Abiotic Factors during Spring and Fall in Ohio: Their Measurement and Shaping of Lettuce Tissue Abundance and Composition

DISSERTATION

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Abstract

The productivity of vegetable cropping systems can be assessed using multiple criteria. Individual criteria tend to reflect the concerns of food suppliers, such as farmers, or consumers. Occasionally, steps to enhance productivity from a food supplier point of view result in losses from a consumer point of view and vice versa. Creating vegetable production systems that most effectively balance the interest of all within the food supply-consumption chain requires a thorough understanding of forces that shape various aspects of system productivity and efficiency. The need for such understanding is particularly high in the evaluation of emerging production systems such as ones operating fall through spring in the Great Lakes Region. Moreover, studies tend to focus on specific aspects of productivity and address only a supply or consumption perspective. In this work, we set out to complete a more comprehensive assessment that would allow us to determine the potential for enhancing the value of so-called ‘off season’ vegetable production as a driver of economy and health.
In two related studies, leaf lettuce crops were exposed to varied aerial and subsurface microclimates and nitrogen nutrition. Red-leaved romaine lettuce (*Lactuca sativa*) cultivars (Outredgeous, Flagship) were direct-seeded into raised beds in fall and spring sowings in Wooster, OH. Multiple harvests were completed across the approximately 4 week experiments. Harvested tissue was frozen, and laboratory measures of anthocyanin, chlorophyll, soluble solids, vitamin C, and total antioxidant power were completed. Microclimate and nutrition impacted both lettuce biomass and composition across different seasons and years. This work encompassed the evaluation of microclimate management treatments using multiple criteria. The effort has improved the record of the impacts of microclimate management approaches and techniques on crops from physiological, farming, and human health points of view.

A third study was completed in order to test the reliability of digital image analysis as a substitute for or supplement to destructive harvest and evaluation in assessing leafy crop yield. A reliable digital image analysis approach could have far-reaching implications for scientists and crop managers as satellite imagery and related approaches have for a range of users. Significant correlations between traditional destructive biomass and leaf area measures of leaf lettuce crops and digital image analysis were noted. Techniques tested in this study were most effective when high color contrast was present between leaves and other material in the image and in images where complete leaf canopy closure had not occurred. We conclude that digital image analysis may be useful in real-time, non-destructive assessments of early-stage leaf lettuce canopy development.

Two additional projects were completed with the assistance of grower-cooperators
around Ohio. The first project comprised on-farm tests of the microclimate management treatments employed in on-station studies in altered, farm-based form. Four farms cooperated. Results generally confirmed those from on-station plots but the data and experience provided key insights into the separate and combined value of on-farm and on-station research designed to improve vegetable production systems.

The second project was designed to train and equip crop managers in the assessment of crop quality on farms using refractometers to measure soluble solids. Crop managers and farmers require simple, cost effective, and reliable methods for assessing crop quality. The measurement of soluble solids by refractometry is an excellent candidate. Eight growers cooperated in the research phase of this work, providing data on approximately 500 samples, while 600 additional samples were collected by the investigator team. In total, over 1100 samples were taken representing approximately 24 vegetable crops. Thereafter, two 2-hr workshops, two videos available online, and a comprehensive °Brix Guide were prepared. Through this project, it became evident that future research and outreach efforts are needed to assist Ohio vegetable producers in enhancing crop quality, including assessment and management.

These studies provide evidence of the potential to alter, through root- and shoot-zone microclimate modification and nitrogen nutrition, and describe, through digital image analysis, leaf lettuce biomass accumulation and composition during fall and spring in the Midwest U.S. Treatments and techniques employed in this work are ready for on-farm use in altered or unaltered forms. Collectively, the results suggest that the capacity and efficiency of vegetable production systems and specific aspects of related research can be
improved through microclimate management and digital analysis. It is also clear that multiple components of crop quality are influenced by crop management.
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In the completion of this research and dissertation, I very gratefully and humbly acknowledge the invaluable contributions of my advisor- Matt; my committee members- Joe, Robert, Mark, and Peter; my lab group- the VPSL crew, and many others in the OARDC family. It is very much their wisdom, guidance, patience, and assistance that I have to thank for the unforgettable journey of the last four and a half years that enabled a grad student to transition into a young scientist.

“He makes grass grow for the cattle, and *plants for the people to cultivate*- bringing forth food from the earth: wine that gladdens human hearts, oil to make their faces shine, and bread that sustains their hearts.” Psalm 104:14-15
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Chapter 1: Introduction

The productivity of vegetable cropping systems can be assessed using multiple criteria. Individual criteria tend to reflect the concerns of food suppliers, such as farmers, or consumers. Occasionally, steps to enhance productivity from a food supplier point of view result in losses from a consumer point of view and vice versa. Creating vegetable production systems that most effectively balance the interest of all within the food supply-consumption chain requires a thorough understanding of forces that shape various aspects of system productivity and efficiency. The need for such understanding is particularly high in the evaluation of emerging production systems such as ones operating fall through spring in the Great Lakes Region. Moreover, studies tend to focus on specific aspects of productivity and address only a supply or consumption perspective. In this work, we set out to complete a more comprehensive assessment that would allow us to determine the potential for enhancing the value of so-called ‘off season’ vegetable production as a driver of economy and health.

Manipulation of the microclimate (e.g., temperature, light) surrounding crops alters their manufacture and use of primary metabolites in ways that may benefit primarily suppliers or consumers. Can vegetable production systems (with tailored microclimates) that increase multiple forms of productivity and efficiency be designed? The three studies
that follow focus on specific but related aspects of microclimate management for the purpose of enhancing fresh market lettuce production in spring and fall seasons.

Lettuce (*Lactuca sativa*) as a spring and fall crop was chosen for these experiments for three reasons. First, lettuce currently ranks second among vegetables in terms of consumption in the US. However, lettuce ranks 26th in nutritional value among common fruits and vegetables in terms of ten key vitamins and minerals. Therefore, small increases in the concentrations of dietary constituents in lettuce may have far-reaching nutrition-health effects for lettuce consumers. Second, harvesting and marketing fresh vegetables -- especially lettuce and other leafy crops - fall through spring in temperate northern latitudes is increasingly popular and valued for local markets. Consumers value access to freshly harvested, locally-grown vegetables while farmers can benefit from fall-to-spring sales. However, current knowledge and description of wintertime production and crop quality is insufficient to be most beneficial to farmers and consumers. Third, light and temperatures profiles diminish fall to winter and increase winter to spring creating contrasting environments. These differing aspects of the production climate suggest that crop production and quality could shift seasonally. Therefore, environments and crops produced fall to spring must be investigated further.

Study 1 (Chapter 2) investigated temperature and nitrogen level effects on key variables, particularly under field conditions during cool seasons of temperate climates. The impact of root-zone heating and nitrogen (N) fertility on fall- and spring-grown lettuce biomass accumulation and composition was documented using a novel, scalable field system. Direct-seeded plots containing a uniform, semi-solid, and nearly inert
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). At the conclusion of the experiment, lettuce plots were harvested to evaluate fresh tissue biomass, and tissue was frozen for subsequent laboratory analysis of anthocyanin, chlorophyll A, vitamin C, soluble solids, and total antioxidant power.

Root-zone heating increased but withholding N decreased biomass accumulation in both years. Low N fertility was also associated with greater anthocyanin and total antioxidant power but lower N and phosphorus tissue levels. Tissue chlorophyll A and vitamin C levels tracked root-zone temperature and N fertility more closely in 2009 and 2010, respectively. 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 nutritional units.

Study 2 (Chapters 3-5), investigated impacts of the separate and combined application of low and high tunnels and root-zone heating on crop yield, composition, and microclimates, which are currently under-reported. These gaps in the literature were addressed by exposing lettuce to four microclimates established with low and high tunnels and root-zone heating during the spring and fall of two years in Wooster, OH. Red-leaved romaine lettuce (‘Outreadeous’, ‘Flagship’) was direct-seeded into raised beds in both outdoor and high tunnel settings in early October and late March and harvested multiple times over 4 weeks. Half of all plots in each setting were underlain by
electric heating cables and half were covered with 0.8-mil, clear, vented, low-tunnels. A growing medium consisting of peat moss, compost, soil, and red clover (*Trifolium pretense*) hay was used, and all plots were overhead-irrigated. Soil and air temperature were monitored throughout the experiments which were repeated four times (2 seasons/year x 2 years). After the approximately 4-week growth period, lettuce plots were harvested to evaluate fresh tissue biomass, and tissue was frozen for subsequent laboratory analysis of anthocyanin, chlorophyll A, vitamin C, soluble solids, and total antioxidant power.

Chapter 3 focused primarily on treatment effects on crop yield and related variables. Root- and shoot-zone conditions and cultivar significantly affected leaf biomass in both settings (outdoor, high tunnel) while population was more often affected in the outdoor experiments. Microclimate main effects were more prevalent than cultivar effects or interactions. Leaf yield was greater in low tunnel-covered and bottom-heated plots than in uncovered and unheated plots. These data provide evidence of the potential to alter lettuce yield through root and shoot-zone microclimate modification, particularly in regions prone to dynamic seasonal and within-season temperature and light conditions. The data also suggest that the relative performance of low and high tunnels in the production of short-statured, quick-cycling crops during fall and spring be more thoroughly evaluated.

Chapter 4 focused on enhancing the record of management strategy effects on abiotic environmental conditions, including temperature and light, and cropping variables in the outdoor and high tunnel settings. Subsurface and aerial temperatures varied consistently
with plot microenvironment management. Relative to control plots, variability in shoot- and root-zone temperatures generally increased and decreased, respectively, with the addition of low tunnels and electric heating cables, regardless of setting. Still, the relative influence of aerial and soil temperature on crop biomass appeared to differ by setting; aerial temperature correlated most strongly with yield in the high tunnel while the combination of aerial and root-zone temperature correlated most strongly with yield in the field. And, the highest thermal energy to plant biomass conversion efficiency was recorded in the high tunnel. Comparing study-wide and historical climatic data collected in Wooster and other locations in the region suggests that results reported here may hold over a larger area and longer time-frame in Wooster, OH.

Chapter 5 focused on the assessment and management of the nutritional or potential health value associated with fresh vegetable composition. Many, including farmers, aim to increase the health value of fresh vegetable, but they face at least three obstacles. First, describing crop composition in terms of its nutritional impact on human health is complex and there are few, if any, accepted processes and associated metrics for assessing and managing composition on-farm. Second, data suggest that primary and secondary metabolism may be 'in conflict' when establishing the abundance versus composition of a crop. Third, fresh vegetable farmers are rarely compensated for the composition of their product. The development and implementation of a fresh vegetable 'nutritional yield' index would be instrumental in overcoming these obstacles. Nutritional yield is a function of crop biomass and tissue levels of health-related properties, including antioxidant power. Data from the outdoor and high tunnel multi-factor studies on leaf
lettuce primary and secondary metabolism and the literature suggest that antioxidant yield is sensitive to genetic and environmental production factors, and that changes in crop production and valuation will be required for fresh vegetable production systems to become more focused and purposeful instruments of public health.

Study 3 (Chapter 6), investigated plant growth and biomass assessments required in horticulture production and research. Such assessments are followed by major decisions (e.g., harvest timing) that channel resources and influence outcomes. This work sought to establish the limits to which image acquisition and analysis may replace standard, destructive measures of fresh lettuce biomass. Outdoor, high tunnel, and greenhouse plantings of three cultivars of red and green leaf lettuce direct-seeded in raised beds and plastic trays were established at the OARDC in Wooster, OH in spring, summer and fall seasons in 2009-10. Overhead images were captured at specific time points after seeding using hand-held and tripod-mounted commercial digital cameras, in addition to subsequent fresh weight and/or leaf area destructive measurements of plants within the digital images. Images were analyzed using user-defined settings in WinCAM software. A reference grid captured within each image allowed for the calculation of crop canopy cover (percent of two-dimensional image area covered by leaves). Calculations of canopy cover require differentiating leaves and rooting medium by color. The rooting medium was dark in color, and differentiating red leaves from it was less reliable than differentiating green leaves from background. Nevertheless, in samples collected in the greenhouse, outdoor, and high tunnel plots, significant correlation coefficients were observed between measures of canopy cover calculated by image analysis software and
biomass and/or leaf area obtained from harvested plant material. We conclude that digital image analysis may be useful in real-time, non-destructive assessments of early-stage leaf lettuce canopy development, particularly in settings dominated by green leaves and in crops where leaf canopy cover is incomplete.

On-farm Study 1 (Appendix A) was designed to address grower needs for reliable information on the relative merits of low tunnel and root-zone heating methods to enhance ‘off-season’ leafy crop production. In Fall and Winter 2010, comparisons of previously tested (Study 1) methods that modified both the root- and shoot-zone microclimates of leafy crops were conducted at four organic farm sites across Ohio in addition to OARDC. Data on growing environments and crops was collected to evaluate the various techniques. We concluded that, although additional management may be needed to optimize these methods of microclimate modification, the use of both shoot- and root-zone temperature altering techniques was shown to increase crop growth and yield in a late fall leaf lettuce in this multi-site, on-farm study.

On-farm Study 2 (Appendix B) was designed to help Ohio growers better assess the quality of vegetable crops on their farms and prepare them to achieve higher produce quality as is increasingly expected of them by their buyers. We sought to achieve these goals through a coordinated research and training program intended to increase the utility of on-farm soluble solid (°Brix) measurement. The research portion of the project focused on the collection of °Brix data with the assistance of cooperating farmers around Ohio. To our knowledge, data on the status of vegetable crop quality in Ohio has not been previously gathered. Our training portion of the project focused on distribution and
demonstration of the use of soluble solid assessment tools as an initial on-farm crop quality indicator. Through this project, over 1100 °Brix samples were collected at 12 sites across Ohio to begin the formation of an Ohio crop quality database. Additionally, multiple training workshops, presentations, along with print and digital outreach publications were completed to assist growers. With knowledge, skills, and appropriate tools targeted to on-farm quality assessment, growers can begin to make informed management decisions to meet crop quality and farm sustainability targets. These five projects comprise our investigation into the potential for enhancing the value of ‘off season’ vegetable production as a driver of farm productivity and profitability and consumer health and well-being.
Chapter 2: Root-Zone Temperature and Nitrogen Affect the Yield and Secondary Metabolite Concentration of Fall- and Spring-Grown, High-Density Leaf Lettuce


Introduction
Modern horticultural systems rely heavily on the ability to alter environments immediately surrounding crops (Lamont, 2005; Wittwer and Castilla, 1995). 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 (Dixon and Paiva, 1995; Kalt, 2005; Tomas-Barberan and Espin, 2001 Treutter, 2010). 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 makeup 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 (Kalt, 2005; Lattanzio et al., 2008; Parr and Bolwell, 2000; Tomas-Barberan and Espin, 2001; van Duyn and Pivonka, 2000). Plant compounds acting as antioxidants and radical scavengers is one of several proposed mechanisms for this plant-based protection (de Pascual-Teresa and Sanchez-Ballesta, 2008; Kalt, 2005; Lattanzio et al., 2008; Parr and Bolwell, 2000; van Duyn and Pivonka, 2000).

The causal mechanisms underlying the protective effects of fruit and vegetable consumption are not all clear; however, there 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 (Dixon and Paiva, 1995; Kalt, 2005; Lattanzio et al., 2008; Parr and Bolwell, 2000; Tomas-Barberan and Espin, 2001; Treutter, 2010; van Duyn and Pivonka, 2000). 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 (Christie
et al., 1994; Dixon and Paiva, 1995; Gazula et al., 2005; Leyva et al., 1995), as phenylalanine ammonia lyase and chalcone synthase mRNA levels increase in response to low temperatures in the presence of light (Leyva et al., 1995). Also, low N levels are reported to increase phenylpropanoid pathway activity or the levels of pathway products (Bongue-Bartelsman and Phillips, 1995; Stewart et al., 2001). Relationships involving N levels and secondary metabolism are only partially characterized but emerging (Bongue-Bartelsman and Phillips, 1995; Fritz et al., 2006; Lillo et al., 2008; Stewart et al., 2001).

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 (Chalker-Scott, 1999; Leyva et al., 1995; Pietrini et al., 2002; Wise, 1995), and they may also protect against low temperature stress by acting as osmotica (Chalker-Scott, 1999). 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 (Bongue-Bartelsman and Phillips, 1995; Guidi et al., 1998; Margna, 1977; Stewart et al., 2001). Still, increases in secondary compound levels may be associated with decreases in primary productivity (Grevsen et al., 2008; Herms and Mattson, 1992; Lattanzio et al., 2008; Le Bot et al., 2009). 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 (Garcia-Macias et al., 2007; Gazula et al., 2005; Lefsrud et al., 2005; Lefsrud et al., 2007; Oh et al., 2009). 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 U.S. 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.

*Materials and Methods*
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 to 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 x 2.4 x 0.15 m) main plots contained all four 0.36 m² randomized N fertility subplots.

Woven plastic ground cover (Hummert International, St. Louis, MO) was attached with staples to 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) 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 m³ main plot⁻¹. Tunnel sides and ends were secured to the frame with wooden lath. Polystyrene foam dividers (0.15 m x 0.6 m x 1.25 cm; Dow Chemical Co., Midland, MI) separated fertility sub-plots within each main plot. A layer of KapMat cloth (BFG Supply Co., Burton, OH) 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) and 40% Primera One calcinated clay field conditioner (Profile Products, LLC, Buffalo Grove, IL). The 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). The two dry components were blended in a Bouldin and Lawson model 12193 mixer (Bouldin and Lawson, McMinnville, TN). 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) 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 dripline with emitters at 15 cm spacing (DIG Irrigation Products, Vista, CA). Each sub-plot contained a 40 x 40 cm square of dripline 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 2 times (10:00, 16:00; 3,600 ml subplot$^{-1}$ total) and 3 times daily (10:00, 12:30, 15:00; 5,400 ml sub-plot$^{-1}$ total) in 2009 and 2010, respectively. A modified Hoagland’s solution (Hoagland and Arnon, 1950), consisting of reagent grade 0.5 mol L$^{-1}$ KCl, 0.5 mol L$^{-1}$ K$_2$SO$_4$, 0.5 mol L$^{-1}$ MgSO$_4$·7H$_2$O, 0.05 mol L$^{-1}$ Ca(H$_2$PO$_4$)$_2$·H$_2$O, 0.01 mol L$^{-1}$ CaSO$_4$·2H$_2$O and 0.5 mol L$^{-1}$ KH$_2$PO$_4$, 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$_2$SO$_4$ 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) 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\(^{-1}\) \(\text{NH}_4\text{NO}_3\) stock solution, diluted and then mixed by the Argus system with the macronutrient solution detailed above prior to delivery to subplots. 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) x N fertility (four levels) experimental units (sub-plots).

*Environmental data.* Air and soil temperatures were recorded continuously at 15-min intervals using Hobo ProV2 data loggers (Onset Computer Co., Pocasset, MA). 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.

<table>
<thead>
<tr>
<th>Year</th>
<th>Above surface</th>
<th>Below surface</th>
<th>Above surface</th>
<th>Below surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>10.17±0.053</td>
<td>10.99±0.094</td>
<td>11.77±0.14</td>
<td>19.11±0.54</td>
</tr>
<tr>
<td>2010</td>
<td>14.87±0.065</td>
<td>14.57±0.14</td>
<td>15.63±0.092</td>
<td>20.52±0.38</td>
</tr>
</tbody>
</table>

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.

*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 x 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) using a Kinematica 10-35 Polytron (Kinematica, Bohemia, NY). A 5 g subsample of the homogenized tissue was then extracted sequentially with 20 mL, 20 mL, and then 10 mL of 1% HCl acidified methanol (Garcia-Macias, 2007; Gazula et al., 2005; Kleinhenz et al., 2003). Each hour-
long extraction took place in the dark at 5 °C and then samples were centrifuged at 7800 x 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) using a Buchner funnel. Samples were then centrifuged a final time and immediately read on a Beckman Coulter DU730 spectrophotometer (Beckman Coulter, Brea, CA). Anthocyanin and Chl A absorbances were obtained at 530 and 420 nm, respectively (Gazula et al., 2005; Kleinhenz et al., 2003). Standard curves for cyanidin-3 glucoside (Chromadex, Irvine, CA) and Chl A (Sigma Aldrich, St. Louis, MO) 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 (Benzie and Strain, 1999). 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 mM sodium acetate buffer at 3.6 pH, 20 mM Fe$_3$Cl, and 10 mM 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 (Benzie and Strain, 1999). 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 x 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 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) in duplicate to determine sucrose sugar content in °BRIX by reading on a Leica Abbe Mark II refractometer (Leica Inc., Buffalo, NY).

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).

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). 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 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 (\(\mathbf{^0\text{BRIX}\)) and total antioxidant power (\(\mu\text{M trolox equivalents g}^{-1}\text{ fw}\)) 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 0 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>
<thead>
<tr>
<th></th>
<th>Sampling one (g m⁻²)</th>
<th>Sampling two (g m⁻²)</th>
<th>Final harvest (g m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2009 Analysis of Variance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fertility</td>
<td>ns</td>
<td>ns</td>
<td>0.0003</td>
</tr>
<tr>
<td>Heat x fertility</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Root-zone heating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>37.5 b</td>
<td>264.9 b</td>
<td>447.6 b</td>
</tr>
<tr>
<td>+</td>
<td>95.1 a</td>
<td>812.7 a</td>
<td>1666.5 a</td>
</tr>
<tr>
<td><strong>2010 Analysis of Variance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat</td>
<td>0.0004</td>
<td>0.0023</td>
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<tr>
<td>Fertility</td>
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</tr>
<tr>
<td>Root-zone heating</td>
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</tr>
<tr>
<td>-</td>
<td>113.3 b</td>
<td>957.5 b</td>
<td>1294.5 b</td>
</tr>
<tr>
<td>+</td>
<td>175.4 a</td>
<td>1626.7 a</td>
<td>2002.2 a</td>
</tr>
<tr>
<td>N Fertility (mg day⁻¹)</td>
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<tr>
<td>0</td>
<td>50.5</td>
<td>424.9</td>
<td>649.8 b</td>
</tr>
<tr>
<td>72</td>
<td>65.3</td>
<td>429.9</td>
<td>937.8 a</td>
</tr>
<tr>
<td>144</td>
<td>56.8</td>
<td>473.3</td>
<td>931.6 a</td>
</tr>
<tr>
<td>576</td>
<td>67.9</td>
<td>536.1</td>
<td>980.1 a</td>
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</tbody>
</table>

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.

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.

