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ARTICLE

Increase in Nutritionally Important Sweet Corn Kernel Carotenoids following Mesotrione and Atrazine Applications

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The herbicide mesotrione inhibits a critical enzyme, phytoene desaturase, in plant carotenoid biosynthesis. Mesotrione is currently labeled for selective weed control in sweet corn (*Zea mays* var. *rugosa*). Mesotrione applied alone, or in mixtures with the photosystem II inhibitor atrazine, acted to increase concentrations of kernel antheraxanthin, lutein, and zeaxanthin carotenoids in several sweet corn genotypes. Kernel lutein and zeaxanthin levels significantly increased 15.6% after mesotrione + atrazine early postemergence applications, as compared to the control treatment. It appears that mesotrione applications resulted in greater pools of kernel carotenoids once the sweet corn genotypes expressing moderate injury overcame the initial herbicidal photo-oxidative stress. This is the first report of herbicides directly up-regulating the carotenoid biosynthetic pathway in corn kernels, which is associated with the nutritional quality of sweet corn. Enhanced accumulation of lutein and zeaxanthin is important because dietary carotenoids function in suppressing aging eye diseases such as macular degeneration, now affecting 1.75 million older Americans.

KEYWORDS: Antheraxanthin; herbicide; HPLC; lutein; zeaxanthin

INTRODUCTION

Mesotrione is a member of the triketone family of herbicides, which are structurally similar to leptospermone, a natural phytotoxin obtained from the Californian bottlebrush plant (*Callistemon citrinus* Stapf.). Mesotrione is a carotenoid biosynthesis inhibitor (CBI), which is currently labeled for pre-emergence and postemergence control of broadleaf and grass weeds in maize (*Zea mays* L.) production (*1*). Mesotrione competitively inhibits the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD), an essential component for the biochemical conversion of tyrosine to plastoquinone and α-tocopherol. Plastoquinone is a critical cofactor for phytoene desaturase, as well as an intermediate electron carrier between the carotenoid desaturase enzyme and the photosynthetic electron transport chain. Maize is tolerant to mesotrione applications; however, differing degrees of sensitivity exist among sweet corn (*Z. mays* var. *rugosa*) genotypes (*2*). Metabolism of mesotrione occurs rapidly in maize through hydroxylation of the active compound. The cyclohexane and phenyl groups of the hydroxyl metabolites are then split hydrolytically into 4-(methylsulfonyl)-2-nitrobenzoic acid (MNBA) and 2-amino-4-(methysulfonyl)benzoic acid (AMBA), two immobile and nonherbicidal breakdown metabolites (*3*).

Carotenoid biosynthesis begins with the condensation of two molecules of geranylgeranyl pyrophosphate to form the first C_{40} carotenoid, phytoene, via phytoene synthase. Two similar desaturase enzymes, phytoene desaturase and ξ-carotene desaturase, create the chromophore present and make the conversions of phytoene to lycopene (**Figure 1**). Carotenoids are secondary plant metabolites that serve antioxidant functions in plant photosynthetic processes, as well as in actions of disease reduction in mammalian systems (*4*). The biosynthesis of carotenoids in plants occurs on membranes of chloroplasts, chromoplasts, and amyloplasts, with the regulating enzymes encoded in the nucleus and targeted to these plastids (*5*). Carotenoids found in endosperm tissue of monocotyledonous maize function as precursors for the phytohormone abscisic acid (ABA), which controls seed dormancy and germination (*6*). The major carotenoid pigments in fresh-market sweet corn are lutein and zeaxanthin, with minor amounts of β-carotene, α-carotene, β-cryptoxanthin, and antheraxanthin (*7*).

Dietary lutein and zeaxanthin are selectively deposited as macular pigment (MP) in the retina, a photoprotective yellow pigmentation (*8*, *9*). Macular pigment filters harmful UV/blue light wavelengths and protects retinal rods and cones, and retinal damage correlates with decreases in MP concentrations (*10*−*12*). Age-related macular degeneration (AMD) is now the leading cause of blindness among people of European descent age 65 and older, affecting more than 1.75 million Americans. Estimates of AMD prevalence are predicted to double, to just over 3 million affected, by 2020 (*13*). Consumption of fruits and vegetables with high lutein and zeaxanthin concentrations can increase MP

