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Effects of Mesotrione on Perennial Ryegrass (*Lolium perenne* L.) Carotenoid Concentrations under Varying Environmental Conditions

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Mesotrione is a carotenoid biosynthesis inhibiting herbicide, which is being evaluated for use in turfgrass. Carotenoids are important light harvesting and photoprotecting pigments that dissipate and quench excess light energy. The effects of mesotrione on carotenoid concentrations in turf and weed species, such as perennial ryegrass (*Lolium perenne* L.), are poorly understood. Mesotrione injury to perennial ryegrass has been reported, and symptomology may differ due to postapplication environmental factors such as irradiance and temperature. Research was conducted to investigate the effects of mesotrione on perennial ryegrass under varying irradiance (600, 1100, or 1600 μmol/m^2/s) at three different temperatures (18, 26, and 34 °C). Postapplication irradiance and temperature levels did not affect visual injury symptoms in perennial ryegrass. Bleaching of treated plants was highest 7 days after treatment (DAT; 8%) and recovered to nontreated levels by 21 DAT. Mesotrione applications did not decrease perennial ryegrass foliar biomass accumulations. Carotenoid concentrations of nontreated plants were similar to those reported in creeping bentgrass and many green leafy vegetable crops. However, chlorophyll a and b, β-carotene, lutein, and violaxanthin concentrations decreased due to mesotrione applications, while phytoene and zeaxanthin, a photoprotecting carotenoid, increased. The photochemical efficiency (F_v/F_m) of treated plants was lower than nontreated plants at 3 and 7 DAT; however, treated plants recovered to nontreated levels 21 DAT. Results indicate that postapplication irradiance and temperature levels may not affect mesotrione efficacy in perennial ryegrass. Preferential accumulation of zeaxanthin following mesotrione applications may be a stress-related response, which may reduce light harvesting complex size and directly quench excess light energy.

KEYWORDS: Mesotrione; *Lolium perenne* L.; carotenoid; HPLC

INTRODUCTION

Mesotrione is a selective preemergence and postemergence herbicide that can control a variety of common turfgrass weeds (*1*−*3*). Mesotrione is a carotenoid biosynthesis inhibitor being evaluated for use in turfgrass. Symptoms in mesotrione sensitive plants are bleaching followed by necrosis within 3−5 days after treatment (DAT; *4*). Tissue whitening is a result of the inhibition of carotenoid biosynthesis and the destruction of existing chlorophyll (*5, 6*). Tolerance to low rates of mesotrione (≤0.28 kg/ha) has been reported in several turfgrass species (*7*−*9*). However, injury can occur when mesotrione is applied to perennial ryegrass (*1*).

Mesotrione competitively inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), the enzyme responsible for the conversion of tyrosine to plastoquinone and α-tocopherol (*10*). Plastoquinone is a cofactor for phytoene desaturase, a crucial enzyme of the carotenoid biosynthesis pathway (*11*). Carotenoids are C_{40} isoprenoid compounds that form lipid-soluble red, orange, and yellow pigments in higher plants, algae, bacteria, and fungi. Carotenoids are associated with photosynthetic light harvesting complexes (LHCs) where they transfer light energy to the photosynthetic reaction center. Carotenoids also play roles in photoprotection by quenching free radicals, singlet oxygen, and other reactive oxygen species (*12, 13*). If carotenoids are not present in the photosystem or they are incapable of quenching excess energy, damage and degradation of thylakoid membranes may occur (*14*).

Carotenoid formation begins with the dimerization of the C_{20} isoprenoid compound geranyl-geranyl pyrophosphate (GGPP)

