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Air Temperature Affects Biomass and Carotenoid Pigment Accumulation in Kale and Spinach Grown in a Controlled Environment

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***Abstract*. Crop plants are adversely affected by a variety of environmental factors, with air temperature being one of the most influential. Plants have developed a number of methods in the adaptation to air temperature variations. However, there is limited research to determine what impact air temperature has on the production of secondary plant compounds, such as carotenoid pigments. Kale (*Brassica oleracea* L.) and spinach (*Spinacia oleracea* L.) have high concentrations of lutein and β-carotene carotenoids. The objectives of this study were to determine the effects of different growing air temperatures on plant biomass production and the accumulation of elemental nutrients, lutein, β-carotene, and chlorophyll pigments in the leaves of kale and spinach. Plants were grown in nutrient solutions in growth chambers at air temperatures of 15, 20, 25, and 30 °C for 'Winterbor' kale and 10, 15, 20, and 25 °C for 'Melody' spinach. Maximum tissue lutein and β-carotene concentration occurred at 30 °C for kale and 10 °C for spinach. Highest carotenoid accumulations were 16.1 and 11.2 mg/100 g fresh mass for lutein and 13.0 and 10.9 mg/100 g fresh mass for β-carotene for the kale and spinach, respectively. Lutein and β-carotene concentration increased linearly with increasing air temperatures for kale, but the same pigments showed a linear decrease in concentration for increasing air temperatures for spinach. Quantifying the effects of air temperature on carotenoid accumulation in kale and spinach, expressed on a fresh mass basis, is important for growers producing these crops for fresh markets.**

Various environmental factors can affect plant growth and development, such as light, water and air temperature. Significant air temperature variations can limit plant growth at both low and high temperature extremes. Environmental conditions between these two extremes provide an optimum air temperature range for plant growth that allows for maximum productivity (Abrami, 1972). Exposing plants to low air temperatures can damage the photosynthetic apparatus, inhibit the synthesis and/or degradation of proteins, damage the thylakoid membrane, and reduce the electron transfer capacity of the plant (Guy et al., 1985; Holaday et al., 1991; Taiz and Zeiger, 1998). An increase in growing air temperatures above optimum levels results in damage to the thylakoid membranes, along with reduction in the rates of photosynthesis and respiration (Arvidsson et al., 1997; Maevskaya et al., 2003; Rokka et al., 2000; Taiz and Zeiger, 1998). At high air temperatures, membrane disruption results from loses in tissue stability and membrane integrity.

Plants have adapted to potential air temperature changes in the growing environment and can usually adjust to conditions slightly above and below optimum air temperature ranges. Under cold air temperatures, plants increase the percentage of unsaturated fatty acid chains in membranes, specifically thylakoid membranes, and increase abscisic acid concentrations (Jun et al., 2001; Shewfelt, 1992; Taiz and Zeiger, 1998). Physiological adaptations to high air temperatures include increased leaf wax, leaf rolling, change in leaf orientation, change in leaf size and the production of heat shock proteins (Taiz and Zeiger, 1998).

Carotenoids are lipid soluble yellow, orange, and red plant pigments. In plants, carotenoids function as light harvesting antennae pigments, as important free radical scavengers, and have photoprotective roles (Demmig-Adam et al., 1996, Miki, 1991; Tracewell et al., 2001). Carotenoids cannot be synthesized in mammals, making plants the primary source of carotenoids in their diet. Two important carotenoids in human health maintenance are lutein and β-carotene. Dietary intake of foods rich in lutein and β-carotene has been associated with reduced risk of lung cancer and chronic eye diseases, such as cataracts and age-related macular degeneration (Ames et al., 1995; Landrum and Bone, 2001; Le Marchand et al., 1993; Semba and Dagnelie, 2003).