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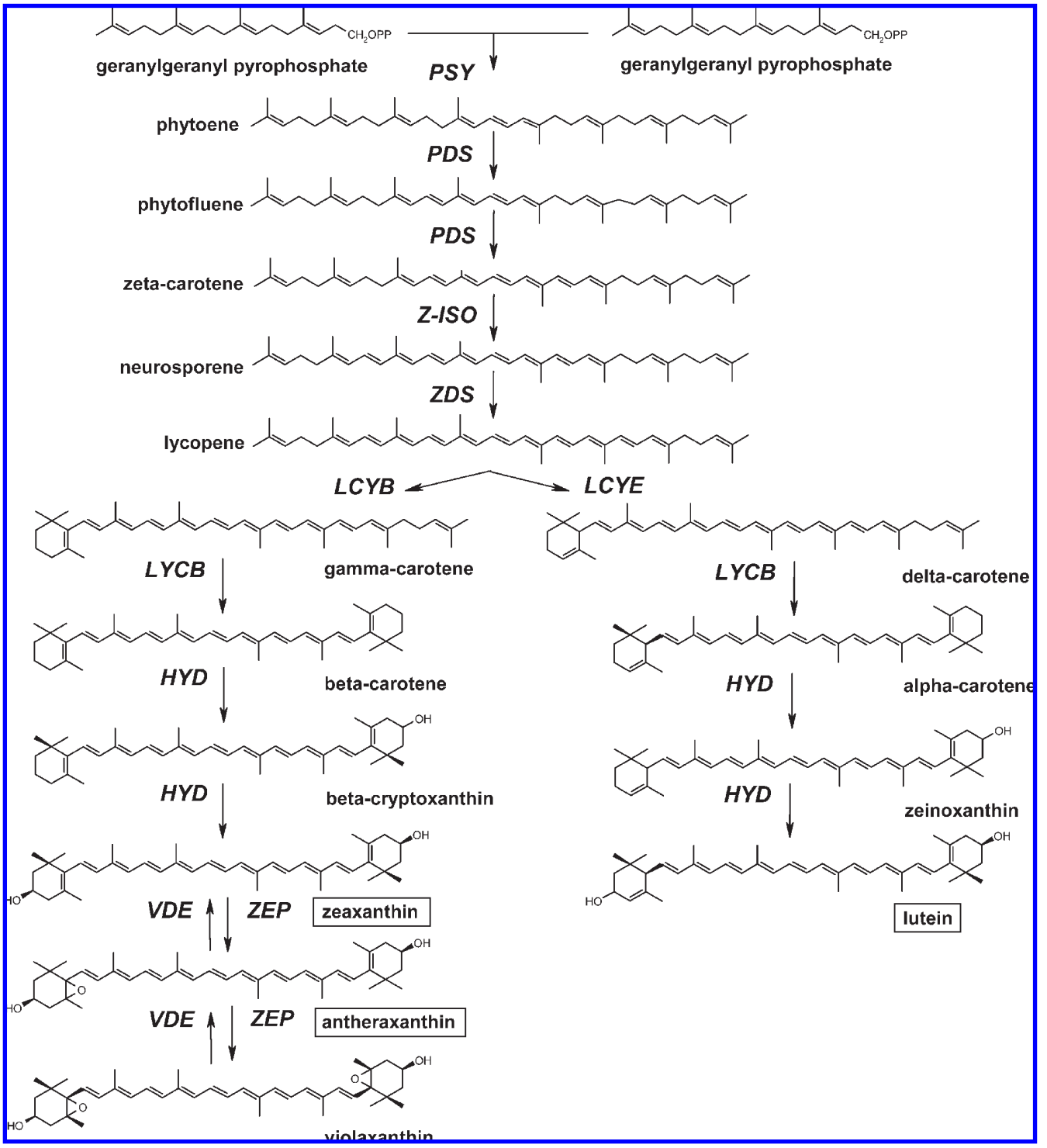

Figure 1. Simplified carotenoid biosynthetic pathway in plants. Substrates evaluated in our studies are boxed in the diagram. Enzymatic reactions throughout the pathway are depicted using solid arrows accompanied by the enzyme abbreviations in capital italics. Enzyme abbreviations: *PSY*, phytoene synthase; *PDS*, phytoene desaturase; *Z-ISO*, ξ-carotene isomerase; *ZDS*, ζ-carotene desaturase; *LCYB*, lycopene β-cyclase; *LCYE*, lycopene ε-cyclase; *HYD*, carotene hydroxylase (both β-ring and ε-ring hydroxylases); *ZEP*, zeaxanthin epoxidase; *VDE*, violaxanthin de-epoxidase.

concentrations in some, but not all, individuals (*14*). Health benefits attributed to consuming fruit and vegetable crops high in carotenoid compounds also include prevention of certain cancers (*15*−*17*) and cardiovascular diseases (*18*). Pro-vitamin A activity is the classical biological function of carotenoids (mostly from β-carotene) in mammalian systems (*19*).

