Research Article

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Root-zone temperature and nitrogen affect the yield and secondary metabolite concentration of fall- and spring-grown, high-density leaf lettuce

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Abstract

BACKGROUND: Understanding the effects of temperature and nitrogen levels on key variables, particularly under field conditions during cool seasons of temperate climates, is important. Here, we document the impact of root-zone heating and nitrogen (N) fertility on the accumulation and composition of fall- and spring-grown lettuce biomass. A novel, scalable field system was employed.

RESULTS: Direct-seeded plots containing a uniform, semi-solid, and nearly stable rooting medium were established outdoors in 2009 and 2010; each contained one of eight combinations of root-zone heating (−/+) and N fertility (0, 72, 144, and 576 mg day^{-1}). Root-zone heating increased but withholding N decreased biomass accumulation in both years. Low N supplies were also associated with greater anthocyanin and total antioxidant power but lower N and phosphorus levels. Tissue chlorophyll a and vitamin C levels tracked root-zone temperature and N fertility more closely in 2009 and 2010, respectively.

CONCLUSIONS: Experimentally imposed root-zone temperature and N levels influenced the amount and properties of fall- and spring-grown lettuce tissue. Ambient conditions, however, dictated which of these factors exerted the greatest effect on the variables measured. Collectively, the results point to the potential for gains in system sustainability and productivity, including with respect to supplying human nutritional units.

Keywords: seasonal production; human nutrition; antioxidant; root-zone; fertility; *Lactuca sativa*

INTRODUCTION

Modern horticultural systems rely heavily on the ability to alter environments immediately surrounding crops.[1,2] Temperature, light and mineral nutrient levels are among the most intensely managed microclimate components. Together, they influence the production and composition of crop biomass beginning with the formation of primary metabolites and concluding with the synthesis, deposition and breakdown of secondary compounds.[3–6] Interest in plant biomass accumulation and composition – specifically, the efficiency with which cropping systems convert natural and often diminishing resources into a selected suite of compounds – is fueled in part by their roles in human nutrition, energy production and other arenas. Overall, the identification of microclimates conducive to abundant harvests of crops with an idealized make-up lags behind the interest to more effectively deploy agricultural systems in the maintenance and enhancement of human well being, e.g. as influenced by the availability of nutritionally dense products. This knowledge gap is particularly wide when considering the design of outdoor systems operating in areas with distinct seasons (e.g. >40°N or S latitude).

Human health benefits associated with fruit and vegetable consumption are increasingly clear and supported by research from many disciplines. Beyond providing essential nutrients, fruit and vegetable intake appears to impact short- and long-term human health factors, including threats of stroke and cancer, heart and other disease.[5–9] Plant compounds acting as antioxidants and radical scavengers is one of several proposed mechanisms for this plant-based protection.[6–10]

The causal mechanisms underlying the protective effects of fruit and vegetable consumption are not all clear; however, there

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is little doubt on other matters, namely: (1) that many plant secondary compounds (e.g. phenolics, vitamin C and various carotenoids) can act as antioxidants, and (2) that phenolics are numerous, widely distributed and often profoundly influenced by environmental conditions.[3–9] The latter point is germane to the heightened design of sustainable production systems as instruments of health and commerce, in part through managing microclimate–yield–tissue composition relationships. The literature provides insight on some of these relationships. For example, anthocyanin biosynthesis and accumulation are up-regulated by low temperature,[3,11–13] as phenylalanine ammonia lyase and chalcone synthase mRNA levels increase in response to low temperatures in the presence of light.[13] Also, low N levels are reported to increase phenylpropanoid pathway activity or the levels of pathway products.[14,15] Relationships involving N levels and secondary metabolism are only partially characterized but emerging.[14–17]

Secondary metabolite levels influence far more than human health, of course. Anthocyanins are thought to protect the photosynthetic apparatus against photo-induced oxidative damage when low temperatures slow photosynthesis,[13,18–20] and they may also protect against low temperature stress by acting as osmotica.[18] Likewise, fluctuations in phenolic metabolism under low N may be due to the need for ammonia released from phenyalanine by phenylalanine ammonia lyase activity and/or for the photoprotection of photosynthetic systems disrupted by low N.[14,15,21,22] Still, increases in secondary compound levels may be associated with decreases in primary productivity.[8,23–25] Given the influence of abiotic conditions (e.g. nutrition, light, temperature) on primary and secondary productivity, setting these conditions in agricultural systems will strengthen their position in health and commerce. Little attention to date has been given to setting environmental conditions during portions of the year with historically low levels of production.

Antioxidant activity and secondary metabolite composition in leafy crops are reported to fluctuate with nutrient, light and temperature levels.[12,26–29] With intervention, agricultural productivity can be maintained when ambient seasonal growing conditions (e.g. low temperature and/or light) otherwise preclude it. However, too little is known regarding the influence of specific microclimate management techniques on crop biomass accumulation and composition during these periods. While tools exist to alter temperature, light, humidity, soil moisture and other abiotic components of the plant environment, this work focuses only on the direct modification of soil temperature and nitrogen fertility and their separate and combined effects on lettuce (*Lactuca sativa* L.) biomass accumulation and composition. Lettuce was chosen as the experimental crop because it: (1) tolerates and responds to a wide range of environmental conditions, (2) is harvested and consumed at a range of developmental stages, (3) is a leading component of a healthy diet, (4) is an increasingly popular commodity among producers and consumers, especially in the US Midwest, and (5) has been included in previous related work conducted indoors and outdoors. Employing a novel rooting medium and reliable nutrient delivery system, we gained valuable insight regarding the interplay of primary and secondary metabolism in lettuce exposed to historically under-studied conditions.

