Changes in ‘Riviera’ Bermudagrass [Cynodon dactylon (L.) Pers.] Carotenoid Pigments after Treatment with Three p-Hydroxyphenylpyruvate Dioxygenase-inhibiting Herbicides

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Abstract. Mesotrione, topramezone, and tembotrione are inhibitors of the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD), which impacts the carotenoid biosynthetic pathway. An experiment was conducted to determine the effects of mesotrione, topramezone, and tembotrione on carotenoid pigment concentrations in common bermudagrass [Cynodon dactylon (L.) Pers.; cv. Riviera] leaf tissues. Bermudagrass plants were treated with three rates of mesotrione (0.28, 0.35, and 0.42 kg ha⁻¹), topramezone (0.018, 0.025, and 0.038 kg ha⁻¹), and tembotrione (0.092, 0.184, and 0.276 kg ha⁻¹). The lowest rate of each herbicide represented the maximum labeled use rate for a single application. Percent visual bleaching was measured at 3, 7, 14, 21, 28, and 35 days after application (DAA). Leaf tissues were sampled on the same dates and assayed for carotenoids. Topramezone and tembotrione bleached bermudagrass leaf tissues to a greater degree than mesotrione. Concomitantly, topramezone and tembotrione also reduced total chlorophyll (chlorophyll a+b), β-carotene, lutein, and total xanthophyll cycle pigment concentrations (zeaxanthin + antheraxanthin + violaxanthin) more than mesotrione. Increases in visual bleaching resulting from application rate were accompanied by linear reductions in lutein, β-carotene, and violaxanthin for all herbicides. Topramezone and tembotrione increased the percentage of zeaxanthin + antheraxanthin in the total xanthophyll pigment pool (ZA/ZAV) 7 days after peak visual bleaching was observed at 14 DAA. Reductions in ZA/ZAV were reported after 21 DAA. This response indicates that sequential applications of topramezone and tembotrione should be applied on 14- to 21-day intervals, because stress induced by these herbicides is greatest at these timings. Increases in photoprotective xanthophyll cycle pigments (ZA/ZAV) at 14 to 21 DAA may be a mechanism allowing bermudagrass to recover from HPPD-inhibiting herbicide injury, because bermudagrass recovered from all treatments by 35 DAA. Data in the current study will allow turf managers to design physiologically validated bermudagrass control programs with HPPD-inhibiting herbicides. Chemical names: mesotrione [2-(4-methysulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione], tembotrione [2-[2-chloro-4-(methylsulfonyl)phenyl]benzoic acid], topramezone [3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl]5-hydroxy-1-nethyl-1H-pyrazol-4-yl)methane.

Materials and Methods

Plant culture. The study was conducted in a greenhouse at the University of Tennessee–Knoxville between Aug. and Sept. 2009. Daytime air temperatures in the greenhouse averaged 29.5 °C.

Cores (6 cm diameter, 5 cm depth) of ‘Riviera’ common bermudagrass were harvested from the East Tennessee Research and Education Center, Knoxville, TN, and transplanted into 10-cm diameter pots filled with a peat moss (55%), perlite (25%), and vermiculite (20%) growing medium (Super Fine Germinating Mix; Conrad Fafard Inc., Agawam, MA). After transplanting, plants were fertilized with a complete fertilizer biweekly (20 N:20 P 2O 5:20 K 2O; Howard Johnson’s Triple Twenty Plus Minors, Milwaukee, WI) at 5.2 kg nitrogen/ha and watered as needed with overhead irrigation to maintain adequate soil moisture.
soil moisture. Hand shears were used to clip plants three times per week to a height of 2 cm. Plants were allowed to resume active growth for 3 weeks before study initiation. 