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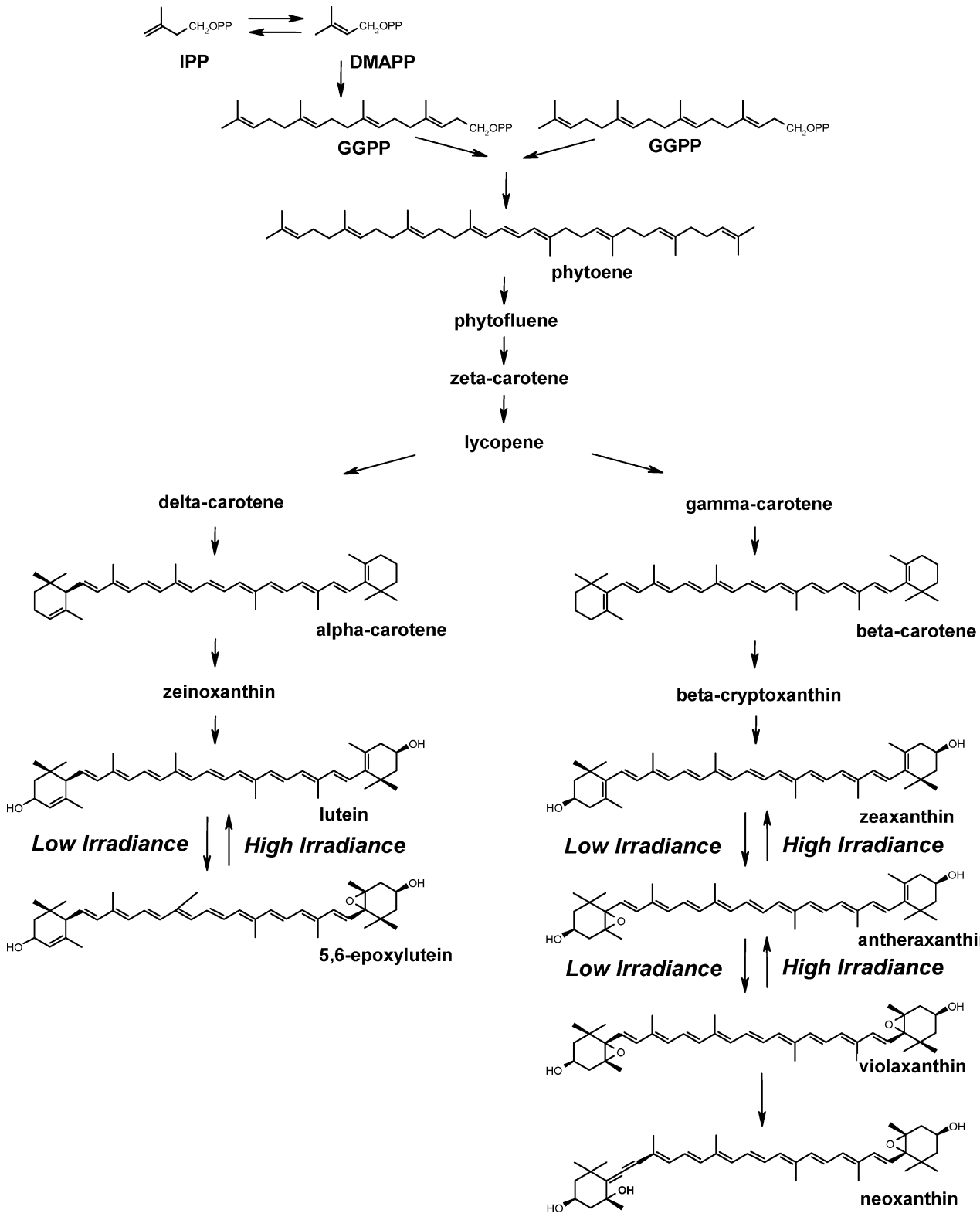

Figure 1. Abbreviated depiction of the carotenoid biosynthesis pathway.

by phytoene synthase to produce phytoene, the first C_{40} compound in the pathway (**Figure 1**). Phytoene desaturase catalyzes the desaturation of phytoene to produce ζ-carotene, which then undergoes two further desaturation reactions to yield lycopene. Branching of the pathway occurs when lycopene is cyclized to form either α-carotene or β-carotene. Lutein and lutein 5,6-epoxide (epoxy-lutein) are derived from α-carotene, while neoxanthin and the xanthophyll cycle pigments zeaxanthin, antheraxanthin, and violaxanthin are formed from β-carotene (*15, 11*).