Kale (*Brassica oleracea* L.) ranks highest, and spinach (*Spinacia oleracea* L.) ranks second among vegetable crops for carotenoid content, including lutein and β-carotene (Holden et al., 1999; U.S. Dept. Agr., 2002). Kale is also an excellent source of Ca, Mg, and K (Mills and Jones, 1996). However, kale has low consumption rates in the United States, with per capita fresh intake of less than 0.33 $kg \cdot year^{-1}$ (Lucier and Plummer, 2003). Spinach has one of the highest rates of consumption among green-leafy vegetables in the United States, with per capita intakes of 0.73, 0.09, and 0.36 $kg \cdot year^{-1}$ for fresh, canned, and frozen product, respectively (Lucier and Plummer, 2003). Spinach is high in Fe, which is bioavailable in human diets (Zhang et al., 1989), and is also high in Ca, Mg, K, and carotenoids (Holden et al., 1999; Mills and Jones, 1996; USDA, 2002).

Environmental air temperatures can have significant impacts on plant performance and metabolism. What remains unclear, however, is the effect of air temperature regimes on the production and accumulation of secondary plant compounds, such as carotenoids. High concentrations of lutein and β-carotene carotenoids are found in both kale and spinach. Carotenoids are membrane-bound compounds that serve important functions in photosynthesis. Both low and high temperature conditions can affect the integrity of thylakoid membranes, and thus affect the stability of carotenoid compounds. Therefore, the goal of this study was to investigate the influences of different air temperatures on plant biomass, elemental concentrations, and accumulation of lutein, β-carotene, and chlorophyll in kale and spinach.

Materials and Methods

Plant culture. 'Winterbor' kale and 'Melody' spinach (Johnny's Selected Seed, Winslow, Maine) were seeded into rockwool growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) and germinated in a greenhouse (22 °C day/14 °C night) under natural lighting conditions (Lat. 43° 09' N, Durham, N.H.). Kale was seeded on 8 and 30 Aug. and 7 Nov. 2002 and spinach was seeded on 12 June 2002 and 19 May and 19 June 2003. Peter's 20N–6.9P–16.6K water-soluble fertilizer (Scotts, Marysville, Ohio) was applied at a rate of 200 $mg \cdot L^{-1}$ every 5 d. After 2 weeks for the kale and 3 weeks for the spinach, the plants were transferred to 38-L plastic containers (Rubbermaid Inc., Wooster, Ohio).

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For each study, eight plants were placed in 2 cm round holes set at 10.6 × 9.5 cm spacing on each container lid. The plants were grown in 30 L of a nutrient solution (Hoagland and Arnon, 1950), with elemental concentrations of ($mg \cdot L^{-1}$): N (105.0); P (91.5), K (117.3), Ca (80.2), Mg (24.6), S (32.0), Fe (0.5), B (0.25), Mo (0.005), Cu (0.01), Mn (0.25), and Zn (0.025). The electrical conductivity (EC) of the starting nutrient solution was 0.7 $mS \cdot cm^{-1}$ and pH was measured at 5.6. Four containers were placed in a growth chamber (E15; Conviron, Winnipeg, Manitoba), with each chamber representing a distinct air temperature treatment.

Individual chambers provided plants with a set point air temperature treatment (T) of 15, 20, 25, or 30 °C for the kale, and 10, 15, 20, or 25 °C for the spinach. Each air temperature treatment was replicated three times in separate studies for each species. Photosynthetically active radiation (PAR) was measured (QSO-ELEC; Apogee Instruments; Logan, Utah) at six locations on top of each container (without plants present) at the four corner plant holes and between the two side middle plant holes and averaged for each of four containers. Irradiance inside each chamber was measured at the beginning and confirmed at the end of each replication and averaged 500 ± 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The photoperiod for all studies was set at 16 h light and 8 h dark. Both cool white fluorescent and incandescent bulbs were used during the experiment. Solutions were aerated with an air blower (25E133W222; Spencer, Winsor, Conn.) connected to air stones. Nutrient solutions were replaced weekly throughout the experiment to refresh the solution to the initial nutrient concentrations.

