Differential Photosynthetic Efficiency and Pigment Content in Two Common Purslane (*Portulaca oleracea*) Biotypes

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Abstract: Studies were conducted to determine how a serine to threonine mutation at position 264 on the Qb binding niche of the D1 protein [urea-resistant/triazine resistant (UR/TR biotype)] in common purslane (*Portulaca oleracea*) impacted carotenoid and chlorophyll pigment pools and measurements of photochemical and non-photochemical quenching (NPQ) following applications of various inhibitors of carotenoid biosynthesis (CBI) and the Photosystem II (PSII) inhibitor diuron when applied alone, or in mixtures, as compared to wildtype (WT) purslane. Non-photochemical quenching decreased 138 to 531% in comparison to the untreated checks following any herbicide application. Most CBI herbicides and diuron did not change chl *a* and chl *b* in the UR/TR biotype, while these same herbicide treatments tended to sharply decrease chlorophyll pigments in the WT population. Zeaxanthin levels were sharply elevated when CBI herbicides were applied alone to both purslane biotypes. β-carotene reduced in both biotypes following herbicide applications in comparison to the untreated check. Neoxanthin, antheraxanthin, and lutein were generally increased or remained similar to the untreated controls in the herbicide treated UR/TR biotype, while levels of these carotenoids tended to decrease in the herbicide treated WT population. Diuron alone increased neoxanthin, antheraxanthin, lutein, and zeaxanthin by 4 to 200% in the UR/TR biotype, but decreased these same carotenoids 25 to 62% in the WT population. The applications of CBI and PSII herbicides demonstrate that redox signaling in response to this mutation in the D1 protein may impact the retention of plant pigment concentrations in the light harvesting complexes of PSII, which would be vital for stress tolerance in this biotype.

Keywords: Antheraxanthin, β-carotene, lutein, neoxanthin, NPQ - non-photochemical quenching, violaxanthin, zeaxanthin.

INTRODUCTION

Carotenoids are plant pigments that expand the light harvesting capacity of chlorophyll (chl) and prevent membrane degradation of chloroplasts by quenching overflow energy from reactive oxygen species and triplet chl [1, 2]. Carotenoid biosynthesis is initiated in the isoprenoid pathway starting with the formation of isopentenyl pyrophosphate (IPP). Molecules are formed from IPP in 5-carbon increments leading to the production of geranylgeranyl pyrophosphate (GGPP), whereby two molecules of GGPP are ultimately merged to form the basic structure of carotenoids [3]. Through a series of desaturations, cyclizations, and epoxidation steps several antioxidant carotenes and xanthophylls are produced which have an important role in preventing photoinhibition by either absorbing excess energy from chl, scavenging for reactive oxygen and chl species, and by performing numerous additional methods of non-photochemical quenching (NPQ) [1, 2].

Currently, carotenoid biosynthesis inhibiting (CBI) herbicides exist that competitively inhibit six of the approxi

mately twenty enzymes throughout the carotenoid biosynthesis pathway [4]. These CBI herbicides are either applied preemergence (prior to emergence of the weeds or crops) or postemergence (POST) (following emergence of the weeds or crops) for control of broadleaf and grass weeds in a variety of non-crop (aquatics and total vegetation management, etc.) and crop (agronomic, fruit, and vegetable crops, and turf) environments depending on the herbicide [5]. When applied POST, bleached tissue generally appears in the meristem within 3 to 5 days after application and is followed by necrosis and plant death within a couple of weeks after application. Since these herbicides rarely provide economical, broad-spectrum weed control at rates safe to most crops they are often applied in mixtures with other herbicides to improve performance.