### Deciduous treatments

Plants were treated with mesotrione (Tenacity®; Syngenta Professional Products, Greensboro, NC), topramezone (Impact®; Amvac Chemical, Los Angeles, CA), and tembotrione (Laudis®; Bayer CropScience, Research Triangle Park, NC) at low, medium, and high application rates. Low, medium, and high rates for mesotrione were 0.2, 0.35, and 0.42 kg·ha⁻¹, respectively. For topramezone, low, medium, and high rates were 0.018, 0.025, and 0.038 kg·ha⁻¹, respectively. Low, medium, and high rates for tembotrione were 0.092, 0.184, and 0.276 kg·ha⁻¹, respectively. Low rates represented the maximum labeled use rate for a single application of each herbicide (Anonymous, 2006, 2009a, 2009b). A non-treated control was also included for comparison. All herbicides were mixed with a methylated seed oil surfactant (MOS; Loveland Industries, Greeley, CO) at 0.25% v/v, because researchers have reported that MOS increases the translatable activity of mesotrione, topramezone, and tembotrione more than nonionic surfactants or crop oil concentrates (Young et al., 2007). Treatments were applied using a CO₂-powered boom sprayer containing four flat-flat nozzles (Teheet 8002 flat fan spray nozzle; Spraying Systems Co., Roswell, GA) calibrated to deliver 280 L·ha⁻¹ with a 4× 6-mm i.d., 5-μm analytical scale polymeric RP-C₁₈ column with a 10× 4.6-mm i.d. guard cartridge and holder (ProntoSIL® MAC-MOD Analytical Inc., Chadds Ford, PA), which allowed for effective separation of chemically similar carotenoid compounds. The column was maintained at 30 °C using a thermostatted column compartment. All separations were achieved isocratically using a binary mobile phase of 11% methyl tert-butyl ether, 88.99% MeOH, and 0.01% triethylamine (v/v/v). The flow rate was 1.0 mL·min⁻¹ with a run time of 53 min followed by a 2-min equilibration before the next injection. Eluted compounds from a 10-μL injection loop were detected at 453 nm (carotenoids, chlorophyll b, internal standard) and 452 nm (chlorophyll a) and data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies) (Demmig-Adams et al., 1996; Frank and Cogdell, 1996). Carotenoids evaluated included: β-carotene, antheraxanthin, lutein, neoxanthin, violaxanthin, and zeaxanthin. These carotenoids were selected based on their active roles in photoprotection and light harvesting. Peak assignments were performed by comparing retention times to internal standards and line spectra (250 to 700 nm) obtained from photodiode array detection with authentic standards (ChromaDex Inc., Irvine, CA). Concentrations of the authentic standards were determined spectrophotometrically using quantitative spectroscopic data (Table 1; Davies and Köst, 1988). Pigments were expressed as mg/100 g fresh weight (FW) of bermudagrass leaf blade tissue.

### Table 1. ‘Riviera’ bermudagrass (Cynodon dactylon) tissue pigments selected for quantification, including their common and IUPAC chemical names, and information used for calibration of authentic standards.