Sweet corn has a significant impact on the U.S. agricultural economy and human nutrition. A total of 94,860 ha of sweet corn were harvested commercially in 2007 in the United States, with a total production value of $625 million (*20*). Because it is unclear whether postemergence applications of CBIs to young sweet corn plants will have an impact on the accumulation of carotenoids in the developing kernels, we evaluated responses of mature kernel concentrations of lutein, zeaxanthin, and antheraxanthin in different sweet corn genotypic sensitivities to mesotrione applications. Field and laboratory studies were conducted in 2008 to

Table 1. Evaluation of Percent Foliar Sweet Corn Visual Injury following Postemergence Applications of Mesotrione, Mesotrione + Atrazine, and Atrazine for the Genotypes 'Merit', 'Incredible', and 'Temptation'

		leaf tissue visual bleaching (%)								
		'Merit'			'Incredible'			'Temptation'		
treatment[a]	timing[b]	7 DAT[c]	14 DAT	21 DAT	7 DAT	14 DAT	21 DAT	7 DAT	14 DAT	21 DAT
untreated		0	0	0	0	0	0	0	0	0
mesotrione	EPOST	60	53	44	16	9	8	6	8	4
mesotrione + atrazine	EPOST	80	79	73	35	19	9	9	9	4
atrazine	EPOST	3	2	0.0	13	13	0	2	3	0
$LSD_{0.05}$[d]		6	5	5	6	8	4	6	5	2
location (L)		ns[e]	ns	$P = 0.006$	ns	ns	ns	ns	ns	ns
treatment (T)		$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P = 0.01$	$P = 0.01$	$P \leq 0.001$
L × T		ns	$P = 0.003$	$P \leq 0.001$	ns	ns	ns	ns	ns	ns
untreated		0	0	0	0	0	0	0	0	0
mesotrione	LPOST	34	29	9	11	9	1	6	6	1
mesotrione + atrazine	LPOST	39	33	13	11	10	2	8	4	0
atrazine	LPOST	0	0	0	0	1	0	0	0	0
$LSD_{0.05}$		5	4	8	6	4	3	5	3	1
location (L)		$P \leq 0.001$	$P \leq 0.001$	ns	ns	ns	ns	ns	ns	ns
treatment (T)		$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	ns	$P = 0.01$	$P \leq 0.001$	ns
L × T		$P \leq 0.001$	$P \leq 0.001$	ns	ns	ns	ns	ns	ns	ns

[a] Values represent four treatment replications grown in two separate locations. Postemergence herbicide application treatments: untreated, no application control; mesotrione at 105 g of ai/ha; mesotrione at 105 g of ai/ha + atrazine at 560 g of ai/ha; atrazine at 560 g of ai/ha. [b] EPOST, early postapplication made to corn 5−10 cm in height 16 days after planting; LPOST, late postapplication made to corn 15−20 cm tall 29 days after planting. [c] DAT, days after herbicide treatment applications for which visual assessments were performed. [d] Fisher's least significant difference among treatments within each application timing at $\alpha = 0.05$. [e] ns, nonsignificant.

evaluate how mesotrione alone and in a mixture with atrazine, a common broadleaf herbicide used in maize production, at two different timings would affect visual bleaching in sweet corn foliar tissues and mature kernel carotenoid concentrations in three different genotypes. Atrazine controls weeds by inhibiting site A of the Q_b binding niche of the D1 protein in photosystem II (PSII) (*3*). Similarly to mesotrione, rapid metabolism of atrazine affords tolerance in maize genotypes. Visual observations of foliar tissue bleaching were made every 7 days following herbicide treatments at both application timings (**Table 1**). Carotenoid levels in mature sweet corn kernels were measured at not less than 45 days after postemergence applications.