The kale plants were cultured for 3 weeks, while the spinach plants were grown for 4 weeks. At harvest, shoot and root tissues were separated and weighted. A fully developed, nonshaded leaf from each of the eight plants was randomly selected and a 4-cm^2 piece of the leaf was removed. This treatment sample was stored at –20 °C before lyophilization. The remaining shoot material was dried at 60 °C for no less than 72 h, at which time shoot dry mass was determined.

Elemental composition. The dried kale and spinach shoot tissues were ground to pass a 0.5-mm screen using a sample mill grinder (1093; Cyclotec-Tector, Höganäs, Sweden). A 0.3-g sample of the ground tissue homogenate was mixed with 10 mL of 70% concentrated nitric acid (HNO_3) and digested in a microwave accelerated reaction system (MARS5, CEM Corp., Matthews, N.C.). The digested solution was cooled to room temperature and deionized water was added to result in a final volume of 40 mL. Elemental analysis was determined by inductively coupled argon plasma–atomic emission spectrometry (ICP–AES) (Vista AX; Varian, Inc., Palo Alto, Calif.).

Carotenoid and chlorophyll content—Tissue extraction. The frozen kale and spinach samples were lyophilized for a minimum of 72 h (6L FreeZone; LabConCo, Kansas City, Mo.). The freeze-dried tissues samples were ground with dry ice in a kitchen grinder (Handy Chopper Plus, HC 3000; Household Products Inc., Shelton, Conn.). Samples were extracted and separated according to the method of Kopsell et al. (2004), which was based on the method of Khachik et al. (1986). A 0.1-g subsample was placed into a Potter-Elvehjem tissue grinder tube (Kontes, Vineland, N.J.) and hydrated with 0.8 mL of deionized water. The sample was placed in a 40 °C water bath for 20 min. After hydration, 0.8 mL of the internal standard, ethyl-β-8-apo-carotenoate (Sigma Chemical Co., St. Louis, Mo.) and 2.5 mL of tetrahydrofuran (THF) stabilized with 25 ppm 2,6-Di-*tert*-butyl-4-methoxyphenol (BHT) were added. The sample was homogenized in the tube with about 25 insertions with a Potter-Elvehjem tissue grinder pestle attached to a drill press (Craftsman 15-inch drill press; Sears, Roebuck and Co., Hoffman Estates, Ill.) at 540 rpm. The sample tube was kept immersed in ice during the grinding process. The tube was placed into a clinical centrifuge for 3 min at 500 g_n. The supernate was removed with a Pasteur pipet, placed into a conical 15-mL test tube, capped and held on ice. The sediment was resuspended in 2.0 mL THF and homogenized with about 25 insertions of the grinding pestle. The tube was centrifuged for 3 min at 500 g_n and the supernate was collected and combined with the first extracted supernate. The extraction procedure was repeated twice more until the supernate was colorless. The sediment was discarded and the combined four supernates were placed in a 40 °C water bath and reduced to 0.5 mL using nitrogen gas (N-EVAP 111; Organomation Inc., Berlin, Mass.). Added to the 0.5 mL reduced sample was 2.5 mL of MeOH and 2.0mL of THF, and the combined sample solution was vortexed. The sample was filtered through a 0.2 µm polytetrafluoroethylene (PTFE) filter (Econofilter PTFE 25/20; Agilent Technologies, Wilmington, Del.) using a 5-mL syringe (Becton, Dickinson and Co., Franklin Lakes, N.J.).

