The Effects of Supra-Optimal Root Zone Temperature and Arbuscular Mycorrhizal Fungi on the Phytonutritional Quality and Growth of Red Onion (Allium cepa L.) cv. ‘Rossa di Milano’ and Strawberry (Fragaria x ananassa Duch.) cv. ‘Chandler’.

THESIS

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By

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Abstract

Phytonutrients (secondary compounds with bioactive properties) are health beneficial secondary metabolites. They also condition plant defenses to various environmental stress as illustrated by current studies. The phytonutritional quality of crops may, in part, be determined by abiotic and biotic interrelationships that are often created by cultural systems such as alteration of soil micro-climates, including changes to soil and root zone temperatures. Only recently have researchers begun to describe the effects of non-optimal root zone temperatures on the phytonutritional quality of horticultural crops. Additionally, arbuscular mycorrhizal fungi (AMF) are thought to improve the health of plants exposed to non-optimal environmental conditions. Evidence suggests that AMF improve the plant stress response through increased phytonutrition and antioxidant capacity. This study examined the combined effects of root zone temperature stress and AMF colonization on crop phytonutritional content of strawberry and red onion. The interactions between AMF and supra-optimal temperature stress were undetected. However, changes in the phytonutritional quality of both strawberry and red onion were observed under all supra-optimal temperature treatments by analysis if phytonutritional content. Additionally, supra-optimal temperatures altered the growth of both strawberry and red onion. Growth was reduced by AMF in strawberry and undetectable in red onion.
These results indicate that root zone temperature stress and AMF can influence the phytonutritional development and growth of strawberry and red onion.
Acknowledgments

Special acknowledgements are given to the late Dewey Bond and his family as well as the Horticulture and Crop Science Endowment for Vegetable Research for the continued financial support during the term of my program. I would also like to thank those who provided countless hours of both physical and intellectual support especially my co-advisors Drs. Joseph C. Scheerens and Matthew D. Kleinhenz, Dr. Ann Chanon, members of the fruit lab and members of the vegetable physiology lab. Additional resources were provided by Dr. A. Ray Miller and Dr. John Cardina.
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May 1994 .................................................. Eldorado High, Albuquerque, NM

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Chapter 1: Root Zone Temperature and Mycorrhizae Influence Phytonutritional Quality of Red Onion and Strawberry

Summary

Growth chamber studies were conducted to determine the effects of supra-optimal root zone temperature (RZT) and arbuscular mycorrhizal fungi (AMF) inoculation on ‘Chandler’ strawberry (Fragaria x ananassa) and ‘Rossa di Milano’ red onion (Allium cepa L.) phytonutritional quality and growth. Four treatments resulted from the supra-optimal RZT, supra-optimal RZT with AMF, optimal RZT, and optimal RZT with AMF. For strawberry phytonutritional quality, the highest values were found in fruit of supra-optimal RZT regardless of AMF inoculation. Furthermore, fruit color reflectance was darker, more red and higher in color saturation for supra-optimal RZT. Values for fruit weight and shape were lowest under supra-optimal RZT. Supra-optimal RZT enhanced vegetative growth of strawberry, while AMF decreased vegetative growth. No interactions were detected between RZT and AMF. For red onion quercetin concentrations, the highest values were found in bulbs of the optimal RZT while the highest values of total monomeric anthocyanins were found in the supra-optimal RZT. Overall plant growth of red onion was highest under optimal RZT. In conclusion, supra-optimal RZTs decreased strawberry fruit size and increased strawberry fruit phytonutrients, but decreased red onion bulb quercetin levels. This research may lead to
more appropriate use of soil micro-environments in both strawberry and red onion production.

**Introduction**

Plant nutritional quality may, in part, be determined by abiotic and biotic interrelationships that are often conditioned by cultural systems and production strategies employed by the producer. Carotenoids and polyphenolics are important secondary compounds that condition the plant defenses to respond to environmental stresses that reduce carbon fixation and/or alter carbon allocation (Davies and Schwinn, 2003; Siegler, 1998). Environmental pressures such as water availability (Castellarin et al., 2007; Csiszar et al., 2007; Jaleel et al., 2008; Kumar et al., 2007; Oh et al., 2009; Terry et al., 2007), irradiance spectrum (Garcia-Macias et al., 2007; Krizek et al., 1998; Tsormpatsidis et al., 2008; Voipio and Autio, 1995) air temperature (Benkeblia, 2003; Coolong and Randle, 2003), soil temperatures (Anttonen et al., 2006; Coolong and Randle, 2006; Locascio et al., 2005; McMichael and Burke, 1998; Tindall et al., 1990; Tulipano et al., 2008; Wang et al., 1998; Yamaguchi et al., 1975) and other environmental factors all appear to influence secondary metabolism, through changes to gene transcription, signal hormone transduction and enzyme activity, which ultimately lead to alterations in plant secondary product concentrations. Physical and biological changes in the root zone can contribute to these processes affecting qualitative content in crops (Fageria and Stone, 2006; Tindall et al., 1990; Yao et al., 2007). Two of these soil attributes are soil temperature and soil microbial population.
Kurets et al., (2003) determined that soil temperature plays a major role in plant productivity by moderating the level of root activity at higher temperatures and the resulting sink demands on photosynthetic organs. In fact, these authors demonstrated that narrow-leaved lupine, white cabbage, tomato and spring wheat were most productive when there was a temperature gradient from the air to the soil ranging from 2-10°C depending on plant species, where the soil temperature was higher than that of the air. It is thought that there is an optimal soil temperature for root growth, function and metabolism, and supra-optimal temperatures result in a reduction of root processes (McMichael and Burke, 1998).