MATERIALS AND METHODS

Plant Culture for Sweet Corn. Sweet corn cultivars selected for the study were 'Merit', a yellow-kernel sensitive genotype (Willhite Seed Inc., Poolville, TX); 'Temptation', a bicolor tolerant genotype (Welter Seed & Honey Co., Onslow, IA); and 'Incredible', a yellow-kernel moderately sensitive genotype (Main Street Seed and Supply Co., Bay City, MI). Sensitivities are based on visual leaf bleaching after mesotrione applications (*2*). Sweet corn cultivars were seeded at the East Tennessee Research and Education Center in Knoxville, TN (35.98 N latitude) on May 1, 2008, in a randomized complete block design split-plot design with four replications at two separate sites 500 m apart. Sweet corn cultivars acted as the main plots, and postemergence herbicide treatments acted as subplots. Seeds were drilled 2.5 cm deep in a Sequatchie silt loam soil (fine-loamy, sili-ceous, thermic, Humic Hapudult) at spacings of 25 cm within rows and 76 cm between rows. Each plot consisted of four rows of corn 9.1 m in length. Pre-emergence applications were made to all plots using *s*-metolachlor (Dual Magnum) at 1070 g of active ingredient per hectare (g of ai/ha) and atrazine (Aatrex, Syngenta Crop Protection, Inc., Greensboro, NC) at 1120 g of ai/ha. The insecticide Warrior (Syngenta Crop Protection, Inc.) was applied preplant at 32 g of ai/ha. Postemergence herbicide treatments included (1) untreated control, no application; (2) mesotrione (Callisto, Syngenta Crop Protection, Inc.) at 105 g of ai/ha as an early post (EPOST); (3) mesotrione at 105 g of ai/ha + atrazine at 560 g of ai/ha as an EPOST; (4) atrazine at 560 g of ai/ha as an EPOST; (5) mesotrione at 105 g of ai/ha as a late post (LPOST); (6) mesotrione at 105 g of ai/ha + atrazine at 560 g of ai/ha as a LPOST; and (7) atrazine at 560 g of ai/ha as a LPOST. Herbicide treatments were applied as EPOST treatment to corn 5−10 cm tall on May 17, 2008, and as LPOST treatment to corn 15−20 cm tall on May 30, 2008.

Plant Harvest. 'Temptation' was harvested 45 days after LPOST applications on July 14, 2008. 'Merit' and 'Incredible' were harvested 56 days after LPOST applications. At harvest, eight uniform ears were collected from the center of the sprayed area of each plot and stored for 24−48 h in a walk-in cooler (4 °C). During processing, a 5 cm section was cut from each ear of the experimental samples and saved for carotenoid analysis. The kernels from the section were cut from the cob and stored at −80 °C. Sweet corn kernels were freeze-dried and ground to a powder in liquid nitrogen prior to extraction of the pigments.

Carotenoid Determination for Sweet Corn. *Tissue Extraction.* Extraction of pigments from the kernels followed the procedure of Kurilich and Juvik (*7*), with slight modifications. A 0.50 g subsample of kernel tissue was placed in a test tube (20 × 150 mm) and rehydrated with 6 mL of EtOH stabilized with 0.1% BHT. Addition of 0.8 mL of the internal standard ethyl-β-8′-apo-carotenoate (Sigma Chemical Co., St. Louis, MO) was used to determine extraction efficiency. Tubes were vortexed for 1 min before being capped and placed in a water bath at 85 °C for 5 min or until the ethanol was brought to boiling. Tubes were removed from the bath, and 0.18 mL of 53% KOH was added for saponification. Tubes were vortexed for 1 min and returned to the bath for 10 min. After saponification, tubes were cooled in an ice bath for 2 min before the addition of 3 mL of cold deionized water and 3 mL of hexane. Tubes were vortexed for 1 min and placed into a clinical centrifuge at $600g_n$ for 10 min. A Pasteur pipet was used to remove the partitioned hexane layer, which was transferred to a separate test tube. The addition of 3 mL of hexane to the sample tubes was made, and the centrifugation step was repeated twice more. The combined hexane fractions were reduced to dryness under a stream of nitrogen gas and brought up to a final volume of 5 mL with 11% methyl *tert*-butyl ether (MTBE), 88.9% MeOH, and 0.1% triethylamine (TEA). A 2 mL aliquot was filtered through a 0.2 μm Econofilter PTFE 25/20 polytetrafluoroethylene filter (Agilent Technologies, Wilmington, DE) using a 5 mL syringe prior to high-performance liquid chromatography (LC) analysis.