Carotenoid and chlorophyll content—HPLC analysis. A HPLC unit with photo diode array detector (Agilent 1100; Agilent Technologies, Palo Alto, Calif.) was used for pigment separation. All samples were analyzed for carotenoid compounds using a Vydac RP-C18 5.0 µm 250 × 4.6 mm column (201TP54; Phenomenex; Torrance, Calif.) fitted with a 4 × 3.0 mm, 7.0 µm guard column compartment. The column was maintained at 16 °C using a thermostatic column compartment. Eluents were A: 75% acetronitrile, 20% methanol, 5% hexane, 0.05% BHT, and 0.013% triethyamine (TEA) in water (v/v) and B: 50% acetonitrile, 25% THF, 25% hexane and 0.013% TEA in water (v/v). The flow rate was 0.7 $mL \cdot min^{-1}$ and the gradient was 100% eluent A for 30 min; 50% A and 50% B for 2 min; 100% B for 2 min; and 50% A and 50% B for 2 min. The eluent was returned to 100% A for 10 min before the next injection. Eluted compounds from a 20 µL injection were detected at 452 (carotenoids and internal standard), 652 (chlorophyll *a*), and 665 (chlorophyll *b*) nm and data were collected, recorded, and integrated using 1100 HPLC ChemStation Software (Agilent Technologies). Peak assignment was performed by comparing retention times and line spectra obtained from the photodiode array detection of authentic standards (lutein from Carotenature, Lupsingen, Switzerland; β-carotene, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) from Sigma Chemical Co.). Recovery rates of ethyl-β-apo-carotenoate during extraction were above 90%.

Statistical analysis. Data were analyzed according to a one-way ANOVA using SAS (SAS Institute, Cary, N.C.). The ANOVA determined the significance of the main effects of the air temperature treatments. The relationship between experimental dependent variables and temperature treatments were determined by regression analysis using SAS. Significance for trend analysis was determined by goodness-of-fit of the regression model (r^2).

Results and Discussion

Tissue biomass accumulation. Shoot tissue fresh mass (FM) was significantly influenced by changes in air temperature for both 'Winterbor' kale ($F = 9.2$, $P = 0.01$) and 'Melody' spinach ($F = 5.2$, $P = 0.03$). Average shoot FM for 'Winterbor' kale increased from 42.3 to 68.6 g/plant as air temperature treatments increased from 15 to 20 °C (Table 1). Average shoot FM increased then decreased quadratically [$FM = -221.1 + 26.6(T) - 0.61(T)^2$; $r^2 = 0.77$] for 'Winterbor' kale with increasing air temperatures. 'Melody' spinach increased linearly in shoot FM from 33.1 to 156.0 g/plant as the air temperature increased from 10 to 20 °C (Table 1). However, average shoot FM increased then decreased quadratically [$FM = 48.8 - 1.2(T) - 333.7(T)^2$; $r^2 = 0.66$] for 'Melody' spinach over the air temperature range investigated (Table 1). Average shoot tissue dry mass (DM) for both 'Winterbor' kale and 'Melody' spinach was not significantly affected by changes in air temperature (Table 1).

Kale can grow successfully under a wider range of air temperatures than spinach, with optimum growth occurring at 20 °C (Decoteau, 2000; Paul, 1991). Optimum air temperatures are lower for spinach, and occur between 16 and 20 °C (Decoteau, 2000). Spinach is also able to withstand very low air temperatures during germination (Roeggen, 1984). Previous research shows that air temperatures exceeding 35 °C will affect the efficiency of spinach metabolism and reduce yields (Foyer et al., 1997; Maevskaya et al., 2003; Rokka et al., 2000). As the air temperature increases beyond an optimum range, decreases in yield are often reported (Abrami, 1972). This decrease in yield can be due to a number of factors including increased dephosphorylation (Rokka et al., 2000), increases in hydrogen peroxide (Foyer et al., 1997), decreases in efficiency of photosystem II of photosynthesis (Karim et al., 2003), and reduction of nitrate reductase (Maevskaya et al., 2003). All of these factors contribute to a reduction in the overall growth of the plant. Results from the current study confirm optimum air temperature conditions for biomass production in both kale and spinach.