CBI herbicides are often applied in mixtures with herbicide inhibitors of Photosystem II (PSII) because these mixtures often result in synergistic herbicidal responses on both annual and perennial weed species [6-8]. In fact, the symptoms produced with these CBI plus PSII mixtures changes from the slow bleaching or chlorotic symptoms normally associated with most CBI or PSII inhibitor applied alone to a rapid necrosis that appears on leaves within 1 to 2 days after application. The level of synergistic herbicidal response ob-

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served with these CBI plus PSII inhibitor mixtures is often dependent on the ratio of herbicide application rates and the inherent level of sensitivity among various weeds to both the CBI and PSII inhibitors applied alone [4,6,9]. It was originally believed that this synergistic response between CBI and PSII inhibitors was mostly attributed to carotenoid inhibitors that target *p*-hydroxyphenylpyruvate dioxygenase (HPPD), whereby the inhibition of this enzyme prevents the conversion of the amino acid tyrosine to homogentisate which is further converted to either the co-factor plastoquinone or the antioxidant α-tocopherol. Plastoquinone has two roles in managing photooxidative stress by shuttling electrons away from the Qb binding niche of the D1 protein (competing with PSII inhibitors for binding to this site) while concurrently assisting the enzyme phytoene desaturase (PDS) in the conversion of phytoene to phytofluene in the steps leading up to the production of various carotenes and xanthophylls [7]. However, similar synergy was observed with PSII inhibitor mixtures with other CBI herbicides that do not inhibit the production of homogentisate and its postcursors plastoquinone and α-tocopherol [4]. Therefore, a better understanding of this synergistic response between CBIs and PSII inhibitors is needed.

One manner in which herbicidal mixtures can be evaluated to determine differences in physiological and biological response is by applying them to herbicide resistant weed biotypes in comparison to wildtype (WT) weed populations. Currently, there are only two weeds resistant to any CBI herbicides, and these weed biotypes are only resistant to inhibitors of the enzyme PDS [10]. There are three known binding sites for on the Qb binding niche of the D1 protein for PSII inhibitors: 1) site A (C1); 2) site B (C2); and 3) site A with a different binding mechanism (C3). Different chemical classes of PSII inhibitors only target one of these binding areas on the D1 protein [11]. Currently, there are 63, 21, and 3 different weed species that are resistant to herbicides that target the Qb binding site on the D1 protein at C1, C2, and C3, respectively [10]. A variety of mutations in the D1 protein of PSII can adversely impact the type of binding observed with different chemical family that inhibit PSII and therefore impact how these herbicides will perform alone, or in mixtures with herbicides with different mechanisms of action [12]. However, [13] found that mixtures of the HPPD inhibitor mesotrione applied in sequential applications or tank mixtures with atrazine provided synergistic responses in a triazine resistant velvetleaf (*Abutilon theophrasti*) biotype with an altered serine to glycine change at position 264 on the Qb binding niche of the D1 protein, but this mixture did not produce synergistic responses in an atrazine resistant velvetleaf population which metabolized atrazine as its mechanism of resistance [13]. To date, these types of studies have only involved applications of the HPPD inhibitors with certain PSII inhibitors and therefore have not evaluated the potential variations that could occur with CBI herbicides that inhibit more than one site in the carotenoid biosynthesis pathway with PSII inhibitors that target multiple sites on the Qb binding niche of the D1 protein. In addition, these types of studies have not incorporated measurements of chl fluo-

rescence and plant pigment levels to physiologically explain visual observations of synergy.

The goal of this research was to evaluate the visual response, levels of carotenoids and chls, and the variations in levels of chl fluorescence in a WT and a unique PSII resistant biotypes of common purslane (*Portulaca oleracea*) following the application of several CBIs applied alone and in mixtures with the PSII inhibitor diuron which targets C2. Since common purslane is a common weed associated with many horticultural crops it is important to fully understand the mechanisms behind resistant weed biotypes to aid in the discovery of alternative strategies for weed management [10].

MATERIALS AND METHODS

Herbicide Assays and Applications

Greenhouse and laboratory studies were conducted in 2008 at the University of Tennessee in Knoxville, TN (35.98°N lat.) to evaluate POST applications of several CBI herbicides alone and in mixtures with the PSII inhibitor diuron. The CBI herbicides chosen and their sites of action are as follows: 1) DFPC- zeta-carotene desaturase (ZDS) inhibitor; 2) norflurazon- PDS inhibitor; 3) clomazone- deoxy-D-xylulose-5-phosphate synthatase (DOXP synthase) (the active metabolite ketoclomazone is actually the active herbicide at this site of action). Herbicides rates were chosen to illicit less than 50% visual response with each individual active ingredient so that synergistic responses might be most pronounced in CBI plus PSII inhibitor mixtures [4].

