Nutritionally Important Pigments in Purslane (*Portulaca oleracea*) Differ between Cultivars and in Response to Nitrogen

Dean A. Kopsell^{1,3}, Kimberly J. Whitlock², and Carl E. Sams¹

Department of Plant Sciences, The University of Tennessee, Knoxville, TN 37996

David E. Kopsell¹

Department of Agriculture, Illinois State University, Normal, IL 61790

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Abstract. Purslane (*Portulaca oleracea*) is a succulent weedy annual in much of the United States. In other parts of the world, purslane is grown as a specialty crop, valued for its nutritional quality. As a leafy crop, purslane contributes carotenoid phytochemicals in the typical Mediterranean diet. Nitrogen (N) influences plant growth and alters pigment composition and accumulation. However, little is known about the impact N fertility may have on pigment concentrations in purslane shoot tissues. The objective of this study was to evaluate the influence of N fertility levels on biomass and concentrations of nutritionally important carotenoid and chlorophyll pigments in purslane. Green Leaf and Golden Leaf purslane cultivars were grown in nutrient solution culture under N concentrations of 13, 26, 52, or 105 mg·L⁻¹. Plants were harvested at 45 days after planting (DAP), and measured for concentrations of shoot pigments using high-performance liquid chromatography (HPLC) methodology. There was no influence of N treatment concentration on purslane shoot tissue fresh weight (FW) accumulation. Nitrogen treatment significantly influenced shoot tissue β -carotene (BC), lutein (LUT), neoxanthin (NEO), total carotenoids, chlorophyll *a*, chlorophyll *b*, total chlorophyll, and the chlorophyll *a* to *b* ratio in purslane shoot tissues. Concentrations of LUT, NEO, violaxanthin (VIO), chlorophyll *b*, total xanthophyll cycle pigments, and the chlorophyll *a* to *b* ratio differed between the purslane cultivars. Increases in N concentrations acted to increase concentrations of nutritionally important shoot tissue carotenoid pigments in only the Green Leaf purslane cultivar. Therefore, N fertility management and cultivar selection should be considered when producing purslane as a nutritious specialty vegetable crop.

Purslane (*P. oleracea*) is a problematic succulent weedy annual in horticultural crop production in the United States (Armél et al., 2012). However, in other parts of the world purslane is grown as a specialty crop, valued for its nutritional quality. Regular consumption of a variety of specialty crops provides the human diet with essential vitamins and minerals, in addition to nutritionally important phytochemicals. Unique phytochemicals within these crops actively participate in chronic disease reductions, and can provide benefits beyond normal health maintenance (Shahidi, 2004). One such important group of phytochemicals is the tetraterpene carotenoids which function as accessory pigments in photosynthesis and prevent photooxidation in

photosynthetic organisms (DellaPenna, 1999; Gross, 1991). Key roles of carotenoids in human nutrition are as vitamin A precursors and as macular pigment which protects against aging eye diseases (Combs, 1998; Johnson et al., 2000). Along with high concentrations of carotenoids, leafy specialty crops also contain high chlorophyll pigment concentrations (Ferruzzi and Blakeslee, 2007; Kopsell et al., 2004). Dietary chlorophyll may be nutritionally important in the reduction of intestinal absorption of potentially harmful chemical mutagens and carcinogens (Ferruzzi and Blakeslee, 2007; Natsume et al., 2004).

The effects of N supply on plant growth are well understood, and most economically important specialty crops have recommended rates for optimal yield. Nitrogen can alter plant composition more than any other essential element, and N is often the most limiting nutrient affecting growth in cropping systems (Barker and Mills, 1980; Hawkesford et al., 2012). Nitrogen can also influence phytochemical quality factors, and recent studies demonstrate that N can alter carotenoid and chlorophyll composition and accumulation in leafy specialty crops (Chenard et al., 2005; Kopsell et al., 2007;

Lefsrud et al., 2007). Purslane contributes nutritionally important phytochemicals, such as omega-3 fatty acids, organic acids, and various flavonoids, in the typical Mediterranean diet (Graça Dias et al., 2009; Su et al., 2002). Purslane can also accumulate high concentrations of carotenoid and chlorophyll pigments (Armél et al., 2012; Graça Dias et al., 2009; Su et al., 2002). However, little is known about the impact N fertility may have on concentrations of nutritionally important pigments in purslane shoot tissues. The objective of this study was to measure impacts of N fertility levels on carotenoid and chlorophyll pigment concentrations between two distinct purslane cultivars.