Carotenoid and chlorophyll pigment accumulation. The accumulation of lutein in

the leaf tissues was influenced by changes in air temperatures. Leaf tissue lutein concentrations responded significantly to changes in air temperatures for 'Winterbor' kale ($F = 10.0$, $P = 0.004$). Leaf tissue lutein increased linearly [lutein = 8.75 + 0.24(T); $r^2 = 0.77$] as the air temperature increased from 15 to 30 °C for 'Winterbor' kale (Table 2). Increases in air temperatures from 10 to 25 °C resulted in a linear decrease in leaf tissue lutein [lutein = 13.0 – 0.25(T); $r^2 = 0.41$) for 'Melody' spinach (Table 2). Maximum tissue lutein accumulation expressed on a fresh mass basis for 'Melody' spinach was 11.2 mg/100 g and occurred at 10 °C (Table 2). Lutein accumulation for 'Winterbor' kale increased with increasing temperature treatments, reaching 16.1 mg/100 g fresh weight at 30 °C (Table 2).

The accumulation of β-carotene in the leaf tissues was also influenced by changes in air temperatures. Leaf tissue β-carotene responded significantly to changes in air temperatures for both 'Winterbor' kale ($F = 14.4$, $P \leq 0.001$) and 'Melody' spinach ($F = 7.7$, $P = 0.01$). Increases in air temperatures for 15 to 30 °C resulted in linear increases in leaf tissue β-carotene [β-carotene = 5.0 + 0.26(T); $r^2 = 0.81$) for 'Winterbor' kale. Leaf tissue β-carotene decreased linearly [β-carotene = 12.8 - 0.27(T); $r^2 = 0.60$) as the air temperature increased from 10 to 20 °C for 'Melody' spinach (Table 2). β-Carotene accumulation for 'Winterbor' kale increased with increasing temperature treatments, reaching 13.0 mg/100 g fresh weight at 30 °C. Maximum β-carotene accumulation for 'Melody' spinach was 10.9 mg/100 g under the 10 °C treatment (Table 2).

Temperature can affect the production of plant secondary compounds. Arvodsson et al. (1997) reported that conversion of violaxanthin to zeaxanthin was highly dependent on air temperature. In spinach, 50% of the violaxanthin was converted to zeaxanthin at 4 °C, but conversion increased to 70% at 25 °C and then to 80% at 37 °C. No further increase in conversion rate was noted at air temperatures >37 °C. Arvidsson et al. (1997) also noted that as the conversion rate increased for violaxanthin to zeaxanthin, the levels of lutein increased significantly while other carotenoids remained unaffected. Kale lutein concentrations in the current study increased as air temperature treatments increased, similar to the results of Arvodsson et al. (1997). However, lutein concentrations decreased in spinach as air temperatures increased, contradicting the results of Arvodsson et al. (1997). The β-carotene concentrations followed a similar trend as the lutein, but Arvodsson et al. (1997) reported no change in the content of this pigment. Increasing air temperatures from 12 to 32 °C also affected the production of glucosinolate compounds and myrosinase enzyme activity in rapid-cycling *B. oleracea* (Charron and Sams, 2004). Changes to plant secondary compounds should be noteworthy when growing *B. oleracea* under the different air temperature ranges provided in this study.

Kale and spinach leaf tissues accumulated high concentrations of chlorophyll (chl) pigments (Table 2). Changes in air temperature

Table 1. Mean value[z] of biomass production in the leaf tissues of 'Winterbor' kale (*Brassica oleracea* L.) and 'Melody' spinach (*Spinacia oleracea* L.) grown under increasing air temperatures in nutrient solution culture.