Mesotrione efficacy may be influenced by environmental factors, such as temperature and relative humidity. Common water hemp (*Amaranthus rudis* Sauer) and large crabgrass (*Digitaria sanguinalis* L.), both C_4 plants, are more susceptible to mesotrione at the cooler temperature of 18 °C than at 32 °C, while cocklebur (*Xanthium strumarium* L.) and velvetleaf

[*Abutilon theophrasti* (L.) Medic.], both C_3 plants, are more susceptible to mesotrione efficacy at the higher temperature of 32 °C rather than at 18 °C. The decreased metabolism of C_4 plants at lower temperatures, as compared to C_3 plants, may decrease the metabolism of mesotrione. However, the role of photosynthetic pathways in herbicide efficacy is not supported consistently by the literature. High temperatures may increase the fluidity of the cuticle and plasma membrane resulting in greater uptake of foliar-applied herbicides; however, as the temperature increases, the plant metabolic activity may increase and be of greater importance for some species (*3*).

Quantification of perennial ryegrass carotenoid composition is limited; therefore, evaluating carotenoid levels may be important for future research using this species. The objective of this research was to investigate the effects of mesotrione on perennial ryegrass carotenoid concentrations under varying irradiance and temperature. Understanding the effects of environmental conditions upon mesotrione efficacy may allow turfgrass managers to more effectively control weeds, while minimizing injury to perennial ryegrass turf.

MATERIALS AND METHODS

Plant Culture of Perennial Ryegrass. Seeds of Palmer IV perennial ryegrass (Proseeds Marketing Jefferson, OR) were planted approximately 0.5 cm deep in 12 cm diameter plastic pots (500 mL volume and 95 cm^2 surface area) containing silt-loam soil [Sequatichie loam soil (fine-loamy, siliceous, semiactive, thermic Humic Hapludult) with pH 6.2 and 2.1% organic matter]. Seeds were germinated at 26 °C and 50% relative humidity in an environmental growth room (Environmental Growth Chambers, Chargrin Falls, OH) at 1100 μmol/m^2/s irradiance under a 16 h photoperiod. Irradiance was provided by a mixture of metal halide and high-pressure sodium lamps. Throughout the experiment, pots were overhead irrigated twice daily to maintain adequate soil moisture, fertilized (5.0 g nitrogen/m^2) with Harrell's Triple Twenty plus minors (Harrells, Inc., Lakeland, FL) on a weekly basis prior to treatment and resuming 7 DAT, and randomized every 2 days to account for potential variation within the environmental growth room.

One week after planting, pots were thinned to five plants per pot. Pots were treated 14 days after emergence with 0.28 kg ai/ha mesotrione plus 0.25% (v/v) nonionic surfactant [X-77 Spreader (alkylphenol ethoxylate, alcohol ethoxylate, tall oil fatty acid, 2,2′ dihydroxydithyl ether, and dimethylpolysiloxane), Loveland Products, Inc., Greeley, CO] applied in a water carrier volume of 280 L/ha with a CO_2-pressurized backpack sprayer equipped with 11002 XR flat fan nozzles (TeeJet Extended Range spray tips, Spraying Systems Co., Wheaton, IL) at 276 kPa. Plants were subsequently placed at 600, 1100, or 1600 μmol/m^2/s irradiance within an environmental growth room at 18, 26, or 34 °C. Irradiance levels were achieved by manipulating the proximity of the plants to the overhead light source. Because of the limited availability of environmental growth rooms, the three temperature regimes could not be conducted simultaneously. For this reason, care was taken to ensure plants were of identical growth stage and size prior to treatment and that they were fertilized and irrigated identically for each temperature studied.

Four treated pots and four nontreated pots were randomly selected from each irradiance level 3, 7, and 21 DAT. Two photochemical efficiency (F_v/F_m) ratings per pot were taken midcanopy as an indication of photoinhibition and overall plant health using a modulated fluorometer (OS1-F1 Modulated Fluorometer, Opti Sciences, Hudson, NH). The percent bleached tissue and percent necrotic tissue were recorded visually as an indication of mesotrione efficacy. All plants were harvested at soil level and immediately placed on ice for transfer to storage at −80 °C. Prior to carotenoid extraction, plant material was weighed to obtain sample fresh weights (FWs).