Materials and Methods

Purslane culture and harvest. Seeds of 'Golden Leaf' and 'Green Leaf' purslane (Richters, Ontario, Canada) were sown into 2.5 × 2.5 cm rockwool growing cubes (Grodan A/S, Hedehusene, Denmark). The first experimental run was initiated on 15 May 2011, and a second experimental run was started on 26 June 2011. Seeds were germinated and greenhouse grown at 29 °C/day and 22 °C/night under natural photoperiods at the University of Tennessee, Knoxville, TN (35°58'N lat.). After the emergence of the first true leaves ≈10 DAP, a 20N–4.4P–16.6K water-soluble fertilizer (Peter's 20–20–20; Scotts, Marysville, OH) was applied every 3 d at a rate of 100 mg·L⁻¹ N. Plantlets were transferred to containers (Rubbermaid Inc., Wooster, OH) filled with 10 L of a modified nutrient solution 17 DAP. Elemental concentrations of the nutrient solutions were (mg·L⁻¹): potassium (117.3), calcium (80.2), magnesium (24.6), sulfur (32.0), iron (0.5), boron (0.25), molybdenum (0.005), copper (0.01), manganese (0.25), and zinc (0.025) (Hoagland and Arnon, 1950). The nutrient solutions were aerated via plastic tubing connected to an air blower (Model VB-007S; Sweetwater, Fort Collins, CO). Plants were grown under increasing N concentrations of 13, 26, 52, and 105 mg·L⁻¹. The N form ratio was kept constant at 1 NH₄-N : 3 NO₃-N. Phosphorus level varied slightly with the N rate, changing from 91, 89, 86, and 80 mg·L⁻¹ for the N treatment concentrations of 13, 26, 52, and 105 mg·L⁻¹, respectively. Deionized (DI) water was added daily to maintain initial solution volumes in each container. The experimental design was a randomized complete block in a factorial arrangement of two cultivars by four N treatment concentrations. Containers acted as experimental units and each contained six individual plants of a single cultivar. Each cultivar/N treatment combination was replicated four times, and solutions were replaced 31 DAP to refresh the solution to the initial nutrient concentrations. Plants were harvested 45 DAP in both experimental repeats and tissues were prepared for pigment analyses. At harvest, shoot and root tissue were separated, six plants in each cultivar/N treatment combination were bulked, and FW was determined. Plant tissues were washed

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¹Professor.

²Undergraduate Research Assistant.

³Corresponding author. E-mail: dkopsell@utk.edu.

with a phosphate-free liquid detergent with neutral pH (Aquet; Bel-art Products, Pequannock, NJ), and were triple-rinsed with DI water. Shoot tissues were equally divided into two groups. One shoot tissue group and one root tissue group was placed in paper bags, and dried at 60 °C for no less than 72 h, at which time dry weight (DW) was calculated, whereas the other shoot tissue group was stored at -80 °C before lyophilization.

Purslane shoot tissue pigment extraction. Plant tissues were freeze-dried (model 6 L FreeZone; LabConCo, Kansas City, MO) at a constant temperature of -20 °C before extraction. Pigments were extracted from freeze-dried tissues and analyzed according to Kopsell et al. (2012). A 0.1-g tissue subsample was rehydrated with 0.8 mL of ultrapure H₂O for 20 min. After incubation, 0.8 mL of the internal standard ethyl-β-8'-apo-carotenolate (ChromaDex, Irvine, CA) 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 tissue grinding tube (Potter-Elvehjem; Kimble Chase-Kontes Glass, Vineland, NJ) using ≈25 insertions with a pestle attached to a drill press set at 540 rpm. During homogenization, the tube was immersed in ice to dissipate heat. The tube was then centrifuged for 5 min at 500 g_n. The supernatant was removed, the sample pellet was resuspended 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 N gas (N-EVAP 111; Organomation, Berlin, MA), and brought up to a final volume of 5 mL with acetone to dissolve all pigments. A 2-mL aliquot was filtered through a 0.2-μm polytetrafluoroethylene (PTFE) filter (Econofilter PTFE 25/20; Agilent Technologies, Santa Clara, CA) before HPLC analysis.