<table>
<thead>
<tr>
<th></th>
<th>Biomass</th>
<th>Antho</th>
<th>Chl A</th>
<th>Antioxidant</th>
<th>Vitamin C</th>
<th>°Brix</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
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<td>.</td>
<td>.</td>
<td>.</td>
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<tr>
<td>Anthocyanin</td>
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<td>Chl A</td>
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<tr>
<td>Vitamin C</td>
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<td>0.66</td>
<td>0.63</td>
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<tr>
<td>°Brix</td>
<td>-0.84</td>
<td>0.62</td>
<td>0.77</td>
<td>0.62</td>
<td>0.45</td>
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</tr>
<tr>
<td>N</td>
<td>ns</td>
<td>-0.65</td>
<td>ns</td>
<td>-0.66</td>
<td>ns</td>
<td>ns</td>
<td>.</td>
</tr>
</tbody>
</table>

2010

<table>
<thead>
<tr>
<th></th>
<th>Biomass</th>
<th>Antho</th>
<th>Chl A</th>
<th>Antioxidant</th>
<th>Vitamin C</th>
<th>°Brix</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
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<td>.</td>
<td>.</td>
<td>.</td>
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<tr>
<td>Anthocyanin</td>
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<td>Chl A</td>
<td>ns</td>
<td>ns</td>
<td>.</td>
<td>.</td>
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<td>.</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>-0.76</td>
<td>0.99</td>
<td>ns</td>
<td>.</td>
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<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>-0.52</td>
<td>0.60</td>
<td>ns</td>
<td>0.60</td>
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<td>.</td>
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<tr>
<td>°Brix</td>
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<td>0.41</td>
<td>ns</td>
<td>0.40</td>
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<td>N</td>
<td>0.57</td>
<td>-0.81</td>
<td>ns</td>
<td>-0.85</td>
<td>-0.80</td>
<td>ns</td>
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</tr>
</tbody>
</table>

Effects of nitrogen fertility on composition

In 2009, anthocyanin and total antioxidant power was highest in tissue taken from plots receiving 0 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 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.
### 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. *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>
<thead>
<tr>
<th></th>
<th>Anthocyanin (g kg(^{-1}) fw)</th>
<th>Chlorophyll A (g kg(^{-1}) fw)</th>
<th>Antioxidant power (µ mol L(^{-1}) trolox eq. g(^{-1}) fw)</th>
<th>Vitamin C (g kg(^{-1}) fw)</th>
<th>°Brix</th>
<th>N (g kg(^{-1}))</th>
<th>P (g kg(^{-1}))</th>
<th>K (g kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2009 Analysis of Variance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>ns</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>0.469 a(^z)</td>
<td>0.663 a</td>
<td>19.8 a</td>
<td>0.074 a</td>
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<td>7.8</td>
<td>62.4</td>
</tr>
<tr>
<td>+</td>
<td>0.305 b</td>
<td>0.530 b</td>
<td>13.7 b</td>
<td>0.034 b</td>
<td>2.4 b</td>
<td>56.9</td>
<td>9.2</td>
<td>71.2</td>
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<tr>
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<td>0.543 a</td>
<td>0.617</td>
<td>22.4 a</td>
<td>3.2</td>
<td>46.8 c</td>
<td>7.5 b</td>
<td>64.4 b</td>
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<tr>
<td></td>
<td>72</td>
<td>0.364 b</td>
<td>0.591</td>
<td>16.2 b</td>
<td>2.8</td>
<td>56.0 b</td>
<td>8.6 a</td>
<td>72.1 a</td>
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<td></td>
<td>144</td>
<td>0.328 bc</td>
<td>0.583</td>
<td>14.7 bc</td>
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<td>64.7 a</td>
<td>9.0 a</td>
<td>61.9 b</td>
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<td><strong>2010 Analysis of Variance</strong></td>
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<td>ns</td>
<td>ns</td>
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<td>ns</td>
<td>ns</td>
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<td>-</td>
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<td>6.8</td>
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<td>+</td>
<td>0.373</td>
<td>0.464</td>
<td>18.8</td>
<td>0.094</td>
<td>2.1</td>
<td>44.6</td>
<td>6.9</td>
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<td>2.4</td>
<td>31.9 c</td>
<td>5.9 b</td>
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<td>0.471 b</td>
<td>19.4 b</td>
<td>2.2</td>
<td>44.4 b</td>
<td>7.1 a</td>
<td>45.5</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>0.320 c</td>
<td>0.479 b</td>
<td>16.6 c</td>
<td>2.1</td>
<td>46.8 b</td>
<td>6.9 a</td>
<td>45.0</td>
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<tr>
<td></td>
<td>576</td>
<td>0.300 c</td>
<td>0.518 a</td>
<td>15.3 c</td>
<td>2.1</td>
<td>58.7 a</td>
<td>7.5 a</td>
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</tbody>
</table>

...
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 (Graves, 1992). Root morphology, in turn, can influence nutrient uptake and other variables (Wang et al., 2006). 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 (Savvas et al., 2004). Overall, the outdoor experimental system integrated a rooting medium with the impedance of soil with temperature and nutrient control typical of indoor soilless systems.

Nitrogen (N) was supplied separately from the other essential plant nutrients in an NH₄NO₃ solution. Supplies of non-target nutrients remained consistent among all treatments. N supplied as NH₄⁺ and NO₃⁻ tends to enhance crop growth and reduce nitrate accumulation in leafy crops (Marschner, 1995; Van Der Boon et al., 1990), 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 NH₄⁺ and NO₃⁻ can be temperature-limited as well (Clarkson and Warner, 1979; Fronta and Tucker, 1972; Kafkafi, 1990). 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; in 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 (Economakis, 1997; Rykbost, 1975; van der Boon et al., 1990). 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 °C 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 (Christie et al., 1994; Gazula et al., 2005; Ozgen et al., 2008). Phenolic, anthocyanin, vitamin C, sugar and Chl levels in lettuce and other crops have risen in response to reduced root or shoot-zone temperatures (Gazula et al., 2005; Kafkafi, 1990; Mahmud et al., 1999; Oh et al., 2009; Voipio and Autio, 1995). 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 (Taiz and Zeiger, 2006).

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; Favallo et al., 2009; Guo et al., 2008), an assertion
consistent with the law of the minimum (Rubel, 1935). 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 (Johnstone et al., 2004). In the presence of *Pythium*, reduced plant growth or mortality have been linked to higher levels of fertility in some crops (Moorman, 1986), 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% to 48% in 2010 but 5% to 23% in 2009. While both ranges overlap previous estimates of 12% to 23% N recovery in lettuce produced outdoors on sand (Sanchez, 2000), 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 (Guo et al., 2008). 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 (Jones et al., 1991), 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 (Filella et al., 1995; Guidi et al., 1998); 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 (Bongue-Bartelsman and Phillips, 1995; Fritz, et al., 2006; Giorgi et al., 2009; Grevesen et al., 2008; Hodges and Nozzolilli, 1996; Lillo et al., 2008; Mozofar, 1996; Stewart et al., 2001), 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.
References


Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil, California Agricultural Experiment Station Circular 347. The College of Agriculture University of California, Berkeley, CA.


Chapter 3: Canopy Cover and Root-Zone Heating Effects on Fall- and Spring-Grown Leaf Lettuce Yield in Ohio


Introduction

Managing the microclimate immediately surrounding plants and products -- comprised of temperature, light, humidity, and other conditions -- is fundamental to horticultural production, post-harvest handling, and research. Microclimate management often involves the use of plastics, for example, increasingly deployed as the cover of low and high tunnels (Lamont, 2005; Wells and Loy, 1985, 1993; Wittwer and Castilla, 1995). Low- and high-tunnel use are popular because they can raise farm productivity and profit potential through direct and indirect effects on crops and crop management (Carey et al., 2009; Lamont, 2005; Waterer, 2003; Wells and Loy, 1993). Low and high tunnels tend to reduce crop stress and often increase yield relative to the uncovered condition. Low and high tunnels differ in size and scope of application but share operational principles of heat and humidity retention, shading and light dispersion, and wind mitigation, especially of aerial environments.

Active root-zone or floor heating is common in year-round greenhouse systems (Elwell et al., 1985; Sachs et al., 1992; Shedlosky and White, 1987; Wai and Newman, 1995).
1992) but less common in low- and high-tunnel systems. In greenhouse systems, root-zone heating is thought to maintain or improve ornamental and vegetable plant growth and quality and lower aerial heating costs (Janes and McAvoy, 1983; Sachs et al., 1992; Shedlosky and White, 1987; Trudel and Gosselin, 1982). Relative to shoot-zone heating and regardless of setting, root-zone heating may also alter broad aspects of crop physiology including root growth, growth regulator production, transport and/or activity, water and nutrient uptake, and photosynthate allocation (Bowen, 1991; Cooper, 1973; Li et al., 1994; Macduff, 1989). As in greenhouse systems, integrating root- and shoot-zone heating in low- and high-tunnel systems may create microclimates that promote specific relationships between primary and secondary metabolism that influence yield, quality, profitability, and sustainability.

Unlike in many greenhouses, temperature modification in low and high tunnels is often a comparatively crude, localized, passive, and sunlight-dependent process unaccompanied by supplemental lighting (Nair and Ngouajio, 2010; Soltani et al., 1995; Waterer, 2003; Wells and Loy, 1985, 1993). Active heating within high tunnels, if used at all, typically is applied intermittently to elevate shoot-zone temperatures, especially for low-temperature protection (Lamont, 2005; Lamont et al., 2003). Moreover, low and high tunnels are routinely deployed in areas characterized by dynamic within- and across-season fluctuations in sunlight and temperature levels. Therefore, it is reasonable to suspect that greenhouse studies, especially those featuring root-zone heating, may have yielded an understanding of temperature effects upon which low- and high-tunnel users cannot rely exclusively, particularly if their system involves a short-statured, quick-
cycling crop grown during fall and spring seasons. We set out to strengthen the record of temperature effects in low- and high-tunnel systems by employing low tunnels, a high tunnel and root-zone-heating cables (alone and in combination) and multi-pronged data collection during spring- and fall-time production of leaf lettuce in 2008-10.

Materials and Methods

Site and experimental growing system. Two experiments were conducted at the Horticulture and Crop Science Department Farm of the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH. The experiments were conducted simultaneously and followed duplicate methodological approaches. The experiments differed only in the setting in which they were completed: one experiment was conducted in a high tunnel, and the second was conducted outdoors in an adjacent open field. Both experiments were repeated in two spring and fall seasons 2008-10.

Both experiments employed a split-plot design containing four replications per treatment. Treatments represented eight combinations of four microclimates and two cultivars also functioning as main and subplot factors, respectively. Eight wood-framed raised beds (2 x 8 ft x 6 inches) contained the four main plot microclimates: 1) control (no low tunnel or subsurface heating), 2) subsurface-heated with heating cable, 3) aerial-covered with low tunnel, and 4) subsurface-heated and aerial-covered. Within these main plots were four randomized subplots (2 x 2 ft) containing the two lettuce cultivars. Each of the eight microclimate x cultivar treatments was replicated four times in each experiment for a total of 32 subplots. The Fall 2008 experiments tested only ‘Outredgeous’ lettuce in the four microclimates, while all subsequent experiments tested
two lettuce cultivars as subplot treatments. Also, in Fall 2008, a 13 x 40 x 6-ft single-layer 6-mil plastic tunnel was used while all subsequent high tunnel experiments were carried out in a 30 x 80 x 13-ft single-bay, gothic style, single-layer 6-mil clear, polyethylene-covered high tunnel.

A woven black weed barrier (Hummert International, Earth City, MO) was placed under all raised beds and covered with a 1 to 1.5-inch layer of sand (Woodland Mulch, Kidron, OH). A second layer of weed barrier (3 x 9 ft) was stapled to the raised bed frame to contain the medium. The growing medium consisted of (v/v) 35% peat (Premier Horticulture, Quakerstown, PA), 35% dairy manure compost (OARDC), 15% shredded organic red clover hay (OARDC), and 15% silt loam field soil (OARDC). A mixer (model 12193; Bouldin and Lawson, McMinnville, TN) was used to completely blend the materials. Each main plot was filled with approximately 5.2 ft³ of new medium (average 5.8 pH, 1.1% Nitrogen, 19.0% Carbon) for each experiment. Polystyrene foam dividers (Dow Chemical Co., Midland, MI) separated the subplots within each main plot.

Control plots consisted of unheated raised beds also lacking a low-tunnel cover. Root-zones in treatments two and four were heated with a 40-ft automatic electric heating cable (Wrap-On Co., Bedford Park, IL) under the rooting medium at about a 4-inch depth and triggered to function at medium temperatures below 23 °C. The heating cable was attached to the upper layer of woven weed barrier at 3-inch spacing to provide even heating and prevent cable contact, and the operation of each cable was governed by a thermostat with its sensing unit in continual contact with the rooting medium at 4-inch depth. Treatment three main plots were covered by a single layer of 0.8-mil slitted
polyethylene (Hummert International) stretched across four wire hoops and attached to the ground with landscape staples to create an 18 x 30-inch low tunnel. Treatment four main plots were polyethylene-covered and cable-heated as described earlier.

Approximately 1000 pre-weighed primed and pelleted seeds of the two red leaf romaine lettuce cultivars, Outredgeous (Johnny’s Selected Seeds, Winslow, ME) and Flagship (Shamrock Seeds, Salinas, CA) were sown in seven parallel rows at 3-inch spacing within each subplot, as recommended for baby leaf lettuce production. Seeds were evenly placed on the substrate and covered by hand on 9 Oct. 2008, 21 Mar. and 10 Oct. 2009, and 16 Mar. 2010. All plots were overhead watered by hand as needed by microclimate treatment, generally twice daily with approximately 1 L/subplot of clear water. No additional fertilizer was added.

*Environmental data.* Air and soil temperatures were recorded continuously at 30-min (Fall 2008, Spring 2009) and 15-min (Fall 2009, Spring 2010) intervals in both experiments using data loggers (Hobo ProV2; Onset Computer Co., Bourne, MA). All main plots were equipped with a separate data logger, and sensors were protected with a radiation shield. Air and soil temperatures were recorded 8 inches above and 1 to 1.5 inches below the medium surface, respectively. Data from the monitoring of each main plot replication are summarized in Table 5. Periodic readings of photosynthetically active radiation (LI250-A; LI-COR Biosciences, Lincoln, NE) were also taken at canopy level in each experiment.
<table>
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<th></th>
<th>Control</th>
<th>Subsurface-heated</th>
<th>Aerial-covered</th>
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<td>Subsurface temp</td>
<td>Aerial temp</td>
<td>Subsurface temp</td>
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<td>15.42±0.031</td>
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<td>11.32±0.030</td>
<td>19.87±0.078</td>
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<td>Fall 2009</td>
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<td>12.06±0.030</td>
<td>10.71±0.081</td>
<td>17.86±0.87</td>
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<td>Spring 2010</td>
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<td>13.17±0.063</td>
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<td>Fall 2008</td>
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<td>9.78±0.57</td>
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<td>8.08±0.12</td>
<td>7.78±0.12</td>
<td>16.13±1.18</td>
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<td>9.19±0.15</td>
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<td>17.80±0.67</td>
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<td>Spring 2010</td>
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<td>10.01±0.13</td>
<td>10.71±0.076</td>
<td>16.43±1.01</td>
</tr>
</tbody>
</table>

Table 5. Shoot- and root-zone temperatures in high-tunnel and outdoor raised beds under four methods of microclimate modification in eight experiments in 2008-10 in Wooster, OH.

* Standard error of shoot- and root-zone temperatures from main plot means in each experiment (n = 2); (1.8 x °C) + 32 = °F.
Crop data. Microclimate effects on plant growth and yield were measured at four developmental stages using destructive and nondestructive sampling. Data are presented for two of these stages. Early stand establishment was measured via nondestructive counts of emerged seedlings taken 5 d after sowing on 10 inches of the center of the third row in each subplot. Plant number and biomass were measured through plant samples collected approximately 2 and 3 weeks after sowing (WAS). In this process, all plants were removed intact and by hand from a 10-inch section of a randomly chosen row (excluding edge rows 1 and 7) in each subplot, placed in a sealed plastic bag and cooled (5 °C) until weighing. All plants from each subplot sample were counted and weighed in bulk. Thereafter, 10 representative plants were selected from the bulk subplot sample and their roots and shoots were separated with a razor blade and weighed. Finally, yield was recorded approximately 4 WAS (7 Nov., 2008, 22 Apr., 2009, 9 Nov., 2009, and 14 Apr., 2010) by collecting all above-ground biomass from a standardized 2-ft² section of each subplot. Leaves and stems were placed in plastic bags and stored on ice in coolers until being processed within 4 h of harvest.

Germination data were collected on a thermogradient table (model DB2000; Van Dok and De Boer, Enkhuizen, The Netherlands). Petri dishes (100 x 15 mm; Fisher Scientific, Waltham, MA) were lined with blotting paper (3.25-inch discs; Anchor Paper Co. St. Paul, MN) and filled with approximately 40 mL of the above described medium. Twenty-five primed and pelleted seeds were evenly spread on the surface of the medium and moistened. Germination was tested at 10 discrete temperatures in 2 °C increments from 10 to 28 °C. A total of 80 petri dishes were placed on the thermogradient table in rows of
eight, with each row containing four replications of each cultivar at each temperature. Emerged and viable seedlings were counted and removed from the petri dishes 3, 5, 7, and 10 d after sowing.

Data analysis. The high-tunnel and outdoor experiments were not replicated in space; therefore, data from these experiments were analyzed separately. Data collected at 2 and 4 WAS were also analyzed separately within experiment to uncover potential treatment effects on growth through time. A Proc Univariate procedure was carried out to test for normality on all data. Normally distributed data were analyzed using untransformed data in Proc Mixed. Data with a nonnormal distribution were log-transformed before analysis (SAS version 9.2; SAS Institute, Cary, NC), a common step in the handling of data displaying a skewed distribution. Means were then back-transformed for inclusion in tables. Microclimate and cultivar were analyzed as fixed effects and replications within years were analyzed as random effects in Proc Mixed. Treatment means were separated using a pdiff difference statement at a $P \leq 0.05$ level of significance when microclimate and cultivar fixed effects were significant at $P \leq 0.05$.

Results
The separate and combined use of low and high tunnels and heating cables established contrasting, characteristic microclimates, as illustrated by air and soil temperature data in Table 5. These microclimates, in turn, were associated with consistent treatment-based differences in lettuce yield (Tables 6 and 7). Yield trends evident at 2 WAS tended to prevail at 4 WAS. And, treatment effects on yield were significant in three of the four high tunnel and all four outdoor experiments (Tables 6 and
Regardless of setting, yield tended to be higher in polyethylene-covered and root-zone cable-heated plots and lower in uncovered, unheated plots. Cultivar significantly affected yield in three experiments with ‘Outredgeous’ tending to register the greatest yield. However, yield was less consistently and dramatically affected by cultivar as a main effect and interactions involving cultivar than by microclimate treatments. When present, statistically significant interactions involving cultivar appeared to result from cultivar-based differences in plant population and growth, themselves driven in part by unknown genetic factors (Table 8).

Microclimate effects on plant biomass

High-tunnel experiments. Yield in the high-tunnel setting was influenced by microclimate treatment in three of four experiments, the lone exception being Fall 2008 (Table 7). Interestingly, plant population recorded 2 WAS was unaffected by microclimate in Fall 2009 and Spring 2010 (Table 6) although treatment effects on biomass production were typically evident at the same and later harvest in 2009 and 2010 (Tables 6 and 7). Yield in the high-tunnel setting was clearly influenced by microclimate and typically lowest in uncovered and unheated plots. The relative impacts of root, shoot and root- and shoot-zone heating on yield also varied by year, season, and crop stage. Still, the data clearly indicate most trends established by 2 WAS tended to hold at 4 WAS. Overall, these results indicate that a slitted polyethylene cover established at sowing, whether alone or in combination with root-zone heating, often resulted in the greatest biomass production.
Table 6. Lettuce plant number and shoot fresh weight from destructive samplings of two red romaine lettuce cultivars taken 2 weeks after sowing in four microclimates in outdoor and high-tunnel settings in eight experiments in 2008-10 in Wooster, OH.

<table>
<thead>
<tr>
<th>Microclimate</th>
<th>Control</th>
<th>Subsurface-heated</th>
<th>Aerial-covered</th>
<th>Subsurface-heated and aerial-covered</th>
<th>Cultivar</th>
<th>Outredgeous</th>
<th>2-week count</th>
<th>2-week wt</th>
<th>2-week count</th>
<th>2-week wt</th>
<th>2-week count</th>
<th>2-week wt</th>
<th>2-week count</th>
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<tbody>
<tr>
<td>High tunnel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fall 2008</td>
<td>Spring 2009</td>
<td>Fall 2009</td>
<td>Spring 2010</td>
<td>Fall 2009</td>
<td>Spring 2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>51</td>
<td>3.68</td>
<td>65 b</td>
<td>3.74 a</td>
<td>72 a</td>
<td>2.09 a</td>
<td>56</td>
<td>1.75 a</td>
<td>56</td>
<td>1.75 a</td>
<td>56</td>
<td>1.75 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subsurface-heated</td>
<td>56</td>
<td>6.07</td>
<td>65 b</td>
<td>7.33 c</td>
<td>77</td>
<td>4.50 b</td>
<td>58</td>
<td>2.61 b</td>
<td>58</td>
<td>2.61 b</td>
<td>58</td>
<td>2.61 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerial-covered</td>
<td>65</td>
<td>5.96</td>
<td>56 ab</td>
<td>5.15 b</td>
<td>70</td>
<td>3.58 b</td>
<td>51</td>
<td>3.02 b</td>
<td>51</td>
<td>3.02 b</td>
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<td>3.02 b</td>
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<tr>
<td>Subsurface-heated and aerial-covered</td>
<td>64</td>
<td>7.19</td>
<td>55 a</td>
<td>8.43 c</td>
<td>69</td>
<td>6.59 c</td>
<td>57</td>
<td>5.54 c</td>
<td>57</td>
<td>5.54 c</td>
<td>57</td>
<td>5.54 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fall 2009</td>
<td>Spring 2009</td>
<td>Fall 2009</td>
<td>Spring 2010</td>
<td>Fall 2009</td>
<td>Spring 2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outredgeous</td>
<td>59</td>
<td>5.56</td>
<td>63</td>
<td>6.41</td>
<td>74</td>
<td>4.01</td>
<td>59</td>
<td>3.33 B</td>
<td>59</td>
<td>3.33 B</td>
<td>59</td>
<td>3.33 B</td>
<td></td>
<td></td>
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<tr>
<td>Flagship</td>
<td>__ x</td>
<td>__ x</td>
<td>57</td>
<td>5.91</td>
<td>70</td>
<td>4.37</td>
<td>53</td>
<td>2.63 A</td>
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<td>2.63 A</td>
<td>53</td>
<td>2.63 A</td>
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Outdoor

<table>
<thead>
<tr>
<th>Microclimate</th>
<th>Control</th>
<th>Subsurface-heated</th>
<th>Aerial-covered</th>
<th>Subsurface-heated and aerial-covered</th>
<th>Cultivar</th>
<th>Outredgeous</th>
<th>2-week count</th>
<th>2-week wt</th>
<th>2-week count</th>
<th>2-week wt</th>
<th>2-week count</th>
<th>2-week wt</th>
<th>2-week count</th>
<th>2-week wt</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>19 a</td>
<td>0.15 a</td>
<td>44 a</td>
<td>0.52 a</td>
<td>54 a</td>
<td>0.56 a</td>
<td>35 ab</td>
<td>0.29 a</td>
<td>35 ab</td>
<td>0.29 a</td>
<td>35 ab</td>
<td>0.29 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subsurface-heated</td>
<td>57 b</td>
<td>1.29 b</td>
<td>63 bc</td>
<td>1.54 b</td>
<td>67 b</td>
<td>1.70 b</td>
<td>26 a</td>
<td>0.34 a</td>
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<td>26 a</td>
<td>0.34 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerial-covered</td>
<td>46 b</td>
<td>1.01 b</td>
<td>56 b</td>
<td>1.38 b</td>
<td>65 b</td>
<td>2.13 c</td>
<td>35 ab</td>
<td>0.41 a</td>
<td>35 ab</td>
<td>0.41 a</td>
<td>35 ab</td>
<td>0.41 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subsurface-heated and aerial-covered</td>
<td>57 b</td>
<td>3.10 c</td>
<td>71 c</td>
<td>5.10 c</td>
<td>68 b</td>
<td>4.68 d</td>
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<td>45 b</td>
<td>1.42 b</td>
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<tr>
<td>Cultivar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fall 2009</td>
<td>Spring 2009</td>
<td>Fall 2009</td>
<td>Spring 2010</td>
<td>Fall 2009</td>
<td>Spring 2010</td>
<td></td>
<td></td>
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<tr>
<td>Outredgeous</td>
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<td>0.88</td>
<td>57</td>
<td>1.49</td>
<td>61</td>
<td>1.61</td>
<td>37</td>
<td>0.50</td>
<td>37</td>
<td>0.50</td>
<td>37</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>__ x</td>
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<td>65</td>
<td>1.91</td>
<td>34</td>
<td>0.48</td>
<td>34</td>
<td>0.48</td>
<td>34</td>
<td>0.48</td>
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</tbody>
</table>

* Means within columns and microclimates (lowercase letters) or cultivars (uppercase letters) followed by the same letter are not significantly different by a pdiff difference statement at $P \leq 0.05$.