In onions, supra-optima soil temperature of 29°C resulted in changes to volatile propyl sulfur compounds and pyruvic acid, which are determiners of pungency and flavor (Yamaguchi et al., 1975). In addition to quality, bulb weight, time to maturity and bulb shape were correlated to root zone temperature. Coolong and Randle (2003; 2006) also demonstrated the influence of elevated root zone temperature on growth and flavor of onions. Onion cultivar ‘Granex 33’, a common field variety, grown hydroponically and in a greenhouse setting exhibited higher fresh bulb weight, increased leaf number, higher soluble solid content, bulb sulfur and sulphate content with a root zone temperature of 21°C (Coolong and Randle, 2003; 2006). Conversely, studies observing a reduction in temperatures resulted in reduced soluble sugar content, phenolics and peroxidase activity (Benkeblia, 2003).

In addition to onion, strawberry also experiences augmented root processes, resulting in lower marketable yields, under increased soil temperatures which affect plant
biochemistry and physiology of both plants (Locascio et al., 2005; Wang et al., 1998). Strawberry root processes are most efficient at soil temperatures ranging from 10-20°C depending on cultivar (McMichael and Burke, 1998). Strawberry has naturally high concentrations of vitamin C and phenolic compounds (Tulipani et al., 2008). Of these compounds, anthocyanins play a major role in the antioxidant capacity. Anthocyanin content has been found to be higher in strawberries grown under brown mulch compared to those grown under white mulch (Anttonen et al., 2006). Soluble solid content and overall pigment concentration was found in high concentrations of plants grown with black polyethylene mulch which is known to elevate soil temperatures (Wang et al., 1998). Additionally, these strawberries ripened a week earlier than those grown without mulch. Black polyethylene mulch has been known to produce higher soluble solid content and the reddest fruit (Wang et al., 1998).

Root zone temperature is not the sole factor influencing root activity; symbiotic relationships with beneficial soil microbes such as arbuscular mycorrhizal fungi (AMF) can also affect growth, productivity and quality of many crops (Gosling et al., 2006; Koide, 2000). Host response to mycorrhizal colonization varies significantly among and within species (Devi and Reddy, 2002; Fan et al., 2008; Guo et al., 2007). Plants that are typically responsive to colonization by AMF possess poorly developed root systems whereas the root systems of “non-responsive” plants have the capacity to successfully mine the soil nutrient supply (Hata et al., 2010). Plants of the former group, like the Liliaceae family including Allium species, exhibit a positive growth response following AMF colonization. Moreover, the application of Glomus species to four vegetable crops
(green pepper, parsley, carrot and tomato) in Slovenia resulted in elevated carotenoids, titratable acids, chlorophyll content as well as plant and root biomass (Regvar et al., 2003). Significant increases in peroxidase and polyphenol oxidase activities, which are important to the plant stress response, following AMF inoculation have been documented (Devi and Reddy, 2002). Studies have concluded that AMF inoculation can also affect phenolic acid concentrations and flavonol glycoside concentrations, specifically quercetin and isorhamnetin, in onions (Guo et al., 2006; Guo et al., 2007; Perner et al., 2008).

Historically, our attention has focused upon the importance of plant-derived vitamins and minerals as nutritional components essential for growth and sustained health. However, public interest in phytonutrients, mainly carotenoids (carotenes and xanthophylls) and polyphenols (e.g., flavonol glycosides, anthocyanins and tannins) as health-beneficial compounds is increasing due to their reported activity as antioxidants and inflammatory regulators that may delay the onset or lessen the severity of many degenerative diseases of aging (Liu, 2003; Seeram, 2008). Methods used to enhance phytonutritional content in commonly consumed, horticultural crops are of great interest to food and crop scientists. Activation of plant defense mechanisms and secondary metabolism may be possible through manipulation of environmental cues but requires additional research.