Purslane shoot tissue pigment HPLC analysis. An HPLC unit with a photodiode array detector (1200 series; Agilent Technologies) was used for purslane pigment separation. Chromatographic separations were achieved using an analytical scale (4.6 i.d. × 250 mm) 5-μm, 200-Å polymeric RP-C₃₀ column (ProntoSIL; MAC-MOD Analytical, Chadds Ford, PA). The column was equipped with a 5-μm guard cartridge (4.0 i.d. × 10 mm) and holder (ProntoSIL), and was maintained at 30 °C using a thermostatted column compartment. All separations were achieved isocratically using a mobile phase of 11% methyl tert-butyl ether, 88.99% methanol, and 0.01% triethylamine (by volume). The flow rate was 1.0 mL·min⁻¹, with a run time of 58 min. Eluted compounds from a 10-μL injection were detected at 453 (carotenoids and internal standard), 652 (chlorophyll *a*), and 665 (chlorophyll *b*) nm; and data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection

using external standards [anthraxanthin (ANT), BC, Chlorophyll *a*, Chlorophyll *b*, LUT, NEO, VIO, and zeaxanthin (ZEA) from ChromaDex, Irvine, CA].

Statistical analyses. Data sets were analyzed using the General Linear Model procedure in SAS (version 9.2; SAS Institute, Cary, NC). The relationship between experimental dependent variables and N treatments were determined by regression analysis using the Regression procedure. Significant treatment by experimental run interactions were not detected in any of the biomass or phytochemical data. Thus, data from both experimental runs were combined and analyzed together.

Results and Discussion

Purslane shoot tissue FW and DW were not influenced by the main effects of N treatment concentration or purslane cultivar. Purslane root tissue FW was not influenced by N treatment concentration, but root FW varied by purslane cultivar ($P = 0.001$). Purslane root tissue DW was not influenced by N treatment concentration, but root DW was impacted by differences in purslane cultivar ($P = 0.006$; Table 1). None of the biomass parameters were impacted by the interaction of N treatment concentration and purslane cultivar. As the concentration of N in the nutrient solution was increased, linear increases were observed in 'Green Leaf' shoot tissue FW [grams per plant = $85.6 + 26.2$ (N trt), $r^2 = 0.15$, $P = 0.029$; Table 1].

Previously, kale (*Brassica oleracea* var. *acephala*) cultivars of Winterbor, Toscano, and Redbor demonstrated linear increases in shoot FW and DW and root FW and DW as N concentrations increased in nutrient solution culture from 6 to 105 mg·L⁻¹ (Kopsell et al., 2007). Moreover, Lefsrud et al. (2007) showed shoot tissue FW and DW in 'Springer F1' and 'Melody' spinach (*Spinacia oleracea*) to also increase in a linear manner in response to increases in N concentrations from 13 to 105 mg·L⁻¹ in nutrient solution culture. Chenard et al. (2005) showed that shoot tissue FW and DW for parsley (*Petroselinum crispum*) increased linearly in response to increases in N from 6 to 105 mg·L⁻¹ in nutrient solution culture. There are several possible reasons why increases in N treatment concentrations did not influence biomass accumulation in the purslane cultivars in this experiment as compared with previous studies, the first being plant biomass at harvest date. Kopsell et al. (2007) reported average biomass values for kale to range from 112 g per plant under 13 mg·L⁻¹ N concentrations to 250 g per plant under 105 mg·L⁻¹ N over 8 weeks; Lefsrud et al. (2007) reported average biomass values for spinach to range from 80 g per plant under 13 mg·L⁻¹ N concentrations to 350 g per plant under 105 mg·L⁻¹ N over 7 weeks; whereas Chenard et al. (2005) reported biomass values for parsley to range from 80 g per plant under 13 mg·L⁻¹ N concentrations to over 500 g per plant under 105 mg·L⁻¹ N over 11 weeks. Purslane in the current experiment was harvested 45 DAP. Plants displayed an upright

