HortScience 59(2):255–257. 2024. https://doi.org/10.21273/HORTSCI17560-23

Effects of Root Zone Temperature of Hydroponic Lettuce Affects Plant Growth, Nutrient Uptake, and Vitamin A Content

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Keywords. β-carotene, controlled environment agriculture, greenhouse, *Lactuca sativa*, soilless production

Abstract. **Water temperature can affect plant growth and quality in hydroponic production. Lettuce 'Antonet', 'Waldman's Dark Green', 'Parris Island', 'Jericho', and 'Rex' were grown using the nutrient film technique with chilled (water temperature set at 21.1 °C) or ambient water. Data were collected on plant growth, foliar nutrient content, and vitamin A content. 'Jericho' had the greatest shoot fresh weight but was only significantly different from 'Antonet', which had the lowest shoot fresh weight but the greatest vitamin A content. SPAD was greatest in 'Paris Island' and was significantly greater in chilled water over ambient for 'Antonet'. Plants grown in ambient water had greater number of leaves and root dry weight, whereas SPAD was greatest with chilled water. Greater nutrient values were observed in 'Rex', 'Jericho', and 'Waldman's Dark Green' in chilled water, whereas no nutrient differences were observed in 'Antonet' and 'Parris Island'.**

Lettuce (*Lactuca sativa* L.) is a cool-season crop grown worldwide but is particularly sensitive to higher temperatures (Turini et al. 2011). Lettuce grows best when ambient temperatures are 15 to 25 °C; higher temperatures cause reduced leaf growth and sudden bolting (Zhao et al. 2022). Because of this temperature sensitivity and other factors, hydroponically grown lettuce is becoming increasingly more common in greenhouse production, and the nutrient film technique (NFT) is the most widely used method (Sharma et al. 2018). Hydroponically grown lettuce tends to have a higher β-carotene content, the most potent form of vitamin A, than soil-grown lettuce (Kobayashi et al. 1989; Mou 2005). Vitamin A is found in leafy greens, yellow-colored vegetables, and orange-colored fruit in multiple forms and is known to promote healthy vision, growth hormone production, embryonic development, and many other roles, whereas a deficiency can lead to blindness and increased mortality (Clagett-Dame and Knutson 2011; Weber and Grune 2012). This study aimed to evaluate chilled vs. ambient root-zone temperatures for plant growth, nutrients, and vitamin A content in lettuce.

Received for publication 20 Oct 2023. Accepted for publication 14 Dec 2023.
Published online 19 Jan 2024.
The work was funded by the US Department of Agriculture (USDA) Research and Extension Experience for Undergraduates (grant no. 2022-67037-36618/project accession no. 1028134) from the US Department of Agriculture, National Institute of Food and Agriculture.
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Materials and Methods

Growth conditions and plant material. The experiment was conducted at the Oklahoma State University Department of Horticulture and Landscape Architecture research greenhouses in Stillwater, OK, USA. No supplemental lighting was used in the greenhouse, and daily light integral levels ranged from 11.3 to 21.8 $mol \cdot m^{-2} \cdot d^{-1}$. The greenhouse temperature was set at 21/18 °C (day/night) but averaged 26.1 °C and relative humidity averaged 71.1%.

Seeds of five diverse lettuce cultivars were obtained from Johnny's Selected Seeds (Winslow, ME). Cultivars of lettuce included Antonet (Lolla loose leaf), Waldman's Dark Green (loose leaf), Parris Island (romaine), Jericho (romaine), and Rex (butterhead). Seeds were sown in oasis cubes (Harris Seeds, Rochester, NY, USA) of size 1.5 cm^3 on 10 May 2023 and were kept under mist.

Seedlings were transplanted into six NFT tables (Hydrocycle Pro; Growers Supply, Dyersville, IA, USA) measuring 10 cm wide × 5 cm deep × 900 cm long with 2.5-cm site holes spaced 20 cm in the center and a slope 2.8% on 1 Jun 2023. Each table had one plant per slot and 10 plants per cultivar randomly arranged. Nutrient solution was prepared using 5N–5.2P–21.6K fertilizer (Jack's; J.R. Peters, Allentown, PA, USA) and calcium nitrate (American Plant Products, Oklahoma City, OK, USA). White plastic water tanks placed below each table were filled to 40-gallon capacity, and 147.4 g of the fertilizer above and 97.5 g of calcium nitrate was added initially. The pH of the nutrient solution was maintained at 5.5 to 6.5, and the EC level was maintained between 1.5 to 2.5 $mS \cdot cm^{-1}$. pH down solution (General Hydroponics, Santa Rosa, CA, USA) was used to lower the pH because of alkaline tap water.

Water temperature. A data logger (HOBO Water Temperature Pro v2; Onset, Bourne, MA, USA) was used to record the water temperature in one tank per treatment throughout the growing period. Water temperature in the NFT nutrient tanks were set at either 21.1 °C or ambient (control). Water chillers (Active Aqua, Hydrofarm, Petaluma, CA, USA) were used to control water temperature.