Carotenoid Determination for Perennial Ryegrass. *Tissue Extraction.* Carotenoids were extracted and quantified according to previously published methods (*16*−*18*). Foliage was first homogenized in liquid nitrogen using a mortar and pestle. A subsample weighing approximately 0.5 g was placed into a Potter−Elvehjem tissue grinder tube (Kontes, Vineland, NJ) with 0.8 mL of ethyl-β-apo-8-carotenoate as an internal standard and 2.5 mL of tetrahydrofuran (THF) stabilized with 2,6-di-*tert*-butyl-4-methoxyphenol (BHT). The sample was further homogenized using 25 insertions with a Potter−Elvehjem tissue grinder pestle attached to a drill press (Sears, Roebuck, and Co., Hoffman Estates, IL) set at 540 rpm while the tube was immersed in ice to dissipate the heat. The tube was then centrifuged for 3 min at 500*g*. Using a Pasteur pipet, the supernatant was placed into a conical 15 mL test tube, capped, and held on ice during the remainder of the extraction. The sample pellet was resuspended in 2.0 mL of THF, and the extraction procedure was repeated five times until the supernatant was clear. The combined supernatants were reduced to 1.0 mL under nitrogen stream (model N-EVAP 111; Orgnomation Inc., Berlin, MA). To each 1.0 mL sample, 4.0 mL of MeOH was added and vortexed. Samples were filtered through a 0.2 μm polytetrafluoroethylene filter (model Econofilter PTFE 25/20, Agilent Technologies, Wilmington, DE) prior to analysis by high-performance liquid chromatography (HPLC).

Carotenoid Liquid Chromatography Analysis. An Agilent 1100 series HPLC unit with a photodiode array detector (Agilent 1100, Agilent Technologies, Palo Alto, CA) was used for sample separation. Samples were analyzed for carotenoids using a 4.6 mm × 250 mm ProntoSIL C_{30} RP column (MAC-MOD Analytical Inc., Chadds Ford, PA) with a 5.0 μm particle size and 200 Å pore size fitted with a guard column (4 mm × 23 mm, 7.0 mm; S-5) (*19*). The column was maintained at 30 °C by a thermostatted column comparment. Pigment separation was performed using an isocratic mixture of methanol/methyl-tert-butyl-ether 89:10.99% (v/v) plus 0.01% triethylamine. Eluted compounds from a 10 μL injection were detected. Data were collected, recorded, and integrated using 1100 HPLC Chem-Station Software (Agilent Technologies, Palo Alto, CA). Carotenoids were selected based upon their active roles in photoprotection and light harvesting. Phytoene was detected at 290 nm. Carotenoids detected at 453 nm included lutein, epoxylutein, violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and β-carotene. α-Carotene was undetectable and therefore not quantified. Chlorophyll a and chlorophyll b were detected at 652 nm. Peak assignment was performed by comparing retention times and line spectra (250−650 nm) obtained from photodiode array detection with authentic standards purchased from a commercial vender (CaroteNature GmbH, Lupsingen, Switzerland). Concentrations of the authentic standards were determined spectrophotometrically using quantitative spectroscopic data (*20*). HPLC recovery rates of ethyl-β-apo-8-carotenoate (53−91%) were used to estimate the extraction efficacy. Carotenoid concentrations quantified in shoot tissues are expressed on a FW basis (mg/100 g FW). Ratios of zeaxanthin plus antheraxanthin to zeaxanthin plus antheraxanthin plus violaxanthin (Z + A/Z + A + V ratio) and epoxy-lutein to lutein (ELU/LU ratio) were calculated for comparison.

Statistical Analysis. The experimental design was completely random with a two by three factorial treatment arrangement (two mesotrione treatments by three irradiance levels). Within each treatment scheme, harvest intervals (0, 7, and 21 DAT) were analyzed as samples. The experiment was conducted at three temperatures (18, 26, or 34 °C) with four replicates within each temperature. A model with equal variance was fit to data, and a likelihood ratio test was used to test if variances were unequal between temperatures. Independent analysis of temperatures was conducted, and visual verification confirmed that results were similar for each temperature. Equal variance among runs allowed for data pooling over temperatures. The Lavene test was used to test for equal variance among treatments. All data were subjected to analysis of variance (ANOVA) ($P = 0.05$). ANOVA results were used to select main effects. Means were separated by Fisher's protected least significant difference (LSD).