Table 1. Mean FW and DW for shoot and root tissues for 'Golden Leaf' and 'Green Leaf' purslane (*Portulaca oleracea*) grown under increasing N concentrations in nutrient solution culture.^z

N (mg·L ⁻¹)	Purslane biomass			
	Shoot tissue		Root tissue	
	FW	DW	FW	DW
(g/plant)				
'Golden Leaf' purslane				
13	23.68	1.04	0.79	0.06
26	27.94	1.03	0.80	0.05
52	25.11	1.09	0.83	0.05
105	30.14	0.99	0.65	0.05
Regression ^y				
Linear	NS	NS	NS	NS
'Green Leaf' purslane				
13	17.94	0.93	1.09	0.08
26	24.21	1.09	1.24	0.08
52	26.98	1.14	1.31	0.09
105	31.56	1.22	1.44	0.09
Regression ^y				
Linear	$P = 0.029$	NS	NS	NS

NS = nonsignificant; FW = fresh weight; DW = dry weight; N = nitrogen.

^zValues represent means for two complete experimental repeats, eight replications of composite shoot tissue samples for each purslane cultivar. SE estimates for 'Golden Leaf': shoot FW (3.63), shoot DW (0.16), root FW (0.10), and root DW (0.01). SE estimates for 'Green Leaf': shoot FW (4.38), shoot DW (0.21), root FW (0.27), and root DW (0.02).

^ySignificance for linear regression over N fertility rates (equations can be found in the text).

growth habit, with limited lateral branching and leaf area. Production cycle timing and growth habit may have reduced the need for N in purslane growth and development and could be the reason that N did not impact biomass accumulation. This information may be helpful in the design of fertility programs for edible purslane production.

Accumulation of chlorophyll *a* ($P \leq 0.001$), chlorophyll *b* ($P \leq 0.001$), total chlorophyll ($P \leq 0.001$), and the chlorophyll *a* to *b* ratio ($P = 0.02$) in purslane shoot tissues varied according to N treatment concentration. Only chlorophyll *b* ($P \leq 0.001$) and the chlorophyll *a* to *b* ratio ($P \leq 0.001$) in purslane shoot tissues were influenced by differences in purslane cultivar. Chlorophyll *a* [mg·100 g⁻¹ FW = $87.8 + 7.0$ (N trt), $r^2 = 0.13$, $P = 0.044$] and total chlorophyll [mg·100 g⁻¹ FW = $117.4 + 8.6$ (N trt), $r^2 = 0.13$, $P = 0.04$] increased linearly in response to increasing N treatment concentrations for 'Golden Leaf' purslane (Table 2). Increasing N treatment concentrations resulted in linear increases in chlorophyll *a* [mg·100 g⁻¹ FW = $39.8 + 23.4$ (N trt), $r^2 = 0.33$, $P \leq 0.001$], chlorophyll *b* [mg·100 g⁻¹ FW = $27.7 + 4.8$ (N trt), $r^2 = 0.34$, $P \leq 0.001$], total chlorophyll [mg·100 g⁻¹ FW = $67.4 + 28.2$ (N trt), $r^2 = 0.33$, $P \leq 0.001$], and the chlorophyll *a* to *b* ratio [mg·100 g⁻¹ FW = $1.6 + 0.3$ (N trt), $r^2 = 0.27$, $P = 0.002$] in 'Green Leaf' purslane shoot tissues (Table 2). Values measured for chlorophyll pigments within 'Green Leaf' and 'Golden Leaf' purslane were similar to data reported for weedy common purslane (*P. oleracea*) (Armel et al., 2012).