| Common name | IUPAC chemical name | Retention time (min) | λ (nm) | Solvent |
|-------------|---------------------|---------------------|--------|---------|
| Violoxanthin | (35,5’R,6S,3’S,5’R,6’S)-5,6,6’-diapoxy-3,5,6,6’-tetrahydro-β, β-carotene-3,3’-diol | 5.36 | 453 | Ethanol |
| Neoxanthin  | (35,5’R,6S,3’R,5’S,6’S)-6,7-didehydro-5,5’-epoxy-5,6,6’-tetrahydro-β, β-carotene-3,3’-diol | 5.67 | 453 | Ethanol |
| Antheraxanthin | (13,45,6’β,1’-([1(E,3E,5E,7E,9E,11E,13E,15E,17E]-18-[(4R)-4-hydroxy-2,6,6-trimethylcylohexa-1,3,5,9,11,13,15,17-nonanoyl]-2,2,6-trimethyl-7-exacyclo[4.1.0]heptan-4-ol | 7.25 | 453 | Ethanol |
| Chlorophyll b | (SP-4’-2’)-[2E,7R,11R]-3,7,11,15-tetramethyl-2-hexadecenyl (35,5’4,21R)-9-ethyl-14-ethyl-13-formyl-21-(methoxyacrylonitrile)-4,8,18-trimethyl-26-oxo-3-phorbinepropanoato(2-)·K₃O₃·C₇O₇ | 8.31 | 453 | Methanol |
| Lutein | [18-[4-hydroxy-2,6,6-trimethyl-1-cyclohexenyl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonanoyl]-3,3’-trimethylcylohexol-2-en-1-ol | 8.88 | 453 | Ethanol |
| Zeaxanthin | [18-[4-hydroxy-2,6,6-trimethyl-1-cyclohexenyl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonanoyl]-3,3’-trimethylcylohexol-2-en-1-ol | 10.66 | 453 | Ethanol |
| Chlorophyll a | (SP-4’-2’)-[2E,7E,11R]-3,7,11,15-tetramethyl-2-hexadecenyl (35,5’4,21R)-9-ethyl-14-ethyl-21-(methoxyacrylonitrile)-4,8,18-trimethyl-26-oxo-3-phorbinepropanoato(2-)·K₃O₃·C₇O₇ | 12.98 | 652 | Methanol |
| β-carotene | (all-β)-1’1’-[(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanoaene-1,18-diyl) bis[2,6,6-trimethylcylohexenyl](2) | 51.25 | 453 | Hexane |

†High-performance liquid chromatography conditions are described in the text.

‡Absorbance wavelength for pigment quantification.
**Statistical analysis.** Two experimental runs were conducted with treatments in each arranged as a 3 × 3 factorial randomized complete block design with three replications. Factors included herbicide (mesotrione, topramezone, and tembotrione) and rate (low, medium, and high). Data from non-treated plants were excluded from statistical analysis to stabilize variance (Corbett et al., 2004); additionally, square-root transformations were also performed before subjecting data to analysis of variance (P = 0.05; Statistical Analysis Software, Inc., Cary, NC) (Ahrens et al., 1990). Interpretations were not different from non-transformed data; therefore, non-transformed means are presented here for clarity. Fisher’s protected least significant difference test (P = 0.05) was used to separate treatment means where appropriate. No significant herbicide-by-rate interactions were detected in bermudagrass carotenoid pigment data. Thus, changes in tissue pigments resulting from herbicide (pooled across rates) are plotted over harvest interval with bars presented as a means of statistical comparison. Trend analyses (P = 0.05) were conducted using orthogonal polynomial contrasts to model significant changes in tissue pigments as a result of application rate as well.

**Results and Discussion.**

**Visual bleaching.** Significant herbicide-by-rate interactions were detected in visual bleaching data. Visual bleaching increased linearly with mesotrione rate at 7 and 14 DAA (Table 2). For mesotrione, the highest level of bleaching occurred at 7 DAA (22%) with the highest rate tested (0.42 kg ha⁻¹) (Table 2). Bleaching never exceeded 10% after treatment with the maximum labeled use rate of mesotrione (0.28 kg ha⁻¹) (Table 2). No differences in bleaching were detected between mesotrione rates after 14 DAA, and by 28 DAA, no visual bleaching was observed for any mesotrione treatment (Table 2). Similar responses have been reported by other researchers investigating bermudagrass bleaching after mesotrione treatment (McCurdy et al., 2008; Willis et al., 2007).

With the exception of 7 DAA, no significant differences were detected between topramezone rates until 28 DAA (Table 2). All rates resulted in greater than 50% visual bleaching at 14 DAA compared with only 19% bleaching for the highest (0.42 kg ha⁻¹) rate of mesotrione (Table 2). Visual bleaching decreased for all topramezone rates after 21 DAA (Table 2). Once visual bleaching began to subside, a linear response was detected among rates with the high (0.038 kg ha⁻¹) rate producing more bleaching than the medium (0.025 kg ha⁻¹) and low (0.018 kg ha⁻¹) rates (Table 2).