* See Table 8 for microclimate x cultivar interaction means separation.

* Fall 2008 experiments compared microclimate treatments without cultivar subplots.
<table>
<thead>
<tr>
<th>Microclimate</th>
<th>Cultivar</th>
<th>Fall 2008</th>
<th>Spring 2009</th>
<th>Fall 2009</th>
<th>Spring 2010</th>
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<tr>
<td>High tunnel</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td></td>
<td>113.1</td>
<td>108.0 a</td>
<td>21.7 a</td>
<td>42.8 a</td>
</tr>
<tr>
<td>Subsurface-heated</td>
<td></td>
<td>111.2</td>
<td>159.6 b</td>
<td>45.0 b</td>
<td>67.9 b</td>
</tr>
<tr>
<td>Aerial-covered</td>
<td></td>
<td>135.5</td>
<td>194.5 c</td>
<td>48.1 b</td>
<td>92.2 c</td>
</tr>
<tr>
<td>Subsurface-heated and aerial-covered</td>
<td></td>
<td>119.5</td>
<td>214.3 c</td>
<td>82.4 c</td>
<td>131.7 d</td>
</tr>
<tr>
<td>Cultivar</td>
<td>Outredgeous</td>
<td>119.8</td>
<td>165.9</td>
<td>44.2</td>
<td>96.2 B</td>
</tr>
<tr>
<td></td>
<td>Flagship</td>
<td>___</td>
<td>172.3</td>
<td>44.5</td>
<td>71.1 A</td>
</tr>
<tr>
<td>Outdoor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>2.9 a</td>
<td>2.0 a</td>
<td>4.0 a</td>
<td>2.6 a</td>
</tr>
<tr>
<td>Subsurface-heated</td>
<td></td>
<td>23.3 b</td>
<td>10.3 b</td>
<td>10.3 b</td>
<td>7.5 b</td>
</tr>
<tr>
<td>Aerial-covered</td>
<td></td>
<td>21.9 b</td>
<td>22.4 c</td>
<td>14.3 c</td>
<td>9.7 b</td>
</tr>
<tr>
<td>Subsurface-heated and aerial-covered</td>
<td></td>
<td>75.0 c</td>
<td>89.1 d</td>
<td>51.3 d</td>
<td>37.4 c</td>
</tr>
<tr>
<td>Cultivar</td>
<td>Outredgeous</td>
<td>18.3</td>
<td>16.2 B</td>
<td>13.6</td>
<td>12.3 B</td>
</tr>
<tr>
<td></td>
<td>Flagship</td>
<td>___</td>
<td>12.5 A</td>
<td>12.8</td>
<td>6.8 A</td>
</tr>
</tbody>
</table>

Table 7. Lettuce shoot fresh weight measured 4 weeks after sowing in two red romaine cultivars exposed to four experimental microclimates in outdoor and high-tunnel settings in eight experiments in 2008-10 in Wooster, OH.

\(^2\) Means within columns and microclimates (lowercase letters) or cultivars (uppercase letters) followed by the same letter are not significantly different by a pdiff difference statement at \(P \leq 0.05\).

\(^3\) See Table 8 for microclimate x cultivar interaction means separation.

\(^x\) Fall 2008 experiments compared microclimate treatments without cultivar subplots.
Outdoor experiments. Biomass production in the outdoor setting was influenced by microclimate treatment in all four experiments (Table 7) but, perhaps, in a manner more mitigated by plant population than in the high-tunnel setting. Plant populations recorded 2 WAS were lowest in uncovered, unheated control plots in three of four experiments, only the rank order and significance of treatment effects on plant population varied slightly by year and season (Table 6). Yield recorded 2 and 4 WAS displayed a similar trend (lowest in the control, variable rank order among the root and shoot alone heated treatments). However, as in the high-tunnel setting, root- and shoot-zone heating tended to result in the greatest yield. Interestingly, the relative impacts of root- and shoot-zone heating when practiced alone varied slightly among year, season, and crop stage. Still, the use of a slitted polyethylene covering alone resulted in statistically higher yield values than using root-zone heating alone in two of four experiments.

Cultivar effects on plant biomass

High-tunnel experiments. Cultivar main effects on biomass production were significant only in Spring 2010 when ‘Outredgeous’ out-yielded ‘Flagship’ 2 and 4 WAS (Tables 6 and 7). A significant microclimate-x-cultivar interaction was detected for shoot fresh weight 4 WAS in Spring 2009 and plant population 2 WAS in Fall 2009 (Table 8).

Outdoor experiments. As in the high-tunnel experiments, cultivar main effects on yield were less common than microclimate effects in the outdoor setting. ‘Outredgeous’ was greater than ‘Flagship’ yield 4 WAS in Spring 2009 and Spring 2010 (Table 7). A
significant microclimate-x-cultivar interaction was detected only at 4 WAS in Spring 2010 (Table 8).

<table>
<thead>
<tr>
<th>Microclimate</th>
<th>Cultivar</th>
<th>Spring 2009 4-week shoot wt</th>
<th>Fall 2009 2-week count</th>
<th>Spring 2010 4-week shoot wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Outredgeous</td>
<td>89.3 a(^2)</td>
<td>70 ab</td>
<td>3.0 ab</td>
</tr>
<tr>
<td></td>
<td>Flagship</td>
<td>126.7 b</td>
<td>74 ab</td>
<td>2.2 a</td>
</tr>
<tr>
<td>Subsurface-heated</td>
<td>Outredgeous</td>
<td>158.0 c</td>
<td>84 b</td>
<td>7.0 c</td>
</tr>
<tr>
<td></td>
<td>Flagship</td>
<td>161.1 c</td>
<td>71 a</td>
<td>8.1 c</td>
</tr>
<tr>
<td>Aerial-covered</td>
<td>Outredgeous</td>
<td>194.7 d</td>
<td>68 ab</td>
<td>19.9 d</td>
</tr>
<tr>
<td></td>
<td>Flagship</td>
<td>194.4 d</td>
<td>72 ab</td>
<td>4.8 bc</td>
</tr>
<tr>
<td>Subsurface-heated and Aerial-covered</td>
<td>Outredgeous</td>
<td>221.7 d</td>
<td>74 ab</td>
<td>55.9 e</td>
</tr>
<tr>
<td></td>
<td>Flagship</td>
<td>207.0 d</td>
<td>64 a</td>
<td>25.0 d</td>
</tr>
</tbody>
</table>

Table 8. Microclimate x cultivar effects on lettuce shoot fresh weight measured 4 weeks after sowing and plant number measured 2 weeks after sowing in two red romaine cultivars exposed to four microclimates in outdoor and high-tunnel settings in 2009-10 in Wooster, OH.

\(^2\) Microclimate x cultivar interaction means within columns followed by the same letter are not significantly different by a pdiff difference statement at \(P\leq0.05\).

Discussion

We set out to document the impacts of root- and shoot-zone heating in low- and high-tunnel production on leaf lettuce yield, a dynamic with scientific and practical consequences. The results suggest that microclimates created by this experimental system profoundly affected leaf yield as measured 2 and 4 WAS, regardless of setting (outdoor, high tunnel). Specifically, the results suggest that a lack of aerial cover and supplemental root-zone heating tends to significantly suppress biomass production relative to all other
microclimates; that the presence of both a cover and supplemental sub-heating can enhance yield, most consistently under outdoor conditions; and that the distinction between aerial cover and root-zone heating in terms of yield potential may be minor in some seasons and settings. The data also suggest that these microclimate effects on yield may begin with differential stand establishment in some settings.

*Interactive and main effects of microclimate and cultivar on plant population*

In this study, the hand sowing of primed and pelleted seeds into an organic medium were used collectively to minimize the potential impact of adverse environmental conditions on stand establishment as outlined previously (Cantliffe, 1989). As hoped, stand differences were less common than microclimate differences. Still, plant populations recorded 2 WAS varied among microclimates in all four outdoor experiments but in only one high tunnel experiment (Table 6). Plant populations did not vary significantly with cultivar 2 WAS in either experimental setting (high tunnel, outdoor). However, cultivar-specific germination temperature optima may partially explain the significant interactive effect of cultivar and microclimate on plant population and yield data obtained in two high-tunnel experiments (Table 8). In the Spring 2009 high-tunnel experiment, yield of ‘Flagship’ was higher than of ‘Outregeous’ in the control, but not in the other three microclimates. Similarly, in Fall 2009, ‘Flagship’ populations were significantly lower than ‘Outregeous’ in some heated root-zone microclimates. Thermogradient table germination tests were then completed to help explain these trends in these two high-tunnel experiments (Fig. 1). In this controlled setting, ‘Flagship’ germination exceeded ‘Outregeous’ germination at lower temperatures while the reverse
was observed at higher temperatures. These cultivar-specific germination impacts may have contributed to microclimate x cultivar interactions in those two experiments.

*High-tunnel experiments.* Plant populations were affected by microclimate in only one of four high tunnel experiments [Spring 2009 (Table 7)]. Plant populations were generally similar among microclimates, suggesting that conditions supported stand establishment and that differences in yield followed growth. These outcomes are supported by previous work suggesting that high-tunnel-field soil temperature differentials may exceed air temperature differentials during some seasons (Both et al., 2007; Knewtson et al., 2010). If true, stand establishment of direct-seeded lettuce could be promoted in high-tunnel systems during periods when low external temperatures would suppress it regardless of whether other strategies are applied.

*Outdoor experiments.* Microclimate treatment, more than cultivar, clearly influenced plant populations recorded 2 WAS in all four outdoor experiments (Table 6). Treatment rank order differences tended to hold across experiments: covering, heating, or covering and heating raised beds generally resulted in greater populations compared to the control as would be suggested by germination data collected in a controlled environment (Fig. 1) but over the temperature range recorded during these experiments (Table 5). However, microclimate effects on population tended to be less consistent than microclimate effects on yield.
Figure 1. Cumulative germination (mean ± SE) of ‘Outredgeous’ and ‘Flagship’ red romaine lettuce 10 d after sowing in an organic medium on a thermogradient table with 10 temperature treatments (10 - 28 °C). Data include emerged and viable seedlings (n = 4).
Microclimate effects on plant biomass

Biomass production is a function of plant population and growth. In this study encompassing a range of stand establishment-growth conditions, biomass production appeared to hinge primarily on growth, our major interest, and less on plant population. Leaf and stem tissue mass also appeared to respond primarily to microclimate treatment more so than to cultivar. Experiments involving microclimate and cultivar treatments were repeated in multiple years, seasons, and settings. Although differences between high-tunnel and outdoor settings and seasonal variation were of secondary interest and largely untestable in this study, further experimentation should investigate their role.

Microclimate effects on yield were similar in the outdoor and high-tunnel experiments in most years. Control, aerial covered or subheated, and covered and heated plots tended to display the lowest, intermediate, and highest levels of biomass production, respectively. Only the magnitude of the variation within settings and experiments varied. In the outdoor setting, biomass production in aerial covered and subheated plots was greater than in all other plots in all experiments. Microclimate effects were more closely associated with microclimate-experiment combinations in the high tunnel, suggesting that the relatively more buffered high-tunnel environment diminished some treatment effects when external conditions were closer to optimal for growth. Taken together, the outcomes of the outdoor and high-tunnel experiments suggest that the cost-benefit relations of investments in low- versus high-tunnel growing systems for short-statured, quick-cycling crops such as baby lettuce are worthy of further focused investigation.
**High-tunnel experiments.** High-tunnel systems are noted for their ability to shield crops against abiotic and biotic stress, especially when a second, interior layer of plastic is used near the crop, as in this study (Lamont, 2005; Lamont et al., 2003). Here, significant differences among four interior microclimates were noted in three of four experiments, Fall 2008 the exception. Air and soil temperature averages were highest in Fall 2008 (Table 5), suggesting that the constant heating capacity of the three treatments was unnecessary or detrimental to optimize growth in that experiment. The warmer high-tunnel environment with potentially lower air volume and circulation was also conducive to plant disease, an additional potential drag on biomass production.

Plant health was sometimes a factor in the high tunnel experiments but less so in the outdoor experiments. Fungal disease, primarily *Rhizoctonia*, was occasionally noted in aerial and subheated plots within the high tunnel, an outcome reported as likely when air movement is reduced, higher temperatures prevail and free moisture is consistently abundant in the root-hypocotyl zone (Grosch and Kofoet, 2003). Electric heating cables surrounded by a high organic matter rooting medium covered with a dense canopy required a consistently high level of soil moisture to be maintained, a condition favoring disease. Upon disease identification, ventilation was increased and in the Fall 2009 and Spring 2010 experiments, etridiazole with thiophanate-methyl (Banrot 40% wettable powder; Scotts, Marysville, OH) was applied to limit disease progression. Variations in root-zone heating method, more frequent ventilation, a rooting medium lower in organic matter and/or other tactics may limit this weakness of our experimental system when applied elsewhere.
Outdoor experiments. Microclimate modification resulted in greater yields, relative to control plots, in all outdoor experiments. Installing plastic covers over horticultural crops is known to increase growth and yield in multiple systems and crops (Nair and Ngouajio, 2010; Rekika et al., 2009; Soltani et al., 1995; Wells and Loy, 1985). However, this approach relies exclusively on passive solar and soil warming. The influence of air- or water-mediated active root-zone heating is most often studied in growth chambers or greenhouses (Economakis, 1997; Elwell, et al., 1985; Li et al., 1994; Shedlosky and White, 1987). Fewer recent studies (Bumgarner et al., 2012; Hunter et al, 2010) have capitalized on earlier reports (e.g., Cameron Brown and Gray, 1957; Canham, 1952; Rykbost et al., 1975) and considered the impacts of direct soil heating in differently engineered low- and high-tunnel systems, especially in temperate climates.

The experimental system used here permitted direct comparisons of three microclimates and a control group in outdoor and high-tunnel settings. Outdoors, aerial-covered and sub-heated plots produced up to 40 times more above-ground biomass than control or other experimental plots. Covering raised beds or heating the root-zone they contain separately also increased yield relative to no action but to lesser extents than combining the two steps. Data reported here suggest that the on-farm value of covering or subheating alone or in combination be examined thoroughly and that such tests account for potential shifts in irrigation and/or disease management that the strategies may involve. Relative to no intervention, covering and subheating appear to be required to maximize fall and spring outdoor, raised bed baby lettuce yield in most years under the experimental conditions experienced here.
Seasonal environmental conditions unquestionably alter the impact of crop management techniques. However, unlike in the high-tunnel experiments, spring and fall seasonal yield averages tended to be similar in outdoor experiments. Interestingly, the average air and soil temperatures for the two lowest yielding outdoor experiments were similar to those recorded for the two higher yielding experiments (Table 7). This observation corroborates earlier assertions that temperature effects on crop growth and tissue composition vary both with the crop stage at which certain temperatures are experienced and the mean temperature of the entire study period (Bumgarner et al., 2012; Gazula et al., 2005; Kleinhenz and Wszelaki, 2003; Radovich et al., 2005). Direct-seeded, quick-cycling crops such as baby lettuce may respond strongly to dynamic environments involving rapid and potentially extreme temperature and light fluctuations over narrow timeframes influencing germination and growth. Growers and scientists accept the challenge of identifying microclimate management techniques that are adaptable and consistently effective and economical. Here, we provide further evidence that root- and shoot-zone microclimate modification can enhance lettuce biomass production, especially when used simultaneously in outdoor growing systems.
References


Chapter 4: Active and Passive Zonal Heating Creates Distinct Microclimates and Influences Spring- and Fall-Time Lettuce Growth in Ohio


Introduction

Microclimates that surround crops impact their yield and quality. Microclimates are, therefore, frequently manipulated (Lamont, 2005; Oebker and Hopen, 1974) but with variable levels of sophistication and success. These manipulations often follow from knowledge gained in tightly controlled studies outlining the role of temperature, light, humidity, and other individual abiotic factors in shaping the productivity and efficiency of horticultural production systems. However, reports from field research tend to emphasize system effects on yield over explanations of the microclimates or mechanisms responsible (Tarara, 2000). Moreover, incomplete descriptions of the microclimates that prevailed during experiments leave open the question -- do the results apply elsewhere? And, they constrain the development of a more complete understanding of plant responses to their environment, however tightly controlled. Both limitations must be addressed with regard to root- and shoot-zone temperatures and their effects within low- and high-tunnel systems, particularly those operating during variable weather periods, characteristic of spring and fall in the Great Lakes region.
In contrast to the sophisticated, active temperature control common in modern greenhouse, "plant factory" and similar systems, temperature control in low and high tunnels is passive and a function of solar radiation (heat source), outside temperature, and ventilation. Low and high tunnels are beneficial in the production of many species in multiple climatic regions (Carey et al., 2009; Lamont, 2005; Lamont et al., 2003; Waterer, 2003; Wells and Loy 1985, 1993; Wien, 2009; Wittwer and Castilla, 1995), but they are not designed to maintain temperatures within narrow ranges typical of actively controlled systems. Though essential in greenhouses, active aerial temperature modification is less effective in tunnels due to their design and is generally implemented only in short-term low-temperature conditions (Lamont et al., 2003). Active root-zone heating, a proven benefit in greenhouse production (Elwell et al., 1985; Shedlosky and White, 1987; Trudel and Gosselin, 1982; Zeroni and Gale, 1987), may also enhance the efficiency of tunnel systems. However, it is incompletely tested in these less controlled environments in temperate climates (Bumgarner et al., 2011, 2012; Hunter et al., 2010; Trudel and Gosselin, 1982). Root-zone heating can increase subsurface temperatures more directly and with greater control than the use of tunnels and/or mulches. That said, the relative impacts of passive and active aerial and root-zone heating within low and high tunnels operating fall and spring in a temperate climate known for unpredictable solar radiation levels are unclear.

The outcomes of combined active and passive temperature control techniques on microenvironments and vegetable crops are also uncertain given that combined systems are less often studied than ones involving passive modification alone (Diaz-Perez, 2009;
Nair and Ngouajio, 2010; Novak and Albright, 1985; Soltani et al., 1995; Wien, 2009; Wolfe et al., 1989). Given economics and crop physiology, incorporating root-zone heating into low- and/or high-tunnel systems may represent an opportunity to tailor microclimates to meet certain production-quality targets, including for short-cycling crops such as leaf lettuce (Bumgarner et al., 2011, 2012). Realizing that potential, however, requires a greater understanding of management effects on crop microclimates that, in turn, influence yield and quality.

Here, low- and high-tunnels undergoing passive and/or active aerial and root-zone heating were employed to meet three objectives: 1) to describe the temperature profiles within eight microclimates established within a field and high-tunnel setting, 2) to calculate thermal energy accumulation and estimate the efficiency with which thermal energy was converted to lettuce biomass in these microclimates, and 3) to compare historical and study-period weather data collected in Wooster and other sites as a first step in estimating the probability that results reported here will hold over larger areas and time frames.

**Materials and Methods**

*Site and experimental growing system.* This study consisted of both root- and shoot-zone microclimate modification treatments concurrently tested in duplicate outdoor and high-tunnel settings in two spring and two fall seasons (2008-10) at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH as reported in Bumgarner et al. (2011). Both experiments employed a split-plot design with four microclimate and two cultivar treatments functioning as main and subplot factors, respectively. Eight
wood-framed raised beds (2 ft x 8 ft x 6 inches) contained the four main plot microclimates: 1) unheated and uncovered control, 2) subsurface heated with soil-heating cable, 3) aerial covered with low tunnel, and 4) subsurface heated and aerial covered. Within these main plots were four subplots (2 x 2 ft) containing the two lettuce cultivars. Each of the eight microclimate x cultivar treatments was replicated four times in each experiment for a total of 32 subplots. In Fall 2008, a 13 x 40 x 6-ft single-layer 6-mil plastic tunnel was used while all subsequent high-tunnel experiments were carried out in one 30 x 80 x 13-ft single-bay, gothic style, single-layer, 6-mil high tunnel. Both tunnels were ventilated manually through doors and/or sidewalls.

The growing medium consisted of (v/v) 35% peat (Premier Horticulture, Quakerstown, PA), 35% dairy manure compost (OARDC), 15% shredded organic red clover (Trifolium pratense) hay (OARDC), and 15% silt loam field soil (OARDC). Control plots consisted of unheated and uncovered raised beds. Root-zones in treatments two and four contained a 40-ft automatic electric heating cable (Wrap-On Co., Bedford Park, IL) at approximately 3-inch spacing and a 4-inch medium depth which was triggered to function at medium temperatures below 23 °C. Treatment three main plots were covered by a single layer of 0.8-mil slitted polyethylene (Hummert International, Earth City, MO) stretched across four wire hoops to create an approximately 18 x 30-inch low tunnel. Treatment four main plots were polyethylene covered and cable heated as described above.

*Biomass data and analysis.* About 1000 preweighed primed and pelleted seeds of the two red-leaf romaine lettuce cultivars, Outredgeous (Johnny’s Selected Seeds, Winslow, ME)
and Flagship (Shamrock Seeds, Salinas, CA) were sown on 9 Oct. 2008, 21 Mar. and 10 Oct. 2009, and 16 Mar. 2010. Biomass yield of a 2-ft² section of each plot was taken approximately 4 weeks after seeding. Yield data was analyzed separately in field and high-tunnel settings in each experiment as described in Bumgarner et al. (2011). Briefly, a Proc Univariate (SAS version 9.2; SAS Institute, Cary, NC) procedure was carried out to test for normality on all data. Normally distributed data were analyzed using untransformed data, while data with a nonnormal distribution were log transformed. All analysis was then performed using Proc Mixed. Microclimate was analyzed as a fixed effect and replications within years were analyzed as random effects. Treatment means were separated using a pdiff difference statement at $P \leq 0.05$ when the fixed effects were significant at $P \leq 0.05$. Means were back transformed for inclusion in tables. Yield data reported in this manuscript will focus on microclimate main effects representing both cultivars. Relationships between biomass, solar radiation, average temperature, and growing degree days (GDDs) were analyzed and described using Pearson correlation coefficients from Proc Corr.