Further studies are needed to further illustrate the influence of supra-optimal root zone temperature and AMF inoculation on secondary metabolism and plant physiology. The objective of this study was to determine the effects of supra-optimal root zone temperature...
temperature and AMF inoculation on the phytonutritional content and growth of strawberry and red onion.

Materials and Methods

Single trials were conducted for each crop during 2009 at the Ohio Agricultural Research Center of the Ohio State University, located in Wooster, OH. Experiments were conducted using growth chambers. A randomized complete block design was used and each growth chamber represented a complete replicate. The strawberry experiment was conducted in a single growth chamber and a completely randomized design was used. A total of four replicates (n=4) were achieved for each experiment.

Four treatments were used: optimal root zone temperature (control), optimal root zone temperature with AMF inoculation, supra-optimal root zone temperature and supra-optimal root zone temperature with AMF inoculation. Soil temperature was controlled by using circulating, heated water baths as described in Appendix A. See Table 1.1 for the environmental parameters maintained in the growth chambers during each experiment.

A steam sterilized, organic potting mixture consisting of sphagnum peat moss, vermiculite, sharp sand, perlite, kelp, humate, rock phosphate and greensand from Ohio Earth Food™ (Hartville, OH) was used as planting media. A mycorrhizal inoculant mixture consisting of three commercially available products was applied by watering in approximately 4 grams per pot immediately following seeding. Also, 4 grams of sterile inoculant, autoclaved for 30 minutes at 100°C, were applied to the control units. The inoculant mixture is as follows: EcoFungi (EcoMicrobilas, LLC, Miami, FL) (Glomus aggregatum, G. intraradices, G. etunicatum and G. mossea, at a concentration of 280
spores per gram), MycoApply® Ultrafine Endo (Mycorrhizal Applications, Inc., Grants Pass, OR) (G.intraradices, G mosseae, G.aggregatum and G. etunicatum) at a concentration of 120 propagules per gram and BIO ORGANICS™ (Santa Maria, CA) Endomycorrhizal Inoculant (BEI) (G. aggregatum, G. clarum, G. deserticola, G. intraradices, G. monosporus, G. mosseae, Gigaspora margarita and Paraglomus brasilianum at a concentration of 77 spores per gram). Approximately 4 grams of either active or sterile inoculant mixture were watered into each respective treatment pot at planting.

Twelve red onion seed Allium cepa cv. Rossa di Milano obtained from Seeds of Change™ (Rancho Dominquez, CA) were planted in each of 8 pots for each of the water baths on April 28, 2009. Single bare-root strawberry crowns obtained from Seeds of Change™ (Rancho Dominquez, CA) were planted in each of 5 pots for each water bath on July 1, 2009. All plants were water daily to container capacity. Onion plants were fertilized with a low concentration (2-1-1 NPK) fish emulsion fertilizer at 72 and 92 days after planting. Strawberry crowns were fertilized with 14.79 mL 12% N (dried blood meal) and 14.79 CaSO₄ (gypsum) at 71 days after planting. Environmental parameters of the growth chambers were monitored and recorded continuously. Soil temperatures were recorded twice daily by visual observation of alcohol thermometers inserted into pots of each treatment. All onions were destructively harvested at 108 days after planting. Hand pollinated strawberry fruit were harvested at 29 days after pollination and full destructive harvesting occurred 123 days after planting.
During the destructive harvest of red onion, leaf number, neck diameter, bulb diameter, bulb diameter, bulb mass, leaf mass, leaf area, root plate diameter and root mass were recorded. Roots were collected and placed in a 4% isopropanol solution for analysis of AMF colonization at a later time. Bulbs were separated from leaves and roots

<table>
<thead>
<tr>
<th>Environmental Parameter</th>
<th>Red Onion</th>
<th>Strawberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Temperature</td>
<td>21°C</td>
<td>18°C</td>
</tr>
<tr>
<td>Relative Humidity</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>14 hours</td>
<td>11 hours</td>
</tr>
<tr>
<td>Optimal Soil Temperature</td>
<td>21°C</td>
<td>18°C</td>
</tr>
<tr>
<td>Supra-Optimal Soil Temperature</td>
<td>32°C</td>
<td>28°C</td>
</tr>
</tbody>
</table>

Table 1.1 Environmental parameters for growth chamber experiments.