Bleaching responses for tembotrione were similar to topramezone. No differences were detected between tembotrione rates until 21 DAA (Table 2). All rates resulted in greater than 32% visual bleaching at 14 DAA, compared with greater than 50% for all rates of topramezone and only 19% bleaching for non-treated control (data not shown).

**Table 2.** Effect of herbicide-by-rate interaction on visual bleaching of ‘Riviera’ bermudagrass treated with mesotrione, topramezone, and tembotrione.

| Herbicide | Rate          | 3 DAA | 7 DAA | 14 DAA | 21 DAA | 28 DAA | 35 DAA |
|-----------|---------------|-------|-------|--------|--------|--------|--------|
| Mesotrione| Low (0.28 kg ha⁻¹) | 2     | 9     | 4      | 1      | 0      | 0      |
|           | Med (0.35 kg ha⁻¹) | 4     | 19    | 14     | 3      | 0      | 0      |
|           | High (0.42 kg ha⁻¹) | 4     | 22    | 19     | 5      | 0      | 0      |
| Linear0.05 |                | NS    | *     | *      | NS     | NS     | NS     |
| Quadratic0.05 |            | NS    | NS    | NS     | NS     | NS     | NS     |
| Topramezone| Low (0.018 kg ha⁻¹) | 6     | 32    | 57     | 46     | 13     | 4      |
|           | Med (0.025 kg ha⁻¹) | 5     | 25    | 57     | 48     | 11     | 3      |
|           | High (0.038 kg ha⁻¹) | 3     | 28    | 50     | 58     | 27     | 14     |
| Linear0.05 |                | NS    | NS    | NS     | NS     | NS     | NS     |
| Quadratic0.05 |            | NS    | NS    | NS     | NS     | NS     | NS     |
| Tembotrione| Low (0.092 kg ha⁻¹) | 5     | 26    | 33     | 15     | 6      | 1      |
|           | Med (0.184 kg ha⁻¹) | 2     | 19    | 41     | 34     | 11     | 2      |
|           | High (0.276 kg ha⁻¹) | 3     | 28    | 45     | 58     | 36     | 22     |
| Linear0.05 |                | NS    | NS    | NS     | ***    | ***    | **     |
| Quadratic0.05 |            | NS    | NS    | NS     | NS     | NS     | NS     |

NS = non-significant at the α = 0.05 level.
* = significant at the P ≤ 0.05, 0.01, and 0.001 levels, respectively.
DAA = days after application.

**Table 3.** Effect of herbicide-by-rate interaction on total chlorophyll (chlorophyll a + chlorophyll b) concentration [mg/100 g fresh weight (FW)] of ‘Riviera’ bermudagrass treated with mesotrione, topramezone, and tembotrione.

| Herbicide | Rate          | 3 DAA | 7 DAA | 14 DAA | 21 DAA | 28 DAA | 35 DAA |
|-----------|---------------|-------|-------|--------|--------|--------|--------|
| Mesotrione| Low (0.28 kg ha⁻¹) | 249.6 | 145.3 | 214.5  | 211.4  | 247.9  | 237.6  |
|           | Med (0.35 kg ha⁻¹) | 255.3 | 130.3 | 223.6  | 251.3  | 269.4  | 271.6  |
|           | High (0.42 kg ha⁻¹) | 241.0 | 107.8 | 179.4  | 217.2  | 276.6  | 274.4  |
| Linear0.05 |                | NS    | NS    | NS     | NS     | NS     | NS     |
| Quadratic0.05 |            | NS    | NS    | NS     | NS     | NS     | NS     |
| Topramezone| Low (0.018 kg ha⁻¹) | 226.7 | 94.2  | 85.4   | 158.9  | 255.9  | 276.8  |
|           | Med (0.025 kg ha⁻¹) | 223.5 | 72.0  | 90.8   | 159.3  | 255.7  | 300.8  |
|           | High (0.038 kg ha⁻¹) | 213.8 | 83.6  | 45.3   | 98.4   | 206.7  | 269.9  |
| Linear0.05 |                | NS    | NS    | NS     | NS     | NS     | NS     |
| Quadratic0.05 |            | NS    | NS    | NS     | NS     | NS     | NS     |
| Tembotrione| Low (0.092 kg ha⁻¹) | 247.2 | 97.3  | 149.9  | 206.3  | 261.4  | 288.1  |
|           | Med (0.184 kg ha⁻¹) | 226.4 | 89.0  | 76.2   | 145.8  | 249.7  | 280.8  |
|           | High (0.276 kg ha⁻¹) | 213.8 | 80.8  | 64.7   | 114.6  | 206.7  | 254.3  |
| Linear0.05 |                | NS    | NS    | NS     | NS     | NS     | NS     |
| Quadratic0.05 |            | NS    | NS    | NS     | NS     | NS     | NS     |