RESULTS AND DISCUSSION

Visual Injury and Foliar Dry Weight. Percent bleaching, percent necrosis, and foliar dry weight did not differ due to measured temperature or irradiance levels; therefore, data were pooled. A treatment by harvest interval interaction was observed

Table 1. Pigment Concentrations, Composition Ratios, Percent Bleaching and Necrosis, and F_v/F_m of Palmer IV Perennial Ryegrass Due to Mesotrione Treatment by Day after Treatment Interaction[a]

		mg/100 g FW			
	DAT	treated	nontreated	LSD[b]	*P* value[c]
% bleaching	3	6.7 a	0.0 b	2.2	0.0001
	7	8.2 a	0.0 b		
	21	0.4 b	0.0 b		
% necrosis	3	0.0 b	0.0 b	1.1	0.01
	7	1.7 a	0.0 b		
	21	2.5 a	0.3 b		
phytoene	3	2.7 a	0.0 d	0.3	0.0001
	7	1.7 b	0.0 d		
	21	1.3 c	0.0 d		
zeaxanthin	3	2.3 a	1.4 cd	0.5	0.02
	7	1.9 ab	1.2 d		
	21	1.7 bcd	1.7 bc		
antheraxanthin	3	2.8 a	2.5 ab	0.3	0.0315
	7	2.5 ab	1.9 c		
	21	2.1 bc	2.2 bc		
Z + A/Z + A + V	3	0.5 a	0.4 b	0.1	0.002
	7	0.5 a	0.4 b		
	21	0.5 a	0.5 a		
F_v/F_m	3	0.633 d	0.723 ab	0.026	0.0003
	7	0.640 d	0.732 a		
	21	0.674 c	0.699 bc		

[a] Abbreviations: Z + A/Z + A + V, ratio of zeaxanthin + antheraxanthin to total xanthophyll cycle pigments. [b] Means are separated by Fisher's protected LSD ($P = 0.05$). [c] Significance of treatment by DAT interaction term.

for percent bleaching and percent necrosis (**Table 1**). When evaluating the perennial ryegrass response to mesotrione, at no time was bleaching of nontreated plants observed. Treated plant bleaching 3 and 7 DAT was 7−8%, respectively, and decreased to less than 1% 21 DAT (**Table 1**). Bleaching was limited to new tissue, mainly leaf tips, and did not result in thinning of the turf canopy. By 7 DAT, some bleached tissue had become necrotic, and by 21 DAT, bleaching was minimal. Because turf is often valued for aesthetics, prolonged bleaching of the turf canopy is undesirable in highly managed turfgrass areas such as golf courses and home lawns. Treated plant necrosis was <3% at all harvest dates (**Table 1**). Perennial ryegrass foliar weights did not differ due to treatment with mesotrione, temperature, or irradiance level (data not shown); however, as expected, weights did increase with DAT.

Pigment Quantification. Pigment concentration, composition ratios, and photochemical efficiency did not vary due to temperature level; therefore, results were pooled across temperature (**Tables 2** and **3**). Differences were observed due to treatment, DAT, treatment by DAT interaction, and irradiance (**Tables 4** and **5**). Observed differences due to DAT trended similarly across all pigment concentrations, decreasing with the age and size of the plant. This effect is thought to be due to the increasing percentage of nonphotosynthesizing stem tissue. Although this tissue type is very limited in perennial ryegrass, this is one possible explanation.

The chlorophyll a to b ratio was slightly lower in treated plants (2.9:1) than in nontreated plants (3.0:1) (**Table 2**). Combined treated and nontreated chlorophyll a to b ratios fluctuated across harvest intervals and were highest 7 DAT (**Table 4**). The treated plant chlorophyll a concentration was less than the nontreated plant concentration (**Table 3**). Similar reductions in Prelude perennial ryegrass chlorophyll a concentrations have previously been observed due to the application of Isoxaflutole, an HPPD inhibitor (*21*). Combined treated and nontreated chlorophyll a concentrations decreased with each harvest interval (**Table 5**). Unlike chlorophyll a, the chlorophyll b concentration did not vary due to treatment with mesotrione; however, the concentration was greater 3 DAT than 7 and 21 DAT (**Table 5**).