and cryopreserved for chemical analysis. As the strawberries were harvested polar length, equatorial diameter, cavity width and cavity length were recorded and reflectance characteristics were measured using a Minolta CR-300 chromameter utilizing (Konica Minolta, Osaka, Japan) CIE values and the Hunter Lab method (Setser, 1984) prior to cryopreservation. During actual destructive harvest, stolon number, daughter number, stolon mass, leaflet number, leaf area and shoot were documented. Similarly, roots were collected by selecting a subsample slice from the media, sieving out root segments and placing in a 4% isopropanol solution for analysis of AMF colonization at a later time.
Both strawberry fruit and red onion bulbs from each experiment were cryomilled. The assays described below were carried out for each of the experiments separately. Using 3 g tissue, phenolic acid extractions (Appendix B) were carried out using a 40 mL of 1% HCl ethanol solvent based on a modified procedure of Proteggente et al. (2002) and Kajdzanoska et al. (2011). The total antioxidant capacity was measured using the ferric reducing antioxidant power (FRAP) method (Appendix C) (Benzie and Strain, 1996). Total phenolics were measured using the Folin-Ciocalteu (FC) reagent method (Appendix D) (Singleton et al., 1999; Singleton and Rossi, 1965; Lee et al., 2003). Total monomeric anthocyanins were measured using a modified pH-differential method (Appendix E) (Giusti and Wrolstad, 2001). Total quercetin in red onion was determined using a modified procedure (Appendix F) by Lombard et al. (2002) using a methanolic extract in a 1:10 dilution for each sample and read using spectrophotometer at a fixed wavelength of 362 nm. The procedure for further extraction of flavonoids was adapted from Maata-Riihinin et al. (2004). Analytical chromatography was performed utilizing high performance liquid chromatography (HPLC-DAD) with an aetric acid: acetonitrile solvent system (Appendix G).

The presence and quantification of arbuscular mycorrhizal fungi (AMF) in red onion and strawberry roots were documented using a modified procedure based on the work of Grace and Stribley (1991) and Barrow and Aaltonen (2001) (Appendix H). Root segments 2 cm in length stored in 4% isopropanol were selected for analysis.

Data means (+ SE, n=4) were subjected to a two-way ANOVA using SAS (The SAS® System for Windows, V9.2; SAS Institute, Cary, NC). Significances between AMF
inoculation, root zone temperature stress or interactions of AMF inoculation and root zone temperature stress were compared.

**Results and Discussion**

After commencing the strawberry experiment, light intensity was adjusted down one level to reflect PAR of 600-610 μmol*m⁻¹*s⁻¹ due to the level of light intensity within the growth chamber. Red onion control units were maintained at 21°C ± 0.5° and supra-optimal temperature units were maintained at 32°C ± 0.5°C. Strawberry control units were maintained at 18°C ± 0.5° and supra-optimal temperature units were maintained at 28°C ± 0.5°.

Table 1.2 displays the concentrations of anthocyanins and quercetin in red onion. Total monomeric anthocyanins have a p=0.07 with a 29% difference in means. Total quercetin was determined spectrophotometrically and individual substituents were identified using HPLC and 3 major quercetin substituents were significantly different between treatments. The identities of peaks B, C, and D are currently unconfirmed and require analysis using liquid chromatographic mass spectrometry, however we believe that the substituent in the highest concentrations (peak C) is quercetin 4’-glucoside as described by Bonaccorsi et al. (2005). The values determined for total phenolic and antioxidant capacity of red onion were not significant.

The exterior of strawberry fruit from the supra-optimal root zone temperature treatment were darker, more red and higher in color saturation whereas the fruit from the optimal temperature treatment were lighter, less red and lower in color saturation (Table 1.3). In this study, total monomeric anthocyanins and as well as total phenolic
compounds were found to be in significantly higher concentrations in the supra-optimal treatment (Table 1.4). Table 1.5 gives the correlations between the total monomeric

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Monomeric Anthocyanins (total mg anthocyanin per 100g FW)</th>
<th>Total Quercetin (mg/Kg FW)</th>
<th>Peak B (mg/Kg FW)</th>
<th>Peak C (mg/Kg FW)</th>
<th>Peak D (mg/Kg FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Temperature</td>
<td>1.7</td>
<td>154.00</td>
<td>15.15</td>
<td>252.84</td>
<td>6.61</td>
</tr>
<tr>
<td>High Temperature</td>
<td>2.4</td>
<td>125.38</td>
<td>11.31</td>
<td>173.81</td>
<td>5.53</td>
</tr>
<tr>
<td>p value</td>
<td>0.0703</td>
<td>0.0136</td>
<td>0.0069</td>
<td>0.0043</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

Table 1.2. Total monomeric anthocyanins, total quercetin and major quercetin compounds in red onion ‘Rossa di Milano’ as influenced by root zone temperature.