Carotenoid Liquid Chromatography Analysis. LC separation parameters and pigment quantification followed procedures of Kopsell et al. (*21*). An Agilent 1200 series LC unit with a photodiode array detector (Agilent Technologies, Palo Alto, CA) was used for pigment separation. The column used was a 250 × 4.6 mm i.d., 5 μm analytical scale polymeric RP-C_{30}, with a 10 × 4.0 mm i.d. guard cartridge and holder (ProntoSIL,

Table 2. Mean Values for Sweet Corn Kernel Carotenoids in the Moderately Sensitive Genotype 'Incredible' in Response to Postemergence Applications of Mesotrione, Mesotrione + Atrazine, and Atrazine

		kernel carotenoid[a] concentrations (mg/100 g of fresh weight)							
treatment[b]	timing[c]	Anth	% change	Lut	% change	Zea	% change	Lut + Zea	% change
untreated		0.188 a[d]		0.328 b		0.448 ab		0.776 ab	
mesotrione	EPOST	0.183 a	−2.7	0.307 b	−6.4	0.415 b	−7.4	0.722 b	−7.0
mesotrione + atrazine	EPOST	0.205 a	+9.0	0.399 a	+21.6	0.498 a	+16.4	0.897 a	+15.6
atrazine	EPOST	0.190 a	+1.1	0.353 ab	+7.6	0.447 ab	−0.01	0.799 ab	+2.9
mesotrione	LPOST	0.179 a	−4.8	0.343 ab	+4.6	0.454 ab	+1.3	0.798 ab	+2.8
mesotrione + atrazine	LPOST	0.162 a	−13.8	0.340 ab	+3.7	0.436 ab	−2.8	0.776 ab	0.0
atrazine	LPOST	0.187 a	−0.1	0.347 ab	+6.0	0.454 ab	+1.3	0.800 ab	+3.0

[a] Table includes the change in concentrations relative to the untreated control. Carotenoid pigments: Anth, antherazanthin; Zea, zeaxanthin; Lut, lutein; Lut + Zea, combined lutein and zeaxanthin. Values expressed represent plots grown in two separate locations. Values represent means of two field sites, four replications per site, eight harvested ears per replication. [b] Postemergence herbicide application treatments: untreated, no application control; mesotrione at 105 g of ai/ha; mesotrione at 105 g of ai/ha + atrazine at 560 g of ai/ha; atrazine at 560 g of ai/ha. [c] EPOST, early postapplication made to corn 5−10 cm in height 16 days after planting; LPOST, late postapplication made to corn 15−20 cm tall 29 days after planting. [d] Means followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.05$.

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 thermostated column compartment. All separations were achieved isocratically using a binary mobile phase of 11% MTBE, 88.9% MeOH, and 0.1% TEA (v/v). The flow rate was 1.0 mL/min, with a run time of 53 min, followed by a 10 min equilibration prior to the next injection. Eluted compounds from a 10 μL injection loop were detected at 453 nm, and data were collected, recorded, and integrated using ChemStation software (Agilent Technologies). Peak assignment for individual kernel pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards (antheraxanthin, lutein, zeaxanthin) (ChromaDex Inc., Irvine, CA). The concentrations of the external pigment standards were determined spectrophotometrically using methods described by Davies and Köst (*22*). Slurried Spinach 2385 standard reference material (National Institute of Science and Technology, Gaithersburg, MD) was used for method validation. Pigment data are presented on a fresh weight basis.

Mesotrione Determination in Sweet Corn Kernels. Mesotrione residues were measured in corn kernels using liquid chromatography − mass spectrometry (LC-MS), with methods based upon those of Freitas et al. (*23*) and Durand et al. (*24*). Ground samples of each sweet corn variety from plots receiving the late postemergent application of mesotrione + atrazine were evaluated. Plant samples (1.0 g) inside 20 mL glass vials were extracted with 10 mL of acetonitrile/water (50:50 v/v) for 2 h on a reciprocating shaker. Samples were removed and allowed to statically equilibrate for 30 min, and the supernatant was filtered through a 0.45 μm filter into a 1.5 mL vial for analysis. Chromatographic analysis used an Agilent 1100 series LC plus an Agilent 6120 series MS system (Agilent Technologies). Mobile phase was 0.5 mL/min of acetonitrile/water (50:50 v/v). The column used was a 150 × 4 mm i.d., 3 μm, Eclipse XDBE-C_{18}, with a 10 × 4 mm i.d. C_{18} guard column (Agilent Technologies). The MS settings included electrospray ionization with single ion monitoring, and specific settings included drying gas flow of 5.0 L/min, nebulizer pressure of 60, drying gas temperature of 250 °C, vaporizing temperature of 150 °C, capillary voltage of 2000, corona current of 4, charging voltage of 2000, and analyzer operating in SIM mode at 338 with fragmentor set to 80, gain set to 1.0, and dwell set at 590. Retention time in our system was 5.98 min. Recoveries from fortified samples indicated recoveries of >91% and a conservative limit of detection of 0.1 μg/L. An external standard technique was used for analysis. No mesotrione residues were detected in any of the sweet corn varieties from the treatments examined.