NS = non-significant at the α = 0.05 level.
* = significant at the P ≤ 0.05, 0.01, and 0.001 levels, respectively.
DAA = days after application.

**Fig. 1.** Total chlorophyll (chlorophyll a + b) concentration [mg/100 g fresh weight (FW)] in ‘Riviera’ bermudagrass [Cynodon dactylon (L.) Pers.] treated with mesotrione, topramezone, and tembotrione at 3, 7, 14, 21, 28, and 35 d after application. Error bars indicate SEs.
the high (0.42 kg ha⁻¹) rate of mesotrione (Table 2). Similar to topramezone, visual tissue bleaching began to subside at 21 DAA. Once bleaching began to subside, linear responses were detected among tembotrione rates suggesting that single applications at higher rates may improve activity against bermudagrass (Table 2).

Chlorophyll. Few statistically significant differences were detected among the low, medium, and high application rates of mesotrione, topramezone, and tembotrione (Table 3). Increases in bleaching at 7 DAA were accompanied by reductions in the concentration of total chlorophyll for all herbicides (Fig. 1). At 7 DAA, total chlorophyll for mesotrione-treated plants measured 127.8 mg/100 g FW compared with 192.9 mg/100 g FW for the non-treated control. By 14 DAA, concentrations of total chlorophyll for mesotrione- and non-treated plants were not significantly different. Topramezone and tembotrione reduced total chlorophyll to a greater extent than mesotrione at 7 DAA and did not rebound to non-treated levels until 28 DAA (Fig. 1).

Lutein and β-carotene. Changes in total carotenoids resulting from herbicide treatment are most likely related to the rise and fall of lutein and β-carotene, because these pigments comprised greater than 53% of the total carotenoids measured on each harvest date. At 7 DAA, topramezone- (5.5 mg/100 g FW) and tembotrione- (5.5 mg/100 g FW) treated plants measured lower in lutein than those treated with mesotrione (7.7 mg/100 g FW) (Fig. 2). By 14 DAA, mesotrione- and non-treated plants had equivalent lutein concentrations; topramezone- and tembotrione-treated plants were not equivalent to the non-treated controls until 28 and 35 DAA, respectively. Topramezone affected lutein to a greater extent than tembotrione; topramezone reduced lutein 67% (compared with the non-treated control) at 14 DAA, whereas tembotrione only led to a 51% reduction. A similar response was observed 21 DAA as well (Fig. 2).

Reductions in β-carotene were greater for tembotrione than mesotrione. Compared with the non-treated control (5.5 mg/100 g FW), tembotrione led to a 56% reduction in β-carotene (2.4 mg/100 g FW), whereas mesotrione only led to a 35% reduction (3.6 mg/100 g FW) at 7 DAA (Fig. 2). β-carotene levels for mesotrione-treated plants were not different from non-treated controls after 7 DAA (Fig. 2). No differences in β-carotene were detected between tembotrione- and topramezone-treated plants on each harvest date.