![HPLC chromatogram](image_url)

Figure 1.1 HPLC chromatogram of the methanolic extract of ‘Rossa di Milano’ red onion at 350nm eluted from 20.0 to 34.0 minutes. From left to right: A) unknown quercetin derivative B) unknown quercetin derivative C) possible quercetin 4’-glucoside, D) unknown quercetin derivative, E) unknown quercetin derivative and F) unknown compound.
anthocyanin concentration and the color reflectance, being positive for chroma and
negative for L and hue angle. Anthocyanin is a red pigment and the primary colorant of
strawberry fruit.

Figure 1.2. Absorbance spectrum (210-470nm) of unknown quercetin
substituent (Peak C) believed to be quercetin 4’glucoside of red onion
‘Rossa di Milano’.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>External L</th>
<th>External Hue (°)</th>
<th>External Chroma</th>
<th>Internal L</th>
<th>Internal Hue (°)</th>
<th>Internal Chroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Temperature</td>
<td>53.5</td>
<td>56.5</td>
<td>35.7</td>
<td>62.6</td>
<td>52.9</td>
<td>24.7</td>
</tr>
<tr>
<td>High Temperature</td>
<td>47.7</td>
<td>41.9</td>
<td>41.6</td>
<td>55.9</td>
<td>66.2</td>
<td>34.2</td>
</tr>
<tr>
<td><em>p value</em></td>
<td>0.0004</td>
<td>0.0015</td>
<td>0.0091</td>
<td>0.0024</td>
<td>0.0022</td>
<td>0.0029</td>
</tr>
</tbody>
</table>

Table 1.3. Internal and external color reflectance of strawberry ‘Chandler’ fruit as
influenced by root zone temperature.

This strong relationship between the two would indicate that as the concentration of
anthocyanins increase, the more intense red the fruit become, the lower the actual hue
angle and L value become. Additionally, the antioxidant capacity of strawberry was not significant.

The supra-optimal temperature treatment resulted in a significant reduction in onion leaf growth (Table 1.6). The mass, shape and size of the onion bulb was also significantly smaller when grown under the supra-optimal root zone temperature treatment (Table 1.7).

Supra-optimal root zone temperatures significantly reduced strawberry fruit fresh weight and shape as estimated by equatorial diameter and polar length (Table 1.8). However, we found that exposure to supra-optimal root zone temperature actually

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Monomeric Anthocyanins</th>
<th>Total Phenolics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(total mg anthocyanin per 100 g FW)</td>
<td>(total mg phenolics per 1 g FW)</td>
</tr>
<tr>
<td>Optimal Temperature</td>
<td>8.3</td>
<td>209.1</td>
</tr>
<tr>
<td>High Temperature</td>
<td>17.1</td>
<td>243.8</td>
</tr>
<tr>
<td>p value</td>
<td>0.0003</td>
<td>0.0138</td>
</tr>
</tbody>
</table>

Table 1.4 Total monomeric anthocyanins and total phenolic compounds of strawberry ‘Chandler’ as influenced by root zone temperature.

<table>
<thead>
<tr>
<th>Color Reflectance Variables</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>External L</td>
<td>-0.90</td>
</tr>
<tr>
<td>External Hue Angle</td>
<td>-0.91</td>
</tr>
<tr>
<td>External Chroma</td>
<td>0.76</td>
</tr>
<tr>
<td>Internal L</td>
<td>-0.92</td>
</tr>
<tr>
<td>Internal Hue Angle</td>
<td>-0.91</td>
</tr>
<tr>
<td>Internal Chroma</td>
<td>0.90</td>
</tr>
</tbody>
</table>

All values were significant at <0.001

Table 1.5. Correlations between color reflectance and total monomeric anthocyanins in ‘Chandler’ strawberries.
increased vegetative growth characteristics (Table 1.9). Of most interest is the increase in
the number of stolons and the number of daughter plants produced by each plant.

Conversely, mycorrhizal colonization was detectable in strawberry roots in most
experimental units with the AMF treatment. Although colonization levels were slightly
variable, this may be a function of random selection in sampling. Failure to detect
mycorrhizal arbuscules in two inoculated treatments resulted in a shift from n=4 to n=5 in
uninoculated control and the presence of inoculum in uninoculated treatments resulted in
a shift from n=4 to n=3 in inoculated treatments. However, as will be discussed further,
this did not have a substantial impact on the results as presented.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf FW (g)</th>
<th>Leaf DW (g)</th>
<th>Leaf Area (cm²)</th>
<th>Leaf Area FW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Temperature</td>
<td>224.9</td>
<td>18.5</td>
<td>616.6</td>
<td>110.6</td>
</tr>
<tr>
<td>High Temperature</td>
<td>174.2</td>
<td>12.9</td>
<td>498.6</td>
<td>86.3</td>
</tr>
</tbody>
</table>