Statistical Analysis. Pigment data were subjected to analysis of variance (ANOVA) using SAS statistical software (v. 9.1, SAS Institute, Cary, NC). Duncan's multiple-range test ($P = 0.05$) was used to separate postemergence CBI treatment means among and within sweet corn cultivars.

RESULTS AND DISCUSSION

In general, mesotrione alone caused bleaching symptoms in the meristematic tissue of "Merit', the sensitive genotype, and 'Incredible', the intermediately sensitive genotype. Atrazine alone caused little to no response on any genotype at any application timing. However, the addition of atrazine to mesotrione treatments caused significant necrotic burn, in addition to the characteristic bleaching symptoms of the mesotrione applied alone (**Table 1**). This synergistic response of mesotrione has previously been reported by several researchers (*3*, *25*) and has been directly attributed to interrelationships of photosynthesis and carotenoid biosynthesis (*26*). However, no direct correlations have previously been made to establish the impacts of these synergistic responses in the accumulation of key carotenoids produced in the fruiting bodies of vegetables. Greater foliar tissue bleaching for the sweet corn genotypes was observed for the EPOST applications (**Table 1**). The moderately sensitive ('Incredible') and tolerant ('Temptation') genotypes recovered from foliar bleaching symptoms from the EPOST applications by 21 days after treatment (DAT), whereas the sensitive ('Merit') genotype did not recover from foliar bleaching by 21 DAT for the EPOST treatments of mesotrione or mesotrione + atrazine. All genotypes had recovered to acceptable tolerances from foliar bleaching symptoms by 21 DAT for the LPOST applications (**Table 1**).

Results from our study demonstrate enhancement of antheraxanthin, lutein, and zeaxanthin kernel concentrations from the mixture of mesotrione + atrazine applied EPOST (**Table 2**−**4**). In addition, carotenoid accumulations in the kernels differed significantly among the sweet corn genotypes. The USDA Nutrient Database lists yellow sweet corn as averaging 0.644 mg/100 g of fresh weight of lutein + zeaxanthin and white sweet corn with a lower value of 0.034 mg/100 g of fresh weight (*27*). Carotenoid values for genotypes in the current study were generally much higher for the yellow-kernel genotypes (**Tables 2** and **3**), possibly due to the presence of dominant alleles of phytoene synthase in these genotypes (*5*). The data show that kernel carotenoid accumulation in the tolerant genotype in our study was unaffected by all postemergence CBI treatments (**Table 4**). No increase in kernel carotenoid levels, coupled with a lack of any visual bleaching symptomology, indicates that the tolerant genotype rapidly metabolized the CBIs and thus did not influence kernel carotenoid concentrations. Furthermore, there was no significant change in kernel carotenoids for the sensitive genotype following CBI applications. It has been established that the sensitivity of 'Merit' to mesotrione applications comes from a homozygous condition of alleles at a single locus (possibly at *Nsf1*/*Ben1* of the short arm of chromosome 5, resulting in sensitivity to P450-metabolized herbicides (*28*). We theorize that the lack of any significant increase in kernel lutein and zeaxanthin concentrations may come from an inability of this genotype to overcome