Environmental data. Total solar radiation was measured continuously at the OARDC weather station approximately 3000 ft from the experimental site. Additionally, photosynthetically active radiation [$PAR$ (LI250-A; LI-COR Biosciences, Lincoln, NE)] and ultraviolet (UV) radiation measurements in the UV-A and UV-B regions (IL1350 radiometer/photometer; International Light, Peabody, MA) in individual microclimates were taken periodically at each experimental site. Air and soil temperatures were recorded continuously in each microclimate plot at 30-min (Fall 2008, Spring 2009) and
15-min (Fall 2009 and Spring 2010) intervals using separate data loggers (Hobo ProV2; Onset Computer Corp., Bourne, MA). Aerial and subsurface temperatures were measured using shielded sensors in each main plot approximately 8 inches above and 1 to 1.5 inches below the media surface. Temperatures from each main plot were averaged to obtain daily treatment means in each experiment. GDDs (5 °C base) were then calculated from daily aerial and subsurface temperature treatment means in each experiment.

Results and Discussion
Aerial and subsurface temperature effects on key plant processes

Temperature contributes to the rate of biochemical reactions, especially those mediated by enzymes, in ways described by the Q10 concept (Campbell, 1977; Krug, 1997) and various researchers (e.g., Burke et al., 1988). Should enzymes in crop species be most efficient within specific temperature ranges, it is reasonable to suggest that crop environments be tailored to maintain these ranges as often as possible. Work outlined here and conducted by many others is consistent with this suggestion.

Aerial and root-zone temperatures impact plant growth; however, the specific modes by which they influence growth in ways that become measurable and discernible at the community or crop level appear to vary. For example, shifts in shoot-zone temperature tend to influence fluxes in the capture and utilization of light, carbon dioxide (CO₂), and water (Hay and Walker, 1989), evident as shifts in photosynthetic productivity in leaves (Oebker and Hopen, 1974). Changes in root-zone temperature can alter nutrient and water uptake, carbon partitioning, and plant hormone synthesis and movement (Bowen, 1991; Cooper, 1973).
This study involved creating and monitoring aerial and root-zone microenvironments and characterizing their effects at the whole plant level. As such, the data represent a summation of processes throughout the root-shoot axis that may have been affected by combinations of the commercially proven active and passive root- and shoot-zone heating methods employed here. Three aspects of these microenvironments are addressed in following sections, namely: 1) aerial and subsurface temperatures: means and variability, 2) thermal energy: its accumulation and conversion to lettuce leaf biomass in low and high tunnels, and 3) study-period specific and georeferenced historical temperature and light data: shaping the scope of inference of project conclusions and management recommendations.

Aerial and subsurface temperatures: means and variability

Temperature profiles within the four microclimates were distinct and internally consistent (Table 1; Bumgarner et al., 2011). Shoot- and root-zone temperature trends held across experiments although ambient solar radiation and temperature varied with season. Average total daily solar radiation levels were 234, 342, 193, and 361 langleys in Fall 2008, Spring 2009, Fall 2009 and Spring 2010, respectively. Slitted low tunnels, high tunnels and low tunnels within high tunnels decreased PAR by approximately 14%, 23%, and 33% relative to ambient, respectively. UV light, reported to influence both crop yield and composition (Tsormpatsidis et al., 2008), was reduced under a high tunnel by more than 60% and 90% in the UV-A and UV-B regions, respectively, relative to outdoor uncovered plots.
Figure 2. Aerial temperature profiles from four microclimates in field (A) and high-tunnel (B) settings in four leaf lettuce experiments in Wooster, OH in 2008-10. Box plots represent fifth and 95th (dots), 10th and 90th (bars), and 25th and 75th percentiles (boxes), median (solid line), and mean (dotted line) of all temperature values logged at 30- and 15-min intervals over the entire experimental period. Numbers represent microclimate treatments. 1 = control, 2 = subsurface heated, 3 = aerial covered, 4 = subsurface heated + aerial covered; (1.8 x °C) + 32 = °F.

Continued
Aerial temperature profiles. Mean aerial temperatures tended to be higher in covered than uncovered plots, regardless of setting (Fig. 2). And, soil heating appeared to have little effect on mean shoot-zone temperatures in uncovered plots; perhaps, heat supplied beneath the soil surface dissipated quickly above it. Still, the mean value is only one aspect of the temperature profile and a potentially weak indicator of possible plant responses to prevailing temperatures. Indeed, cumulative temperature-based plant growth models incorporate temperature variation using daily maximum and minimum values in order to calculate GDD (Diaz-Perez, 2009; Wolfe et al., 1989). In the current work, the
aerial temperature range was usually wider in passively heated (aerial covered) plots than in bottom-heated but uncovered plots or in unheated and uncovered (control) plots (Fig. 2). Also, the fact that mean values often exceeded median values indicates that maximum air temperatures were elevated more by shoot-zone heating strategies than minimum temperatures.

Temperature variability can also be described by calculating the average difference between consecutive logged values in each plot across entire experiments. By averaging the difference between temperature values recorded at 30-min intervals, a single number can be used to represent the variability depicted in Fig. 2. Field aerial temperature differentials (Fig. 3A) are larger and clearly more strongly influenced by aerial covering than by subsurface heating. A similar trend was noted in the high tunnel (Fig. 3B); however, the high-tunnel setting tended to dampen the overall effect of aerial covering on air temperature differentials relative to the outdoor setting.
Figure 3. Aerial temperature differentials from four microclimates in field (A) and high-tunnel (B) settings in four leaf lettuce experiments in Wooster, OH in 2008-10. Differential represents the mean ± SE of the difference in absolute value between consecutive data logger values representing 30-min intervals in all main plots throughout the experiment. A lower value suggests a more consistent temperature profile; \((1.8 \times ^\circ C) + 32 = ^\circ F\).
Figure 4. Subsurface temperature profiles from four microclimates in field (A) and high-tunnel (B) settings in four leaf lettuce experiments in Wooster, OH in 2008-10. Box plots represent fifth and 95th (dots), 10th and 90th (bars), and 25th and 75th percentiles (boxes), median (solid line), and mean (dotted line) of all temperature values logged at 30- and 15-min intervals over the entire experimental period. Numbers represent microclimate treatments: 1= control, 2= subsurface heated, 3= aerial covered, 4= subsurface heated + aerial covered; (1.8 x °C) + 32 = °F.
Figure 5. Subsurface temperature differentials from four microclimates in field (A) and high-tunnel (B) settings in four leaf lettuce experiments in Wooster, OH in 2008-10. Differential represents the mean ± SE of the difference in absolute value between consecutive data logger values representing 30-min intervals in all main plots throughout the experiment. A lower value suggests a more consistent temperature profile; $1.8 \times ^\circ C + 32 = ^\circ F$. 
Subsurface temperature profiles. Mean soil temperature values tended to increase by electric heating cables and low tunnels in outdoor and high-tunnel settings (Fig. 4). This effect was most pronounced when cables were used in conjunction with low tunnels (treatment 4). And, cable use alone (treatment 2) tended to increase root-zone temperatures more than low tunnel use alone (treatment 3). Moreover, cable use demonstrated a potential to reduce the variability in root-zone temperature relative to treatments in which cables were not used (Fig. 4). This trend was most evident in both the field and high tunnel when lower levels of solar radiation led to limited increases in daytime subsurface temperature.

Like aerial temperature differentials, field subsurface temperature differentials (Fig. 5A) tended to be greatest in aerial covered plots. In the field, subsurface heating in addition to aerial cover tended to reduce subsurface temperature differentials compared to aerial covering alone in all experiments. This trend was clearly observed in two of four high-tunnel experiments (Fig. 5B). Less ventilation of the high tunnel in Fall 2008 and Spring 2009 (Bumgarner et al., 2011) may have muted temperature variability and subsurface temperature differentials across microclimates in these experiments.

Plastic coverings applied as mulches, low tunnels or rowcovers are commonly and effectively used to increase soil temperature and heat unit accumulation in horticultural systems (Diaz-Perez, 2009; Moreno et al., 2002; Nair and Ngouajio, 2010; Soltani et al., 1995; Wolfe et al., 1989). Still, data reported here underscore that increases in soil and, possibly, aerial temperatures may be lower and more variable when only a plastic covering is used compared to when soil heating is used alone or in conjunction with a
plastic covering such as a low tunnel (Figs. 2-5). Passive heating (heat entrapment) hinging on solar radiation is less consistent and predictable than active heating provided via electric cables, warm water lines, or other approaches. Soil temperature is a key factor in lettuce emergence and early growth, (Bierhuizen and Wagenvoort, 1974; Krug, 1997; Wien, 1997; Scaife, 1973) and active root-zone heating can facilitate both, especially when ambient temperature and solar radiation levels are low (Bumgarner et al., 2011, 2012). Warmer root-zone temperatures may stabilize plant growth processes when cooler air temperatures would otherwise disrupt them (Zeroni and Gale, 1987), but this hypothesis is largely untested in tunnel settings. Should the data continue to suggest that root-zone heating has separate and unique impacts on crop physiology important to growers, investments in scalable approaches to root-zone heating may be more appealing to incorporate with existing aerial covering systems.

*Thermal energy: its accumulation and conversion to lettuce leaf biomass in low and high tunnels*

Thermal time, heat unit, or GDD calculations can help describe or predict cumulative, growth-related outcomes associated with continuous exposure to biologically relevant, crop-specific temperatures (Diaz-Perez, 2009; Villordon et al., 2009; Waterer, 2003; Wolfe et al., 1989). In so doing, GDDs and related calculations bridge physical and crop science. These calculations are typically based on air or soil temperatures only. Here, we employed a 5 °C base temperature in calculating soil and aerial GDDs, in part to compare the relative associations between average temperature and GDD values as independent variables and yield as the dependent variable.
Table 9. Correlations between biomass yield of leaf lettuce in field and high-tunnel settings and average aerial temperature, average subsurface temperature, aerial growing degree days (GDDs) base 5 °C (41 °F), subsurface GDDs, total GDDs, and total accumulated solar radiation (ASR) in Wooster, OH, in 2008-10.

<table>
<thead>
<tr>
<th></th>
<th>Aerial temp</th>
<th>Aerial GDDs</th>
<th>Subsurface temp</th>
<th>Subsurface GDDs</th>
<th>Total GDDs</th>
<th>Total ASR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Field Yield</strong></td>
<td>0.60 (0.015)</td>
<td>0.65 (0.0065)</td>
<td>0.72 (0.0016)</td>
<td>0.75 (0.0009)</td>
<td>0.80 (0.0002)</td>
<td>-0.19 (0.49)</td>
</tr>
<tr>
<td><strong>High tunnel Yield</strong></td>
<td>0.60 (0.015)</td>
<td>0.68 (0.034)</td>
<td>0.47 (0.068)</td>
<td>0.53 (0.033)</td>
<td>0.64 (0.008)</td>
<td>0.39 (0.14)</td>
</tr>
</tbody>
</table>

Accumulated solar radiation for specific microclimate treatments were calculated using daily solar radiation measurements from the Ohio Agricultural and Development Center weather station (Ohio State University, 2012) and adjusted using point PAR measurements from microclimate treatments.

Number in parentheses represents P value of Pearson correlation coefficient (r), N=16.

Yield and microclimate temperature values reported here and previously (Bumgarner et al., 2011) were positively related, regardless of season or setting (Table 9). GDDs tended to be more strongly correlated with yield than average temperature values in both experimental settings. In the high tunnel, aerial GDDs correlated most strongly with yield; yield associations with soil GDDs were significant but numerically lower. In the outdoor setting, total GDDs (Table 9) registered the highest correlation with lettuce biomass. Correlations with average soil temperature and soil GDDs were intermediate but greater than correlations with average aerial temperature and aerial GDDs. These
relationships in outdoor plots are reasonable given that lettuce growth is heavily influenced by the temperature of the growing point which remains close to the soil and, thereby, affected more by shallow soil than air temperature (Scaife, 1973; Wien, 1997).

The effectiveness or efficiency of heating strategies could be defined in part by the ratio of cumulative thermal energy applied to growth observed. Such calculations could be made for various crop stages and units of time. Here, we combine yield (grams per square foot) recorded 4 weeks after sowing and aerial and subsurface GDDs into grams per GDD to estimate the efficiency of the conversion of heat units to marketable yield outdoors and in the high tunnel (Table 10). In both settings, grams per GDD values were generally greater in modified than control microclimates. Also, grams per aerial GDD values tended to be higher than grams per soil GDD values. However, it is important to note that grams per aerial GDD tended to be greater when root-zone heating was applied, and this was most apparent in the field setting. These results suggest that a synergy involving simultaneous increases in aerial and subsurface temperature, inseparable in these calculations, may be at work. This speculation is supported by other authors who have reported that the utility of soil heating is linked with levels of other factors, such as light and aerial temperatures (Trudel and Gosselin, 1982; Zeroni and Gale, 1987).
<table>
<thead>
<tr>
<th></th>
<th>Field</th>
<th>High tunnel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall 2008</td>
<td>Fall 2009</td>
</tr>
<tr>
<td>Control</td>
<td>Shoot fresh wt</td>
<td>2.9 a</td>
</tr>
<tr>
<td></td>
<td>Aerial GDD conversion efficiency</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Subsurface GDD conversion efficiency</td>
<td>0.02</td>
</tr>
<tr>
<td>Subsurface heated</td>
<td>Shoot fresh wt</td>
<td>23.3 b</td>
</tr>
<tr>
<td></td>
<td>Aerial GDD conversion efficiency</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Subsurface GDD conversion efficiency</td>
<td>0.06</td>
</tr>
<tr>
<td>Aerial covered</td>
<td>Shoot fresh wt</td>
<td>21.9 b</td>
</tr>
<tr>
<td></td>
<td>Aerial GDD conversion efficiency</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Subsurface GDD conversion efficiency</td>
<td>0.10</td>
</tr>
<tr>
<td>Subsurface heated + aerial covered</td>
<td>Shoot fresh wt</td>
<td>75.0 c</td>
</tr>
<tr>
<td></td>
<td>Aerial GDD conversion efficiency</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Subsurface GDD conversion efficiency</td>
<td>0.16</td>
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<tr>
<td>Control</td>
<td>Shoot fresh wt</td>
<td>113.1</td>
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<td></td>
<td>Aerial GDD conversion efficiency</td>
<td>0.44</td>
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<tr>
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<td>Subsurface GDD conversion efficiency</td>
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<td>Subsurface heated</td>
<td>Shoot fresh wt</td>
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<td></td>
<td>Aerial GDD conversion efficiency</td>
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<td>Subsurface GDD conversion efficiency</td>
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<td>Aerial covered</td>
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<td>Subsurface GDD conversion efficiency</td>
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<td>Subsurface heated + aerial covered</td>
<td>Shoot fresh wt</td>
<td>119.5</td>
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<tr>
<td></td>
<td>Aerial GDD conversion efficiency</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Subsurface GDD conversion efficiency</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Table 10. Lettuce shoot fresh weight and aerial and subsurface growing degree days (GDD; 5 °C (41 °F) base) conversion efficiency measured approximately 4 weeks after sowing in both field and high-tunnel setting raised beds containing red romaine leaf lettuce in Wooster, OH, in 2008-10.

Grams per aerial GDD and grams per soil GDD calculated by dividing leaf lettuce yield (grams per square foot) by respective aerial and soil GDD for each microclimate in each experiment.

Continued
Table 10 continued

Means within settings and experiments followed by the same letter are not significantly different by a pdiff difference statement at $P \leq 0.05$ in Proc Mixed.

In the outdoor setting, grams per GDD calculations illustrate that the conversion of both aerial and soil GDD units into plant biomass was less efficient in the field than in the high tunnel in most plots (Table 10). However, it is apparent that in some outdoor experiments, the combination of root- and shoot-zone heating resulted in grams per GDD efficiencies similar to some high-tunnel microclimates. Therefore, concurrent modification of root- and shoot-zone temperatures may be the most viable approach for producing a direct-seeded baby leaf lettuce crop outdoors under conditions experienced in this study. The separate and combined value of passive aerial and active root-zone heating should be investigated in high tunnels, especially since relative yield gains in modified environments were influenced by ambient conditions.

While temperature was the focus of this study, it alone does not shape lettuce yield or quality. Other environmental factors, such as light, also have major roles in this process. Furthermore, temperature profiles and solar radiation levels can be closely associated. Although monitored less vigorously than temperature in this study, the light readings recorded beneath low and high tunnels help illustrate system effects on yield. For example, higher solar radiation levels contributed to increased GDD accumulation under low and high tunnels (relative to GDD accumulation in control plots) in Spring 2009.
compared to lower relative increases in GDD accumulation in Fall 2009 with lower ambient solar radiation.

Light may most affect lettuce growth when other factors, such as temperature, are less limiting (Wien, 1997). Here, as noted previously (Moreno et al., 2002; Nair and Ngouajio, 2010), yield was greatest in plots in which measured \( PAR \) levels were lowest, suggesting that temperature may have been more limiting to growth than light. Greater growth in warmer but dimmer microclimates could follow from an increased rate of reactions driving growth (Campbell, 1977; Krug, 1997) and/or reduced photoinhibition (Heatherington et al., 1989). Using correlations from the separate field and high-tunnels experiments, both average temperatures and GDDs appeared to have a stronger impact on yield than light (Table 9). In contrast to the field \((r = -0.19)\), the correlation \((r = 0.39)\) between light and yield was positive in the high-tunnel setting where temperatures may have been less limiting. However, neither correlation was statistically significant (Table 9). These correlations are based on cumulative data; therefore, they are incomplete indicators of whether light was limiting in specific instances. The correlations suggest that, overall, light is unlikely to have been the most consistently limiting factor (Elwell et al., 1985). A more complete description of solar radiation and physiological measures, such as chlorophyll fluorescence and carbon dioxide assimilation, is needed to further elucidate the relationships between plant productivity, light, and shoot- and root-zone temperature in these settings.

*Study-period-specific and georeferenced historical temperature and light data: shaping the scope of inference of project conclusions and management recommendations*
Reliable recommendations for managing crop microclimates hold across locations and seasons. Developing such recommendations requires testing microclimate-crop relationships across space and time, a challenging and resource-intensive process. Comparing abiotic conditions recorded during an experiment to historical weather data may aid in this process in three ways. First, it may help establish the probability of achieving similar results at other times and locations. Second, when coupled with yield data, the same exercise may help identify the relative influence of various factors (e.g., temperature, light) on system productivity at given locations and over specific periods. And, third, identifying yield-limiting factors may assist farmers in targeting investments designed to correct them.

Figure 6 depicts historical and experimental period ambient GDDs for OARDC in Wooster, OH (OARDC Weather Station, lat. 40.78°N), Alma, MI [National Climatic Data Center (NCDC) Station 200146, lat. 43.23°N], and Frankfort, KY (NCDC Station 153028; lat. 38.14°N). Alma, MI and Frankfort, KY are each approximately 2.5° north and south latitude, respectively, of an east-west transect set by Wooster, OH. We draw the following tentative conclusions from Fig. 6. First, GDD accumulation (pattern, total) was similar to historical trends in Wooster in two of four experimental periods (Fall 2008 and Spring 2009). This outcome suggests that results reported here are somewhat likely to recur there in similarly managed systems. And, second, given that historical trends in Michigan and Kentucky generally fall within the variability recorded during the project in Wooster, we hypothesize that temperature and light effects at those locations may mirror those in Wooster.
Figure 6. Experimental period and average spring (A) and fall (C) solar radiation accumulation and spring (B) and fall (D) aerial growing degree days [GDD; 5 °C (41.0°F) base] as calculated for Wooster, OH, Alma and Ithaca, MI, and Frankfort, KY. Experimental period GDD were calculated from data gathered in this study. Historical GDD were calculated using data from Wooster, OH collected by the Ohio Agricultural Research and Development Center (OARDC) weather station (lat. 40.78°N; The Ohio State University, 2012) for 1982-2011 and 1971-2000 National Climatic Data Center (NCDC) data for Alma, MI (lat. 43.23°N; Station 200146), and Frankfort, KY (lat. 38.14°N; Station 153028); (1.8 x °C) + 32 = °F. Accumulated solar radiation (ASR) data in langleys (Ly) were obtained from the OARDC weather station (2002-11) in Wooster, OH. Solar radiation for KY and MI were obtained from the nearest locations possible to the Frankfort, KY.
Figure 6 continued

and Alma, MI, NCDC weather stations. Frankfort solar radiation data were calculated from 2009-11 data (lat. 38.12°N; Western Kentucky University, 2012). Solar radiation data from Ithaca, MI, approximately six miles from Alma, were calculated from 2002-11 data (lat. 43.32°N; Michigan State University, 2012); 1 Ly = 41.8400 kJ m$^{-2}$. 

![Graph showing accumulated GDD and solar radiation data over time.](image)
Collectively, these data suggest that the relative value of both shoot- and root-zone microclimate management in high-tunnel and outdoor settings should be more thoroughly examined, especially as they may be applied in producing a short-season, cool-tolerant crop such as lettuce in transitional seasons. High tunnels alone can increase soil temperatures beneficially (Both et al., 2007; Knewtson et al., 2010), so commercial application of root-zone heating should maintain efficiency by avoiding overinvesting and producing temperatures higher than necessary, as has been suggested in previous leafy crop production under lower light conditions (Hicklenton and Wolynetz, 1987). In our high tunnel, cables combined with solar radiation may have increased root-zone temperatures more than needed to spur growth in some experiments (e.g., Fall 2008), thereby reducing efficiency. Further testing should involve greater control over the level, timing and/or duration of root-zone heating than possible here especially since temperature effects vary with crop growth stage. Future studies could incorporate crop microclimate, crop yield and georeferenced, real-time, and historical climate data to help identify optimal aerial and/or root-zone heating strategies that result in more predictable outcomes, especially in low- and high-tunnel settings.
References


Chapter 5: The Crop Growth-Nutritional Value Paradox as Illustrated by Lettuce Antioxidant Yield


Introduction

Suppliers and consumers tend to employ different criteria when assessing the value or worth of fresh vegetables. Intrinsic crop properties such as size, weight, appearance and, occasionally, biochemical composition tend to be important among consumers. More importantly, food choices of consumers signal characteristics they value most from farmers and suppliers. To date, the consumer value system generally appears to emphasize appearance, flavor, price, convenience and other factors (Scheerens, 2001). However, interest in the composition of fresh vegetables and other food products as it relates to nutritional or dietary value as a cornerstone of health is increasing (Bruckner, 2008; Dorias and Ehlert, 2008; Goldman, 2003; Gray et al., 2003; Liu, 2003; Milner, 2000; Premier, 2002).