Table 1.6. Vegetative characteristics of red onion ‘Rossa di Milano’ as influenced by root zone temperature.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neck diameter (mm)</th>
<th>Bulb diameter (mm)</th>
<th>Bulb Length (mm)</th>
<th>Bulb FW (g)</th>
<th>Root Plate diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Temperature</td>
<td>14.3</td>
<td>21.6</td>
<td>64.1</td>
<td>59.6</td>
<td>11.7</td>
</tr>
<tr>
<td>High Temperature</td>
<td>13.6</td>
<td>19.8</td>
<td>56.4</td>
<td>46.8 b</td>
<td>7.9</td>
</tr>
</tbody>
</table>

Table 1.7. Bulb characteristics of red onion ‘Rossa di Milano’ as influenced by root zone temperature.
No effect of AMF inoculation was observed in the physical characteristics or quality of the strawberry fruit. However, significant differences were documented on runner dry weight (Table 1.10) which illustrates decreased plant growth under mycorrhizal

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit FW (g)</th>
<th>Equatorial Diameter (mm)</th>
<th>Polar Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Temperature</td>
<td>10.9</td>
<td>26.4</td>
<td>34.1</td>
</tr>
<tr>
<td>High Temperature</td>
<td>8.5</td>
<td>24.2</td>
<td>31.9</td>
</tr>
<tr>
<td><em>p</em> value</td>
<td>0.0049</td>
<td>0.0160</td>
<td>0.0592</td>
</tr>
</tbody>
</table>

Table 1.8. Physical characteristics of the strawberry ‘Chandler’ fruit as influenced by root zone temperature.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stolon No.</th>
<th>Daughter No.</th>
<th>Leaflet No.</th>
<th>Total Leaf Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Temperature</td>
<td>4.9</td>
<td>12.5</td>
<td>104.7</td>
<td>1540</td>
</tr>
<tr>
<td>High Temperature</td>
<td>10.8</td>
<td>16.5</td>
<td>134.5</td>
<td>1868</td>
</tr>
<tr>
<td><em>p</em> value</td>
<td>0.0001</td>
<td>0.0581</td>
<td>0.0006</td>
<td>0.0139</td>
</tr>
</tbody>
</table>

Table 1.9. Vegetative growth of strawberry ‘Chandler’ as influenced by root zone temperature.

inoculation indicating a possible change in source-sink relations during symbiosis. The results of daughter number, stolon fresh weight and leaflet number are just above the significance level of *p*<0.05 and may warrant further examination since the magnitude of differences are relatively large. Differences in anthocyanin and total phenolic compounds were not found to be significant between treatments.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stolon No.</th>
<th>Daughter No.</th>
<th>Stolon FW (g)</th>
<th>Stolon DW (g)</th>
<th>Leaflet No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ AMF</td>
<td>7.2</td>
<td>12.5</td>
<td>88.21</td>
<td>23.9</td>
<td>134.5</td>
</tr>
<tr>
<td>- AMF</td>
<td>8.3</td>
<td>16</td>
<td>121.68</td>
<td>35.5</td>
<td>104.7</td>
</tr>
</tbody>
</table>

$p$ value 0.0554 0.0773 0.0759 0.0397 0.0630

Table 1.10. Plant vegetative growth of strawberry ‘Chandler’ as influenced by AMF.

The circulating, heated water baths used to impose root zone temperature treatments were unique to this study and were effective in maintaining relatively constant root zone temperatures with minimal maintenance. Environmental settings were held constant and experienced only a few minor disruptions with cooling systems but any effects were confined to blocks as evidenced in our statistical analysis.

These results support multiple findings. First, that data indicated that the observed red onion quercetin decrease can be attributed to supra-optimal root zone temperatures while total monomeric anthocyanins increased. No studies have been uncovered that have examined the influence of root zone temperature stress on the accumulation of phenolic compounds in onion bulbs, including total anthocyanins. However, the effects of other forms of root stress such as drought stress have been documented for the changes in defense related compounds such as superoxide dismutase and catalase (Csiszar et al., 2007), yet few studies have recorded the effects of abiotic stress on the anthocyanin and quercetin concentration in onions. Further research is needed to determine if this is a common phenomenon or a genotypic function of this cultivar.