Table 3. Mean Values for Sweet Corn Kernel Carotenoids in the Sensitive Genotype 'Merit' in Response to Postemergence Applications of Mesotrione, Mesotrione + Atrazine, and Atrazine

		kernel carotenoid[a] concentrations (mg/100 g of fresh weight)							
treatment[b]	timing[c]	Anth	% change	Lut	% change	Zea	% change	Lut + Zea	% change
untreated		0.183 b[d]		0.632 ab		0.509 a		1.141 a	
mesotrione	EPOST	0.231 ab	+26.2	0.681 ab	+7.8	0.570 a	+12.0	1.251 a	+9.6
mesotrione + atrazine	EPOST	0.264 a	+44.3	0.706 a	+11.7	0.583 a	+12.7	1.290 a	+13.1
atrazine	EPOST	0.213 ab	+16.4	0.692 ab	+9.5	0.564 a	+10.8	1.255 a	+10.0
mesotrione	LPOST	0.185 b	+1.1	0.577 b	−8.7	0.490 a	−3.7	1.068 a	−6.4
mesotrione + atrazine	LPOST	0.200 ab	+9.3	0.672 ab	+6.3	0.582 a	+14.3	1.254 a	+10.0
atrazine	LPOST	0.228 ab	+24.6	0.664 ab	+5.1	0.550 a	+8.1	1.215 a	+6.5

[a] Table includes the change in concentrations relative to the untreated control. Carotenoid pigments: Anth, antherazanthin; Zea, zeaxanthin; Lut, lutein; Lut + Zea, combined lutein and zeaxanthin. Values expressed represent plots grown in two separate locations. Values represent means of two field sites, four replications per site, eight harvested ears per replication. [b] Postemergence herbicide application treatments: untreated, no application control; mesotrione at 105 g of ai/ha; mesotrione at 105 g of ai/ha + atrazine at 560 g of ai/ha; atrazine at 560 g of ai/ha. [c] EPOST, early postapplication made to corn 5−10 cm in height 16 days after planting; LPOST, late postapplication made to corn 15−20 cm tall 29 days after planting. [d] Means followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.05$.

Table 4. Mean Values for Sweet Corn Kernel Carotenoids in the Resistant Genotype 'Temptation' in Response to Postemergence Applications of Mesotrione, Mesotrione + Atrazine, and Atrazine

		kernel carotenoid[a] concentrations (mg/100 g of fresh weight)							
treatment[b]	timing[c]	Anth	% change	Lut	% change	Zea	% change	Lut + Zea	% change
untreated		0.124 a[d]		0.359 a		0.203 a		0.562 a	
mesotrione	EPOST	0.137 a	+10.5	0.381 a	+6.1	0.212 a	+4.4	0.594 a	+5.4
mesotrione + atrazine	EPOST	0.124 a	0.0	0.335 a	−6.7	0.183 a	−9.9	0.519 a	−7.7
atrazine	EPOST	0.124 a	0.0	0.346 a	−3.6	0.198 a	−2.5	0.544 a	−3.2
mesotrione	LPOST	0.134 a	+8.1	0.355 a	−1.1	0.193 a	−4.9	0.547 a	−2.7
mesotrione + atrazine	LPOST	0.109 a	−12.1	0.351 a	−2.2	0.199 a	−2.0	0.550 a	−2.1
atrazine	LPOST	0.129 a	+4.0	0.346 a	−3.6	0.194 a	−4.4	0.550 a	−2.1

[a] Table includes the change in concentrations relative to the untreated control. Carotenoid pigments: Anth, antherazanthin; Zea, zeaxanthin; Lut, lutein; Lut + Zea, combined lutein and zeaxanthin. Values expressed represent plots grown in two separate locations. Values represent means of two field sites, four replications per site, eight harvested ears per replication. [b] Postemergence herbicide application treatments: untreated, no application control; mesotrione at 105 g of ai/ha; mesotrione at 105 g of ai/ha + atrazine at 560 g of ai/ha; atrazine at 560 g of ai/ha. [c] EPOST, early postapplication made to corn 5−10 cm in height 16 days after planting; LPOST, late postapplication made to corn 15−20 cm tall 29 days after planting. [d] Means followed by the same letter are not significantly different based on Duncan's multiple range test at $\alpha = 0.05$.

the initial stress (foliar bleaching) associated with herbicide applications (**Table 3**).

The most interesting response in these studies came from the moderately sensitive genotype 'Incredible'. For all herbicide treatments applied to 'Incredible', EPOST applications (corn plants 5−10 cm) resulted in greater increases in kernel carotenoid concentrations when compared to LPOST applications (corn plants 15−20 cm). In addition, EPOST applications of mesotrione + atrazine significantly increased kernel lutein + zeaxanthin by >15% when compared to the untreated control (**Table 2**). This significant increase in carotenoid levels is likely due to an orderly stress response that did not occur in the tolerant genotype due to rapid metabolism of the herbicides or in the sensitive genotype due to the overwhelming hypersensitive response associated with the herbicide applications.