The greater interest in horticultural crops as vehicles that may influence health creates three related dilemmas for farmers and scientists. First, describing crop composition in terms of its impact on human health via nutrition is complex and there are few, if any, accepted processes and associated metrics for assessing and managing composition on-farm. This is in direct contrast to the availability of protocols, recommendations and
decision-aides for measuring and increasing biomass yield (Dorias and Ehlert, 2008). Second, data suggest that primary and secondary metabolism may be 'in conflict' when establishing the abundance versus composition of a crop through agronomic management. Although beneficial phytochemical accumulation can be enhanced on-farm (Poiroux-Gonord et al., 2010; Crosby et al., 2008; Kalt, 2005; Parr and Bolwell, 2000; Schreiner, 2005; Treutter, 2010), high rates of growth and secondary product formation often do not co-occur (Grevesen et al., 2008; Herms and Mattson, 1992; Jones and Hartley, 1999; LeBot et al., 2009). Third, fresh vegetable farmers are rarely compensated based on the phytochemical composition of their product.

Faced with a lack of standard indices related to the nutritional quality and health-promoting potential of fresh vegetable crops, seemingly intractable crop physiology-based obstacles for managing crops to improve health-related aspects of crop quality while maintaining yield, as well as markets which rarely reward management of this tradeoff to benefit the consumer, farmers experience two types of loss. First, they cannot profit from rising consumer interest in crop composition and the desire to benefit maximally from each serving. Second, they are prevented from contributing fully to the advancement of food systems or meeting the challenges of crop production related to food security (Morris and Sands, 2006; Sands et al., 2009; Schneeman, 2001; Zhao and Shewry, 2011). Likewise, scientists operate less efficiently in evaluating benefits of experimental alterations in crop management.

In contrast to fresh market crops, processed vegetable production has established crop quality indices and targets to help optimize the relationship between fresh biomass yield
and processed product yield, especially for the benefit of the processor. For example, management may be guided by fruit soluble solid values (°Brix) in attempts to maximize °Brix yield in processing tomato (Solanum lycopersicum). This involves measures of biomass yield and °Brix levels to calculate the amount of soluble solids that can be harvested from an area and sold (Johnstone, 2005; Nichols, 2006). Intrinsic parameters of crop quality, such as °Brix, are a function of genetics and environment, both of which are partially controlled by farmers. Thus, tracking the components of °Brix yield allows crop managers to manipulate the relationship between abundance and quality through various means. Similar indices and approaches are used in other processed or industrial crops, including sugarcane (Saccharum sp) (Bressiani et al., 2002), grapes (Vitis vinifera) (Sharma and Upadhyay, 2004), and crops where pigments, antioxidants, and other compounds are important to the quality of products (Kassara and Kennedy, 2011; Mercurio et al., 2010); or can be extracted for commercial applications (Giusti et al., 1998; Greven et al., 2008; Piccaglia et al., 2002; Reyes et al., 2004). Collectively, these examples provide a pathway for positioning fresh vegetable production systems as focused and purposeful instruments of public health.

We employ an industrial crop perspective in proposing that the potential nutritional value of fresh market vegetables -- as described by their content of constituents known to positively influence human health -- can be measured and managed. 'Nutritional yield' is chosen to connote the yield (on an area basis) of these constituents, including vitamins, minerals, and phytochemicals either required for optimal human development or possessing the potential to enhance human health. As a component of 'nutritional yield',
'antioxidant yield' is calculated from crop tissue total antioxidant potential and crop biomass. Leafy crops, specifically red leaf lettuce (*Lactuca sativa*), were chosen as a model system to test this hypothesis. Leafy crops, including lettuce, are a major category worldwide and a worthwhile test crop in production-nutrition studies. Lettuce is consumed at high rates as it is currently second among fresh vegetables (Mou, 2009), although it offers comparatively less nutritional value. For example, crisphead lettuce ranked 26th among common fruits and vegetables in the levels of 10 key vitamins and minerals (Stevens, 1974). Also, lettuce phytochemical content tends to respond to genetic and environmental manipulation (e.g., Bumgarner et al., 2012; Garcia-Macias, 2007; Oh et al., 2009, 2010). Therefore, small increases in the concentrations of dietary constituents in lettuce may have far-reaching effects. At minimum, tracking the effects of treatments on biomass abundance and composition can provide important insights into the physiology and potential health value of major world crops. Just as important, documenting such treatment effects will also help chart a path toward optimizing biomass abundance and composition to the benefit of farmers and consumers. The specific objective of this project was to document environmental effects on red leaf lettuce biomass accumulation and composition. However, the greater purpose of documenting these effects was to develop a preliminary crop-specific antioxidant yield index. Implications and future related research associated with the use of such an index are also outlined.

*Materials and Methods*
Site description and experimental setup. All experiments were conducted at the Horticulture and Crop Science Department Farm of the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH. This study consisted of eight experiments conducted in four (two spring and two fall) seasons in two (outdoor, high tunnel) settings as previously described (Bumgarner et al., 2011). Within both the high tunnel and outdoor setting, the split-plot experiment consisted of four environments (three experimental and one control) and two red romaine lettuce cultivars as main and subplot factors, respectively. The four main plot (0.6 x 2.4 x 0.15 m) environments included: 1) unheated and uncovered control, 2) subsurface heated with soil heating cable, 3) aerial heated with plastic low tunnel covering, and 4) subsurface heated and aerial covered with soil heating cable and low tunnel covering. Within these main plots were four randomized subplots (0.6 x 0.6 m) with the ‘Outredgeous’ (Johnny’s Selected Seeds, Winslow, ME) and ‘Flagship’ (Shamrock Seeds, Salinas, CA) lettuce cultivars. Each of the eight environment x cultivar treatments was replicated four times in each experiment. Fresh biomass yield was recorded approximately 4 weeks after sowing by collecting all above-ground biomass from a standardized 0.19 m² section of each subplot. Leaves and stems were stored on ice in coolers until being weighed and then flash frozen in liquid N₂ within 4 h of harvest and kept at -20 or -80 °C, depending on laboratory analysis to be conducted.

Tissue composition analysis. Anthocyanin (Antho) concentrations were determined after pigment extraction from frozen tissue (-20 °C) using a method adapted from prior publications (Kleinhenz et al., 2003; Gazula et al., 2005) that we have previously
described (Bumgarner et al., 2012). Briefly, frozen tissue was homogenized with distilled, deionized water and then three separate and sequential extractions were completed with 20 ml, 20 ml, and 10 ml of 1% HCl acidified methanol. Extracts were filtered and centrifuged before being read on a Beckman Coulter DU730 spectrophotometer (Beckman Coulter, Brea, CA). Anthocyanin absorbances were obtained at 530 nm, and a standard curve of kuromanin chloride (Chromadex Inc., Irvine, CA) was used to calculate tissue pigment concentrations (mg 100/g fresh weight (fw)). The Ferric Reducing Antioxidant Power (FRAP) (Benzie and Strain, 1999) test was used to determine total antioxidant power (TAP). The above described extracts were combined with 3 ml of a working solution, incubated for precisely one hour at room temperature, and read at 593 nm in a Beckman Coulter DU730 spectrophotometer. The FRAP working solution contained 30 mM sodium acetate buffer at 3.6 pH, 20 mM Fe$_3$Cl, and 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ; Sigma Aldrich, St. Louis, MO) in a 10:1:1 ratio. A 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox; Sigma Aldrich, St. Louis, MO) standard curve was used to convert spectrophotometric absorbances to TAP (µM trolox equivalents/g fw). A modified FRAP procedure (FRASC; Benzie and Strain, 1999) was also used to quantify vitamin C (Vit C) levels in the frozen lettuce tissue (-80 °C) using the same procedure except that a water extract was used as previously described (Bumgarner et al., 2012).

Methanol extractions described above were stored in a -20°C freezer until they were analyzed by HPLC to determine quercetin glycoside contents. HPLC analysis was completed on all samples from ‘Outredgeous’ plots as that cultivar was repeated in all
eight experiments (Bumgarner et al., 2011). Methanol extract (10 ml) from each sample was combined with 10 ml HPLC water and 20 ml of 0.2 M sodium acetate (7.0 pH) solution. Three separate, sequential phase separations of 10, 10, and 5 ml ethyl acetate volumes were completed in a separatory funnel. Ethyl acetate fractions were then evaporated to dryness using a nitrogen evaporation system (N-Evap 111 unit, Organomation Assoc., Inc., Berlin, MA). Dry samples were re-dissolved prior to analysis in 0.5 ml or 1.0 ml methanol with 2% acetic acid depending on sample concentration and filtered through a 0.45 µm nylon filter (Fisher Scientific, Waltham, MA). Quercetin quantification was carried out on a reverse-phase HPLC System Gold 406A (Beckman Coulter) using a Phenomenex (Torrance, CA) Gemini 5 µ C6-Phenyl column (4.6 x 250 mm) equipped with a SecurityGuard column (4 x 3.0 mm), of similar material. The mobile phase consisted of two solvents: Solvent A = 0.2% acetic acid; Solvent B = acetonitrile. The mobile phase flow rate was 0.7 mL/min and the solvent gradient was as follows: initial conditions, 22% B; 0-1 min, hold at 22%; 1-8 min, ramp from 22% to 30% B; 8-13 min, ramp from 30% to 60% B; 13-18 min, hold at 60% B; 18-21 min ramp from 60% to 22% B; 21-24 min; hold at 22% B. The injection volume was 30 or 50 µl depending on sample concentration. The detection wavelength was 256 nm, and quercetin glycosides were quantified in mg/g fw using a quercetin-3-glucoside (Sigma Aldrich, St. Louis, MO) standard curve.

Data analysis. Data from each of the eight experiments were analyzed separately. Antioxidant yield, used as an initial measure of crop phytochemical composition per unit area, was calculated by multiplying tissue fresh weight per unit area (m²) with the
concentration of TAP (g/m² x TAP/g fw). A Proc Univariate procedure (SAS version 9.2; SAS Institute, Cary, NC) was carried out to test for normality, and normally distributed data were analyzed using untransformed data in Proc Mixed, while data with a non-normal distribution were natural log-transformed before analysis. Environment and cultivar were analyzed as fixed effects and replications within seasons were analyzed as random effects in Proc Mixed. Treatment means for antioxidant yield were separated using a pdiff difference statement at a $P<0.05$ level of significance when microclimate and cultivar fixed effects were significant at $P<0.05$. Figs. 7 and 8 contain untransformed data, while all transformed means were back-transformed for inclusion in appropriate tables.

Results and Discussion

Leaf lettuce composition was affected by genetic and environmental factors (Figs. 7 and 8). Seasonal weather conditions, experimental combinations of shoot- and root-zone heat treatment and cultivar influenced the makeup of leaf lettuce grown in outdoor and high tunnel settings (Figs. 7 and 8). Interactions occurred occasionally but generally constituted differences in magnitude, not direction, of treatment effects. We previously reported (Bumgarner et al., 2011) that biomass accumulation was impacted by the same factors. More importantly, the present (Figs. 7 and 8) and previous results reveal that the highest yielding varieties and environments in each experimental setting (outdoor, high tunnel) typically displayed the lowest density of constituents of dietary interest. In fact, these data and others in Table 11 suggest that a generally consistent tradeoff exists.
between enhancing biomass yield (growth) and the levels of compounds or properties associated with nutritional value and health promoting benefits for humans.

*Genetic and environmental effects on leaf lettuce composition*

Significant differences in tissue composition were noted in all six experiments involving cultivar comparisons and in six of eight experiments involving manipulations of root- and shoot-zone environments. Anthocyanin, TAP, Vit C, and quercetin were positively correlated in all experiments, indicating that TAP data presented here also represents trends in the other measured secondary compounds (Table 1). Amounts of Antho and TAP levels followed cultivar in all six experiments in both outdoor and high tunnel settings, whereas cultivar affected Vit C in only one outdoor experiment. ‘Flagship’ tissue tended to register greater levels of Antho and TAP (Figs. 7C and 8C) than ‘Outredgeous’. Root- and shoot-zone heat treatment influenced Antho and TAP levels in six of eight outdoor and high tunnel experiments. Quercetin glycoside content also followed heat treatment in five of eight experiments, although Vit C levels were unaffected by the crop microenvironment. Seasonal environmental conditions (Bumgarner et al., 2012b) altered Antho, TAP, and quercetin concentrations (Figs. 7B and 8B) and the impact of exposure to the eight experimental crop environments. However, the overriding pattern of these impacts on tissue mass and composition was consistent. That is, exposure to environments lacking subsurface heating or aerial covering tended to result in lower tissue yield that was 'phytochemically dense' whereas exposure to environments with subsurface heating and aerial covering tended to result in a heavier but 'less phytochemically dense' tissue (Figs. 7A and 8A).
Exposure to higher aerial and root-zone temperatures tended to lower the content of potentially beneficial secondary metabolites (Figs. 7 and 8) while increasing biomass yield (Table 1). The potential for tradeoffs between primary and secondary plant products has been reported previously and theoretical models are available to describe their relationships (Herms and Mattson, 1992; Jones and Hartley, 1999; Le Bot et al., 2009). Primary metabolism is responsible for utilizing photosynthetic products for biomass production, while secondary metabolism produces phytochemicals, such as phenolics, from the same plant products that drive growth (Jones and Hartley, 1999; Le Bot et al., 2009). Therefore, management to increase beneficial phytochemical levels can reduce saleable yield. Should fresh-market vegetable suppliers aim to enhance the health or nutritional value of their products within business climates that reward primarily saleable biomass yield, a greater command of and comfort with this tradeoff will be needed.

Here, the experimental objective was to document the influence of genetic and environmental factors on lettuce biomass accumulation and composition. The overall goal, however, was to employ these data in the development of a preliminary index of antioxidant yield. That genetic, environmental, and cultural practices influence fresh market leafy vegetable crop yield, pigment, and phytochemical levels is well documented (Borowski and Michalek, 2008; Bumgarner et al., 2011, 2012; Bunning et al., 2010; Garcia-Macias et al., 2007; Gazula et al., 2005; Kleinhenz et al., 2003; Lefsrud et al., 2005; Oh et al., 2009, 2010; Pulgar et al., 2001; Voipio and Autio, 1995; Zhao et al., 2007). However, few reports integrate yield (area basis) and tissue composition (weight
basis) as in processing, industrial, or less perishable crops (Bokanga et al., 1994; Bressiani et al., 2002; Guisti et al., 1998; Johnstone et al., 2005; Nichols, 2006; Piccaglia et al., 2002; Reyes et al., 2004; Sharma and Upadhyay, 2004). To our knowledge, the nutritional yield index reported here is the first of its type in the fresh market vegetable category. It is presented to help advance the science and practice of managing the paradox between crop growth and nutritional value.

Figure 7. Total antioxidant power (TAP) and biomass of red romaine leaf lettuce harvested 4 weeks after sowing as impacted by microclimate (A), season (B) and cultivar (C) in outdoor experiments in 2008-10 in Wooster, OH.
Figure 7 continued

B

C
Figure 8. Total antioxidant power (TAP) and biomass of red romaine leaf lettuce harvested 4 weeks after sowing as impacted by microclimate (A), season (B) and cultivar (C) in high tunnel experiments in 2008-10 in Wooster, OH. Continued
Nutritional yield is a function of biomass and composition

Nutritional yield may be calculated using any relevant variable of composition. Here, antioxidant capacity is emphasized with TAP selected for the calculation as a result of its significant correlation with other measured elements with health-promoting properties, including Antho, Vit C, and quercetin (Table 11). In outdoor experiments, antioxidant yield varied by cultivar in only the Fall 2009 experiment when ‘Flagship’ produced more antioxidant potential per area than ‘Outredgeous’ (Table 12). Microclimate impacts on antioxidant yield were observed in all four outdoor experiments. In all outdoor
experiments, antioxidant yield was highest in the combined subsurface heated and aerial covered plots and lowest in the unheated and/or uncovered plots. Antioxidant yield in individually aerial covered plots was similar to the subsurface heated plots in three of four experiments, but was always lower than that in plots with combined aerial covered and root-zone heating.

<table>
<thead>
<tr>
<th></th>
<th>Yield</th>
<th>Antho</th>
<th>TAP</th>
<th>Vit C</th>
<th>Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outdoor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antho</td>
<td>-0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAP</td>
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<td>0.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit C</td>
<td>-0.26</td>
<td>0.61</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
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<td>0.87</td>
<td>0.91</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td><strong>High tunnel</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
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<td>0.94</td>
<td></td>
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<td></td>
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<tr>
<td>Vit C</td>
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<td>0.43</td>
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</tr>
<tr>
<td>Quercetin</td>
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<td>0.65</td>
<td>0.75</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

Table 11. Correlation coefficients of leaf lettuce biomass yield, anthocyanin (Antho), total antioxidant power (TAP), vitamin C (Vit C), and quercetin in outdoor and high tunnel settings.

Number represents Pearson correlation coefficients ($r$) while $P$ value is contained within parentheses.
Table 12. Antioxidant yield from two cultivars of leaf lettuce harvested 4 weeks after sowing exposed to four experimental microclimates in outdoor and high tunnel settings in 2008-10 in Wooster, OH.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Outredgeous</th>
<th>Flagship</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outdoor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microclimate</td>
<td>Control</td>
<td>Subsurface heated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>296 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1108 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>880 a</td>
</tr>
<tr>
<td><strong>High tunnel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microclimate</td>
<td>Control</td>
<td>Subsurface heated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4810</td>
</tr>
<tr>
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<td>5978</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5210</td>
</tr>
</tbody>
</table>

a Denotes insufficient tissue for antioxidant power measurements.
b Letters denote significant main effects treatment differences as separated by pdiff at \( P < 0.05 \).
c TAP/m² was calculated by multiplying leaf fresh weight (fw) per unit area (g/m²) and total antioxidant power (µM trolox equivalents/g fw).

w Fall 2008 experiments were completed with only the cultivar Outredgeous.
In the high tunnel, microclimate and cultivar significantly influenced antioxidant yield in three of four experiments (Table 12). Antioxidant yield was greater in ‘Flagship’ than ‘Outredgeous’ in all three experiments. And, antioxidant yield was lowest in control plots and highest in subsurface heated and aerial covered plots, although the relative, individual effects of active subsurface and passive aerial heating varied by experiment.

Calculations of nutritional yield based on data reported here are worthwhile but what of values based on data in the literature? Data describing the effects of various factors on biomass yield and composition can be used to further evaluate the fresh market nutritional yield index. For example, Zhao et al. (2007) documented the influence of high tunnel and open field production environments on pac choi (Brassica rapa). Yield increased under high tunnel production but antioxidant capacity tended to be greater under open field conditions. From their data, we calculated that antioxidant yield (Table 13; TAP/plant) was higher in plots exhibiting the lowest tissue antioxidant levels but highest mass. This result is similar to ours. Similar trends were noted in reports considering biomass and carotenoid levels in spinach (Spinacea oleracea; Lefsrud et al., 2005).

Our data suggest that antioxidant yield (TAP/m²) tends to more closely follow tissue mass rather than composition. In local studies, the concentration of pigments and other secondary metabolites varied with crop environment (Figs. 7 and 8). However, as one component of nutritional yield, these concentrations appeared to be less influenced by environment than tissue mass, setting the stage for antioxidant yield to follow primary metabolism more strongly than antioxidant level (secondary metabolism) (Table 12).
These trends can vary by crop and environment, and there are examples in the literature that suggest it may be possible to enhance nutritional yield while maintaining biomass yield. One example is found in a study by Pulgar et al. (1999) describing Chinese cabbage (*Brassica pekinensis*) produced outdoors uncovered and covered with two different row cover materials. These researchers reported that carotene concentrations were greatest in uncovered plots where calculated carotene yields (mg carotene/plant) were lowest. However, under polypropylene cover, carotene concentrations decreased only slightly while plant mass remained high, resulting in the highest carotene yield of the three environments (Table 13). Secondly, Oh et al. (2009; Table 13) reported that short-term stress increased secondary metabolites in leafy crops while still producing biomass similar to untreated plants, thus increasing antioxidant yield. Finally, although not statistically comparable due to the experimental design in our study, antioxidant yield in outdoor, subsurface heated and aerial covered plots were often similar to or higher than antioxidant yield in unheated and/or uncovered plots in the high tunnel (Table 12). These data and those of Pulgar et al. (1999) and Oh et al. (2009) suggest that a more farmer- and consumer-friendly balance of abundance and quality may be possible under some conditions and management practices.

The calculation of nutritional yield must account for a number of variables, including tissue sampling and analytical methodology. Regardless of analytical approach, the levels of nutritionally relevant compounds exhibit spatio-temporal variability, differing by tissue type, crop stage, and time after harvest. Aspects of this variability are evident in nutritional yield values calculated from data reported earlier (Garcia-Macias, 2007).
Nutritional yield values calculated from Lollo Rosso lettuce varied according to whether whole heads or leaf sub-samples were analyzed and which secondary metabolic was included. Consistent methodology and a command of underlying physiological and metabolic systems (Poiroux-Gonord et al., 2010) will be required to increase the capacity to maintain yield and enhance nutritional value and develop production systems that optimize the abundance-quality relationship.

 Challenges associated with employing nutritional yield as a guide in crop management

The management of fresh market vegetable crops must allow products to meet consumer-based quality criteria different from those employed in the evaluation of industrial or processing crops. Therefore, enhancing nutritional yield must heighten, not undermine, interactions between farmers and buyers. For example, lettuce from plots exhibiting the highest antioxidant yield in this study was larger and more green in color than lettuce from plots displaying higher antioxidant density levels. Going forward, consumer evaluation must be paired with analytical procedures to ensure that crops with higher nutritional value also appeal to consumers.
### Nutritional yield described by antioxidant and carotene yield per plant

<table>
<thead>
<tr>
<th>Spinach carotene yield (Lefsrud et al., 2005)(^z)</th>
<th>Air temperature</th>
<th>(\beta) carotene (mg/g fw)</th>
<th>Biomass (g/plant)</th>
<th>Carotene yield (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
<td>0.11</td>
<td>33.1</td>
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<tr>
<td>15°C</td>
<td>0.08</td>
<td>119.6</td>
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<tr>
<td>20°C</td>
<td>0.07</td>
<td>156.0</td>
<td>11.1</td>
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<td>25°C</td>
<td>0.07</td>
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<td>8.0</td>
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</table>

<table>
<thead>
<tr>
<th>Pac choi antioxidant yield (Zhao et al., 2007)(^y)</th>
<th>Growing System</th>
<th>Antioxidant potential (µM trolox eq./g fw)</th>
<th>Biomass (g/plant)</th>
<th>Antioxidant yield (TAP/plant)</th>
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</thead>
<tbody>
<tr>
<td>Open field</td>
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<td>122.7</td>
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<td>High tunnel</td>
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</table>

<table>
<thead>
<tr>
<th>Chinese cabbage carotene yield (Pulgar et al., 1999) (^x)</th>
<th>Aerial covering</th>
<th>Total carotene (mg/g fw)</th>
<th>Biomass (g/plant)</th>
<th>Carotene yield (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncovered</td>
<td>0.23</td>
<td>523.6</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Polyethylene</td>
<td>0.13</td>
<td>1106.1</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>Polypropylene</td>
<td>0.19</td>
<td>1088.3</td>
<td>207</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lettuce antioxidant yield (Oh et al., 2009) (^w)</th>
<th>Stress treatment</th>
<th>Antioxidant potential (µM TEAC/g fw)</th>
<th>Biomass (g/plant)</th>
<th>Antioxidant yield (TAP/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.4</td>
<td>10.2</td>
<td>289</td>
<td></td>
</tr>
<tr>
<td>Heat (10 min)</td>
<td>32.7</td>
<td>9.2</td>
<td>300</td>
<td></td>
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<tr>
<td>Chilling (1 d)</td>
<td>37.1</td>
<td>8.6</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Light (1 d)</td>
<td>39.3</td>
<td>8.7</td>
<td>341</td>
<td></td>
</tr>
</tbody>
</table>

Table 13. Nutritional yield as described by antioxidant and carotene yield (biomass x composition) in leafy crops.