Data collection. Plant greenness using a soil plant analysis development meter (SPAD) (Konica Minolta, Tokyo, Japan), plant height, plant width (average of two perpendicular measurements), and number of leaves of each plant were measured 28 d after transplanting. SPAD was taken using one mature leaf in the middle of the plant, and three readings were taken from the leaf tip. Shoots and roots were separated to get fresh weights, and then the material was dried for 7 d at 53.9 °C for dry weight. At the end of each study, leaf nutrient concentration was measured on one plant per replication per treatment by the Soil, Water, and Forage Analytical Laboratory in Stillwater, OK, and analyzed as outlined by Zhang and Henderson (2018).

Determination of vitamin A content. Vitamin A was determined using an Elisa Kit [CSBE07038Pl, Plant vitamin A (VA) ELISA Kit; Cusabio, Houston, TX, USA]. Fresh samples from a mature leave were cut into small pieces, and 400 mg was used, whereas frozen samples stored at –18 °C for 2 d were used the second time. Samples were homogenized with 3.6 mL phosphate-buffered saline buffer (ThermoFisher, Waltham, MA, USA) at a 1:9 ratio of buffer to plant sample (3.6 µL per 400-mg sample). Homogenous extract solutions were centrifuged at 4 °C and 4600 g_n for 7 min. Then, 50 µL of the extract solution was used, following the manufacturer's instructions. Two sets of standards were employed to calibrate the assay. The absorbance at 450 nm was measured with a spectrophotometer microplate reader (Epoch; Bio-TEK, Instruments Inc., Winooski, VT, USA).

Experimental design and statistical analysis. The study was conducted in a split-plot design with three replications. Factors were water temperature (two levels) as the whole plot and cultivars of lettuce as the subplot. Three samples per treatment were analyzed in the vitamin A tests with two replications. Statistical analysis was performed using SAS/STAT software (Version 9.4; SAS Institute, Cary, NC, USA). Tests of significance were reported at the 0.05, 0.01, and 0.001 levels. The data were analyzed using generalized linear mixed model methods. Tukey multiple comparison methods were used to separate the means.

Table 1. Least square means treatment differences between lettuce cultivars and water temperature (chilled at 23 °C or ambient) for plant growth and quality of five lettuce cultivars in nutrient film technique hydroponic systems at research greenhouses in Stillwater, OK, USA.

Cultivar	Treatment	SPAD (unitless)	Plant ht (cm)	Plant width (cm)	No. of leaves	Shoot fresh wt (g)	Shoot dry wt (g)	Root fresh wt (g)	Root dry wt (g)	Vitamin A (ng·mL^{-1})
Antonet	23	20.2 d[i]	14.3 d	19.9 d	11.5 e	53.3 b	2.4 d	9.9 d	0.6 b	2.3 a
	Ambient	15.0 e	15.1 d	20.0 d	13.1 cde	54.3 b	4.2 abc	13.7 cd	2.1 a	2.5 a
Rex	23	28.9 b	14.9 d	23.6 cd	21.8 a	96.4 a	3.4 cd	12.4 d	0.6 b	1.3 c
	Ambient	27.8 bc	14.8 d	23.5 cd	23.0 a	95.9 a	3.7 bc	15.4 bcd	2.3 a	1.5 bc
Jericho	23	25.2 bc	21.7 bc	30.4 a	15.1 bc	110.6 a	4.1 abc	19.0 abc	0.7 b	1.4 bc
	Ambient	23.9 cd	21.5 c	29.0 ab	17.5 b	105.6 a	4.4 abc	19.8 ab	2.3 a	1.5 bc
Paris Island	23	37.3 a	23.4 abc	24.2 c	14.5 bcd	98.3 a	3.7 bc	18.6 abc	0.8 b	1.2 c
	Ambient	34.1 a	23.4 abc	25.1 bc	15.2 bc	96.0 a	4.0 abc	20.8 ab	2.3 a	1.5 bc
Waldman's Dark Green	23	25.8 bc	25.5 ab	32.2 a	11.5 de	86.2 a	4.7 ab	13.2 cd	0.8 b	1.4 c
	Ambient	25.0 bc	27.4 a	32.9 a	14.1 cde	108.5 a	5.2 a	23.7 a	2.5 a	2.0 ab
Cultivar		***[ii]	***	***	***	***	***	***	NS	*
Treatment		***	NS	NS	***	NS	**	***	***	*
Cultivar × Treatment		**	NS	NS	NS	NS	*	**	NS	NS

[i] Means (n = 10 except for vitamin A where n = 6) within a column followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

[ii] Main effects and interactions significant at * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), or nonsignificant (NS).