Two seedling plants of two biotypes of common purslane were transplanted into individual 6.35-cm by 6.35-cm pots containing a high organic matter potting media. The WT biotype was purchased from a seed supplier (Herbiseed, Twyford, England), while a UR/TR resistant biotype was acquired from Michigan State University, whereby the mechanism of weed resistance is a serine to threonine change at position 264 on the Qb binding niche of the D1 protein which confers resistance to both triazine herbicides (targeting C1) and urea herbicides (targeting C2) [20]. Common purslane plants were not greater than 16 cm at the time of herbicide application. Herbicides were applied using a CO_2-charged backpack sprayer calibrated to deliver 215 L/ha through 4 flat fan nozzles (Teejet, Wheaton, IL).

Herbicide treatments were replicated three times and arranged in a randomized complete block design. All treatments were repeated in time. Weed control was visually rated after treatment on a scale of 0 to 100% where 0% = no injury or weed control and 100% = crop death or complete weed control. Weed control was evaluated at 5 days after treatment as this timing highlighted the most prominent differences in visual symptomology between herbicides applied alone and in mixtures. Following visual evaluations weeds were harvested and immediately analyzed for measurements of chl fluorescence and photosynthetic efficiency. After these measurements, plant samples were then stored in a -80 ºC freezer prior to analyzing tissue for carotenoid and chl pigments.

Measurements of Chlorophyll Fluorescence and Photosynthetic Efficiency

After a dark acclimation period of 60 min, whole plants of each biotype x herbicide treatment combination were measured for chl fluorescence parameters using a pulse-amplitude-modulated (PAM) fluorimeter (Photon Systems Instruments, Brno, Czech Republic). Measurements started with dark-adapted leaf tissues characterized by low, minimum fluorescence emission signal, F_0. The F_0 value is interpreted as the fluorescence signal from open PSII reaction centers with their primary quinone acceptor, Q_A, fully oxidized. The open reaction centers have the maximum photochemical quenching and the minimum fluorescence yield. At the same time, non-photochemical quenching (*NPQ)* is fully relaxed with the effective antenna size at its maximum. Dark-adapted leaf tissues were then exposed to strong flash of light (3,000 µmol×m^{-2}×s^{-1} for 0.1 µs) that transiently reduces plastoquinone pool and the primary quinone acceptor, Q_A. The quenching by photochemistry of the reaction centers is eliminated and the fluorescence yield reaches its maximum. *NPQ* remains relaxed because the illumination is transient and the quenching mechanisms cannot respond on such a short time-scale. The effective antenna size thus remains at its maximum. The measured fluorescence signal, F_M, is now at its maximum. This F_M signal is often called maximum fluorescence (yield). The variable fluorescence, F_V, signal defined as the difference $F_M - F_0$ combined with the maximum fluorescence, F_M, are used to calculate the maximum quantum yield of PSIII photochemistry F_V/F_M (or *QYmax*). The dark relaxation following the saturating flash leads to a re-oxidation of the plastoquinone pool and of the primary quinone acceptor, Q_A. The decline of fluorescence signal from the F_M level back to F_0 dark-adapted level reflects kinetics at which the reaction centers re-open. The computer program decreased the light intensity of a series of saturating flashes to eliminate transiently photochemical quenching by reducing the plastoquinone pool and the Q_A acceptor. The fluorescence signal reached local maxima, which reveals the dynamics of *NPQ* during light adaptation. The value of *NPQ* is quantified by $F_M - F_M\_Ln/F_M$ [14]. All value presented for F_V/F_M, *NPQ*, and Q_P are presented as a percent reduction in comparison to the untreated check for each weed biotype.