The total quantified carotenoids did not vary due to treatment with mesotrione; however, total concentrations were greater 3 DAT than 7 and 21 DAT (**Table 5**). When evaluating individual carotenoid concentrations as affected by mesotrione treatment, changes occurred in phytoene, β-carotene, lutein, zeaxanthin, antheraxanthin, and violaxanthin (**Table 3**). Mesotrione did not influence neoxanthin or epoxy-lutein concentrations (data not shown). Phytoene was undetectable in nontreated plants; however, phytoene accumulated to 1.9 mg/100 g FW in mesotrione-treated plants (**Table 3**). A treatment by DAT interaction was observed for phytoene concentration. Treated plant phytoene concentrations were highest 3 DAT and decreased 3 and 7 DAT (**Table 1**). Mesotrione results in HPPD inhibition, which increases phytoene concentrations due to the indirect inhibition of phytoene desaturase (*5, 22*). The phytoene concentration was also affected by irradiance level. Phytoene concentrations of plants grown at 600 μmol/m^2/s were greater than those grown at 1200 and 1600 μmol/m^2/s (**Table 6**). Phytoene synthase, the enzyme that generates phytoene from two molecules of GGPP, is believed to be closely associated with the chloroplast membrane (*21*). High light conditions will cause up-regulation of phytoene mRNA; however, light-induced expression of phytoene synthase gene may be under photosynthetic control (*22*−*24*). Phytoene is a precursor to lycopene, which is cyclized to form either α-carotene, a precursor to lutein, or β-carotene, a precursor to the xanthophyll cycle (**Figure 1**). α-Carotene was undetectable and therefore not quantified; however, the treated plant β-carotene concentration was less than that of nontreated plants, presumably due to the decrease in the precursor phytoene.

Despite carotenoid biosynthesis inhibition by mesotrione, perennial ryegrass preferentially accumulated photoprotecting pigments zeaxanthin and antheraxanthin at the expense of the light harvesting pigment violaxanthin. Similar results due to treatment with mesotrione have not previously been reported. The xanthophyll cycle pigments (zeaxanthin, antheraxanthin, and violaxanthin) participate as antioxidants in LHCs (*15, 25*). Within the xanthophyll cycle, zeaxanthin is the primary carotenoid responsible for preventing photoinhibition; however, antheraxanthin has been reported to have similar photoprotective properties (*26*). Increases in photoprotection are linked to zeaxanthin quenching of singlet oxygen and free radicals in chloroplast membranes (*27*). The ratio of zeaxanthin and antheraxanthin to total xanthophyll cycle pigments (Z + A/Z + A + V) varied due to both treatment and irradiance level, while total xanthophylls (Z + A + V) were similar in both treated and nontreated plants (**Table 1**). Z + A/Z + A + V was lower in plants grown at 600 μmol/m^2/s than in plants grown at higher irradiance levels (**Table 6**). Zeaxanthin and antheraxanthin accumulate in high irradiance conditions due to the increased activity of the pH-dependent enzyme violaxanthin de-epoxidase (*15, 25*). Increased binding of zeaxanthin to photosystem II proteins allows for more efficient quenching of excess energy, a process known as nonphotochemical quenching (*28, 29*). In the current study, Z + A/Z + A + V ratio was also greater in treated plants than nontreated plants (**Table 2**). Mesotrione-induced photoinhibition may influence violaxanthin de-epoxidase activity, thus increasing the conversion of violaxanthin to the intermediate antheraxanthin and subsequently zeaxanthin. However, we did not directly measure violaxanthin

Table 2. Percent Bleaching and Necrosis, F_v/F_m, Pigment Concentration, and Pigment Composition Ratios of Mesotrione Treated and Nontreated Palmer IV Perennial Ryegrass[a]

	% bleaching	% necrosis	F_v/F_m	chlorophyll a	chlorophyll b	chlorophyll a/b	Z + A/Z + A + V
treated	5.1	1.4	0.649	177.4	62.5	2.9	0.53
nontreated	0.0	0.1	0.718	191.0	64.0	3.0	0.41
LSD ($P = 0.05$)[b]	1.2	0.6	0.015	12.6		0.1	0.03
P value[c]	0.0001	0.0001	0.0001	0.034	NS	0.0001	0.0001

[a] Abbreviations: Z + A/Z + A + V, ratio of zeaxanthin + antheraxanthin to total xanthophyll cycle pigments; NS, nonsignificant. [b] Means are separated by Fisher's protected LSD ($P = 0.05$). [c] Significance of treatment main effect pooled across DAT.