EXPERIMENTAL

Site and nutrient delivery system

The experiment was conducted in duplicate runs in 2009 and 2010 at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH, USA (latitude: 40° 46′ N; longitude: 81° 55′ W). The study was located in an outdoor gravel-bed laboratory operated by the Department of Food, Agricultural and Biological Engineering. OARDC is a beta testing site for an Argus Titan Nutrient Delivery System (White Rock, British Columbia, Canada) which was used throughout the study. The Titan system is capable of executing highly tailored nutrient delivery regimens, in part through single element dosing. The multi-feed system has been designed for small-scale experimental and commercial application and is capable of timed and accurate nutrient solution delivery rates of 0.25–10 L min^{-1}. Here, fertility treatments were implemented by calculating and programming the timed injection of stock solutions at dilution ratios resulting in each chosen fertility level. Valves and separate irrigation lines then delivered each specifically mixed treatment solution to targeted outdoor raised beds at the study site approximately 30 m from the injection point. All aspects of the nutrient delivery system were controlled electronically through a software interface connected to sensors which continuously monitored the flow volume, electrical conductivity (EC), pH and temperature of the irrigation solution (fertigation) to ensure delivery of the selected nutrient levels.

Experimental and growing system design

This factorial experiment included two levels of root-zone heating (yes/no) and four levels of nitrogen (N) supply (0, 72, 144 and 576 mg day^{-1}). For both experimental runs, treatments were replicated four times and arranged in a split-plot design with root-zone heating and N fertility functioning as the main and sub-plots, respectively. All eight wood-framed, randomized, raised bed (0.6 × 2.4 × 0.15 m) main plots contained all four 0.36 m^2 randomized N fertility sub-plots. Woven plastic ground cover (Hummert International, St Louis, MO, USA) was attached with staples to the main plot frames to contain the inorganic media. Root-zone heated main plots included a 12.2 m automatic electric heating cable (Wrap-On Co., Bedford Park, IL, USA) triggered to function at medium temperatures below 23 °C. The operation of each cable was governed by an integrated thermostat in continual contact with the rooting medium. The cable was securely attached with zip ties to the plastic ground cover to assure even heating and prevent cable contact. All main plots were covered with a 0.02 mm slitted polyethylene low tunnel (Hummert International) stretched over four wire hoops to create partially enclosed aerial volumes of approximately 0.50 m^3 main $plot^{-1}$. Tunnel sides and ends were secured to the frame with wooden lath. Polystyrene foam dividers (0.15 m × 0.60 m × 1.25 cm; Dow Chemical Co., Midland, MI, USA) separated fertility sub-plots within each main plot. A layer of KapMat cloth (BFG Supply Co., Burton, OH, USA) was installed on the sides and bottom of each sub-plot to enhance drainage and prevent cross-contamination between N fertility treatments. The growing medium contained the following two components (by volume): 60% industrial grade 20 : 40 silica sand (Best Sand, Chardon, OH, USA) and 40% Primera One calcinated clay field conditioner (Profile Products, LLC, Buffalo Grove, IL, USA). Medium contained 50.1% total pore space (32.0% air, 18.1% capillary) with a bulk density of 1.32 g cm^{-3} (Brookside Laboratories, Inc., New Knoxville, OH, USA). The two dry components were blended in a Bouldin and Lawson model 12 193 mixer (Bouldin and Lawson, McMinnville, TN, USA). Sub-plots were loaded with approximately 0.04 m^3 of medium and new medium was used in 2009 and 2010.

Crop establishment

Organically primed and pelleted but conventionally produced 'Outredgeous' lettuce seed (Johnny's Selected Seeds, Winslow, ME, USA) were sown on 1 October 2009 and 1 April 2010. Approximately 1000 pre-weighed seeds were sown in each of the 32 sub-plots in seven rows separated by 7.5 cm, an anticipated plant density recommended by the supplier for baby leaf lettuce production. Seeds were evenly placed by hand on the formed substrate and covered with approximately 1 cm of vermiculite.