Results and Discussion

Daily water temperature in the ambient treatment ranged from 19.3 to 30.6 °C and averaged 24.3 °C, whereas it ranged from 20.0 to 25.3 °C and averaged 22.7 °C in the chilled treatment (data not shown). A significant cultivar × treatment interaction was observed for SPAD, shoot dry weight, and root fresh weight (Table 1). SPAD was greatest in 'Paris Island' chilled but was not different from ambient but significantly greater than any other cultivar. SPAD values were significantly greater in chilled than ambient for 'Antonet'. Shoot dry weight was greatest for 'Waldman's Dark Green' ambient but was not different from 'Waldman's Dark Green' chilled, 'Paris Island' ambient, 'Jericho' at either temperature or ambient 'Antonet'. For 'Antonet', shoot dry weight was significantly greater under ambient than chilled conditions. 'Waldman's Dark Green' ambient was significantly greater than chilled for root fresh weight and also different from 'Antonet' and 'Rex' at either temperature.

Plant height, plant width, number of leaves, shoot fresh weight, and vitamin A had a significant cultivar affect. Plant height and width were greatest for 'Waldman's Dark Green'. 'Rex' had the greatest number of leaves. 'Jericho had the greatest shoot fresh weight but was only different from 'Antonet'. 'Antonet', a red loose-leaf type, had the greatest vitamin A content. This is in contrast to what is known as romaine type lettuce, which was thought to have a higher β-carotene content than other varieties of lettuce (US Department of Agriculture 2020). Significant treatment effects were seen for number of leaves and root dry weight. The number of leaves and root dry weight were greater in ambient conditions. Thakulla et al. (2021) reported greater °Brix, plant width, shoot and root fresh weight, and shoot and root dry weight with chilled (21.1 °C) water of 17 lettuce cultivars grown over three seasons.

Phosphorus (P), magnesium (Mg), calcium (Ca), sulfur (S), iron (Fe), zinc (Zn), and manganese (Mn) were significantly greater in the chilled than ambient temperature for 'Rex' (Table 2). For 'Jericho', P, K, Mg, Ca, S, Fe, Zn, and Mn were significantly greater in the chilled than ambient treatment. For 'Waldman's Dark Green', the chilled treatment increased the concentration of each measured nutrient except calcium, which showed no difference. Air and root temperatures have been found to influence nutrient uptake in lettuce 'Red Wave' (Sakamoto and Suzuki 2015). No significant elemental concentration differences were found for 'Paris Island' and 'Antonet' between ambient and chilled treatments.

Conclusion

Chilled water did not lead to increased shoot fresh weight but did increase most leaf nutrients in 'Rex', 'Jericho', and 'Waldman's Dark Green'. Vitamin A content was unaffected by water temperature except 'Waldman's Dark Green', which was lower in chilled water. 'Antonet' was the smallest plant but had the greatest concentration of vitamin A content. Future research could investigate the relationship between air and water temperature and how influential each variable is on plant growth and vitamin A content alone or in combination at different levels.

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Table 2. Least square means treatment differences between lettuce cultivars and water temperature (chilled at 23 °C or ambient) for nutrient content for five lettuce cultivars in nutrient film technique hydroponic systems at research greenhouses in Stillwater, OK, USA.

Cultivar	Treatment	TN[i] (%)	P (%)	K (%)	Mg (%)	Ca (%)	S (%)	Fe (mg·L^{-1})	Zn (mg·L^{-1})	Mn (mg·L^{-1})
Antonet	23	5.4 a[ii]	0.9 a	3.3 a	0.5 a	0.8 a	0.3 a	444.9 a	67.9 a	168.0 a
	Ambient	5.2 a	0.8 a	2.5 a	0.4 a	0.8 a	0.2 a	125.9 a	51.1 a	184.9 a
Rex	23	5.9 a	1.7a	5.3 a	0.7 a	1.5 a	0.4 a	189.9 a	96.8 a	293.8 a
	Ambient	5.7 a	0.2 b	0.5 a	0.1 b	0.2 b	0.1 b	37.7 b	9.1 b	27.6 b
Jericho	23	5.1 a	1.6 a	2.9 a	0.7 a	1.5 a	0.3 a	285.8 a	97.3 a	346.6 a
	Ambient	5.0 a	0.2 b	0.4 b	0.1 b	0.2 b	0.1 b	30.1 b	9.3 b	33.7 b
Paris Island	23	5.7 a	0.7 a	1.0 a	0.3 a	0.7 a	0.1 a	101.2 a	31.6 a	153.0 a
	Ambient	5.3 a	0.2 a	0.4 a	0.1 a	0.2 a	0.1 a	21.0 a	8.2 a	34.1 a
Waldman's Dark Green	23	6.0 a	1.2 a	3.9 a	0.6 a	1.1 a	0.4 a	228.1 a	84.4 a	297.5 a
	Ambient	0.1 b	0.1 b	0.4 b	0.1 b	0.1 a	0.1 b	19.6 a	8.9 b	30.5 b

[i] TN = total nitrogen.

[ii] Means (n = 3) within a column for a cultivar followed by same lowercase letter are not significantly different by pairwise comparison in mixed model ($P \leq 0.05$).

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