Table 3. Pigment Concentrations of Mesotrione Treated and Nontreated Palmer IV Perennial Ryegrass

	mg/100 g FW					
	phytoene	β-carotene	lutein	zeaxanthin	antheraxanthin	violaxanthin
treated	1.9	0.1	19.4	2.0	2.4	4.4
nontreated	0.0	1.4	20.8	1.4	2.2	4.9
LSD ($P = 0.05$)[a]	0.2	0.7	1.2	0.3	0.2	0.3
P value[b]	0.0001	0.0002	0.0	0.0007	0.0095	0.0019

[a] Means are separated by Fisher's protected LSD ($P = 0.05$). [b] Significance of treatment main effect pooled across DAT.

Table 4. Pigment Composition Ratios and Totals and Percent Bleaching and Necrosis Pooled over Treatments as Affected by Harvest Interval[a]

DAT	Chl a/b	Elu/Lu	Z + A + V	% bleaching	% necrosis
3	2.9 b	0.1 a	9.5 a	3.4 a	0.0 b
7	3.0 a	0.5 b	8.5 b	4.1 a	0.8 a
21	2.9 b	0.1 b	7.9 b	0.0 b	1.4 a
LSD ($P = 0.05$)[b]	0.1	0.004	0.7	1.5	0.8
P value[c]	0.03	0.0001	0.0001	<0.0001	0.002

[a] Abbreviations: Chl a/b, ratio of chlorophyll a to chlorophyll b; Z + A + V, total xanthophyll cycle pigments zeaxanthin, antheraxanthin, and violaxanthin. [b] Means of significant effects are separated by Fisher's protected LSD ($P = 0.05$). [c] Significance of DAT reaction terms when data were subjected to ANOVA ($P = 0.05$).

de-epoxidase activity. Therefore, it is still undetermined how mesotrione may influence the activity of this enzyme.

Lutein functions as an integral subunit of LHCs. In plant mutants void of the xanthophyll cycle carotenoids, lutein functions in nonphotochemical quenching as a photoprotectant against oxidative damage (*25*). Treated plant lutein concentrations were less than nontreated plant concentrations (**Table 3**). Decreases in lutein through mesotrione applications may be responsible for reduced size and quantity of LHCs. Epoxy-lutein can also function as a light harvesting pigment under low irradiance conditions, with shifts from lutein to epoxy-lutein occurring in low irradiance (*25, 30*). The epoxy-lutein concentration was not affected by treatment with mesotrione; however, concentrations did vary due to harvest date. The epoxy-lutein concentration was highest 3 DAT (**Table 5**).

The Palmer IV perennial ryegrass chlorophyll a to b ratio (approximately 3:1) is similar to that of Prelude perennial ryegrass; however, chlorophylls a and b were both measured at lower concentrations in this study (near 200 and 70 mg/100 g FW, respectively) than previously reported (near 500 and 150 mg/100 g FW, respectively). Similarly, the Palmer IV total carotenoid concentration was lower than those previously reported for Prelude (*21*). Carotenoid concentrations were comparable to Crenshaw creeping bentgrass (*Agrostis stolonifera* L.) grown at 554 μmol/m^2/s (*18*). Palmer IV perennial ryegrass produced higher concentrations of β-carotene, lower concentrations of violaxanthin, and similar concentrations of neoxanthin, lutein, and epoxy-lutein as compared to Crenshaw creeping bentgrass. Concentrations were also similar to many green leafy vegetable and herbal crops. Concentrations of β-carotene, violaxanthin, lutein, epoxy-lutein, and neoxanthin in raw unprocessed spinach (*Spinacia oleracea* L.) have been reported as 8.9, 7.4, 9.5, 0.5, and 2.4 mg/100 g FW, respectively (*31*). Perennial ryegrass in the current study produced similar levels of β-carotene, violaxanthin, epoxy-lutein, and neoxanthin but higher levels of lutein as those reported for spinach. Perennial ryegrass produced higher levels of all carotenoids than various *Brassica* sp. whose levels of β-carotene, zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, lutein, and epoxy-lutein have been reported as 3.5, 0.4, 0.6, 1.6, 1.8, 8.0, and 0.4 mg/100 g FW (*32*).