Second, the data indicated that the observed anthocyanin concentration and color reflectance increase can be attributed to supra-optimal root zone temperatures while the fruit size and shape decreased. The values for fruit weight are considerably lower than
those published by Scheerens and Brenneman (1991) for ‘Chandler’. Similar to color reflectance, fruit weight may also be lower due to growth chamber simulated environmental conditions. Based on this reduction in fruit fresh weight and pigment concentration, we conjecture that fruit obtained from plants of the supra-optimal root zone temperature ripen precipitately before fruit have reached developmental maturity and may infer that higher accumulations of anthocyanin concentrations may occur in plants undergoing exposure to supra-optimal root zone temperatures if allowed to further mature. Overall pigment concentrations in strawberry are higher under elevated soil temperatures compared to those of optimal soil temperatures according to one study (Wang, et al., 1998). Furthermore, data presented by Wang et al. (1998) indicate a similar trend for fruit surface color (external) and a slightly different trend for fruit flesh color (internal) for cultivars ‘Northeaster’ and ‘Primetime’. However, results provided by Wang et al. (1998) are indicative of field grown strawberries and have considerably lower reflectance values than those found in our study as an outcome of growth chamber simulated environmental conditions. Additionally, L, a, b measurements are also a preliminary indication that the ripening times for strawberries undergoing supra-optimal root zone temperatures are shorter. These observations are different from the results found in a study performed by Kadir et al. (2006) in which strawberry fruit of ‘Chandler’ had the greatest degree of redness when grown in an air temperature of 20°C versus 30°C. Furthermore, a reduction in reproductive growth and fruit development has been documented in plants exposed to high aerial temperatures (Ledesma et al., 2008). This may indicate that there is a relationship between ripening of fruit and pigmentation
development with both aerial temperature and root zone temperature. A shift in metabolic networks of the fruit may occur (Fait et al., 2008) based on changes to the growing environment and subsequent activation of the plant defense mechanism. Further research is required to determine how the ripening mechanism is affected in developing fruit.

This research may lead to greater consideration of soil micro-environments in production strategies. Current research is already demonstrating the effects of certain production strategies on soil properties. For instance, synthetic mulches can change or maintain soil and root zone temperatures in fields utilizing net solar radiation and influencing radiative flux between mulch cover and soil surface (Liakatas et al., 1986) and specific effects of these mulches depends upon their color. Research conducted over the last several decades has proven that controlling soil temperatures using organic or synthetic mulch types can improve plant growth, quality and overall productivity and may alter additional soil properties including water relations, fertility, permeability and soil aggregation that influence plant performance (Diaz-Perez et al., 2005; Diaz-Perez and Batal, 2002; Moreno et al., 2009; Schonbeck and Evanylo, 1998; Wang et al., 1998).
Chapter 2: Literature Review

As the relationship between diet and lifelong health is validated by an overwhelming body of medical research, horticultural investigation to understand environmental influences on crop physiochemical properties is increasingly warranted. Plant nutritional quality may, in part, be determined by abiotic and biotic interrelationships that are often conditioned by cultural systems and production strategies employed by the producer. Historically, our attention has focused upon the importance of plant-derived vitamins and minerals as nutritional components essential for growth and sustained health. However, public interest in phytonutrients, mainly carotenoids (carotenes and xanthophylls) and polyphenols (e.g., flavonol glycosides, anthocyanins and tannins) as health-beneficial compounds is increasing due to their reported activity as antioxidants and inflammatory regulators that may delay the onset or lessen the severity of many degenerative diseases of aging (Liu, 2003; Seeram, 2008).

Carotenoids and polyphenolics are important secondary compounds that condition the plant defenses to respond to environmental stresses that reduce carbon fixation and/or alter carbon allocation (Davies and Schwinn, 2003; Siegler, 1998). Environmental pressures such as water availability (Castellarin et al., 2007; Csiszar et al., 2007; Jaleel et al., 2008; Kumar et al., 2007; Oh et al., 2009; Terry et al., 2007), irradiance spectrum (Garcia-Macias et al., 2007; Krizek et al., 1998; Tsormpatsidis et al., 2008; Voipio and
Autio, 1995) air temperature (Benkebelia, 2003; Coolong and Randle, 2003), soil temperatures (Anttonen et al., 2006; Coolong and Randle, 2006; Locascio et al., 2005; McMichael and Burke, 1998; Tindall et al., 1990; Tulipano et al., 2008; Wang et al., 1998; Yamaguchi et al., 1975) and other environmental factors all appear to influence secondary metabolism, through changes to gene transcription, signal hormone transduction and enzyme activity, which ultimately lead to alterations in plant secondary concentrations.

Alteration of soil environments have also been shown to affect a collection of nutritive compounds (Devi and Reddy, 2002; Guo et al., 2006; Guo et al., 2007; Revgar et al., 2003; Perner et al., 2008). Current quality studies have focused on polyphenolics, titratable acids, soluble solid concentrations and flavor precursors that are attributed to antioxidant capacities as well as protective and preventative abilities (Anttonen et al., 2006; Del Pozo-Insfran et al., 2006; Devi and Reddy, 2002; Tulipano et al., 2008; Wang et al., 2002). Physical and biological changes in the root zone can attribute to the processes affecting qualitative content in crops (Frageria and Stone, 2006; Tindall et al., 1990; Yao et al., 2007). Two of these important soil attributes are soil temperature and soil microbial population.