Data are mounting on the role of dietary chlorophyll in the protection against harmful chemical mutagens and carcinogens (Ferruzzi and Blakeslee, 2007; Natsume et al., 2004). Therefore, additions of chlorophyll in diets may be nutritionally beneficial. Increasing N treatment concentrations acted to increase concentrations of valuable chlorophyll in both purslane cultivars in the current experiment. Increases were most dramatic for the Green Leaf cultivar (Table 2). Previously, Kopsell et al. (2007) demonstrated that increasing N concentrations from 6 to 105 mg·L⁻¹ in nutrient solution culture had no impact on the accumulation of chlorophyll pigments in three kale cultivars. Lefsrud et al. (2007) reported that increases in N concentrations in hydroponic production significantly increased chlorophyll pigments in only one of two spinach cultivars. Increasing N concentrations in nutrient solution culture caused significant increases in shoot tissue chlorophyll concentrations for 'Dark Green Italian' parsley (Chenard et al., 2005). Ammonia-N assimilation, the enzymatic activity of glutamine synthase isozymes (GS1 and GS2) and other intracellular transporters are linked with the chlorophyll biosynthesis pathway (Chatterjee and Kunda, 2015). Nitrogen is also a structural component of the chlorophyll molecule. Higher N treatment concentrations may have resulted in greater N uptake and translocation for chlorophyll biosynthesis within the purslane cultivars. High chlorophyll concentrations would improve the nutritional functionality of purslane.

Accumulation of BC ($P = 0.01$), LUT ($P = 0.003$), NEO ($P = 0.002$), and total carotenoid pigments ($P = 0.006$) in purslane shoot tissues varied according to N treatment concentration. Shoot tissue concentrations of LUT ($P \leq 0.001$), NEO ($P \leq 0.001$), total xanthophyll cycle pigments [ZEA + ANT + VIO (ZAV)] ($P = 0.008$), and the ratio of ZEA + ANT/ZEA + ANT + VIO (ZA/ZAV) ($P = 0.009$) were influenced by differences in purslane cultivar. Increases in N treatment concentrations did not impact accumulation of any carotenoid pigment within shoot tissue of 'Golden Leaf' purslane (Table 3). However, BC [mg·100 g⁻¹ FW = 4.2 + 0.7 (N trt), $r^2 = 0.26$, $P = 0.003$], LUT [mg·100 g⁻¹ FW = 7.5 + 1.2 (N trt), $r^2 = 0.30$, $P = 0.001$], NEO [mg·100 g⁻¹ FW = 2.2 + 0.4 (N trt), $r^2 = 0.31$, $P = 0.001$], and total carotenoid pigment concentration [mg·100 g⁻¹ FW = 17.1 + 2.7 (N trt), $r^2 = 0.27$, $P = 0.002$] all increased in a linear response to increases in N treatment concentrations in the 'Green Leaf' purslane (Table 3). Values measured for carotenoid pigments within 'Green Leaf' and 'Golden Leaf' purslane were similar to data reported for weedy common purslane (Armell et al., 2012); however, concentrations were higher for data previously reported for edible purslane (Graça Dias et al., 2009; Su et al., 2002).

The nutritional contributions of dietary carotenoid phytochemicals are as provitamin A compounds, photoprotection against aging eye diseases, and as antioxidants in chronic disease prevention. Purslane in the current

Table 2. Mean values for chlorophyll shoot tissue pigments in 'Golden Leaf' and 'Green Leaf' purslane (*Portulaca oleracea*) grown under increasing N concentrations in nutrient solution culture.^z

N (mg·L ⁻¹)	Purslane shoot tissue chlorophyll pigments			
	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll <i>a</i> + <i>b</i>	<i>a</i> to <i>b</i> ratio
	(mg·100 g ⁻¹ fresh wt)			
<i>'Golden Leaf' purslane</i>				
13	92.28	29.93	122.22	3.06
26	107.25	34.59	141.83	3.12
52	105.28	34.99	140.27	3.07
105	116.16	35.22	151.38	3.30
Regression ^y				
Linear	<i>P</i> = 0.044	NS	<i>P</i> = 0.040	NS
<i>'Green Leaf' purslane</i>				
13	68.18	32.81	100.99	1.97
26	86.82	38.47	125.29	2.24
52	94.34	38.57	132.92	2.42
105	143.64	48.78	192.43	2.94
Regression ^y				
Linear	<i>P</i> ≤ 0.001	<i>P</i> ≤ 0.001	<i>P</i> ≤ 0.001	<i>P</i> = 0.002

NS = nonsignificant; N = nitrogen.