Nutrient application

Fertigation was delivered using 0.6 cm diameter soaker drip-line with emitters at 15 cm spacing (DIG Irrigation Products, Vista, CA, USA). Each sub-plot contained a 40 × 40 cm square of drip-line with a center row with a total of 12 individual emitters. At the measured 160–190 kPa, these 12 emitters were capable of delivering approximately 36 000 mL h^{-1}. All irrigation was applied in automatic, programmed 3-minute cycles which delivered approximately 1,800 mL sub-$plot^{-1}$ of nutrient solution at each watering. Irrigation was applied twice (10 : 00, 16 : 00; 3600 mL sub-$plot^{-1}$ total) and three times daily (10 : 00, 12 : 30, 15 : 00; 5400 mL sub-$plot^{-1}$ total) in 2009 and 2010, respectively. A modified Hoagland's solution,[30] consisting of reagent grade 0.5 mol L^{-1} KCl, 0.5 mol L^{-1} K_2SO_4, 0.5 mol L^{-1} $MgSO_4 \cdot 7H_2O$, 0.05 mol L^{-1} $Ca(H_2PO_4)_2 \cdot 2H_2O$, 0.01 mol L^{-1} $CaSO_4 \cdot 2H_2O$ and 0.5 mol L^{-1} KH_2PO_4, was diluted as programmed by the Argus system for each scheduled watering. Municipal water was used to dilute the nutrient solution and possibly provide additional cations. Fertigation pH was monitored by the Argus system and H_2SO_4 was automatically added to maintain pH near 6.0. Additionally, 250 mL sub-$plot^{-1}$ of a micronutrient mix (STEM; Scotts Company LLC, Marysville, OH, USA) containing 0.0105 g kg^{-1} S, 0.001 g kg^{-1} B, 0.0024 g kg^{-1} Cu, 0.0056 g kg^{-1} Fe, 0.006 g kg^{-1} Mn, 0.00003 g kg^{-1} Mo, and 0.0034 g kg^{-1} Zn was hand-delivered weekly to each sub-plot.

The major nutrients of phosphorus (P), potassium (K), magnesium (Mg), sulfur (S), and calcium (Ca) were provided at consistent and sufficient levels throughout both experimental runs. In 2009, the modified Hoagland's solution was diluted at 20 : 1 to deliver 155 mg P, 529 mg K, 108 mg Mg, 292 mg S, and 22 mg Ca daily (0.043, 0.147, 0.030, 0.081 and 0.006 g kg^{-1}) in the fertigation solution. When irrigation volume was increased in 2010, dilution ratios were adjusted to 30 : 1 (0.029, 0.098, 0.020, 0.054, 0.004 g kg^{-1}) to ensure the application of the same total daily quantity of each nutrient as in 2009.

Nitrogen was supplied in a separate 0.11 mol L^{-1} NH_4NO_3 stock solution, diluted and then mixed by the Argus system with the macronutrient solution detailed above prior to delivery to sub-plots. Prior to the initiation of N fertility treatments in sub-plots, an establishment phase of 20 days in 2009 and 15 days in 2010 ensured adequate plant germination and growth before beginning N fertility treatments. During the establishment phase, sufficient levels of N, 288 mg day^{-1} in 2009 and 144 mg day^{-1} in 2010, were delivered to all sub-plots. In 2010, establishment phase N levels were decreased due to the concern that delayed effect of N treatment in 2009 were due to residual N in sub-plots from establishment phase fertilization. In both experimental runs, this establishment period was followed by a treatment period of 21 days (2009) and 18 days (2010) where four levels of N (0, 72, 144 and 576 mg day^{-1}) were applied to individual root-zone heating (yes/no) × N fertility (four levels) experimental units (sub-plots).

Table 1. Average above and below surface temperatures (°C) and standard errors in 2009 and 2010 in outdoor, plastic-covered raised beds with or without the incorporation of root-zone heating cables

	Root-zone heating			
	No		Yes	
Year	Above surface	Below surface	Above surface	Below surface
2009	10.17 ± 0.053	10.99 ± 0.094	11.77 ± 0.14	19.11 ± 0.54
2010	14.87 ± 0.065	14.57 ± 0.14	15.63 ± 0.092	20.52 ± 0.38

Environmental data

Air and soil temperatures were recorded continuously at 15-min intervals using Hobo ProV2 data loggers (Onset Computer Co., Pocasset, MA, USA). Each of the main heating plots was equipped with a separate data logger attached to a wooden stake and covered with a radiation shield. Air temperature was recorded 20 cm above the media surface and soil temperature was recorded 4–5 cm below the surface. Table 1 provides the average soil and air temperature from 2009 and 2010. Data for pH, EC and system flow volume for each watering cycle were provided by the Argus system while an outdoor weather station provided measures of total solar radiation.

Crop data

Treatment effects on plant status were assessed at four stages using destructive and non-destructive measures. Assessments were completed on days 5, 14, 28 and 41 after seeding in 2009 and on days 5, 14, 25 and 33 after seeding in 2010. Stand establishment as a function of root-zone heating was calculated from counts of emerged seedlings taken five days after planting on 25 cm of the center of the third row in each sub-plot. Subsequent assessments involved destructive sampling of portions of each sub-plot and were scheduled to either precede or follow the initiation of N fertility treatments (20 days and 15 days after seeding in 2009 and 2010, respectively). Samples collected on days 14, 25 and 28 after seeding were taken from 25 cm of a randomly chosen row (excluding edge rows 1 and 7) in each sub-plot. Whole plants were removed gently by hand and placed in a sealed plastic bag and cooled (5 °C) until measurements were taken. All plants from each sub-plot sample were counted and weighed. Thereafter, ten representative plants were selected and the roots were removed from the shoots with a razor blade and both shoot and root portions were weighed collectively. The final assessment involved removal of nearly all above-ground biomass from a 30 × 60 cm section of each sub-plot and placing it on ice until being processed. After weighing, separate sub-samples were dried and flash frozen for tissue elemental and biochemical composition analysis. Laboratory sub-samples were flash-frozen within 3 h of harvest and stored at −20 °C or −80 °C until being processed in the laboratory.

