₁ played an important role in increasing the shoot elongation of turfgrass grown under low light conditions.

GA₂₀ content. There were no differences in GA₂₀ content among control cultivar plants under either level of irradiance (Fig. 4). Pooled data of all cultivars indicated that a light reduction of 73% increased GA₂₀ content by 30%, although no differences were found when analyzed by individual cultivars. Gawronska et al. (1995) found that as light intensity decreased from 386 to 193 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the level of GA₂₀ increased 3.7 fold in pea plants. Likewise, Potter et al. (1999) reported a 4-fold increase

Fig. 3. GA₁ content in shoots of 'Kenblue', 'Livingston', and 'NuGlade' Kentucky bluegrass as influenced by irradiance and trinexapac-ethyl treatment. Vertical line represents Fisher's protected LSD.

of GA₂₀ as light intensity decreased from 500 to 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in *Brassica*.

Application of TE dramatically increased GA₂₀ content for all cultivars at both irradiance levels (Fig. 4). The reduced GA₁ and increased GA₂₀ by TE treatment support a conclusion that TE inhibited GA₁ biosynthesis by blocking the conversion of GA₂₀ to GA₁.

Shoot elongation was correlated with GA₂₀ content with a correlation coefficient of 0.42 for the control and 0.54 for TE-treated plants (Fig. 5). However, correlation analysis of pooled data of both with and without TE treated plants resulted in no relationship between GA₂₀ and canopy height. This is because GA₂₀ itself does not promote shoot elongation, but its product (GA₁) does.

In summary, the results from this study indicated that both GA₁ and GA₂₀ increased under low irradiance in KBG. The concentration of GA₁ played an important role in increasing the shoot elongation of turfgrass grown under low light conditions. Previous research with zoysiagrass suggested that turf quality negatively correlated with shoot vertical growth under dense shade conditions (Qian et al., 1998). Several implications may be drawn from these results with respect to selection and management of KBG under low light irradiance: 1) KBG cultivars that perform better under shade conditions should be those that minimize vertical growth under shade and divert energy to maintaining horizontal growth; 2) GA manipulation through using

Fig. 4. GA₂₀ content in shoots of 'Kenblue', 'Livingston', and 'NuGlade' Kentucky bluegrass as influenced by irradiance and trinexapac-ethyl treatment. Vertical line represents Fisher's protected LSD.

Fig. 5. Correlation of canopy height vs. (A) GA₁ and (B) GA₂₀ content.

anti-GA plant growth regulators may favorably affect turf stature, thereby increasing the shade tolerance of turf.

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