At the point of maximum visual bleaching (14 DAT), linear reductions in lutein were observed among the low, medium, and high rates of each herbicide (Table 4); lutein concentrations after treatment at low and medium rates measured 9.7 mg/100 g FW compared with 6.3 mg/100 g FW after treatment at high rates (Table 4). Comparatively, the lutein concentration of non-treated plants at 14 DAA measured 15.3 mg/100 g FW (Table 4). Linear reductions in β-carotene were also observed among the low, medium, and high application rates by 21 DAA (Table 4).

Xanthophyll cycle pigments. Xanthophyll cycle pigments (zeaxanthin + antheraxanthin + violaxanthin) participate as antioxidants in light-harvesting complexes (Demmig-Adams et al., 1996; Niyogi et al., 1997). Mesotrione, topramezone, and tembotrione lowered the concentration of total xanthophyll cycle pigments compared with the non-treated control at 7 DAA (Fig. 3). Concentrations of total xanthophyll cycle pigments for mesotrione and non-treated controls were equivalent at 14 DAA, whereas topramezone- and tembotrione-treated plants were not equivalent to the non-treated controls until 28 and 21 DAA, respectively (Fig. 3).

After peak visual bleaching occurred (14 DAA), topramezone and tembotrione appeared to affect xanthophyll cycle function, because the percentage of zeaxanthin + antheraxanthin in the total xanthophyll pigment pool (ZA/ZAV) increased in topramezone- and tembotrione-treated plants from 14 to 21 DAA (Fig. 3). Pooled across all herbicides, ZA/ZAV concentrations compared with the non-treated control remained greater than 53% of the total xanthophyll cycle pigments for mesotrione- and non-treated plants at 14 DAT, linear reductions in lutein were observed 21 DAA as well (Fig. 2).

Table 4. Effects of application rate (pooled across herbicide) on lutein and β-carotene concentrations [mg/100 g fresh weight (FW)] in ‘Riviera’ bermudagrass [Cynodon dactylon (L.) Pers.] treated with mesotrione, topramezone, tembotrione, or non-treated at 3, 7, 14, 21, 28, and 35 d after application. Error bars indicate S.E.

| Herbicide rate | Lutein (mg/100 g FW) | β-carotene (mg/100 g FW) |
|---------------|---------------------|-------------------------|
|               | 3 DAA | 7 DAA | 14 DAA | 21 DAA | 28 DAA | 35 DAA | 3 DAA | 7 DAA | 14 DAA | 21 DAA | 28 DAA | 35 DAA |
| Low           | 15.3  | 6.9   | 9.7    | 12.6   | 15.8   | 18.2   | 8.8   | 5.5   | 6.2   | 5.4    | 8.9    | 10.2   |
| Medium        | 14.8  | 5.9   | 9.7    | 11.7   | 15.7   | 18.9   | 8.4   | 2.7   | 4.7   | 7.1    | 8.9    | 10.6   |
| High          | 14.0  | 5.7   | 6.3    | 9.6    | 13.6   | 17.8   | 7.8   | 2.6   | 4.1   | 5.2    | 7.4    | 9.6    |
| Linear         | NS    | NS    | NS     | NS     | NS     | NS     | NS    | NS    | NS    | NS     | NS     | NS     |
| Linear         | NS    | NS    | NS     | *      | **     | **     | NS    | NS    | NS    | NS     | NS     | *      |
| Linear         | NS    | NS    | NS     | NS     | NS     | *      | NS    | NS    | NS    | NS     | NS     | NS     |
| Linear         | NS    | NS    | NS     | NS     | NS     | NS     | NS    | NS    | NS    | NS     | NS     | NS     |

Lutein concentrations in non-treated plants measured 15.1, 11.2, 15.3, 13.9, 15.5, and 17.4 mg/100 g FW at 3, 7, 14, 21, 28, and 35 DAA, respectively.