Tissue pigment composition

Anthocyanin and chlorophyll a (Chl A) concentrations were determined after pigment extraction from flash-frozen tissue samples stored at −20 °C. A 10 g sample of frozen tissue was homogenized with 5 g distilled, deionized water in a 50 mL falcon tube (Thermo Fisher Scientific, Pittsburgh, PA, USA) using a Kinematica 10–35 Polytron (Kinematica, Bohemia, NY, USA). A

5 g sub-sample of the homogenized tissue was then extracted sequentially with 20 mL, 20 mL, and then 10 mL of 1% HCl acidified methanol.[12,27,31] Each hour-long extraction took place in the dark at 5 °C and then samples were centrifuged at 7800 × *g* for 15 min at approximately 20 °C in a Sorvall Legend RT (Thermo Fisher Scientific). Following the final extraction, the leaf tissue and all three extraction volumes were combined and vacuum filtered through 297 μm polypropylene mesh (Spectrum Laboratories, Rancho Dominguez, CA, USA) using a Buchner funnel. Samples were then centrifuged a final time and immediately read on a Beckman Coulter DU730 spectrophotometer (Beckman Coulter, Brea, CA, USA). Anthocyanin and Chl A absorbances were obtained at 530 nm and 420 nm, respectively.[12,31] Standard curves for cyanidin-3-glucoside (Chromadex, Irvine, CA, USA) and Chl A (Sigma Aldrich, St Louis, MO, USA) were then used to calculate tissue pigment concentrations from spectrophotometric absorbances.

Tissue antioxidant power, and vitamin C and sugar content

The ferric reducing antioxidant power (FRAP) test was used to determine total antioxidant power.[32] Duplicate samples of the above described extracts were combined with 3 mL of a working solution, incubated for precisely 1 h at room temperature, and read at 593 nm in a Beckman Coulter DU730 spectrophotometer. The FRAP working solution contained 30 mmol L^{-1} sodium acetate buffer at 3.6 pH, 20 mmol L^{-1} Fe_3Cl, and 10 mmol L^{-1} 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) in a 10 : 1 : 1 ratio. A 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) standard curve was used to convert spectrophotometric absorbances to trolox equivalents (TE) g^{-1} fresh weight (fw). A modified FRAP procedure (FRASC) was also used to quantify vitamin C levels in the frozen lettuce tissue.[32] Samples used for vitamin C quantification were stored at −80 °C from harvest. A 7 g frozen tissue sample was homogenized with distilled, deionized water as described above in a 1 : 1 ratio. Samples were then vacuum filtered with a Buchner funnel as described above and centrifuged at 7800 × *g* for 20 min at 4 °C. Sets of duplicate 100 μL samples were then combined with either 40 μL of water or 40 μL of 4 U mL^{-1} ascorbate oxidase to degrade the vitamin C. The working solution (3 mL) detailed above was added to each sample and incubated at room temperature for precisely 1 h. Samples were then read at 593 nm and the absorbances of the ascorbate oxidase samples were subtracted from the water samples to estimate the vitamin C content in each sample. An ascorbic acid standard curve was then used to calculate vitamin C content. Frozen lettuce samples of approximately 2–3 g were thawed and juiced through cheesecloth (American Fiber and Finishing Inc., Ablemarle, NC, USA) in duplicate to determine sucrose sugar content in °BRIX by reading on a Leica Abbe Mark II refractometer (Leica Inc., Buffalo, NY, USA).

Tissue elemental composition

Leaf tissue was dried at 55 °C for 72 h and ground with a mortar and pestle to pass a 2 mm screen. Total plant nitrogen was analyzed by combustion (AOAC method 990.03). For other tissue elements, tissue was dry ashed and acid digested and elemental composition was determined by inductively coupled plasma spectrometry (OSU Service Testing and Research Laboratory, Wooster, OH, USA).

Data analysis

The experiment was repeated in 2009 and 2010 using nearly identical methods. However, slight differences between these repetitions or runs in plot establishment-phase duration and fertility, the timing of sampling episodes and total run duration prompted us to analyze data from the two runs separately. In addition, data from each of the three destructive sampling points within each run were analyzed separately due to the relative calendar positions of sampling points and the initiation of fertility treatments in each year. Moreover, the total quantity of sampled plant material was larger at the third than at the previous two harvests. A Proc Univariate procedure was carried out to test for normality on all data; data with a non-normal distribution were analyzed further using the Proc Glimmix model (SAS version 9.2; SAS Institute, Cary, NC, USA). This model was employed on data which displayed skewed distributions that were more accurately analyzed following a log-normal transformation. Means were back transformed for inclusion in tables. Normally distributed data were analyzed using Proc Mixed (1) 2009 (Chl A, N, P, and K) and (2) 2010 (25-day and 33-day biomass, vitamin C, N, P and K). Temperature and N fertilization were analyzed as fixed effects and replications within years were analyzed as random effects in Proc Mixed and Glimmix analyses. Treatment means were separated using diff statements at a $P < 0.05$ level of significance. Proc Corr was used to calculate Pearson correlation coefficients.