Carotenoid Tissue Extraction and HPLC Analysis for Plant Pigments

Leaf tissues were lyophilized for 96 h (model 12 L Free-Zone; LabConCo, Kansas City, MO) and stored at –80 ºC prior to extraction and analysis. Pigments were extracted from freeze-dried tissues according to Kopsell *et al*. [15] and analyzed according to Emenhiser *et al*. [16]. A 0.1 g tissue subsample was re-hydrated with 0.8 mL of ultra pure H$_2$O and placed in a water bath set at 40 °C for 20 min. After incubation, 0.8 mL of the internal standard ethyl-β-8'-apo-carotenoate (Sigma Chemical Co., St. Louis, MO) was added to determine extraction efficiency. The addition of 2.5 mL of tetrahydrofuran (THF) was performed after sample hydration. The sample was then homogenized in a Potter-Elvehjem (Kontes, Vineland, NJ) tissue grinding tube using

~20 insertions with a pestle attached to a drill press (Sears, Roebuck and Co., Hoffman Estates, IL) set at 540 rpm. During homogenation, the tube was immersed in ice to dissipate heat. The tube was then placed into a clinical centrifuge for 3 min at 500 g_n. The supernatant was removed and the sample pellet was re-suspended in 2 mL THF and homogenized again with the same extraction technique. The procedure was repeated for a total of four extractions to obtain a colorless supernatant. The combined supernatants were reduced to 0.5 mL under a stream of nitrogen gas (N-EVAP 111; Organomation Inc., Berlin, MA), and brought up to a final volume of 5 mL with methanol (MeOH). A 2 mL aliquot was filtered through a 0.2-µm polytetrafluoroethylene (PTFE) filter (Model Econofilter PTFE 25/20, Agilent Technologies, Wilmington, DE) using a 5-mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) prior to high-performance liquid chromatography (HPLC) analysis.

An Agilent 1200 series HPLC unit with a photodiode array detector (Agilent Technologies, Wilmington, DE) was used for pigment separation. Chromatographic separations were achieved using an analytical scale (4.6 mm i.d. x 250 mm) 5 µm, 200 Å polymeric C$_{30}$ reverse-phase column (ProntoSIL, MAC-MOD Analytical Inc., Chadds Ford, PA), which allowed for effective separation of chemically similar pigment compounds. The column was equipped with a guard cartridge (4.0 mm i.d. x 10 mm) (ProntoSIL), and was maintained at 30 ºC using a thermostatted column compartment (Agilent Technologies, Wilmington, DE). All separations were achieved isocratically using a binary mobile phase of 11% methyl *tert*-butyl ethanol (MTBE), 88.9% MeOH, with 0.1% triethylamine (TEA) (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 prior to the next injection. Eluted compounds from a 10 µL injection were detected at 453 nm (carotenoids, chl *b*, and internal standard) and 652 nm (chl *a*) and data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies, Wilmington, DE). Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards (antheraxanthin, β-carotene, chl *a*, chl *b*, lutein, neoxanthin, violaxanthin, zeaxanthin) (ChromaDex Inc., Irvine, CA). Spinach standard reference material (Slurried Spinach 2385, National Institute of Science and Technology, Gaithersburg, MD) was used for method validation. Due to the small amount of tissue generated from the purslane plants all material harvested from all three replicates of each run were combined and both runs were averaged for statistical analysis. All pigment values were calculated on a fresh weight basis.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and means were separated using Fisher's protected LSD test at the α = 0.05 significance level. When ANOVA revealed no significant study by treatment interaction, data were pooled over studies. For measurements of chlorophyll fluorescence and non-photochemical quenching mean values are also presented with standard deviations.

RESULTS

Visual Ratings and Fresh Weights

No study by treatment interaction occurred for injury ratings for the UR/TR biotype (Table **1**). Therefore, only the injury ratings for the WT population are presented by studies. All CBI herbicides provided 33 to 45% control of common purslane across all biotypes. Diuron provided 40 to 57% control of the WT biotype and similarly controlled the UR/TR biotype 48%. Mixtures of all CBI herbicides with diuron provided 42 to 63% control of the WT biotype, but only 43 to 44% control of the UR/TR biotype.

Carotenoid and Chlorophyll Content

Zeaxanthin levels were sharply elevated in CBI treatments applied alone to both common purslane biotypes (Table **2**). β-carotene was reduced in both biotypes following herbicide application in comparison to the untreated check. Neoxanthin, antheraxanthin, and lutein were generally increased or remained similar to the untreated controls in the herbicide treated UR/TR biotype, while levels of these carotenoids tended to decrease in the herbicide treated WT population. Diuron alone increased neoxanthin, antheraxanthin, lutein, and zeaxanthin by 4 to 200% in the UR/TR biotype, but decreased these same carotenoids 25 to 62% in the WT population. CBI herbicides applied in mixtures with diuron decreased amounts of β-carotene by 48 to 84%. In general, most mixtures of CBI herbicides plus diuron increased antheraxanthin and zeaxanthin in the UR/TR biotypes, but decreased these same carotenoids in the WT population when compared to the untreated check.