Mesotrione is a potent inhibitor of the HPPD enzyme downstream of the shikimate pathway (*1*). However, it does not affect the biosynthesis of isopentenyl diphosphate (IPP) in isoprenoid biosynthesis. Therefore, reduction in phytoene desaturase (*PDS*) production caused by mesotrione stops the carotenoid biosynthetic pathway at the conversion of phytoene to phytofluene, resulting in bioaccumulation of the colorless pigment phytoene (**Figure 1**) (*29*). This accumulation of phytoene may continue until the corn plant metabolizes mesotrione to its nonherbicidal metabolites, which removes HPPD inhibition. Renewed biosynthesis of plastoquinone results in further catalytic activities associated with *PDS*. This enzyme now acts on this larger pool of phytoene by moving the substrate into the carotenoid biosynthetic pathway, thus resulting in greater pools of carotenoids.

There is evidence for two isoforms of phytoene synthase (*PSY*) being present in tomato (*Lycopersicon esculentum* Mill.) that mediate conversion of geranylgeranyl pyrophosphate (GGPP) to phytoene, with the *PSY1* isoform responsible for carotenoid accumulation in ripening fruit and the *PSY2* isoform controlling carotenoid biosynthesis in immature green fruit and leaf tissues (*30*). It is also possible that two isoforms of *PDS* are present in other plants (*31*). Mesotrione was not detectable in mature kernel tissues in our studies (data not shown). Thus, any impact of mesotrione on kernel carotenoid development must be indirect, as it is unlikely that kernels would actively metabolize the intact herbicide. The regulating enzymes for carotenoid biosynthesis are encoded in the nucleus and targeted to specialized plastids (*5*). It may be possible that mesotrione inhibition of HPPD stops production of all plastid *PDS* isoforms in the plant, both chloroplastic and chromoplastic; moreover, there is evidence that all CBI herbicides inhibit noncompetitively with respect to the target enzyme phytoene desaturase (*32*). Suppression of chloroplastic *PDS* is apparent in the visual leaf tissue bleaching following foliar applications of mesotrione to sensitive maize genotypes (**Table 1**). Previously, mesotrione applications to the monocot perennial ryegrass (*Lolium perenne* L.) resulted in significant foliar chlorophyll and carotenoid pigment decreases, but increasing phytoene (*29*), thus providing clear evidence that mesotrione will suppress *PDS* production in monocot leaf tissues. In the current study, data may provide evidence of possible suppression of chromoplastic *PDS* in maize following foliar mesotrione applications. We believe mesotrione + atrazine applications suppressed chromoplastic *PDS* and caused stress in early corn plant development, and once this stress is alleviated

through metabolism of the active (mesotrione and atrazine) chemicals, the corn plants emerge from the stress with greater pools of kernel carotenoids (**Tables 2** and **3**).

On the basis of our study results, we believe that the synergistic response associated with mesotrione + atrazine applications at specific timings caused significant stress in certain genotypes and resulted in up-regulation of key carotenoids immediately following the plants' recovery from this herbicidal response. We suspect that this carotenoid enhancement could be duplicated with other mixtures of CBIs that target HPPD or other enzymes in the pathway, plus PSII inhibitors. Results would depend on specific timings and application rates that could mimic the level of stress response placed on the intermediately sensitive genotype observed in our studies, assuming enough time would be allowed to recover from this response prior to fruit set. As stated earlier, our hypothesis is that the extra 14 days between EPOST and LPOST applications may have allowed this moderately sensitive genotype ('Incredible') to fully recover from mesotrione injury with greater pools of kernel carotenoids following these early applications.

Mesotrione is currently labeled for pre- and postemergence applications to field corn, sweet corn, and popcorn. Maize has the ability to rapidly metabolize mesotrione to nonherbicidal byproducts. Moreover, maize has the ability to outgrow visual sensitivity of leaf tissue bleaching, which results from suppression of *PDS* activity in the carotenoid biosynthetic pathway. Data from this study suggest the possibility to increase concentrations of nutritionally important kernel carotenoid in sweet corn genotypes through applications of HPPD-inhibiting herbicides such as mesotrione. The exact mechanisms of these increases are still unclear and warrant further study. However, results further emphasize the ability to enhance valuable phytochemicals in crop plants through careful management of cultural growing practices.

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