RESULTS

Within years, main effects predominated and interactions were rare. Root-zone heating tended to increase biomass accumulation, withholding N after crop establishment tended to reduce it (Table 2), and biomass accumulation and enrichment (e.g. anthocyanin, total antioxidant power) were negatively related (Table 3). Close inspection of the yearly data reinforces these trends while also highlighting interesting exceptions.

Effects of root-zone heating on biomass

Shoot fresh weight in 2009 was significantly greater in root-zone heated versus unheated plots when measured 14 days, 28 days and 41 days after sowing (6 days before the initiation of N fertility treatments and 8 days and 21 days thereafter) (Table 2). In 2010, shoot fresh weight was greater in root-zone heated plots than in unheated plots when measured 14 days, 25 days and 33 days after sowing (1 day before the initiation of N fertility treatments and 10 days and 18 days thereafter).

Effects of root-zone heating on composition

In 2009, anthocyanin (g kg^{-1}), Chl A (g kg^{-1}), vitamin C (g kg^{-1}), sugar (°BRIX) and total antioxidant power (μmol L^{-1} trolox equivalents g^{-1} fw) levels were higher in unheated plots than in heated plots while in 2010 root-zone heating had no effect on N (g kg^{-1}), P (g kg^{-1}), K (g kg^{-1}), anthocyanin, Chl A, vitamin C, sugar or total antioxidant power levels (Table 4).

Effects of nitrogen fertility on biomass

In 2009, N fertility had little effect on shoot fresh weight 8 days after treatment initiation; however, shoot fresh weight was lower in zero-N plots than in all treatments after 21 days exposure to varying N levels (Table 2). In 2010, N fertility significantly affected shoot fresh weight measured 10 days after the initiation of N fertility treatments. Biomass was greatest at 576 mg N, intermediate at 72 and 144 mg N and least at 0 mg N. At 18 days after N treatment initiation, biomass was least at 0 mg N and similar in the 72, 144 and 576 mg N plots.

Table 2. Fresh shoot weight (g m^{-2}) of leaf lettuce grown with under four levels (0, 72, 144, 576 mg day^{-1}) of N fertility from destructive samples taken 14 days, 28 days, and 41 days after sowing in 2009 and 14 days, 25 days, and 33 days after sowing in 2010

Factor		Sampling one (g m^{-2})	Sampling two (g m^{-2})	Final harvest (g m^{-2})
2009 Analysis of variance				
Heat		<0.0001	<0.0001	<0.0001
Fertility		NS	NS	0.0003
Heat × fertility		NS	NS	NS
Root-zone heating	−	37.5[b]	264.9[b]	447.6[b]
	+	95.1[a]	812.7[a]	1666.5[a]
N Fertility (mg day^{-1})	0	50.5	424.9	649.8[b]
	72	65.3	429.9	937.8[a]
	144	56.8	473.3	931.6[a]
	576	67.9	536.1	980.1[a]
2010 Analysis of variance				
Heat		0.0004	0.0023	0.0067
Fertility		NS	0.0004	<0.0001
Heat × fertility		NS	NS	NS
Root-zone heating	−	113.3[b]	957.5[b]	1294.5[b]
	+	175.4[a]	1626.7[a]	2002.2[a]
N Fertility (mg day^{-1})	0	131.4	940.0[c]	977.9[b]
	72	155.7	1286.5[b]	1726.1[a]
	144	133.9	1268.5[b]	1899.1[a]
	576	144.0	1673.5[a]	1990.3[a]

Letters denote root-zone heating and N fertility levels separated by difference statements at $P < 0.05$ in Proc Mixed and Glimmix when fixed main effects were significant at $P < 0.05$. NS denotes not significant at $P = 0.05$.

Table 3. Pearson correlation coefficients among fresh shoot biomass and tissue composition measured 41 days after sowing in 2009 and 33 days after sowing in 2010

	2009							2010						
	Biomass	Antho	Chl A	Antioxidant	Vit. C	Sugar	N	Biomass	Antho	Chl A	Antioxidant	Vit. C	Sugar	N
Biomass	–	−0.70	−0.86	−0.74	−0.60	−0.84	NS	–	−0.76	NS	−0.76	−0.52	−0.51	0.57
Anthocyanin	−0.70	–	0.77	0.99	0.67	0.62	−0.65	−0.76	–	NS	0.99	0.60	0.41	−0.81
Chl A	−0.86	0.77	–	0.77	0.66	0.77	NS	NS	NS	–	NS	NS	NS	NS
Antioxidant power	−0.74	0.99	0.77	–	0.63	0.62	−0.66	−0.76	0.99	NS	–	0.60	0.40	−0.85
Vitamin C	−0.60	0.67	0.66	0.63	–	0.45	NS	−0.52	0.60	NS	0.60	–	0.51	−0.80
Sugar	−0.84	0.62	0.77	0.62	0.45	–	NS	−0.51	0.41	NS	0.40	0.51	–	NS
N	NS	−0.65	NS	−0.66	NS	NS	–	0.57	−0.81	NS	−0.85	−0.80	NS	–

$P < 0.05$. NS, not significant.