^zValues represent means for two complete experimental repeats, eight replications of composite shoot tissue samples for each purslane cultivar. SE estimates for 'Golden Leaf': chlorophyll *a* (7.56), chlorophyll *b* (2.09), chlorophyll *a* + *b* (9.13), and *a* to *b* ratio (0.16). SE estimates for 'Green Leaf': chlorophyll *a* (12.19), chlorophyll *b* (2.70), chlorophyll *a* + *b* (14.48), and *a* to *b* ratio (0.21).

^ySignificance for linear regression over N fertility rates (equations can be found in the text).

Table 3. Mean values for carotenoid shoot tissue pigments in 'Golden Leaf' and 'Green Leaf' purslane (*Portulaca oleracea*) grown under increasing N concentrations in nutrient solution culture.^z

N (mg·L ⁻¹)	Purslane shoot tissue carotenoid pigments							
	BC	ZEA	LUT	ANT	NEO	VIO	Total CAR	ZA/ZAV
	(mg·100 g ⁻¹ fresh wt)							
	<i>'Golden Leaf' purslane</i>							
13	5.65	0.38	8.02	1.83	2.39	2.73	21.01	0.44
26	6.37	0.34	9.20	1.74	2.81	3.45	23.91	0.39
52	6.20	0.36	9.16	1.49	2.79	2.77	22.77	0.41
105	6.25	0.38	9.18	1.64	2.75	2.96	23.15	0.41
Regression ^y								
Linear	NS	NS	NS	NS	NS	NS	NS	NS
	<i>'Green Leaf' purslane</i>							
13	5.07	0.33	8.96	1.42	2.64	1.94	20.36	0.50
26	5.71	0.33	10.23	1.50	3.11	2.13	22.99	0.49
52	5.69	0.37	10.20	1.39	3.03	1.90	22.56	0.50
105	7.40	0.41	13.12	1.89	3.98	2.80	29.60	0.45
Regression ^y								
Linear	<i>P</i> = 0.003	NS	<i>P</i> = 0.001	NS	<i>P</i> = 0.001	NS	<i>P</i> = 0.002	NS

NS = nonsignificant; BC = β-carotene; ZEA = zeaxanthin; LUT = lutein; ANT = antheraxanthin; NEO = neoxanthin; VIO = violaxanthin; Total CAR = total integrated carotenoid pigments; ZA/ZAV = ratio of ZEA + ANT/ZEA + ANT + VIO; N = nitrogen.

^zValues represent means for two complete experimental repeats, eight replications of composite shoot tissue samples for each purslane cultivar. SE estimates for 'Golden Leaf': BC (0.38), ZEA (0.06), LUT (0.53), ANT (0.17), NEO (0.17), VIO (0.33), Total CAR (1.26), and ZA/ZAV (0.04). SE estimates for 'Green Leaf': BC (0.48), ZEA (0.05), LUT (0.78), ANT (0.15), NEO (0.24), VIO (0.34), Total CAR (1.78), and ZA/ZAV (0.03).

^ySignificance for linear regression over N fertility rates (equations can be found in the text).

study accumulated high concentrations of carotenoid phytochemicals. Increasing N concentrations in nutrient solution culture did not impact carotenoid concentrations in the shoot tissue of 'Golden Leaf' purslane. However, concentrations of BC, LUT, and total carotenoid pigments within the shoot tissues of 'Green Leaf' purslane all increased in response to increasing N treatment concentrations. Data support the presence of genetic variation for carotenoid metabolism in response to N fertility within available purslane germplasm. Therefore, N fertility management and cultivar selection should be considered when producing purslane as an edible specialty crop.

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