\(^z\) \(\beta\)-carotene and biomass values from Tables 2 and 1, respectively.

\(^y\) Field and high tunnel total antioxidant potential values are averaged from both organic and conventional total ORAC values from Table 2 and biomass from Table 4.

\(^x\) Carotene and biomass values from Tables 4 and 2, respectively.

\(^w\) Antioxidant potential and biomass from Fig. 2 and Table 1, respectively.
Table 14. Total antioxidant power (TAP; µM trolox equivalents/g fw) per serving (28 g) and servings/m² of growing area for ‘Flagship’ red romaine leaf lettuce grown in four experimental microclimates in Spring 2010 in Wooster, OH.

Rates of fresh vegetable consumption also suggest that change on the part of consumers and farmers will be required to ensure that efforts to enhance nutritional yield continue. For example, a 0.24 L (1 cup) shredded serving of leaf lettuce is estimated at 28 g (USDA). Using biomass and TAP data from the Flagship cultivar in the Spring 2010 experiment (data not shown), Table 14 portrays the antioxidant content per serving of lettuce and the number of servings produced in 1 m² of production area for four of the eight microclimate treatments tested. Microclimate treatment clearly impacted the amount of antioxidants in a serving -- e.g., a serving from outdoor control plots would contain nearly 4x the TAP contained in a serving taken from the high tunnel subsurface-heated and aerial-covered plots. However, the relative difference in the number of servings produced in 1 m² of these plots is much larger (approximately 50x; 0.84 versus 44.1 servings). Clearly, crop valuation and production (supported by integrated research and education) must change in order for nutritional yield to increase. As this work proceeds, it will be important to keep in mind that freshly consumed products tend to
have many attributes important to consumers. Therefore, a nutritional yield index useful to farmers and consumers will have to incorporate multiple diet/health-related variables (McCarty, 2004; Yao et al., 2004). This complex accounting could require a weighted index including several beneficial compounds or properties, the agronomic or genetic factors that control them, and their relationship.
References


Liu, R.H. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Amer. J. Clinical Nutr. 78:517S-520S.


Chapter 6: Digital Image Analysis to Supplement Direct Measures of Lettuce Biomass

Bumgarner, N.R., W. Miller, and M.D. Kleinhenz. Digital image analysis to supplement direct measures of lettuce biomass. HortTechnology (In review)

Introduction
Crop production and research involve descriptions and predictions of plant growth and biomass accumulation and distribution. Both processes tend to involve destructive and nondestructive sampling. Destructive sampling is most common and typically precedes direct measures of many variables. Still, direct sampling consumes time, effort, crop tissue, space, and other resources. It may also produce misleading results, such as inaccurate estimates extrapolated from sub-sample biomass values (Catchpole and Wheeler, 1992; Im and Jensen, 2008). Destructive sampling also tends to disrupt repeated measures of experimental plants or plots through time (Baker et al., 1996; Casadesus et al., 2007; Tucker, 1980). Reliable, resource-saving tools are required to enhance the effectiveness of destructive sampling in commercial and research settings.

Nondestructive assessment methods (e.g., remote sensing), which rely on estimates made without destroying or removing tissue are an alternative for scientists and producers. Remote sensing includes photography, machine vision, thermal imaging, laser scanning, and multi-spectral imaging. Regardless of form, the use of remote sensing is increasingly common in agriculture and ecology (Campbell, 2002; Im and Jensen, 2008; Thomas et al., 1988; Tucker, 1980). Photography, using film and digital formats, is one
of the most common, versatile, and cost-effective methods of nondestructively gathering information on a variety of crops (Campbell, 2002; Casadesus et al., 2007; Gerard et al., 1996; Hunt et al., 2011; Stewart et al., 2007; Yang et al., 2009). Still, estimates made via remote sensing and other approaches to nondestructive assessment are often adjusted based on validated measures or models built from on-site duplicate sampling or ground-truthing (Catchpole and Wheeler, 1992; Hatfield et al., 2008; Im and Jensen, 2008).

Digital images are gathered with various types of equipment. Inexpensive, consumer-based digital cameras capture wide bands of reflected light primarily in the blue, green, and red regions. Hyper- or multi-spectral, special-use cameras capture a wider portion of the spectrum and/or more narrow wavebands across the spectrum (Hunt et al., 2011; Li et al., 2010; Trout et al., 2008; Yang et al., 2009). Image analysis typically follows acquisition, regardless of method, and often involves software. Like cameras, image analysis software includes common-use commercially available and project-specific programs. Image analysis publications have listed these software programs: Adobe Photoshop, (Adobe Systems, San Jose, CA; Aide et al., 2001; Klassen et al., 2003; Stewart et al., 2007), GIMP 2.2 and Image J 1.33 freeware (Campillo et al., 2008), MATLAB (MathWorks, Natick, MA; Lati et al., 2011; Rasmussen et al., 2007), ENVI (Exelis Visual Information Solutions, Boulder, CO; Hunt et al., 2011), Sigma Scan (Systat Software, San Jose, CA; Olmstead et al., 2001; Olmstead et al., 2004), and WinCAM (Regent Instruments; Chai et al., 2010; Mkandawire et al., 2005; Roturier and Bergsten, 2009).
Image analysis is reported to be effective in assessing vegetative cover, nutrient status, crop maturity, and vegetation indices in many agronomic and forage crops (Adamsen et al., 1999, 2000; Casadesus et al., 2007; Ewing and Horton, 1999; Gerard et al., 1997; Li et al., 2010; Lukina et al., 1999; Olmsted et al., 2004; Stewart et al., 2007). Image analysis has also proven useful in describing weed populations and plant diseases (Lamb and Weedon, 1998; Lati et al., 2011; Nesser et al., 2000; Ngouajio et al., 1999; Nilson, 1995). And, digital images are used in ecology to monitor plant- or landscape-level changes (Bold et al., 2010; Booth et al., 2008; Ide and Oguma, 2010). Image assessment and other methods of remote sensing have been used in horticultural crop applications (Campillo et al., 2008; Davenport et al., 2005; Yang et al, 2008), although further investment in techniques and applications are needed (Lee et al., 2010; Trout et al., 2008).

With testing and calibration, nondestructive sampling -- e.g., image acquisition and analysis using commercially available equipment and software -- may effectively supplement or replace destructive sampling in horticultural crop production and science. Digital image analysis has often been tested and optimized with the use of sophisticated or proprietary image manipulation tools and/or algorithms that may be better suited to research than production settings (Adamsen et al., 1999; Lati et al., 2011; Rasmussen et al., 2007). Ease of use, cost, utility, and accuracy are important considerations in the development of nondestructive assessment methods regardless of application (Aide et al., 2007; Hatfield et al., 2008). Overall, digital image acquisition and analysis must be repeated under a range of conditions in order to establish the limits of their application.
We grew leaf lettuce in outdoor, high-tunnel, and greenhouse settings and used commercially available cameras and software to acquire and analyze digital images of these crops at various stages. Our goal was to document relationships between calculated values of crop variables obtained via this nondestructive approach and values obtained via destructive sampling and direct measurement. We also aimed to identify steps in the image acquisition-analysis process where these relationships are weakened.

**Materials and Methods**

*Case Study 1: Eight microclimates in outdoor and high-tunnel settings.* This study consisted of experimentally altered microclimates in duplicate outdoor and high-tunnel settings in two spring and one fall season (2009-10) at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH as reported in Bumgarner et al. (2011). High-tunnel experiments were carried out in a single 30 x 80 x 13-ft single-bay, gothic style, single-layer 6-mil high tunnel while outdoor experiments were in an immediately adjacent open field. Both experiments consisted of split-plot designs with the four microclimate and two cultivar treatments functioning as main and subplot factors, respectively. The eight microclimate x cultivar treatments were replicated four times in each experiment. Eight wood-framed raised beds (2 x 8 ft x 6 inches) contained the four main plot microclimates: 1) unheated and uncovered control, 2) subsurface heated with soil heating cable, 3) aerial covered with low tunnel, and 4) subsurface heated and aerial covered. Four subplots (2 x 2 ft) containing the two tested lettuce cultivars were contained within each main plot.
Control plots consisted of unheated and uncovered raised beds. Treatments two and four contained a 40-ft automatic electric heating cable (Wrap-On Co., Bedford Park, IL) at approximately 4-inch medium depth, triggered to function at temperatures below 23 °C, to provide root-zone heating. Low tunnels in treatment three main plots were covered by a single layer of 0.8-mil slitted polyethylene (Hummert International, Earth City, MO). Treatment four contained main plots with both polyethylene-covering and cable-heating as described in treatments two and three. The growing medium consisted of (v/v) 35% peat (Premier Horticulture, Quakerstown, PA), 35% dairy manure compost (OARDC, Wooster, OH), 15% shredded organic red clover (Trifolium pratense) hay (OARDC), and 15% silt loam field soil (OARDC). Approximately 1000 pre-weighed primed and pelleted seeds of the two red leaf romaine lettuce cultivars [‘Outredgeous’ (Johnny’s Selected Seeds, Winslow, ME) and ‘Flagship’ (Shamrock Seeds, Salinas, CA)] were sown on 21 Mar. and 10 Oct. 2009, and 16 Mar. 2010 in seven parallel rows within each plot.

Digital images (3264 x 2448 pixels) of a 1-ft² quadrant in the center of each plot were gathered in the field using a handheld camera with a focal length of 6.3 to 18.9 mm (Olympus FE-360; Olympus Corp., Tokyo, Japan) positioned directly above the target at a distance of approximately 2 ft. Camera aperture and focus were placed on the automatic setting to prevent variable operator-influenced focus adjustments and the flash was turned off. The camera-subject distance and viewing angle was standardized across images as much as possible. Images (576 in total) were gathered approximately 16, 23, and 30 DAS and acquisition was followed by destructive sampling in each plot. Destructive sampling
(10 inches of random row) included removal of all plants followed by counting and weighing the whole sample prior to measuring shoot and root weight of a representative subsample. Final yield data were taken on a standardized half (2-ft² section) of each plot approximately 30 DAS.

Case Study 2: Greenhouse setting. Leaf lettuce was produced in a 400 ft² glass greenhouse room in the Horticulture and Crop Science greenhouse facility in Summer 2009 at the OARDC in Wooster, OH. Destructive shoot biomass and leaf area measurements were compared with analyzed digital images across several time points of plant growth. ‘Outredgeous’, a red-leaf romaine lettuce, and green leaf ‘Two-Star’ were used to ensure measurements across varying leaf color, shape, and plant growth habit. Half flats [11 x 11 inches (BFG Supply, Burton, OH)] were filled with moistened media (Pro-Mix BX; Premier Horticulture) and three rows of primed and pelleted seeds (~70 seeds/ft) were placed by hand in three rows at 3-inch spacing on 24 Jun., 2009. Four replicate flats of each cultivar, one in each of the four blocks, were seeded for harvest at each of the six harvest dates for a total of 48 flats. Flat harvest date was randomized within each block.

Digital images (3648 x 2736 pixels) of all flats were gathered in the greenhouse using a commercial camera with a 36 to 216 mm focal length (Canon Powershot A2000; Canon USA, Lake Success, NY). Images were generally taken between 13:00 and 16:00 using a stationary tripod at a vertical distance of approximately 30 inches and a consistent camera angle. Camera aperture and focus were placed on the automatic setting to prevent variable, operator-influenced focus adjustments, and the flash was turned off. Images (48...
in total) were taken 7, 10, 14, 16, 21, and 28 DAS to correspond to pre-harvest destructive samples and the final harvest. Destructive plant data were also collected on the center of three rows in each half flat at each harvest date. The center row of each flat was removed by hand, roots were washed to remove media particles, whole plants were counted and weighed as a group, and shoot and root weights were recorded for a representative subsample. Shoot leaf subsamples were then measured with a leaf area meter (LI-COR 3100-C; LI-COR Biosciences, Lincoln, NE). These procedures were duplicated at each harvest 7, 10, 14, 16, and 21 DAS. For the final harvest date at 28 DAS, digital images were captured, and plants in the center row of each flat were harvested just above media level to represent leaf lettuce shoot biomass yield.

*Digital image analysis.* Image analysis was carried out using WinCAM 2009 Regular (Regent Instruments) which is designed to estimate specified colored regions in images. Images were saved from the camera onto a desktop computer (Dell Optiplex GX270 2.6 GHz; Dell, Round Rock, TX) as JPEG files. The analyzed region of each image was standardized for analysis to include only the area inside the 1-ft² quadrant or the 11 x 11-inch half flat. Operator-assisted color selection was used to designate colors in the image as background or leaf by selecting a specific set of colors from within the image to define color classes. All pixels chosen in the color class as leaf were used in the leaf calculation while all other pixels were classified as background color by WinCAM. The percentage of pixels was then used to calculate canopy cover compared to the area of media background in every image (Figs. 9-11). The accuracy of the software program was also assessed by comparing digitally traced and uniformly colored outlines of the total plant...
cover in a subset of selected images in Adobe Photoshop prior to analysis with WinCAM. These manually enhanced high contrast images were used as a reference leaf area in the image and were compared to the same raw image analyzed by WinCAM (Fig. 12).

Data analysis. Analysis by Proc Corr (SAS Version 9.2; SAS Institute, Cary, NC) was employed specifically to describe relationships between data collected on leaf lettuce crops using destructive and non-destructive approaches. Overall, destructive plant measures and WinCam estimates of canopy cover from 624 digital images in outdoor, high-tunnel, and greenhouse settings were correlated (Tables 15 and 16), providing opportunities for further more specific analysis. In the field and high-tunnel settings, biomass yield data (Bumgarner et al., 2011) and WinCAM canopy cover estimates collected approximately 30 DAS were analyzed separately within each experiment to uncover the ability of the image analysis method to describe potential treatment effects on growth (Table 17). A Proc Univariate procedure was carried out on all data to test for normality. All data were analyzed in Proc Mixed. Data with a nonnormal distribution were log transformed before analysis and then back-transformed for inclusion in tables. Microclimate and cultivar were analyzed as fixed effects and replications within years were analyzed as random effects in Proc Mixed. Treatment means were separated using a pdiff difference statement at a $P \leq 0.05$ level of significance when microclimate and cultivar fixed effects were significant at $P \leq 0.05$.

Results

Relationships between calculated values of crop variables obtained via nondestructive image acquisition-analysis approaches and values obtained via destructive sampling and
direct measurement were tested using correlation. Correlations were significant and positive at all time points in the field and high-tunnel experiments (Table 15; Case Study 1) and at five of six time points in the greenhouse experiment (Table 16; Case Study 2). Plant age did not consistently impact correlations within Case Study 1 (field, high tunnel) but age was a factor in Case Study 2 (greenhouse). Analysis of variance of microclimate treatment effects in the field and high tunnel at approximately 30 DAS using data from analyzed digital images was often similar to analysis using fresh biomass data (Table 17). However, leaf color (cultivar-based) also emerged as a factor that can influence the reliability and efficiency of digital images used as a partial proxy for direct measurement.

**Case study 1**

Pearson correlation coefficients between shoot fresh biomass measurements taken at approximately 16, 23, and 30 DAS and digital image leaf cover estimates were significant for both the outdoor and high-tunnel settings in all experiments (Table 15). When both cultivars were included in the analysis (N = 32), correlations were 0.71 to 0.95 outdoors and 0.72 to 0.85 in the high tunnel. Time points with the highest correlation coefficients varied by experiment and no consistent trend between correlations and DAS was detected. While correlations were slightly lower in the high tunnel than the field overall, results in both settings were similar. While correlations were significant at all time points in all experiments, correlations across all sampling points tended to be strongest in Spring 2009 in both settings.

Correlations between direct measures of shoot biomass and estimates of leaf cover from image analysis were also calculated on a cultivar-specific basis (Table 15).
Correlations were significant \((P \leq 0.05)\) at all points of measurement and major trends tended to hold for both cultivars. Still, outdoors, correlations for ‘Outredgeous’ were stronger at all sampling points in all three experiments (Table 15). Numerically, correlations between destructive and nondestructive measures outdoors were greater for ‘Outredgeous’ alone than for the average of the two cultivars although fewer observations \((N = 16)\) were included in the single-cultivar analysis (Table 15). Similar trends were observed in the high tunnel but less consistently and in a manner mediated by season.

A second approach involving pre-existing data and images was also used to describe relationships between direct and indirect measures of biomass. Specifically, we set out to determine if the outcomes of image analysis after the fact resembles outcomes obtained previously via destructive sampling and direct measurement. Would image analysis arrive at the same treatment effects on plant biomass as previous direct measurement? Analysis of variance was completed to address this question, the answer to which appears to be yes under these conditions (Table 17). Significant treatment effects on direct measures of biomass and indirect measures of canopy cover were noted in all six experiments. Trends in treatment means separation outcomes were similar between direct and indirectly obtained estimates of biomass, particularly in the outdoor setting. However, cultivar effects were similar between approaches in only three of six experiments.
<table>
<thead>
<tr>
<th></th>
<th>Spring 2009</th>
<th></th>
<th>Fall 2009</th>
<th></th>
<th>Spring 2010</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Out +Flag(^z)</td>
<td>Out(^y)</td>
<td>Flag(^y)</td>
<td>Out +Flag</td>
<td>Out</td>
<td>Flag</td>
</tr>
<tr>
<td><strong>Outdoor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 16 CC:FSW</td>
<td>0.91</td>
<td>(0.00001)</td>
<td>0.95</td>
<td>(0.00001)</td>
<td>0.71</td>
<td>(0.00001)</td>
</tr>
<tr>
<td>Day 23 CC:FSW</td>
<td>0.90</td>
<td>(0.00001)</td>
<td>0.91</td>
<td>(0.00001)</td>
<td>0.82</td>
<td>(0.00001)</td>
</tr>
<tr>
<td>Day 30 CC:FSW</td>
<td>0.87</td>
<td>(0.00001)</td>
<td>0.89</td>
<td>(0.00001)</td>
<td>0.95</td>
<td>(0.00001)</td>
</tr>
<tr>
<td><strong>High tunnel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 16 CC:FSW</td>
<td>0.85</td>
<td>(0.00001)</td>
<td>0.91</td>
<td>(0.00001)</td>
<td>0.77</td>
<td>(0.00005)</td>
</tr>
<tr>
<td>Day 23 CC:FSW</td>
<td>0.77</td>
<td>(0.00001)</td>
<td>0.77</td>
<td>(0.00006)</td>
<td>0.76</td>
<td>(0.00006)</td>
</tr>
<tr>
<td>Day 30 CC:FSW</td>
<td>0.80</td>
<td>(0.00001)</td>
<td>0.91</td>
<td>(0.00001)</td>
<td>0.77</td>
<td>(0.00005)</td>
</tr>
</tbody>
</table>

Table 15. The relationship between canopy cover (CC) from Win CAM analyzed digital images and fresh shoot weight (FSW) from destructive harvests of ‘Outredgeous’ (Out) and ‘Flagship’ (Flag) leaf lettuce in spring and fall outdoor and high-tunnel experiments (Case Study 1) in 2009-10 in Wooster, OH, represented by Pearson correlation coefficients (r).

\(^z\)N = 32
\(^y\)N = 16
<table>
<thead>
<tr>
<th>CC:LA</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
<th>Day 16</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 7-28</th>
<th>Outredgeous Day 7-28</th>
<th>Two-Star Day 7-28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.85</td>
<td>0.91</td>
<td>0.96</td>
<td>0.90</td>
<td>0.75</td>
<td>0.04</td>
<td>0.82</td>
<td>0.73</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>(0.0076)</td>
<td>(0.0017)</td>
<td>(0.0025)</td>
<td>(0.032)</td>
<td>(0.93)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td>CC:FSW</td>
<td>0.84</td>
<td>0.86</td>
<td>0.95</td>
<td>0.84</td>
<td>0.77</td>
<td>0.22</td>
<td>0.86</td>
<td>0.78</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>(0.0084)</td>
<td>(0.0055)</td>
<td>(0.004)</td>
<td>(0.0096)</td>
<td>(0.026)</td>
<td>(0.60)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>FSW:LA</td>
<td>0.99</td>
<td>0.89</td>
<td>0.91</td>
<td>0.99</td>
<td>0.93</td>
<td>0.88</td>
<td>0.96</td>
<td>0.99</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.003)</td>
<td>(0.0018)</td>
<td>(&lt;0.0001)</td>
<td>(0.0008)</td>
<td>(0.004)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
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Table 16. The relationship between canopy cover (CC) from WinCAM analyzed digital images, fresh shoot weight (FSW) and leaf area (LA) from destructive harvests of ‘Outredgeous’ and ‘Two-Star’ leaf lettuce in a summer greenhouse experiment (Case Study 2) in 2009 in Wooster, OH. Pearson correlation coefficients (r) are followed by P value in parentheses.

\(^a\) N = 8
\(^b\) N = 48
\(^c\) N = 24
<table>
<thead>
<tr>
<th>Shoot fresh wt (g/ft^2) and Canopy cover (in^2)</th>
<th>Spring 2009</th>
<th>Fall 2009</th>
<th>Spring 2010</th>
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</thead>
<tbody>
<tr>
<td>FSW</td>
<td>CC</td>
<td>FSW</td>
<td>CC</td>
</tr>
<tr>
<td><strong>Outdoor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microclimate</td>
<td>Control</td>
<td>2.0 a^2</td>
<td>25.1 a</td>
</tr>
<tr>
<td>Subsurface heated</td>
<td>10.3 b</td>
<td>46.5 b</td>
<td>10.3 b</td>
</tr>
<tr>
<td>Aerial covered</td>
<td>22.4 c</td>
<td>56.5 c</td>
<td>14.3 c</td>
</tr>
<tr>
<td>Subsurface heated + aerial covered</td>
<td>89.1 d</td>
<td>84.0 d</td>
<td>51.3 d</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cultivar</td>
<td>Outredgeous</td>
<td>16.2 B^2</td>
<td>56.7 B</td>
</tr>
<tr>
<td>Flagship</td>
<td>12.5 A</td>
<td>49.2 A</td>
<td>12.8</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.013</td>
<td>0.0002</td>
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</tr>
<tr>
<td><strong>High tunnel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microclimate</td>
<td>Control</td>
<td>108.0 a</td>
<td>72.0 a</td>
</tr>
<tr>
<td>Subsurface heated</td>
<td>159.6 b</td>
<td>88.8 b</td>
<td>45.0 b</td>
</tr>
<tr>
<td>Aerial covered</td>
<td>194.5 c</td>
<td>93.3 b</td>
<td>48.1 b</td>
</tr>
<tr>
<td>Subsurface heated + aerial covered</td>
<td>214.3 c</td>
<td>98.1 b</td>
<td>82.4 c</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cultivar</td>
<td>Outredgeous</td>
<td>165.9</td>
<td>91.9 B</td>
</tr>
<tr>
<td>Flagship</td>
<td>172.3</td>
<td>84.2 A</td>
<td>44.5</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.23</td>
<td>0.0091</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Table 17. Lettuce fresh shoot weight (FSW) from destructive harvest and canopy cover (CC) as estimated using WinCAM digital image analysis approximately 30 days after sowing in two red romaine cultivars exposed to four experimental microclimates in six outdoor and high-tunnel experiments in 2009-10 in Wooster, OH.