Effects of nitrogen fertility on composition

In 2009, anthocyanin and total antioxidant power was highest in tissue taken from plots receiving zero N, while plots receiving 0 and 72 mg N recorded statistically higher values than the 576 mg plots with intermediate values at 144 mg N (Table 4). The statistical interaction between root-zone heating and N was significant ($P = 0.022$) only for anthocyanin; however, root-zone heating affected only the magnitude, not direction, of the N fertility effect. Chl A, vitamin C and sugar content were not significantly affected by N fertility as a main effect (Table 4). Tissue N concentrations 21 days after N treatment initiation were highest and similar at 576 and 144 mg N with 0 and 72 mg lower than the 576 mg plots. Phosphorus concentrations were similar at 576, 144 and 72 mg N but higher than in the 0 mg N control group. Potassium levels at 0 and 576 mg N were similar but lower than at 72 and 144 mg N (Table 4). Biomass at 21 days after N treatment initiation was negatively correlated with anthocyanin, Chl A, total antioxidant power, vitamin C and sugar content (Table 3).

In 2010, tissue composition was similar to 2009 in that anthocyanin and total antioxidant power levels were highest in tissue taken from plots receiving 0 mg N after establishment. However, a more graded response to 72, 144 and 576 mg N was present in 2010 (Table 4). Unlike 2009, vitamin C in 2010 in the 0 mg treatment was higher than all other N treatments and the 72 mg treatment was higher than the similar 144 and 576 mg treatment (Table 4). Chl A was higher in the 576 mg treatment than the 0, 72 and 144 mg treatments, which were similar. Sugar and potassium levels were not significantly affected by N exposure. Tissue N concentrations measured 18 days after N treatment initiation followed a graded response to N exposure, being greatest at 576 mg N, intermediate at 72 and 144 mg N and least at 0 mg

Table 4. Tissue composition of leaf lettuce grown under varying root-zone heating and nitrogen fertility treatments 41 days after sowing in 2009 and 33 days after sowing in 2010

Factor		Anthocyanin (g kg^{-1} fw)	Chlorophyll A (g kg^{-1} fw)	Antioxidant power (μmol L^{-1} trolox equivalents g^{-1} fw)	Vitamin C (g kg^{-1} fw)	Sugar (°BRIX)	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
2009 Analysis of variance									
Heat		0.0003	<0.0001	0.0021	0.0084	0.0015	NS	NS	NS
Fertility		<0.0001	NS	<0.0001	NS	NS	0.002	0.011	0.0052
Heat × fertility		0.022	NS	NS	NS	NS	NS	NS	NS
Root-zone heating	−	0.469^{a}	0.663^{a}	19.8^{a}	0.074^{a}	3.8^{a}	56.9	7.8	62.4
	+	0.305^{b}	0.530^{b}	13.7^{b}	0.034^{b}	2.4^{b}	56.9	9.2	71.2
N Fertility (mg day^{-1})	0	0.543^{a}	0.617	22.4^{a}	0.057	3.2	46.8^{c}	7.5^{b}	64.4^{b}
	72	0.364^{b}	0.591	16.2^{b}	0.043	2.8	56.0^{b}	8.6^{a}	72.1^{a}
	144	0.328^{bc}	0.583	14.7^{bc}	0.047	2.9	60.2^{ab}	9.0^{a}	68.9^{a}
	576	0.316^{c}	0.595	13.8^{c}	0.055	3.1	64.7^{a}	9.0^{a}	61.9^{b}
2010 Analysis of variance									
Heat		NS	NS	NS	NS	NS	NS	NS	NS
Fertility		<0.0001	0.032	<0.0001	<0.0001	NS	0.0002	0.022	NS
Heat × fertility		NS	NS	NS	NS	NS	NS	NS	NS
Root-zone heating	−	0.403	0.511	19.8	0.114	2.3	46.3	6.8	43.4
	+	0.373	0.464	18.8	0.094	2.1	44.6	6.9	42.8
N Fertility (mg day^{-1})	0	0.610^{a}	0.481^{b}	27.9^{a}	0.158^{a}	2.4	31.9^{c}	5.9^{b}	42.3
	72	0.386^{b}	0.471^{b}	19.4^{b}	0.122^{b}	2.2	44.4^{b}	7.1^{a}	45.5
	144	0.320^{c}	0.479^{b}	16.6^{c}	0.066^{c}	2.1	46.8^{b}	6.9^{a}	45.0
	576	0.300^{c}	0.518^{a}	15.3^{c}	0.071^{c}	2.1	58.7^{a}	7.5^{a}	39.5

Letters denote root-zone heating and N fertility levels separated by difference statements at $P < 0.05$ in Proc Mixed and Glimmix when fixed main effects were significant at $P < 0.05$. NS denotes not significant at $P = 0.05$.

N. As a group, tissue P concentrations following exposure to 72, 144 and 576 mg N were similar but greater than in plots given no N. Final yield was positively and significantly correlated (Table 3) with elemental leaf N and negatively correlated with anthocyanin, vitamin C, sugar, and total antioxidant power.