Most CBIs applied alone and in mixtures with diuron decreased the amount of chl *a* and chl *b* in the WT population in comparison to the untreated check (Table **3**). Furthermore, the ZDS inhibitor DFPC decreased chl *a* and chl *b* in both biotypes by 33 to 100%. Diuron decreased chl *a* and chl *b* in the WT population by 56 to 70%.

Measurements of Chlorophyll Fluorescence and Photosynthetic Efficiency

NPQ values decreased by 138 to 531% in the herbicide treated WT and UR/TR biotypes when compared to the untreated check (Table **4**). Measurements of Q_P severely decreased in CBI plus diuron treatments when compared to the untreated check for both biotype populations. Decreases in Q_P were greater in the WT population than the UT/TR biotypes. These decreases in Q_P appeared to coincide most frequently with those treatments that caused the most visual injury to common purslane (Table **1**, **4**). F_V/F_M values were decrease 11 to 97% regardless of herbicide treatment in both the WT and UR/TR biotypes (Table **4**).

DISCUSSION

In general, the visual injury observed from these CBI and diuron applied alone or in mixtures did not always provide obvious differences in visual symptom between the WT and UR/TR biotypes (Table **1**). However, the manner in which each biotype responded to the photooxidative stress caused by these herbicide inhibitors was to produce different levels of various plants pigments [17-19]. Previous research with this UR/TR biotype uncovered decreases in fresh weight, F_V/F_M, and CO_2 assimilation following applications of both

Table 1. **Percent Injury for Two Common Purslane (*Portulaca oleracea*) Biotypes [wildtype (WT) and Urea/Triazine-resistant (UR/TR)] Treated with Mixtures of Carotenoid Biosynthesis Inhibitors Alone and in Mixtures with the Photosystem II Inhibitor Diuron at 5 Days After Treatment**

Herbicide Treatment[b]		Injury		
		WT		UR/TR [a]
	Rate	Study 1	Study 2	Study 1 + 2
	g ai/ha	——————————— % ———————————		
Norflurazon	500	37	40	42
DFPC	62	37	33	37
Clomazone	125	35	33	45
Diuron	448	40	57	48
Norflurazon + diuron	500 + 448	63	42	43
DFPC + diuron	62 + 448	57	48	43
Clomazone + diuron	125 + 448	43	47	44
Untreated check[c]	—	0	0	0
LSD$_{0.05}$		16	13	8

[a] No study by treatment interaction occurred for percent control visual ratings for the UR/TR common purslane populations, therefore these data were pooled over studies.
[b] All treatments included 1% (v/v) crop oil concentrate.
[c] The untreated check was not included in the statistical analysis.

Table 2. Mean Values ± Standard Variation of Key Carotenoids for Two Common Purslane (*Portulaca oleracea*) Biotypes [wild-type (WT) and Urea/Triazine-resistant (UR/TR)] Treated with Mixtures of Carotenoid Biosynthesis Inhibitors Alone and in Mixtures with the Photosystem II Inhibitor Diuron at 5 Days After Treatment