^2 Leaf canopy cover as estimated by digital image analysis using WinCAM software. ^2 Means within columns and microclimates (lowercase letters) or cultivars (uppercase letters) followed by the same letter are not significantly different by a pdiff difference statement at P≤0.05.
Case Study 2

Destructive-nondestructive measure correlations were positive and significant at most time points in the greenhouse experiment (Table 16). However, plant age had a stronger influence on these correlations when tested in the greenhouse than when tested in field or high-tunnel settings. For example, in the greenhouse, correlations were strongest 14 DAS and decreased thereafter, showing as not significant 28 DAS (Table 16). Correlations in the greenhouse were moderately influenced by cultivar with ‘Two-Star’ WinCAM:destructive harvest correlations tending to be higher than those of ‘Outredgeous’. However, cultivar effects appeared to be diminished in the summer greenhouse relative to fall and spring field and high-tunnel settings.

Discussion

In digital image analysis, samples are comprised of two-dimensional images representing three-dimensional objects. Plant canopies, such as those studied here, include both visible or emergent and hidden or occluded leaves. We sought to assess the strength of the association between leaf and biomass data obtained via nondestructive digital image analysis and related data obtained through standard destructive means. The strength of that association hinges on the extent to which two-dimensional images represent three-dimensional canopies. The data suggest that the association may be strong enough to warrant further attention in research and other settings. More specifically, the data suggest that digital image acquisition-analysis may be a suitable replacement for or supplement to destructive sampling and direct measurement of leafy vegetable crop canopies provided cultivar-background color contrast, growth setting, and plant age do
not interfere. Correlations between direct measures of lettuce leaf biomass taken on harvested plant samples and indirect measures obtained with a camera and software were consistent and statistically significant. This major result held for crops of two-three cultivars grown in a range of conditions established in field, high tunnel, and greenhouse settings in spring, fall, and summer seasons.

In research, these correlations suggest that fewer resources (e.g., seed, space, crop inputs) may be required to document treatment effects, that the benefits of repeated sampling can be realized more often, and that new hypotheses can be tested. In commercial production, these correlations suggest that yield models that aid scheduling and other processes may be developed, as reported previously (Kleinhenz and Radovich, 2003; Radovich et al., 2004). Results reported here also suggest that image acquisition-analysis be considered more seriously, particularly as improved hardware and software become available and as the need for efficiency increases in all sectors. That said, there is a wealth of opportunity and need with regard to follow-up related research designed to facilitate the greater application of digital image acquisition-analysis as outlined here.

**Image acquisition**

*Instrumentation.* Cameras vary in cost, performance, and ease of use, so the ideal camera type will vary with application. We tested the utility of images acquired with commercial digital cameras which may appeal to scientists and farmers due to cost, availability, and features. This type of camera has assets but may have limits in measured wavebands and some parameters of color (Casadesus et al., 2007; Hunt et al., 2011) relative to specialized multi- or hyper-spectral units which others may prefer. Comparing cameras or
types of cameras was beyond the scope of this work but the relative benefits of various units for use in the process described here should be explored. Potential users will likely prefer a balance of key features.

*Environmental and plant conditions.* Controlling for or overcoming ambient environmental conditions can be challenging in remote sensing, regardless of the instrument(s) used (Baker et al., 1996; Lati et al., 2011; Lukina et al., 1999; Rasmussen et al., 2007; Tucker, 1980). For example, cloud cover exhibits a high level of spatio-temporal variation and complicates image acquisition and analysis. Controlling for cloud cover has involved numerous improvements in equipment, software, and process over time. In the two case studies described here, images were collected under spring, summer, and fall conditions in a temperate climate but on an experimental plan based on crop stage. There was comparatively little freedom in altering sampling date (e.g., per sky conditions). Case Study 1 was carried out in spring and fall when light levels varied. Shadows were occasionally present during image acquisition and difficult to avoid. Shadows were also occasionally visible in the data images and difficult to correct at the analysis stage using software and methods employed here. Case Study 2 was completed in a relatively constant, high light environment (summer greenhouse room) and presented fewer shadow issues than observed in the field and high tunnel.

Ambient conditions, especially light, complicate the use of image acquisition-analysis by scientists and field production personnel. Supplemental lighting was unavailable in Case Study 1 (outdoor, high tunnel). Also, use of the camera flash, when tried experimentally, further reduced contrast in the images as bright sunlight may due to glare
(Lukina et al., 1999). To reduce experimental error, images for each experiment were analyzed and compared separately by time point rather than between time points. This step standardized the impact of ambient light conditions and eliminated the need to calibrate for light conditions across time, as described previously (Hunt et al., 2011). This approach is not optimum for all users, especially farmers and field managers. Follow-up efforts may benefit from including portable illumination to standardize light conditions and reduce the need for complicated or time-consuming adjustments during analysis.

Plant status and stand must also be considered during image acquisition. Digital images quantify leaf cover as a percentage of image area; therefore, leaf orientation is important (Klassen et al., 2003). Leaf orientation is influenced by light and moisture conditions, which vary in time and space. Not surprisingly, leaf position can change the amount of canopy closure on diurnal cycles (Mullen et al., 2006; van Zanten et al., 2012). Therefore, images should be acquired at similar times of day for comparison across dates (Giacomelli et al., 1998). And, plant populations should be even for reliable extrapolation from image canopy cover to larger field cropping areas.

*Camera angle and height.* The outcomes of image analysis may also be affected by the orientation and height of the camera during image acquisition. Inconsistencies in orientation can be due to altered pixel resolution in space within and between digital images (Lati et al., 2011). Two methods were employed in attempts to standardize camera orientation in this work: free-hand in Case Study 1 and a tripod in Case Study 2. Additionally, all images were taken from stationary rather than moving camera locations. Concerns over camera orientation and light levels are often justified, but their influence
on image analysis can be reduced by consistency (Rasmussen et al., 2007). Earlier work has also shown that increasing the distance at which images are taken, resulting in a larger field of view or larger surface area in each image, can reduce variability (Ngouagio et al., 1999). Here, images were gathered from distances that required no additional equipment. Future field application could incorporate techniques to increase area in images by increasing camera height. Regardless, taking specific steps to standardize camera orientation and evaluate height-resolution relationships is prudent.

Image analysis

Plant size and growth effect. Data reported here (e.g., Case Study 2) indicate that plant age, through its effect on canopy closure and leaf area index, can influence the relationship between digital image-based and direct estimates of biomass. Canopy closure tends to signal that LAI has reached one and that additional growth is unlikely to be captured in digital images and their analysis with the same reliability as during pre-closure (Stewart et al., 2007). Thus, correlations as studied here are likely to weaken after canopy closure and LAI exceeds one, especially in the absence of crop differences due to experimental treatments. In response, some propose the use of side-view images for tall crops, either alone or in conjunction with overhead images (Baker et al., 1996; Tackenberg, 2007). Integrating data from two views is more complex and likely to appeal primarily to scientists, however. Repeated evaluation of indirect-direct biomass estimate relationships -- in target cropping systems, over specific timeframes and as drawn with overhead images analyzed with improved commercial software -- may be a suitable alternative for many.
A) Approximately 17% canopy cover
11.5 g fw 237.5 cm² leaf area

B) Approximately 53% canopy cover
51.9 g fw 1528.0 cm² leaf area

C) Approximately 97% canopy cover
145.4 g fw 6212.6 cm² leaf area

Figure 9. Black and white images illustrating WinCAM identification of leaf (white) or background (black) material paired with original digital images in greenhouse-grown ‘Two-Star’ leaf lettuce at 10 days (A), 16 days (B) and 28 days (C) after seeding.
Separation of background material from leaf material. Image analysis relies on an adequate and consistent differentiation between plant material (leaf canopy) and soil or media background (Olmsted et al., 2004; Thomas et al., 1988), and a lack of contrast lowers the precision and potential utility of image analysis (Olmstead et al., 2001). In this work, canopy-soil separation at the analysis stage was occasionally inadequate. Growing media were dark-colored and contrasted strongly only with green leaves (Figs. 9 and 10), sometimes complicating the analysis of images of red-leaved cultivars (Fig. 11). Others (e.g., Trout et al., 2008) have faced the same challenge, especially if images were collected in low light.

Four methods were employed to enhance background-canopy contrast, two during image acquisition and two during image analysis. First, during acquisition, solid blue-colored paper strips were inserted between plant rows to limit background interference. This method enhanced background-canopy contrast and saved time during analysis. Second, red and green lens filters were used in a series of test images. However, in our tests, the value of lens filters was reduced because ambient light levels were often insufficient and resulted in dark images that were difficult to analyze well. Third, during analysis, color settings were manually adjusted during processing in WinCAM for each set of 'troublesome' images. And, fourth, images were manually adjusted before analysis. Using Adobe Photoshop (Adobe Systems), blackout regions were inserted in images in between-row regions where background interference could occur. This process was completed on all images within a time-point sample to limit bias. This method was effective when plants were small, but decreasingly effective as plant cover increased. It
was also very time-consuming. Follow-up work would do well to identify constitutive steps or camera or software features that enhance background-canopy contrast. These steps or features will significantly increase the appeal of image acquisition-analysis in research and crop production. Software and camera advances will likely increase the ability to differentiate canopies and backgrounds under varied conditions and reduce these challenges in the future (Lati et al., 2011; Stewart et al., 2007).

The impact of incomplete background-canopy separation by WinCAM on correlations was quantified by further analysis of a subset of images. Images varying in canopy size and leaf color were chosen, the canopies were manually colored blue in Adobe Photoshop, the edited images were re-processed by WinCAM, and data from edited and non-edited images were compared. Data in Fig. 12 help illustrate that plant age and leaf color may influence the accuracy of image analysis as conducted here. Overestimation of leaf area was more common on small, young canopies, especially when background-canopy differentiation was inadequate. Interestingly, in test images, levels of overestimation were similar in green- and red-leaved cultivars at early stages of crop development. As canopies increased, image accuracy varied more clearly by leaf color with green more accurately identified than red and the latter being underestimated due to misclassification of leaf area as background.

In conclusion, inadequate leaf-background color differentiation limited the ease and reliability with which digital image acquisition and analysis substituted for destructive sampling and direct measure of lettuce leaf biomass. Nevertheless, correlations between direct and indirect estimates of biomass were high under a range of circumstances and
image analysis led to statistical results closely resembling a standard harvest-weigh routine. Advances in equipment, software and technique will increase the appeal of digital image acquisition-analysis routines as partial substitutes for standard routines in research and production. Similarly, if modeling and/or prediction are goals, system scaling factors must be determined so that relative gains in real leaf area or biomass versus those in calculated values can be identified and employed in formulae. These relative gains are unlikely to be linear in all cases.

Figure 10. Black and white images illustrating WinCam identification of digital images of high-tunnel grown ‘Outredgeous’ leaf lettuce with primarily green leaves where separation between leaf (white) and background (black) material was relatively efficient.
Figure 11. Black and white images illustrating WinCAM identification of digital images of high-tunnel grown ‘Flagship’ leaf lettuce with primarily red leaves where separation between leaf (black) and background (white) material was less efficient.
Figure 12. Potential impact of cultivar and age on separation of leaf and background material in red (‘Outredgeous’) and green (‘Two-Star’) lettuce in an outdoor summer experiment as illustrated by the percent leaf canopy (±SE) cover as classified by the WinCAM software program ($n = 2$).
References


Chapter 7: Conclusion

Commercial vegetable crops are evaluated using metrics related to their abundance and quality. Yield, the primary unit of abundance, can be measured objectively at multiple scales and is used to calculate productivity and efficiency. Quality is not a unit but rather a condition described with objectively and subjectively measured terms usually based more on the market's interest than the supplier's. Crop quality terms can be placed into physical, chemical, biological, sensory, social, economic, and other categories. And, the perceived quality of crops influences their value to suppliers and users.

Vegetables contain constituents that contribute to human health when eaten -- this fact is one aspect of vegetable quality. Perhaps as a result, interest in vegetable composition has risen. Many have asked if it can be improved, thereby increasing the positive impact of vegetable consumption.

Scientists have long known that key aspects of vegetable nutritional value can be derived from their content of secondary metabolites. And, they understand that secondary metabolism is shaped by genetics and growing conditions. What is less clear, and what we set out to discover, is whether primary and secondary metabolism can be harnessed so that crop yield and quality are improved simultaneously. To test this hypothesis, we employed lettuce as a model crop, root- and shoot-zone heating, and fertility levels as treatments, and fall- and spring-time production as experimental periods. Lettuce ranks high in vegetable consumption but much lower in nutritional value, so improving its
secondary metabolite content could have far-reaching effects. And, fall- and spring-time production in the Great Lakes Region is increasingly popular but hampered by an incomplete understanding of the effects of root- and shoot-zone conditions (temperature, fertility) on crop physiology.

Assessments of crop growth and quality are also important to scientists and crop managers. In commercial settings, these assessments are the first step in maintaining or improving crop status (and equating production techniques with outcomes). In research, assessments of crop status are required to resolve treatment effects. We set out to test the use of digital imagery as a whole or partial proxy for destructive harvests in estimating yield. And, we cooperated with growers in developing baseline estimates of the soluble solids content of Ohio vegetables and related grower-based educational interests.

In total, four studies were completed over four years at the OARDC in Wooster, OH and on the farms of grower-cooperators. Major findings and recommendations for further work include:

1. Future studies should focus on developing accessible and accurate methods for on-farm, nondestructive vegetable crop yield and quality assessment.

The use of nondestructive methods in crop evaluation has been gaining ground in recent decades. Reasons for this increase include the fact that nondestructive methods can be very resource-efficient, readily standardized, applied in multiple settings in real time, and reliable in the assessment of an ever-increasing range of attributes. That said, too few of these methods and their associated technologies are accessible to farmers due, for
example, to cost or technical-managerial requirements. This low accessibility appears to be pronounced in vegetable production where the need may be greatest. Vegetable crop yield and quality fluctuate significantly with environmental conditions pre- and post-harvest and involve significant amounts of labor and coordination to maintain. Also, crop quality can strongly influence sale price, creating situations where crops which are very expensive to produce earn little to no return. Optical methods, such as canopy reflectance, NIR spectroscopy, and fluorescence, are prime candidates for application in horticultural enterprises much like a similar suite of remote sensing technologies have become commonplace in many agronomic operations. Still, vegetable crop producers need instruments with features adaptable to the diversity of crops, cultivars, scales of production, etc. common in horticultural operations. While the technology for nondestructive assessment is often available, it is too infrequently 'packaged' in ways useful and economically feasible for vegetable (horticultural) growers. Future work should focus on making such tools more accessible in all facets.

2. An enhanced understanding and management of the physiological responses of vegetable crops to off-season production in northern latitudes is needed to enhance their role in local food production systems.

Field and tunnel vegetable production in northern latitudes stands to increase, especially if growers manage temperature-light relationships more effectively. A greater understanding of the underlying physiology of these relationships and plant response to biotic and abiotic factors and a greater capacity to manage them -- including through
system engineering -- will allow growers to become more efficient on the farm and effective at penetrating local, specialized, and 'off-season' markets. Gains in efficiency (including through crop and cultivar selection and system setup and management) may be particularly relevant as premiums paid for organic and local products may erode. Decisions incorporating research-based information specific to northern latitudes and vegetable operations serving local, specialized and 'off-season' markets must be more commonplace. At minimum, this requires that the information be developed and made available. To begin, university-industry partnerships must remain viable and continue to provide targeted research for the spectrum of vegetable crop producers.

3. Management of production factors appears to be an appropriate and viable means of enhancing nutritional and health-promoting compounds in vegetables, but long-term incorporation of genetic advancements will likely be needed as well.

Genomic, metabolomic, human nutrition, management-oriented, and other methods and approaches have not yet revealed a clear, optimal path to enhancing crop nutritional value. It can be improved through genetic and management-based approaches but each involve risk, for example in terms of crop acceptance and environmental stewardship, and operate on different timelines as well as development and implementation investments. Alternative management approaches can be implemented which are capable of producing crop improvements immediately, but they may increase farming's environmental footprint. Breeding, genetics, and other related approaches may take years and improve crops dramatically but have the potential to lower their rate of acceptance. Moreover,
there is scant evidence that enhancing nutritional value is a major focus in genetic-based crop improvement since other traits such as biomass yield and disease resistance tend to be priority. Therefore, in the short term, the enhancement of crop nutritional value may remain mostly in the domain of crop managers as they manipulate crop microenvironments (including through the application of research-based information). Going forward, however, insights on how to better integrate genetic and management-based approaches will be needed.

4. Short-term management of temperature, nutrient, or light stress may be most effective in off-season leafy vegetable production for enhancing health-promoting secondary compounds while retaining an adequate biomass yield.

Agronomic management techniques, such as water, light, temperature, and nutrition management have been shown effective in increasing crop productivity as well as altering composition and potentially human health value. However, in terms of agronomic adjustments to fall and spring leafy crop production systems utilized in this work, it appears that conditions ‘stressful’ enough to enhance secondary metabolite accumulation occur after conditions ‘stressful’ enough to significantly decrease yield. The volatility of off-season production in temperate northern latitude climates makes managing stresses, especially temperature-related, during production difficult. These results indicate that while static alterations to growing systems were effective in altering crop composition, the drastic reductions in saleable yield suggest that targeted short-term stress may be the most effective approach. These recommendations are specific to leafy crops where the
saleable unit and the photosynthesizing unit are the same – therefore, physiological and biochemical composition changes that have the potential to impact the crop can occur quickly. Such approaches would also potentially fit better into producer schemes and allow growers to focus on certain aspects of crop production. Fruiting crops, which are in production longer and typically grow in more traditional seasons would likely be crops where longer-term management schemes would be potentially more necessary to provide agronomic enhancement of nutritional parameters.

5. To enhance future utility and crop management decisions, the nutritional yield index will need to be able to incorporate multiple measures of nutritional composition and health-promoting properties.

The nutritional yield concept has the potential to link consumer and producer valuation metrics of vegetable crop quality and value. Nutritional yield can be calculated using biomass and any number of nutritional components but we have proposed that a set of indicators be employed, creating the potential for an index by which crops can be compared. Such indices or related calculations are common in processed crops but more rare in fresh market ones. One key difference between processed and fresh market crops is that the wide array of composition factors native to the crop that influence taste, visual appeal, nutritional density, and other attributes are present and critical to the value of fresh market crops. It is also important to recall that nutritional components and properties are shaped by different biological pathways that can be differentially regulated by agronomic management practices and environmental conditions. Conditions that raise
the level of one compound may lower another. Therefore, metrics and means of valuation related to impacts on human health will need to incorporate multiple elements of composition and will likely be complex and crop specific. Therefore, the application of nutritional yield concepts and indices will need to adequately describe and account for these potential contradictions and complexities, particularly if indices are to assist in setting crop management and value.
References


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Appendix A: Warner Grant Report for 2010 On-farm Lettuce Microclimate Project: Fall through Spring Sustainable- Organic Lettuce Production in Ohio

Project Description

In the past several decades, vegetable crop production has changed through the use of agricultural plastics (plasticulture) that allow the immediate environment surrounding crops (microclimates) to be altered to more closely achieve optimum growing conditions. Methods of microclimate modification have the ability to alter both root- and shoot-zone growing conditions through the use of row covers, low and high tunnels, plastic mulches, and drip irrigation. These methods, also known as season extension techniques, have the ability to increase production within traditional temperate growing seasons and also to extend the productive season for crop growth and harvest. This extension of the growing and marketing season can have many economic advantages for horticultural producers because of the potential for a more local supply of vegetable products over the widest possible portion of the calendar year. Yet, Ohio farmers -- across the range of experience and operational size -- require additional information on these tools and techniques in order to employ them most effectively.

Considering the uncertainty that some Ohio growers have displayed in evaluating low and high tunnel purchases and integration into their operations, we have concluded that research-based information, workshops, and other educational resources could improve grower success. This project was designed to address grower requests for reliable information on the relative merits of season extension tools. In fall to spring 2010-11, we initiated comparisons of season extension techniques that modified both the root- and shoot-zone microclimate of leafy crops at five sites across Ohio and collected data on crop productivity and cost of treatment application to evaluate the potential of the
various techniques. Our aim was to help Ohio farmers be more successful, especially in local markets through the use of season-extending tools and techniques. Our specific objectives were to: 1) document the productivity of season-extending systems in on-farm tests across Ohio, and 2) assist farmers in applying the knowledge gained in these and companion tests underway at OARDC.

Description of Activities

Experimental setup

These on-farm experiments were all completed using three to six levels of root- and shoot-zone microclimate modification each replicated three times in a randomized complete block design. Microclimate treatments including (1) control, (2) root-zone microclimate modification, (3) continuous shoot-zone microclimate modification, (4) root- and continuous shoot-zone microclimate modification, (5) nighttime shoot-zone microclimate modification, and (6) root- and nighttime shoot-zone microclimate modification. The control (1) plots contained no additional heating or coverings. Estimated materials costs listed below correspond to all materials needed for each treatment in excess of the control. The root-zone heating treatment (2) included a 20 ft electric heating cable (Wrap-On Co., Bedford Park, IL) equipped with automatic thermostats set to heat below 23°C buried 3 to 4 inches under the soil surface. The heating cable was attached to a layer of woven ground cover (Hummert Horticultural, Earth City, MO) and was directly in contact with the soil. Hot-water sub-soil heating was also used at one site. The shoot-zone heating treatment (3) included the addition of a 0.8 mil slitted polyethylene low tunnel (Hummert Horticultural) stretched across three wire hoops and secured with lath and landscape staples to hold the covering in place. The root- and shoot-zone heating treatment (4) included both the electric soil heating cable and the polyethylene low tunnel as described above. The nighttime shoot-zone heating treatment (5) included the addition of Agribon Pro-50 (1.5 oz./yd²) polypropylene spunbonded row cover (Yoder’s Produce, Millersburg, OH) stretched across three wire hoops and secured with clothespins. The row cover was used to cover the plots each evening and
removed each morning. The root- and nighttime shoot-zone heating treatment (6) included both the electric soil heating cable and the polypropylene row cover used nightly as described above.

Organically certified primed and pelleted ‘Outredgeous’ red romaine and ‘Roxy’ red butterhead lettuce seeds were used. Three experimental sites were direct seeded with pre-weighed seed while two sites used transplants. Direct-seeded plots were sown by hand at 70 seeds/ft in with 6 inches between row spacing. Transplanted plots contained approximately 4 week old plants at 6-inch spacing.

*Environmental Data Collection*

Air and soil temperatures and relative humidity were collected at 15-minute intervals using Hobo ProV2 data loggers (Onset Computer Co., Bourne, MA). Each of the main heating plots in 2 or 3 blocks of the experiment were equipped with a separate data logger. Loggers were attached to wooden stakes and covered with radiation shields with air temperatures recorded 8 inches above the media surface and soil temperatures recorded 1.5 inches below the media surface.