DISCUSSION

Growing system and nitrogen supply

The primary goal of this work was to document root-zone temperature and nitrogen fertilization effects on lettuce biomass accumulation and composition. These effects have been tested in highly controlled indoor settings but rarely in environments resembling field conditions. The specialized and costly infrastructure common to highly controlled systems is prohibitive in typical production settings. And, root and shoot parameters can vary in liquid versus solid rooting media-based culture systems. Therefore, our secondary goal was to impose the temperature and fertility treatments using a cultural system offering high levels of experimental control but with physical properties more similar to field soil, thereby promoting root growth that may be expected under field conditions. The system used here is scalable and suitable for use in experimental and limited commercial settings and its main components include electric heating cables (replaceable with hot water) and an inorganic, chemically stable and potentially reusable rooting medium. Use of the Argus Titan Nutrient Delivery System also served our specific experimental objectives.

Root morphology and architecture can vary with rooting medium (e.g. liquid versus solid), primarily due to the impedance it imposes.[33] Root morphology, in turn, can influence nutrient uptake and other variables.[34] Here, sand was mixed with calcinated clay to provide a solid, inorganic medium that physically supported the root system but lacked the nitrogen mineralization potential of organic substrates. Calcinated clay was also included to provide cation exchange and buffering capacity reported to improve vegetable production in inorganic mediums.[35] Overall, the outdoor experimental system integrated a rooting medium with the impedance of soil with temperature and nutrient control typical of indoor soil-less systems.

Nitrogen (N) was supplied separately from the other essential plant nutrients in an NH_4NO_3 solution. Supplies of non-target nutrients remained consistent among all treatments. N supplied as NH_4^+ and NO_3^- tends to enhance crop growth and reduce nitrate accumulation in leafy crops,[36,37] which can be a human health hazard. Also, the presence of both N forms may have equalized the opportunity for plant N uptake across seasons since ammonium and nitrate uptake appear to be preferred at low and high soil temperatures, respectively, although translocation of NH_4^+ and NO_3^- can be temperature-limited as well.[38–40] In follow-up work, this system would allow N delivery amount and form to be set according to root-zone temperature.

Effect of root-zone heating on biomass

Overall, trends in leaf lettuce biomass and composition were similar across years. However, ambient conditions appeared to dictate whether temperature or N levels were most limiting to biomass accumulation and composition in any one year. Conditions in 2009 were less conducive to growth than in 2010; as a result, root-zone heating effects on biomass and composition were enhanced in 2009 relative to 2010. In 2010, however, root-zone heating effects were diminished and N treatment effects were enhanced.

For leaf biomass specifically, the presence of root-zone heating increased shoot fresh weight in both years, an outcome consistent with previous reports.[36,41,42] However, the effect was greatest in 2009 and corresponded to the potential impact of soil heating given ambient temperatures. Root-zone heating increased shoot weight relative to unheated plots by 270% in 2009 and 55% in 2010. Average air temperatures were nearly 5 °C warmer in 2010 than in 2009 and, therefore, generally supported growth regardless of root-zone heating. Likewise, root-zone heating appeared to have a more pronounced effect on temperature profiles in 2009 than in 2010 since the average sub-surface temperature of heated plots exceeded that of unheated plots by more than 8 °C in 2009 but by less than 6 °C in 2010. Temperatures in fall 2009 resembled long-term values provided by the National Climatic Data Center (http://www.ncdc.noaa.gov/oa/mpp/freedata.html); however, temperatures in spring 2010 averaged approximately 5° warmer than historical values. Results here suggest that rising spring-time temperatures facilitated the onset and progression of N treatment effects more so than declining fall-time temperatures. However, this hypothesis requires further testing in field settings, possibly with additional variables such as light.

Effects of root-zone heating on composition

Root-zone temperature also affected leaf tissue composition. Anthocyanin, Chl A, vitamin C and sugar levels, and total antioxidant power were higher in unheated than heated plots in 2009. However, in the warmer season of 2010, root-zone heating did not influence these leaf tissue composition parameters. These results are consistent with previous findings outlining the influence of low and high temperature on secondary metabolism and the levels of anthocyanins specifically.[11,12,43] Phenolic, anthocyanin, vitamin C, sugar and Chl levels in lettuce and other crops have risen in response to reduced root or shoot-zone temperatures.[12,29,40,44,45] Light levels also could have contributed to the often higher levels of Chl recorded in 2009 versus 2010 as greater pigment concentrations may have allowed for maximal levels of light interception and photosynthesis under the lower light conditions of 2009.[46]

Effects of nitrogen fertility on biomass

Biomass accumulation among the four N fertility treatments showed similar trends in 2009 and 2010. However, data from samplings taken 28 and 41 days after sowing (2009) and 25 and 33 days after sowing (2010) suggest that shoot growth patterns varied across years. Higher N levels during stand establishment and lower growing temperatures throughout the entire study period typified 2009. These conditions may have increased the time necessary for fertility effects to become apparent since biomass levels varied with N treatment at 41 days (21 days after N treatment initiation) but not at 28 days (8 days after N treatment initiation). Shoot biomass measures, therefore, varied little among treatments for the majority of the experimental period. And, by its conclusion, biomass values for all N treatments differed significantly from the zero-N control. These findings agree with previous reports suggesting that fertility impacts can diminish when other environmental factors, such as temperature, are more limiting to plant growth and productivity (e.g. as in 2009),[47,48] an assertion consistent with the law of the minimum.[49] Still, the presence of N-fixing microbial associations on the surface of the growing medium may have also contributed to the fact that providing 72, 144 or 576 mg N had little influence on biomass yield in 2009 (B. McSpadden-Gardener, personal communication).