Temp	Biomass (g/plant)	
(°C)	Fresh mass (%)	Dry mass (%)
Winterbor kale		
15	42.3 ± 3.9	4.9 ± 1.6
20	68.6 ± 3.0	6.5 ± 1.0
25	65.0 ± 9.9	8.5 ± 2.6
30	30.2 ± 4.9	4.4 ± 1.4
Contrasts[y]		
L	NS	NS
Q	**	NS
Melody spinach		
10	33.1 ± 15.5	3.1 ± 1.6
15	119.6 ± 25.5	6.6 ± 1.8
20	156.0 ± 28.9	6.8 ± 1.6
25	120.0 ± 19.4	4.4 ± 1.0
Contrasts		
L	*	NS
Q	**	NS

[z]Mean leaf tissue mass of three replications, eight plants each ± standard deviation.
[y]Significance for linear (L) and quadratic (Q) orthogonal contrasts.
NS,*,**,***Nonsignificant or significance at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Mean value[z] of pigment accumulation expressed on a fresh mass basis in the leaf tissues of 'Winterbor' kale (*Brassica oleracea* L.) and 'Melody' spinach (*Spinacia oleracea* L.) grown under increasing air temperatures in nutrient solution culture.

Temp	Pigment concn (mg/100 g fresh mass)			
(°C)	Lutein	β-Carotene	Chl *a*[y]	Chl *b*[x]
Winterbor kale				
15	12.6 ± 0.6	9.3 ± 0.5	204.4 ± 15.1	50.5 ± 4.0
20	13.2 ± 0.1	9.8 ± 0.3	201.4 ± 13.8	49.5 ± 4.3
25	14.8 ± 0.5	11.8 ± 0.5	223.8 ± 31.4	56.1 ± 1.0
30	16.1 ± 0.5	13.0 ± 0.5	255.1 ± 3.5	61.1 ± 0.4
Contrasts[w]				
L	***	***	NS	*
Q	***	***	NS	*
Melody spinach				
10	11.2 ± 1.6	10.9 ± 1.0	199.0 ± 17.8	47.5 ± 5.6
15	8.2 ± 0.8	7.7 ± 0.3	147.6 ± 7.8	35.8 ± 1.4
20	7.7 ± 1.1	7.1 ± 0.8	134.9 ± 14.8	29.9 ± 1.3
25	7.3 ± 0.6	6.7 ± 0.5	125.7 ± 13.3	30.5 ± 3.2
Contrasts				
L	*	**	**	**
Q	*	**	**	**

[z]Mean composition of sampled leaf tissue of three replications, eight plants each ± standard deviation.
[x]Chl a = chlorophyll a.
[y]Chl b = chlorophyll b.
[w]Significance for linear (L) and quadratic (Q) orthogonal contrasts.
NS,*,**,***Nonsignificant or significance at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 3. Mean value[z] of percent dry mass, and pigment concentration as expressed on a dry mass basis in the leaf tissues of 'Winterbor' kale (*Brassica oleracea* L.) and 'Melody' spinach (*Spinacia oleracea* L.) grown under increasing air temperatures in nutrient solution culture.

Temp	Concn ($mg \cdot g^{-1}$ dry mass)		
(°C)	Dry mass (%)	Lutein	β-Carotene
Winterbor kale			
15	14.3 ± 0.7	0.89 ± 0.08	0.65 ± 0.06
20	14.6 ± 1.4	0.92 ± 0.08	0.68 ± 0.06
25	15.3 ± 1.6	0.99 ± 0.10	0.80 ± 0.10
30	16.0 ± 1.4	1.02 ± 0.06	0.83 ± 0.05
Contrasts[y]			
L	NS	NS	NS
Q	NS	NS	NS
Melody spinach			
10	13.2 ± 0.1	0.59 ± 0.05	0.43 ± 0.05
15	9.1 ± 0.5	0.76 ± 0.04	0.57 ± 0.04
20	8.2 ± 0.8	0.90 ± 0.07	0.68 ± 0.04
25	8.4 ± 0.6	0.88 ± 0.08	0.67 ± 0.06
Contrasts			
L	**	NS	NS
Q	***	NS	NS

[z]Mean composition of sampled leaf tissue of three replications, eight plants each ± standard deviation.
[y]Significance for linear (L) and quadratic (Q) orthogonal contrasts.
NS,**,***Nonsignificant or significance at $P \leq 0.01$ or 0.001, respectively.