*Plant Data Collection*

Plant stand counts of direct seeded plots were taken pre-harvest while stand counts on transplanted plots were taken at harvest. Final yield measurements were taken on representative rows or plants in each plot 4 to 5 weeks after planting. Leaves were cut close to ground level and placed in plastic bags and generally stored on ice in coolers until weighing. Weights were recorded for the entirety of harvested leaf material. Additional plant subsamples were taken to measure average individual plant weight and leaf area. These subsamples were then dried at 32°C for 72 hours and weighed to obtain percent dry weight.
Production Results

Farm 1

Research on this site was carried out to determine the effect of six root- and shoot-zone microclimate modification treatments (8 ft² plots) on direct seeded ‘Outredges’ leaf lettuce (Johnny’s Selected Seeds, Winslow, ME) grown in a (~) 20 x 96-ft double layer 6-mil high tunnel in native field soil for a period of 5 weeks between 10/8/10 and 11/12/10.

Crop Production and Financial Investment

<table>
<thead>
<tr>
<th>Proc mixed P≤0.1</th>
<th>Control (1)</th>
<th>Root-zone modified (2)</th>
<th>Continuous shoot-zone modified (3)</th>
<th>Root-zone + continuous shoot-zone modified (4)</th>
<th>Nighttime shoot-zone modified (5)</th>
<th>Root-zone + nighttime shoot-zone modified (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (g/8 ft²)</td>
<td>97.7 c</td>
<td>308.5 b</td>
<td>300.5 b</td>
<td>707.9 a</td>
<td>83.9 c</td>
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<td>Air temperature °C</td>
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<td>13.15</td>
<td>14.51</td>
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<tr>
<td>Estimated materials cost (8 ft² plot basis)</td>
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<td>$48.18 (all reuseable)</td>
<td>$3.81 ($2.51 reuseable)</td>
<td>$51.99 ($50.69 reuseable)</td>
<td>$4.71 (all reuseable)</td>
<td>$52.89 ($50.69 reuseable)</td>
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</table>
**Farm 2**

Research at this site was carried out to determine the effect of four root- and shoot-zone microclimate modification treatments (8ft² plots) on transplanted outdoor fall ‘Roxy’ butterhead lettuce (High Mowing, Wolcott, VT) grown in native soil for a period of 4 weeks between 10/12/10 and 11/11/10.

**Crop Production and Financial Investment**

<table>
<thead>
<tr>
<th>Proc mixed</th>
<th>Control (1)</th>
<th>Root-zone modified (2)</th>
<th>Continuous shoot-zone modified (3)</th>
<th>Root-zone + continuous shoot-zone modified (4)</th>
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<tr>
<td>P≤0.1</td>
<td>222.2 c</td>
<td>308.2 bc</td>
<td>424.0 a</td>
<td>348.6 ab</td>
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<td>Yield (g/8 ft²)</td>
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<td>Soil temperature °C</td>
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<td>$51.99 ($50.69 reusable)</td>
</tr>
<tr>
<td>Estimated materials cost (8 ft² plot basis)</td>
<td>$0.00</td>
<td>$48.18 (all reusable)</td>
<td>$3.81 ($2.51 reusable)</td>
<td>$51.99 ($50.69 reusable)</td>
</tr>
</tbody>
</table>

**Farm 3**

Research on this site was carried out to determine the effect of three shoot-zone microclimate modification treatments (8ft² plots) on direct-seeded ‘Outredgeous’ grown in native soil under black plastic mulch in a (~) 24 x 48-ft single layer 6-mil high tunnel for 5 weeks between 10/12/10 and 11/17/10.

**Crop Production and Financial Investment**

<table>
<thead>
<tr>
<th>Proc Mixed</th>
<th>Control (1)</th>
<th>Continuous shoot-zone modified (3)</th>
<th>Nighttime shoot-zone modified (6)*</th>
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<td>Nonsignificant at P≤0.1</td>
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<td>151.5</td>
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<tr>
<td>Yield (g/8 ft²)</td>
<td>$0.00</td>
<td>$3.81 ($2.51 reusable)</td>
<td>$4.71 (all reusable)</td>
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<tr>
<td>Estimated materials cost (8 ft² plot)</td>
<td>$0.00</td>
<td>$3.81 ($2.51 reusable)</td>
<td>$4.71 (all reusable)</td>
</tr>
</tbody>
</table>

* Inconsistently applied throughout the experiment
Research at this site was carried out to determine the effect of four root and shoot zone microclimate modification treatments (5.3 ft² plots) on a direct-seeded ‘Outredgeous’ red romaine leaf lettuce crops grown in a medium composed of peat, compost, clover hay, and field soil (35:35:15:15 v/v) from 10/2/2010 through 11/18/2010. Three sequential runs were grown for a period of 4 weeks each. Final yield measurements of the entire plot for each time group were taken on 29 Oct., 8 Nov., and 18 Nov. 2010. Leaves were placed in plastic bags and then stored on ice in coolers until weighing. Weights were recorded for the entirety of harvested leaf material and subsamples were taken to determine individual plant weight and leaf area. Yields and temperatures reported below are averages of the three runs.

**Production Results**

<table>
<thead>
<tr>
<th>Microclimate</th>
<th>Control (1)</th>
<th>Root-zone modified (2)</th>
<th>Continuous shoot-zone modified (3)</th>
<th>Root-zone + continuous shoot-zone modified (4)</th>
</tr>
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<td></td>
</tr>
<tr>
<td>Yield (g/8ft² plot basis)</td>
<td>13.7 c</td>
<td>59.0 b</td>
<td>53.6 b</td>
<td>155.8 a</td>
</tr>
<tr>
<td>Air temperature °C (10/2-11/18)</td>
<td>9.42</td>
<td>9.86</td>
<td>11.13</td>
<td>11.74</td>
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<tr>
<td>Soil temperature °C (10/2-11/18)</td>
<td>10.35</td>
<td>21.41</td>
<td>12.55</td>
<td>22.03</td>
</tr>
</tbody>
</table>
**Farm 5 (final yield data outstanding)**

Research on this site was carried out to determine the effect of root- and shoot-zone microclimate modification treatments (8 ft² plots) on approximately 4-week-old transplanted red leaf lettuce grown in field soil in a (~) 48 x 96-ft high tunnel for a period of ~16 weeks between 11/10/10 and 2/28/2011.

<table>
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<th>Microclimate 11/10-12/23</th>
<th>Nighttime shoot-zone modified</th>
<th>Root-zone + nighttime shoot-zone modified</th>
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**Combined Results and Financial Investment (3 sites)**

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<th>Root-zone modified (2)</th>
<th>Continuous shoot-zone modified (3)</th>
<th>Root-zone + continuous shoot-zone modified (4)</th>
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<td>Yield (g/8ft² plot basis)</td>
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<td>Estimated materials cost (8 ft² plot basis)</td>
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<td>$48.18 (all reusable)</td>
<td>$3.81 ($2.51 reusable)</td>
<td>$51.99 ($50.69 reusable)</td>
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**Project Discussion and Conclusions**

*Microclimate modification treatment effects on temperature profiles*

Temperature data collection illustrates the consistent impact of modification methods in altering the air and soil microclimates of leafy crops. At all sites, air and soil temperatures were increased by the use of agricultural plastics, either continuously or only at night and by the addition of soil heating. These temperature trends were consistent whether employed in outdoor plots or in a high tunnel, with or without the addition of raised bed, and in both mineral field soil and an organic growing medium. The highest air temperature averages were seen when slitted polyethylene was used as a low tunnel to cover crops continuously. Polypropylene covers used at night did increase average air temperatures, but these average increases were less than plots covered with
polyethylene during both the day and night. Soil temperatures were increased by the application of low tunnels alone and more dramatically by sub-soil heating methods. However, the highest air and soil temperatures were recorded when shoot- and root-zone microclimate modification methods were combined illustrating a synergistic effect of many of the microclimate modification methods employed in this study.

*Microclimate modification effects on biomass yield*

Yield analysis was carried out on a data set with three sites (two field growing system sites and one high tunnel site) combined. The effect of growing system was not significant showing that trends tended to be similar in the field and the high tunnel. Patterns of biomass yield mirrored differences seen in the air and soil temperature data with control (1) plots typically resulting in the lowest harvested yield and treatments that employed both root- and shoot-zone microclimate modification (4) resulting in the largest harvested yield. Microclimate modification systems incorporating root-zone (2) or shoot-zone (3) heating alone tended to be similar to each other, higher than the control, and less than the combined root- and shoot-zone modification treatments. Although carried out on fewer sites, it is also interesting to note that plots covered continuously with slitted polyethylene displayed higher yield trends than those covered only at night with a heavy polypropylene cover. Assuming that adequate light and air movement are present for growth and disease management, these results suggest than fall production could be most productive when daytime temperature is optimized.

While the trend of combined root- and shoot-zone modification methods increasing yields more than either method applied alone held true across two of the three sites in the combined analysis, one site showed slightly different trends. This site reported the highest yield in the (3) treatment which was similar to the (4) treatment and higher than the (1) and (2) plots. Treatment (4), while higher than the control was similar to the (2) treatment. These results display a yield pattern where the root-zone microclimate modification system increased yield to a lesser degree than was seen in the other sites. This reduced impact of root-zone heating could have been due to warm fall conditions that were generally conducive to plant growth leading to fewer benefits being derived
from additional heating. Another potential contributor was the difference between direct seeded and transplanted lettuce crops. The greatest impact of root-zone heating was seen in direct-seeded sites where increased media temperatures led to faster germination which contributed to increased yield due to a longer growth period for some microclimate treatments. However, another potential explanation for these differences at this specific site was the difference in water usage between the plots with and without sub-soil heating. Irrigation was applied equally to all plots and rainfall was sparse in the fall growing period causing the root-zone heated plots to dry out more quickly than the unheated plots. This soil drying led to some observed plant decline which decreased the impact of the root-zone microclimate modification method. Moisture was also shown to be crucial at the OARDC experimental site where the earliest of the three seeding dates received heavy rainfall that keep the medium saturated for several days after sowing. This moist medium contributed to increased disease pressure in the sub-soil heated plots where conditions were very conducive to the growth of plant pathogens. Disease pressure was not detected in crops seeded when soil moisture levels were lower and drying was able to occur.

The examples discussed above illustrate potential challenges of microclimate modification because techniques to alter plant environments can require additional management by the grower to prevent moisture and disease issues from reducing potential yield gains. Whether in an open field setting or a high tunnel, some management alterations will likely be needed to most beneficially use these and other methods of microclimate modification. These season-extending tools are increasingly common in the grower community and to achieve the greatest benefit in terms of capturing local markets and maintaining farm profitability, further research in the area of appropriate management needs to be a priority. In conclusion, although there can be an additional layer of management needed to optimize many methods of microclimate modification, the use of both shoot- and root-zone temperature altering techniques were shown to increase crop growth and yield in a late fall growing season for leafy lettuce crops in this multi-site study.
Dissemination of Project Results to Grower Communities

To facilitate dissemination of the information gained in this project, a Wiki site was created following the on-farm study (http://coldfingersclub.wikispaces.com/). This site is divided into a general overview page that discusses the overview and goals of the project and the combined analysis of multiple sites. Additionally, each individual site (identified only by number, not grower name) has a page with site-specific data and pictures at multiple points in the experiment. Final harvest pictures are included to visually display differences in yield measured. Initially, this site was distributed only to grower-cooperators, but as presentations have been made at producer conferences and meetings, this site has been further distributed. It is our hope that a site for the sharing and discussion of information and experiences on season extension can continue to be augmented and become a grower resource for Ohio producers.

In addition to the digital distribution of results from this microclimate study on the Wiki page, presentations on this project have been carried out throughout the winter and spring 2010-11. The project culminated in a 2-hour season extension workshop presented by the Co-PI’s by invitation at the Ohio Ecological Food and Farm Conference on Feb. 19th, 2011 in Granville, Ohio. An estimated 65 Ohio growers were in attendance at this combined lecture and discussion workshop. A presentation of the Warner grant on-farm microclimate modification study was a central part of this workshop. Yield, temperature, and material cost data was presented and growers were also encouraged to visit the Wiki site for additional information. It was an opportunity to demonstrate how root and shoot-zone microclimate modification can increase yield in fall and winter leafy cropping systems along with a discussion of the costs and benefits of such season extending techniques. Similar presentations will also be given at upcoming OSU Extension workshops to further assist Ohio farmers in incorporating techniques and methods tested in this study to optimum benefit in their operations.

This workshop also served as an additional step in grower education on season extension techniques as many present were relatively inexperienced in the use of these
methods. It became clear in the discussion incorporated in this season extension workshop that many newer sustainable and organic growers were unfamiliar with the principles and practices underlying the extension of growing seasons. Additionally, many growers seemed unsure of the location of resources to obtain additional information. We are considering the development of a resource guide to assist new and transitional growers in locating information and technical assistance in season extension techniques. This capstone resource publication could represent a significant step in linking growers to valuable information on season extension tools and techniques.
Appendix B: Warner Grant Report for 2011 On-farm Crop Quality Assessment Project: Preparing Farmers to Meet Crop Quality Goals

Project Overview

Our aim is to help Ohio farmers be more successful, especially in local markets through the use of crop quality assessment and management. In this project, our goal was to help Ohio growers better assess the quality of vegetable crops on their farms and prepare them to achieve higher produce quality as is increasingly expected of them by their buyers. We sought to achieve these goals through a coordinated research and training program. Our long-term goal was to begin a program of data collection that described aspects of the quality status of vegetables from Ohio farms with the assistance of cooperating farmers. To our knowledge, data on the status of vegetable crop quality in Ohio has not been previously gathered. This on-farm research was undertaken in conjunction with our goal of training and equipping growers to meet quality targets. Our education and outreach goal was to distribute and demonstrate use of quality assessment tools that focus on °Brix as an initial on-farm crop quality indicator useful. °Brix represents an ideal place to begin on-farm vegetable quality assessment because it can be reliably measured and is recognized throughout the value chain. With such methods of on-farm quality assessment, growers can begin to make informed management decisions to meet crop quality and farm sustainability targets. **Our research objective was to:** 1) document the crop quality status on Ohio farms through novel °Brix data collection. **Our training objectives were to:** 1) instruct and assist growers in on-farm measures of quality through assessment of soluble solids, and to 2) help growers identify and apply management techniques that optimize quality outcomes.
Background and Rationale

One of the key benefits of measuring °Brix levels in vegetable tissue is the availability of affordable and relatively precise field measurement devices, which is not the case for many other factors that contribute to crop quality. Sensitive analytical approaches and sensory panels are often recognized as critical in the description of crop quality and consumer appeal, but the level of investment and training needed makes these techniques impractical in many settings. Therefore, on-farm quality assessment and management must utilize tools and techniques that are not prohibitively expensive or technically demanding. Assessing °Brix through handheld refractometers represents one such method. Therefore, appropriate use of °Brix as an indicator of crop quality can be a ‘gateway’ for farmers allowing quality goals to be targeted in production systems. However, it is clear that such measures are currently underutilized and incompletely understood by Ohio vegetable growers and those were the critical gaps addressed by both the data collection and grower training goals in this project.

Description of Activities

Recruitment of Grower Cooperators for Crop Quality Assessment Project

Experienced and inexperienced farmers operating on small and large parcels are often interested but ill-equipped to tailor production systems and decisions to crop quality. To address these limitations in the grower community, the eight grower-cooperators involved in this project included farms representing a range of experience, geographic location, and operation size and production practices. Cooperators represented primarily organic/sustainable farm operations and/or educational institutions. Data was also collected and analyzed at OARDC from four additional farm locations, which did include farms with conventional production practices. Cooperators were initially drawn from participants of a crop quality workshop at the OEFFA 2011 conference that included both growers and marketers. Names of voluntary participants were collected at this February, 2011 workshop and contacted after notification that the grant was funded. Three growers in the project were attendees at this workshop. Three additional cooperators were
recruited through the OEFFA email list serve, while the remaining cooperators were recruited through personal contact by the co-PIs.

Grower Training and Project Implementation

Our two central goals were gathering Ohio crop quality data and training and equipping growers to assess vegetable crop quality on their own farms. Addressing both of these goals began in Summer 2011 with cooperator contact and initial education in the area of °Brix and crop quality. The training portion of the project was initiated with grower-cooperator training visits by PI-Bumgarner to assist growers with the use of handheld refractometers and distribute equipment and materials. These on-farm training sessions with each cooperator included a demonstration of methods and techniques and a discussion of crop selection and data collection methods throughout the season. Emphasis was placed on gathering data that represented replicated measures across potential seasonal, variety, farm management, and maturity impacts on soluble solid contents in vegetable crops. Visits also included the distribution of a refractometer (if the grower did not already own one), a garlic press to prepare samples, datasheets, and materials to properly clean and care for the refractometer.

Results and Data Summary

On-farm quality data collection, the central element of our research goal, began on each farm as soon as the training visit occurred and materials were in hand. An interactive web portal was available to grower-cooperators to allow for the real-time uploading and viewing of soluble solid data recorded during the project. Growers were also able to directly send or give data to the CO-PIs, and this seemed to be the simplest, most direct, and generally preferred method for growers. Data was compiled by farm (in a single-blind method) by the PIs as it was turned in by growers. Additionally, °Brix data was collected on-site at some farms. Data from these locations was recorded by the PIs and also immediately returned to the cooperating farm. In total, data from 24 different crops across 12 locations was gathered from July through November, 2011. All data was
compiled at the end of the season for dissemination through reports, presentations, and other publications. Below is a synopsis of data from the 2011 project on a several tested vegetable crops.

<table>
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<tr>
<th>Crop</th>
<th>°Brix average of all observations</th>
<th>Range of °Brix values</th>
<th># Observations in average</th>
<th># Farms reporting</th>
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<td>Beet</td>
<td>7.8</td>
<td>2.8 - 13.6</td>
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<tr>
<td>Bean</td>
<td>6.9</td>
<td>2.9 - 15.7</td>
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<tr>
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<td>2.6 - 6.5</td>
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<td>2</td>
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<td>3.5 - 5.3</td>
<td>43</td>
<td>4</td>
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<tr>
<td>Sweet corn</td>
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<td>9.5 - 26.5</td>
<td>66</td>
<td>2</td>
</tr>
<tr>
<td>Cherry tomato</td>
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<td>4.5 - 11.7</td>
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</table>

Dissemination of Project Results to Grower Communities:

Dissemination of results at the conclusion of the project was the second approach used to reach our training and equipping goal. These broader educational activities followed our in-person training visits to grower cooperators completed earlier in the project. Both of these training and educational approaches were designed to reach growers through a combination of in-person presentations and demonstrations, group workshops, and widely accessible print and digital educational materials.

In September of 2011, a °Brix demonstration and presentation of the project was given at the Ohio Food and Farm Education and Research Program annual field day. This project and accompanying activities and data were also presented by PI-Bumgarner to the
Great Lakes Vegetable Working Group at their annual meeting and drew interest and comment from vegetable production faculty and extension personnel from across the Midwest region. In February of 2012, a 2-hr training and demonstration workshop was held at the OARDC to share and expand upon knowledge and practices emphasized throughout the project. A similar workshop was also presented at the 2012 Ohio Ecological Food and Farming Association conference in February. In total, approximately 70 Ohio vegetable producers and educators were in attendance at these two workshops. In both workshops, information on the background and utilization of °Brix measurements to describe vegetable crop quality was discussed, and a demonstration of proper methods in gathering °Brix data and proper use of refractometers was presented. This overview and demonstration was followed by discussions of the impact of production practices on soluble solids content and overall crop quality. Data from the study was used to illustrate key points that could assist growers in the optimum utilization of °Brix measurements. Additionally, a sensory evaluation was carried out to allow growers to better understand human evaluations of crop taste and quality and both the utility and limitations of soluble solid measures.

Evaluations were conducted at both of these workshops. Results from both workshops combined showed a 37% increase in knowledge of what °Brix is, a 43% increase in knowledge of °Brix measurement, a 38% increase in knowledge of °Brix as an indicator of quality, and a 35% increase in knowledge of the use of °Brix in the farm business. The evaluations also indicated that 96% of participants would recommend the program to others while 94% answered that the workshop would influence the use of °Brix. On the question of whether the workshop would help them be more successful in measuring and using °Brix, 83% indicated it would. A final important indicator of usefulness was that 62% of respondents indicated that they would do something different in their operation as a result of something they learned in the workshop. These evaluations all indicate that the workshops were generally well received and that attendees felt they took valuable information away from the workshop. Open-ended questions in the evaluation also
indicated that areas remain to be explored and that more specifics about management remain for future projects.

To further facilitate dissemination of the information gained in this project, a °Brix guide (approximately 25 pages) has already been prepared. This guide will proceed through internal review and will be published as soon as possible as an OSU Extension publication. This publication focuses on four critical areas of °Brix assessment for farmers including: 1) The background and use of °Brix, 2) Discussion and pictorial guide to °Brix measurement, 3) Farm management decisions that can impact °Brix levels in crops, and 4) Using °Brix in farm management. Attendees at the two workshops were given a handout that also represented a shortened version of the larger °Brix guide. We also plan to divide the larger guide into sections that will address specific aspects of soluble solids measurement. These smaller versions of the guide will be available at our lab website (http://hcs.osu.edu/vpslab/).

In addition to the °Brix guide extension publication, two educational video segments have been prepared. A 5-min video entitled “What is °Brix?” as well as a 10-min demonstration video of °Brix sampling methods are currently available at the VPSL facebook page (http://www.facebook.com/osuvpslab). By March, 2012, our Facebook page reported that 211 people have been reached by the combination of these two videos. Both the guide and the demonstration videos will magnify the educational impact of the project by reaching a larger number of growers across a wider geographical area than would be possible with only workshops and presentations.

**Project Contributions**

This project has contributed to current knowledge and benefits Ohio producers in several ways:

- °Brix data was collected on many crops, both leafy and fruit, throughout the growing season to contribute to the formation of a novel Ohio crop quality database.
Ohio growers were given necessary tools and training to carry out on-farm crop quality assessment, which will allow future farm management decisions to be connected to quality outcomes.

Information on quality data and assessment techniques were disseminated to the Ohio grower community to assist producers in better understanding and achieving crop quality goals.

Project findings have been/and or will be presented and discussed …

- During annual field days, farm tours, and stakeholder meetings,
- During annual conferences and meetings (e.g., as organized by OEFFA, GLVWG and others),
- During workshops and training programs hosted by OSUE,
- In project reports available at our website,
- In Extension handouts, publications, and video segments, and
- On the OSU Vegetable Production System Laboratories facebook page and website.
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<th>Microclimate</th>
<th>Control</th>
<th>Subsurface heated</th>
<th>Aerial covered</th>
<th>Subsurface heated + aerial covered</th>
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* denotes insufficient tissue to complete replicated biochemical analysis

Letters denote significant main effects treatment differences as separated by pdiff at < 0.05
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<td>0.78</td>
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<tr>
<td>Control</td>
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<td>8.72 b</td>
<td>60.76 b</td>
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<td>0.226 b</td>
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<td>6.10 a</td>
<td>54.89 a</td>
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Letters denote significant main effects treatment differences as separated by pdiff at < 0.05