Herbicide [b]	Carotenoids (mg/100 g Fresh weight)[a]											
	Violaxanthin		Neoxanthin		Antheraxanthin		Lutein		Zeaxanthin		β-carotene	
	WT	UR/TR	WT	UR/TR	WT	UR/TR	WT	UR/TR	WT	UR/TR	WT	UR/TR
Norflurazon	0.42 ± 0.07	0.71 ± 0.21	1.19 ± 0.30	1.28 ± 0.31	0.83 ± 0.13	1.25 ± 0.01	4.23 ± 1.02	4.65 ± 0.95	0.35 ± 0.24	0.61 ± 0.19	1.69 ± 0.35	1.75 ± 0.31
DFPC	0.86 ± 0.08	0.86 ± 0.08	nd	nd	0.83 ± 0.07	0.90 ± 0.04	4.96 ± 0.56	3.16 ± 0.74	0.75 ± 0.80	1.64 ± 0.16	2.63 ± 0.52	1.04 ± 0.44
Clomazone	1.34 ± 0.58	0.59 ± 0.21	2.13 ± 0.23	1.13 ± 0.22	1.27 ± 0.32	0.58 ± 0.22	6.98 ± 0.63	3.51 ± 0.56	0.12 ± 0.04	0.08 ± 0.01	3.94 ± 0.41	1.82 ± 0.41
Diuron	0.49 ± 0.22	0.54 ± 0.12	1.01 ± 0.30	1.93 ± 0.07	0.64 ± 0.35	1.13 ± 0.14	3.38 ± 1.14	6.31 ± 0.04	0.06 ± 0.01	0.15 ± 0.02	1.03 ± 0.32	2.03 ± 0.01
Norflurazon + diuron	1.15 ± 0.56	1.18 ± 0.19	1.16 ± 0.49	1.16 ± 0.24	0.71 ± 0.39	0.69 ± 0.29	3.54 ±1.30	3.58 ± 0.58	0.10 ± 0.02	0.18 ± 0.04	1.51 ± 0.71	1.40 ± 0.19
DFPC + diuron	0.67 ± 0.30	2.19 ± 0.00	0.37 ± 0.11	nd	0.28 ± 0.13	2.10 ± 0.00	2.35 ± 0.88	8.38 ± 0.00	0.07 ± 0.02	0.13 ± 0.00	0.63 ± 0.23	1.02 ± 0.00
Clomazone + diuron	0.60 ± 0.20	1.17 ± 0.04	0.41 ± 0.22	0.94 ± 0.01	0.36 ± 0.17	1.15 ± 0.01	2.45 ± 0.90	4.74 ± 0.01	0.07 ± 0.00	0.13 ± 0.01	0.80 ± 0.27	1.44 ± 0.08
Untreated check	1.21 ± 1.30	1.45 ± 1.20	1.75 ± 0.02	1.24 ± 1.10	1.71 ± 0.21	1.09 ± 1.16	8.46 ± 0.71	5.61 ± 4.46	0.08 ± 0.02	0.05 ± 0.00	4.10 ± 0.01	2.75 ± 1.95
LSD$_{0.05}$	1.30	1.20	0.65	1.19	0.57	1.16	2.13	4.44	0.80	0.28	0.92	1.97

[a] Plants from all three repetitions of each common purslane biotype were pooled together from study 1 and study 2 for analysis of carotenoid content. nd = below detection limits.
[b] Rate, g ai/ha: Norflurazon, 500; DFPC, 62; Clomazone, 125; Diuron, 448; Norflurazon + diuron, 500 + 448; DFPC + diuron, 62 + 448; Clomazon + diuron, 125 + 448. All treatments included 1% (v/v) crop oil concentrate.

atrazine and linuron (another substituted urea herbicide similar to diuron and this injury dissipated by 2 weeks after treatment) [20]. However, these researches did not report how carotenoid content varied during that period of time after PSII inhibitor application. Concentrations of violaxanthin, neoxanthin, antheraxanthin, lutein, β-carotene, and zeaxanthin often varied between the two biotypes depending on the applied composition of herbicides in our studies (Table **2**). In general, CBI applications increased concentrations of neoxanthin, antheraxanthin, and lutein in the UR/TR biotype, while all of these pigments were generally decreased in the WT population with most CBI applications. In addition, zeaxanthin was increased, while β-carotene was decreased by most CBI applications in both weed biotypes. Violaxanthin tended to increase in the WT population and decrease in the UR/TR biotype following most CBI applications.