Table 4. Mean value[z] of macronutrient accumulation in the leaf tissues of 'Winterbor' kale (*Brassica oleracea* L.) and 'Melody' spinach (*Spinacia oleracea* L.) grown under increasing air temperatures in nutrient solution culture.

Temp	Macronutrient (% dry mass)				
(°C)	P	K	Ca	Mg	S
Winterbor kale					
15	1.07 ± 0.12	3.53 ± 0.37	3.81 ± 0.63	0.72 ± 0.14	0.67 ± 0.07
20	1.23 ± 0.05	4.92 ± 0.41	4.74 ± 0.23	0.89 ± 0.06	0.85 ± 0.09
25	1.10 ± 0.13	3.55 ± 0.57	3.15± 0.57	1.27 ± 0.20	0.71 ± 0.14
30	1.07 ± 0.05	3.83 ± 0.29	2.95 ± 0.28	0.70 ± 0.04	0.61 ± 0.06
Contrast[y]					
L	NS	NS	NS	NS	NS
Melody spinach					
10	1.74 ± 0.36	10.86 ± 0.43	1.37 ± 0.22	1.66 ± 0.15	0.33 ± 0.08
15	2.58 ± 0.43	11.7 ± 0.59	1.18 ± 0.09	1.79 ± 0.12	0.33 ± 0.07
20	2.44 ± 0.44	11.3 ± 1.16	1.23 ± 0.11	1.68 ± 0.09	0.33 ± 0.07
25	1.90 ± 0.19	11.7 ± 0.65	0.98 ± 0.04	1.65 ± 0.08	0.31 ± 0.05
Contrast					
L	NS	NS	NS	NS	NS

[z]Mean composition of sampled leaf tissue of three replications, eight plants each ± standard deviation.
[y]Significance for linear (L) orthogonal contrast.
[NS]Nonsignificant.

Table 5. Mean value[z] of micronutrient accumulation in the leaf tissues of 'Winterbor' kale (*Brassica oleracea* L.) and 'Melody' spinach (*Spinacia oleracea* L.) grown under increasing air temperatures in nutrient solution culture.

Temp	Micronutrient ($mg \cdot kg^{-1}$ dry mass)					
(°C)	B	Cu	Fe	Mn	Mo	Zn
Winterbor kale						
15	47.4 ± 8.4	3.0 ± 1.1	76.0 ± 19.9	150.2 ± 28.4	0.77 ± 0.16	73.3 ± 51.6
20	55.7 ± 11.2	20.6 ± 17.7	59.5 ± 32.6	168.1 ± 9.1	0.77 ± 0.13	52.0 ± 24.1
25	46.0 ± 0.5	16.3 ± 9.8	32.4 ± 9.7	127.0 ± 20.5	0.81 ± 0.11	42.8 ± 7.9
30	55.2 ± 7.2	17.2 ± 14.8	45.7 ± 20.0	108.8 ± 11.7	0.59 ± 0.15	110.3 ± 73.8
Contrast[y]						
L	NS	NS	NS	NS	NS	NS
Melody spinach						
10	37.3 ± 12.4	9.6 ± 6.5	159.4 ± 19.9	296.6 ± 64.6	0.71 ± 0.21	149.3 ± 24.1
15	41.8 ± 7.3	8.2 ± 2.9	172.9 ± 20.2	309.5 ± 21.8	0.92 ± 0.08	78.5 ± 19.2
20	46.3 ± 7.5	6.8 ± 1.2	138.1 ± 21.8	297.2 ± 40.0	0.68 ± 0.09	131.2 ± 42.5
25	47.4 ± 8.5	7.0 ± 0.6	115.7 ± 32.8	342.0 ± 68.9	0.87 ± 0.08	135.1 ± 32.4
Contrast						
L	NS	NS	NS	NS	NS	NS