In 2010, the establishment period N fertility level was reduced and warmer ambient temperatures prevailed; together, these conditions sped the onset of clear N treatment effects. Significant differences in crop biomass were evident 25 days and 33 days after sowing (10 days and 18 days after N treatment initiation, respectively). Interestingly, differences in biomass between the four N fertility treatments were greater at 25 days than at 33 days. In the final week of the 2010 experimental run, plant wilting was observed and *Pythium* spp. was identified. Banrot 40WP (Scotts Company LLC) was applied as a drench at a label rate. *Pythium* is a common root pathogen capable of reducing growth in soilless lettuce production systems.[50] In the presence of *Pythium*, reduced plant growth or mortality have been linked to higher levels of fertility in some crops,[51] and this may be due to increased susceptibility to root disease. The presence of warmer temperatures and root pathogens coupled with higher levels of soluble salts in the medium may have limited the response to N level as measured 33 days after sowing.

Nitrogen fertilization effects on plant biomass present interesting implications for early and late season production. Nitrogen utilization calculations revealed that plants exposed to unheated root zones but high fertility incorporated the lowest percentages of N applied while incorporation rates were generally higher in heated root zones and at low to intermediate N levels. Calculated incorporation rates were 14–48% in 2010 but 5–23% in 2009. While both ranges overlap previous estimates of 12–23% N recovery in lettuce produced outdoors on sand,[52] variation in these calculated estimates of N usage reinforces the need to further reduce N losses by more closely matching N fertilization rates with plant growth and temperature patterns.[47] Lower N utilization during early growth stages and low temperature periods can be offset by tailored fertigation regimes. Regardless, yield values reported here suggest maximum production is possible under reduced N in the spring and fall in temperate climates.

Effects of nitrogen fertility on composition

Elemental composition levels followed similar patterns in 2009 and 2010. Plant tissue N and P levels (g kg^{-1}) at final harvest tended to increase with N application in both years. Tissue N concentration tracked N treatment more closely than P concentrations since P levels, although greater than in the control group, did not differ significantly among the three N treatment groups. Nitrogen deficiency has prompted variable responses in P and K levels,[53] a result noted here.

Unlike N, P and other measures of tissue composition, the response of Chl A to N treatment differed by year. Chl A was not significantly affected by N treatment in 2009 but the opposite was true in 2010 when Chl A levels were greatest at the highest N treatment level. Chlorophyll levels are thought to follow N availability;[22,54] however, data here suggest that temperature and light may affect Chl A levels more strongly than N under some conditions.

Like for biomass yield, N supply effects on indices relevant to human nutrition were greatest in 2010. Yet, annual N effects on these aspects of tissue composition varied in magnitude, not direction. As in other reports on the impact of N on secondary metabolites,[14–17,24,55–57] vitamin C, anthocyanin, and total antioxidant power levels tended to increase when N was removed. Moreover, anthocyanin, vitamin C and total antioxidant capacity values were consistently positively correlated in 2009 and 2010 (Table 3), but only anthocyanin and total antioxidant power levels were significantly greater at low N levels in both

years. Vitamin C was impacted by N level only in 2010. Results here suggest that phenolic compounds, like anthocyanin, responded more consistently to N fertility alterations than vitamin C.

A number of plant secondary metabolites directly impact plant and human health. *In situ*, these metabolites often protect against environmental stresses, especially oxidative. When consumed, the same compounds can retain their antioxidant activity and, thereby, capacity to enhance or maintain consumer health. Steps to increase plant secondary metabolite levels (i.e. nutritionally enhanced products) are appealing from a human health standpoint but they present a conundrum for producers, consumers and others in the food supply value-chain: namely, as shown here, secondary metabolite levels tend to be highest when biomass yield – most farmers' fundamental economic unit – is lowest. Resolving this dilemma may require shifting emphases within research, crop production–marketing and product valuation.

Much research remains focused on maximizing production efficiency, typically calculated as the broad ratio of total inputs and marketable yield (simple biomass). Reporting paired measures of biomass and nutritional yield (product of biomass yield and nutritional component levels) will further explain their relationship under varying environmental conditions. Such data are required to establish farm-stage parameters for balancing economic yield, sustainability and human health concerns.

Products must meet quality minima to reach the market but these criteria rarely include nutritional properties. Moreover, producers are usually not rewarded for providing more nutritionally dense products, perhaps at the expense of biomass yield. Monitoring food properties in the marketplace, raising off-farm awareness of on-farm limits and opportunities in shaping them and recognizing superior products may provide further direction and incentive to incorporate nutritional yield into calculations of cropping system productivity.

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