Table 6. Palmer IV Perennial Ryegrass Phytoene Concentration and Z + A/Z + A + V Concentration Ratios as Affected by Irradiance Level[a]

	mg/100 g FW	
μmol/m^2/s	phytoene	Z + A/Z + A + V
600	1.1 a	0.4 b
1100	0.9 b	0.5 a
1600	0.9 b	0.5 a
LSD ($P = 0.05$)[b]	0.1	0.1
P value[c]	0.002	0.0001

[a] Abbreviations: Z + A/Z + A + V, ratio of zeaxanthin + antheraxanthin to total xanthophyll cycle pigments. [b] Means are separated by Fisher's protected LSD ($P = 0.05$). [c] Significance of treatment main effect pooled across DAT.

Photochemical Efficiency. Photochemical efficiency, measured as the chlorophyll fluorescence parameter F_v/F_m, varied due to treatment and treatment by DAT interaction (**Table 1**). Treated plant photochemical efficiency was slightly less than that of nontreated plants (**Table 2**). While nontreated plant photochemical efficiency decreased from 0.732 at 7

Table 5. Pigment Concentrations (mg/100g FW) Pooled over Treatments as Affected by Harvest Interval

	mg/100 g FW							
DAT	totals	phytoene	β-carotene	antera-xanthin	viola-xanthin	epoxy-lutein	Chl a	Chl b
3	46.0 a	1.4 a	9.2 a	2.6 a	5.1 a	1.1 a	204.4 a	70.8 a
7	41.4 b	0.8 b	8.5 ab	2.2 b	4.8 a	0.9 b	183.4 b	61.9 b
21	40.7 b	0.7 b	7.8 b	2.2 b	4.1 b	0.9 b	166.3 c	57.1 b
LSD ($P = 0.05$)[a]	3.0[a]	0.2	0.8	0.3	0.4	0.1	15.3	4.9
P value[b]	0.002[b]	0.0001	0.007	0.0004	0.0001	0.0001	0.0001	0.0001

[a] Means of significant effects are separated by Fisher's protected LSD ($P = 0.05$). [b] Significance of DAT reaction terms when data were subjected to ANOVA ($P = 0.05$).

DAT to 0.699 at 21 DAT, treated plant photochemical efficiency recovered to levels observed in the nontreated plants by 21 DAT (**Table 1**). Decreased photochemical efficiency may be due, in part, to the observed increase in photoprotecting pigments. Zeaxanthin accumulation, accompanied by decreases in violaxanthin, may decrease photochemical efficiency by decreasing the size of light harvesting antenna (*27, 33*). Additionally, the role of lutein in LHC quantity may also lead to reduced light harvesting capability.

Results for the current study indicate that postapplication irradiance and temperature levels may not affect mesotrione efficacy in perennial ryegrass. The perennial ryegrass response to mesotrione did not vary due to the tested temperature levels; however, mesotrione efficacy may vary at lower or higher than tested temperature extremes. Similarly, perennial ryegrass injury did not vary between the tested irradiance levels; however, levels in the current study may not have represented irradiance extremes of field conditions. In many instances, shaded turfgrass receives less than 600 μmol/m^2/s of irradiance; moreover, irradiance levels have exceeded 2100 μmol/m^2/s irradiance on the University of Tennessee, Knoxville campus in mid-July.

Previous quantification of perennial ryegrass carotenoid composition is limited; therefore, the reported levels in this study may be important for future research. Clearly bleaching of perennial ryegrass turf is a major concern to turfgrass managers; however, injury was limited to bleaching with no reduction in foliar biomass, and visual quality ratings recovered to an acceptable level within 21 DAT. Temperature and irradiance levels did not influence visual mesotrione injury; however, turfgrass managers should use precaution when applying any herbicide to turf undergoing summer heat stress.

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