[z]Mean composition of sampled leaf tissue of three replications, eight plants each ± standard deviation.
[y]Significance for linear (L) contrast.
[NS]Nonsignificant.

did not significantly affect 'Winterbor' kale chlorophyll concentrations. However, Chl *a* (F = 5.5, $P = 0.02$) and Chl *b* (F = 5.9, $P = 0.02$) concentrations responded significantly to changes in air temperatures for 'Melody' spinach. Similar to the carotenoid pigments, maximum chlorophyll concentrations occurred at 30 °C for 'Winterbor' kale and 10 °C for 'Melody' spinach (Table 2). The accumulation of both chl *a* [Chl $a = 233.2 - 4.65(T)$; $r^2 = 0.57$) and chl *b* [Chl $b = 55.8 - 1.13(T)$; $r^2 = 0.56$) pigments decreased linearly as the air temperature increased from 10 to 25 °C for 'Melody' spinach (Table 2). Previously, positive correlations between chlorophyll and carotenoid pigments have been reported for several crop species (Terry and Abadía, 1986), including kale (Kopsell et al., 2004) and Swiss chard (*Beta vulgaris* L.; Ihl et al., 1994). Chlorophyll and carotenoid pigment concentrations followed a similar trend for the kale and spinach grown in the current study.

Percentage dry matter accumulation for the spinach responded to increases in air temperatures (F = 17.0, $P \leq 0.001$). The spinach percent DM increased, then decreased [$\%DM = 26.6 - 1.78(T) + 0.04(T)^2$; $r^2 = 0.85$) as the air temperatures increased from 10 to 25 °C (Table 3). Growing environment, handling, storage, or food processing can cause changes in tissue water content, resulting in changes in the concentration of carotenoids in the plant when expressed on a fresh mass basis (Ezell and Wilcox, 1959; Gil et al., 1999).

The carotenoid concentration of the kale and spinach calculated on a dry mass basis was not significantly affected by changes in air temperature (Table 3). Maximum lutein and β-carotene dry mass accumulation in kale and spinach occurred at the temperature treatments (25 and 20 °C, respectively) of maximum dry mass accumulation. Overall, dry mass carotenoid concentrations increased with increasing total dry matter production in both kale and spinach. Maximum lutein and β-carotene fresh mass accumulation for both kale and spinach occurred at the temperature treatments (30 and 10 °C, respectively) of minimum fresh mass accumulation. Overall, there tended to be a dilution of fresh mass carotenoid concentrations in both kale and spinach with increasing total fresh mass production.

Macro- and micronutrient accumulation. None of the essential macro- or micronutrients responded to changes in air temperatures for 'Winterbor' kale or 'Melody' spinach (Tables 4 and 5). Marschner (1985) reported that mineral uptake is only slightly affected by air temperature, with changes in root zone temperature having a larger effect on ion uptake.

Previous research shows the potential for genetic differences in carotenoid accumulations within plant species (Howard et al., 2000; Kopsell et al., 2004; Kurilich et al., 2003; Nicolle et al., 2004). The current study demonstrates that air temperatures can also influences the production of carotenoid pigments in the leaves of kale and spinach. Carotenoid concentrations in the leaves of kale increased as the air temperatures increased from 15 to 30 °C, while the carotenoid concentrations decreased in spinach as the air temperature increased from 10 to 25 °C. Changes in the growing air temperatures of these cool-season crops resulted in changes in fresh biomass production, and the accumulation of lutein, β-carotene, and chl *b* pigments, when calculated on a fresh mass basis. In many parts of the country, cool-season crops such as kale and spinach can be planted in both spring and fall. Air temperatures in field conditions can be modified by growing kale and spinach at different times of the year. Therefore, the influence of air temperatures on kale and spinach carotenoid concentrations should be considered when selecting appropriate planting dates.

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