Other researchers have reported on how differential carotenoid content impacts photoprotection and plant develop-ment. The xanthophyll cycle pigments antheraxanthin, violaxanthin, and zeaxanthin play an important role in photoprotection, light harvesting, and the assembly of light harvesting antenna complexes (LHC) [21]. The xanthophyll cycle pigments are integral in allowing plants to fluctuate between light harvesting complexes under low light environments, while affording the flexibility to convert to dissipative activities in high light environments [22]. In addition, LHC of photosynthesis are primarily composed of conserved concentrations of neoxanthin, violaxanthin, and lutein, whereby each xanthophyll plays a unique functional role [19, 21]. However, either by conversion from violaxanthin and antheraxanthin through violaxanthin de-epoxidase, or by conversion from β-carotene, zeaxanthin may substitute for violaxanthin and lutein and thereby maintain the integrity of the D1 protein during periods of stress [23,24]. However, in our studies the treatments that produced the most visual damage to purslane generally created higher concentrations

Table 3. Mean values ± standard deviation for chlorophyll *a* (chl *a*) and *b* (chl *b*) for two common purslane (*Portulaca oleracea*) biotypes [wildtype (WT) and urea/triazine-resistant (UR/TR)] with mixtures of carotenoid biosynthesis inhibitors alone and in mixtures with the Photosystem II inhibitor diuron at 5 days after treatment.

Herbicide Treatment[b]	Chlorophyll (mg/100 g Fresh Weight)[a]			
	Chl *a*		Chl *b*	
	WT	UR/TR	WT	UR/TR
Norflurazon	55.24 ± 12.50	50.85 ± 13.05	15.22 ± 3.07	21.08 ± 4.47
DFPC	2.94 ± 1.81	nd	19.93 ± 1.47	6.42 ± 7.78
Clomazone	90.32 ± 8.17	35.13 ± 4.36	26.62 ± 2.98	12.83 ± 1.26
Diuron	25.98 ± 11.35	50.93 ± 4.47	13.08 ± 4.99	22.52 ± 1.79
Norflurazon + diuron	33.44 ± 15.36	28.74 ± 2.16	13.19 ± 5.42	12.29 ± 1.72
DFPC + diuron	19.46 ± 8.60	63.35 ± 0.00	9.23 ± 3.71	39.67 ± 0.00
Clomazone + diuron	20.16 ± 8.78	32.98 ± 1.57	8.57 ± 3.39	15.13 ± 0.46
Untreated check	87.45 ± 4.94	54.82 ± 48.76	29.80 ± 1.75	20.23 ± 17.00
$LSD_{0.05}$	22.57	54.39	8.28	18.42

[a] Plants from all three repetitions of each common purslane biotype were pooled together from study 1 and study 2 for analysis of carotenoid content. nd = below detection limits.
[b] Rate, g ai/ha: Norflurazon, 500; DFPC, 62; Clomazone, 125; Diuron, 448; Norfluszon + diuron, 500 + 448; DFPC + diuron, 62 + 448; Clomazon + diuron, 125 + 448.
All treatments included 1% (v/v) crop oil concentrate.

of antheraxanthin, lutein, and neoxanthin in the UR/TR biotype, while decreasing these two pigments in the WT population (Table **2**). Other researchers have demonstrated that lutein can also substitute for zeaxanthin in order to restore the functionality of non-photochemical quenching in plants [25]. In general, plant pigments are in a constant state of flux responding to both abiotic and biotic stressors alike and no one pigment configuration is always favored among individual biotypes of various species in response to the same stressors.

Differences in plant response to stressors are often best observed when comparing mutants or resistant weed biotypes to the WT. For example, a serine to threonine mutation at site 264 on the Qb binding niche of the D1 protein in potato cells led to no reductions in photosynthetic electron transport or n-cell growth penalties when compared to the WT [26]. However, a serine to glycine mutation at the same site caused limited Q_P and *NPQ* and a hypersensitivity to photoinhibition [27]. This hypersensitivity was likely due to a greater concentration of Qb-non-reducing (inactive) binding centers that are produced in this mutant when compared to the WT that led to reductions in electron transport [28]. While this mutation may provide tolerance to herbicides which would normally kill the WT population, the mutation may also provide fitness penalties that are not desirable in overcoming other plant stressors [29]. However, some mutations in the D1 protein may offer some advantages that are not always obvious. For example, Narusaka *et al.* [30] theorized that certain mutations in Qb may increase or decrease hydrophilic interactions on the D1 protein thereby initiating a redox signaling through protein phosphorylation which triggers gene expression and facilitates the retention of certain pigment combinations in the LHC. In our studies, applications of the PSII inhibiting herbicide diuron to the UR/TR

biotype with a serine to threonine mutation increased neoxanthin, antheraxanthin, zeaxanthin, and lutein concentrations by 82 to 258% when compared to the untreated check (Table **2**). These xanthophyll cycle pigments, especially zeaxanthin, are directly involved in energy dissipation.