β-carotene concentrations in non-treated plants measured 8.9, 5.5, 9.5, 8.5, 8.9, and 9.5 mg/100 g FW at 3, 7, 14, 21, 28, and 35 DAA, respectively.

NS = non-significant at the α = 0.05 level.

** = significant at the P ≤ 0.01 and 0.001 levels, respectively.

DAA = days after application.
well (Table 5). By 21 DAA, reductions in bleaching for all herbicides were accompanied by reductions in ZA/ZAV (Table 2; Fig. 3). Stress induced by these herbicides from 14 to 21 DAA may have influenced violaxanthin de-epoxidase activity, thus increasing the conversion of violaxanthin to the intermediate antheraxanthin and subsequently zeaxanthin, the primary carotenoid responsible for preventing photoinhibition (Baroli et al., 2003).

When peak visual bleaching occurred (14 DAT), violaxanthin was reduced by more than 50% in topramezone- and tembotrione-treated plants and linear reductions in violaxanthin were also detected among application rates (Fig. 3; Table 5). Polle et al. (2001) reported that zeaxanthin can replace violaxanthin and lutein in light-harvesting antennae of photosystem II and photosystem I. Moreover, Depka et al. (1998) reported a conversion of \( \beta \)-carotene to zeaxanthin under conditions of high light stress and surmised that this conversion was required to maintain the integrity of the D1 protein of photosystem II. On each date that linear increases in ZA/ZAV were observed in the current study, linear reductions in \( \beta \)-carotene were observed as well (Tables 2 and 3).

**Conclusion**

This research outlines several findings. First, temporary cessation of phytoene desaturase activity from mesotrione, topramezone, and tembotrione did not result in equivalent decreases in all carotenoids. Topramezone and tembotrione resulted in greater visual bleaching than mesotrione and consequently greater reductions in total chlorophyll, lutein, \( \beta \)-carotene, and xanthophyll cycle pigments as well. Considering the roles \( \beta \)-carotene and the xanthophyll cycle pigments play in photosystem II functionality and non-photochemical quenching, responses in this study suggest topramezone and tembotrione are more herbicidally active against bermudagrass than mesotrione.

Few differences in bleaching were detected between rates of topramezone and tembotrione before 21 DAA. Linear increases in bleaching were reported with increasing rates of mesotrione as soon as 7 DAA. This information supports the concept that topramezone and tembotrione are more active against bermudagrass than mesotrione. Furthermore, current maximum labeled use rates of mesotrione, topramezone, and tembotrione may need to be increased to provide improved herbicidal activity against bermudagrass, because concentrations of most carotenoid pigments decreased linearly with increased application rate.

Third, topramezone and tembotrione increased the percentage of zeaxanthan + antheraxanthin in the total xanthophyll pigment
pool (ZA/ZAV) 7 d after peak visual bleaching was observed (14 DAA). ZA/ZAV increased linearly with application rate from 14 to 21 DAA as well. Considering that zeaxanthin + antheraxanthin are vital for energy dissipation of excess absorbed light (Demmig-Adams, 1999), these increases indicate that bermudagrass leaf tissue is still under considerable stress even after visual bleaching begins to subside. After 21 DAA, reductions in ZA/ZAV were reported for herbicides. Thus, sequential applications of HPPD-inhibiting herbicides for bermudagrass control should be applied on 14- to 21-d intervals, because the stress imposed by these herbicides appears to be greatest 14 to 21 DAA. Additionally, increases in photoprotective xanthophyll cycle pigments (ZA/ZAV) may be a mechanism by which bermudagrass recovers from HPPD-inhibiting herbicide injury. These findings will allow turf managers to design physiologically validated bermudagrass control programs with HPPD-inhibiting herbicides. Further research should investigate how responses of the HPPD inhibitors in the current study are affected by application methods, surfactants, bermudagrass growth stage, climatic conditions, or by applying HPPD inhibitors in mixtures with other herbicides, including inhibitors of photosystem II and other graminicides.

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