CONCLUSIONS

Harsh impacts of environmental stressors and applications of certain herbicides which inhibit photosynthesis and/or carotenoid biosynthesis can reduce photosynthetic rates and increase the need for increased energy dissipation *via* xanthophyll cycle pigments [31]. Therefore, it may be possible that mutations to the Qb binding niche of the D1 protein may result in the retention of optimal pigment combinations that would improve plant stress tolerance, and ultimately ensure survival for reproduction. These findings demonstrate that the selection of herbicide resistant weed biotypes might not only limit weed control options for growers, but may allow for easier proliferation of weed biotypes that are better able to deal with environmentally stressful conditions that would normally limit the competitiveness of their wildtype relatives in similar crop environments.

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Table 4.   **Chlorophyll fluorescence parameters ± standard deviation for two common purslane (*Portulaca oleracea*) biotypes [wild-type (WT) and urea/triazine-resistant (UR/TR)] treated with mixtures of carotenoid biosynthesis inhibitors alone and in mixtures with the Photosystem II inhibitor diuron at 5 days after treatment.**

	Chlorophyll fluorescence parameters[a]							
	F_V/F_M			NPQ		Q_P		
	WT	UR/TR		WT	UR/TR	WT	UR/TR	
Herbicide treatment[b]	Study 1+2	Study 1	Study 2	Study 1+2	Study 1+2	Study 1+2	Study 1	Study 2
Norflurazon	0.351 ± 0.131	0.528 ± 0.068	0.487 ± 0.043	0.041 ± 0.006	0.041 ± 0.040	0.548 ± 0.068	0.668 ± 0.048	0.547 ± 0.043
DFPC	0.687 ± 0.042	0.667 ± 0.032	0.557 ± 0.098	0.069 ± 0.031	0.055 ± 0.025	0.739 ± 0.049	0.608 ± 0.103	0.603 ± 0.041
Clomazone	0.592 ± 0.058	0.550 ± 0.033	0.553 ± 0.099	0.006 ± 0.009	0.035 ± 0.034	0.710 ± 0.049	0.635 ± 0.063	0.638 ± 0.033
Diuron	0.043 ± 0.019	0.088 ± 0.015	0.098 ± 0.037	0.010 ± 0.003	0.010 ± 0.000	-0.061 ± 0.096	0.110 ± 0.056	0.138 ± 0.028
Norflurazon + diuron	0.036 ± 0.019	0.070 ± 0.005	0.136 ± 0.012	0.011 ± 0.002	0.011 ± 0.002	-0.118 ± 0.097	0.112 ± 0.089	0.208 ± 0.012
DFPC + diuron	0.025 ± 0.015	0.098 ± 0.008	0.092 ± 0.038	0.010 ± 0.000	0.010 ± 0.000	-0.415 ± 0.546	0.165 ± 0.085	0.170 ± 0.030
Clomazone + diuron	0.028 ± 0.019	0.065 ± 0.018	0.197 ± 0.121	0.010 ± 0.000	0.014 ± 0.006	-0.237 ± 0.240	0.075 ± 0.056	0.285 ± 0.103
Untreated check	0.768 ± 0.008	0.757 ± 0.003	0.750 ± 0.005	-0.016 ± 0.037	-0.023 ± 0.011	0.747 ± 0.071	0.628 ± 0.006	0.653 ± 0.013
$LSD_{0.05}$	0.068	0.053	0.120	0.018	0.026	0.252	0.120	0.080

[a] Plants from all three repetitions of each common purslane biotype were pooled together from study 1 and study 2 for analysis of carotenoid content.
[b] Rate, g ai/ha: Norflurazon, 500; DFPC, 62; Clomazone, 125; Diuron, 448; Norflurazon + diuron, 500 + 448; DFPC + diuron, 62 + 448; Clomazon + diuron, 125 + 448.
All treatments included 1% (v/v) crop oil concentrate.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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