Net solar radiation effects soil surface temperatures that eventually are redistributed through conduction among the vertical layers of soil (Renaud et al., 2001). Other physical properties such as soil color, vegetative cover, volumetric water content and time of day/year affect soil temperatures secondarily. Kures et al., (2003) determined that soil temperature plats a major role in plant productivity by moderating the level of root
activity at higher temperatures and the resulting sink demands on photosynthetic organs. In fact, these authors demonstrated that plants were most productive when there was a temperature gradient from the air to the soil, where the soil temperature was much higher than that of the air. It is thought that there is an optimal soil temperature for root growth, function and metabolism, and supra-optimal temperatures result in a reduction of root processes (McMichael and Burke, 1998).

Research concluded over the last several decades has proven that controlling soil temperatures using organic or synthetic mulch types can improve plant growth, quality and overall productivity and may alter additional soil properties including water relations, fertility, permeability and soil aggregation that influence plant performance (Diaz-Perez et al., 2005; Diaz-Perez and Batal, 2002; Moreno et al., 2009; Schonbeck and Evanylo, 1998; Wang et al., 1998). Synthetic mulches can change or maintain soil and root zone temperatures in fields utilizing net solar radiation and influencing radiative flux between mulch cover and soil surface (Liakatas et al., 1986). The specific effect of these mulches depends upon their color. Liakatas et al. (1986) demonstrated that black colored mulches could raise mean daily temperatures by a minimum of 3-4°C and maintain radiation flux in the soil. Additionally, clear plastic mulches demonstrated an increase of 6-8°C over bare soil (Liakatas et al., 1986). Conversely, reflective colored mulches showed decreased soil temperatures over bare soil and air temperatures, the most significant growth and productivity was achieved using black colored mulch. Studies conducted in growth chambers agreed with the field studies that increasing soil temperature to an optimum does increase root processes (Tindall, et al., 1990).
In onions, supra-optimal soil temperature of 29°C resulted in changes to volatile propyl sulfur compounds and pyruvic acid, which are determiners of pungency and flavor (Yamaguchi et al., 1975). In addition to quality, bulb weight, time to maturity and bulb shape were correlated to root zone temperature (Yamaguchi et al., 1975). Coolong and Randle (2003; 2006) also demonstrated the influence of elevated root zone temperature on growth and flavor of onions. Onions grown hydroponically and in greenhouse settings exhibited higher fresh bulb weight, increased leaf number, higher soluble solid content, bulb sulfur and sulphate content at 21°C (Coolong and Randle, 2003; 2006). Conversely, studies observing a reduction in temperatures resulted in reduced soluble sugar content, phenolics and peroxidase activity (Benkeblia, 2003).

In addition to onion, strawberry also experiences augmented root processes, resulting in lower marketable yields, under increased soil temperatures which affect plant biochemistry and physiology of both plants (Locascio et al., 2005; Wang et al., 1998). Strawberry root processes are most efficient at an optimum soil temperature of 10-20°C depending on cultivar (McMichael and Burke, 1998). Strawberry has naturally high concentrations of vitamin C and phenolic compounds (Tulipani et al., 2008). Of these compounds, anthocyanins play a major role in the antioxidant capacity. Anthocyanin content has been found to be higher in strawberries grown under brown mulch compared to those grown under white mulch (Anttonen et al., 2006). Soluble solid content and overall pigment concentration was found in higher concentrations under elevated soil temperatures (Wang et al., 1998). Additionally, these strawberries ripened a week earlier.
than those grown without mulch. Black polyethylene mulch has been known to produce higher soluble solid content and the reddest fruit (Wang et al., 1998).

Root zone temperature is not the sole factor influencing root activity; symbiotic relationships with beneficial soil microbes such as arbuscular mycorrhizal fungi (AMF) can also affect growth, productivity and quality of many crops (Gosling et al., 2006; Koide, 2000). AMF are accredited with providing many services to their symbiotic plant hosts such as increased daily photosynthetic rates, stomatal conductance, increased numbers of vascular bundles, alteration of leaf folding and orientation (Allen, 1991), increased inorganic phosphate mobilization and acquisition (Gosling et al., 2006; Raghothama and Karthikeyan, 2005), increased growth, yield and quality (Devi and Reddy, 2002; Fan et al., 2008; Guo et al., 2007; Perner et al., 2008; Revgar et al., 2003) protection from pests, drought tolerance, improved nutrient uptake as well as enhanced resistance to abiotic stress (Gosling et al., 2006; Koide, 2000).

The physiological process that governs the relationship between plants and mycorrhizae has been most recently described in a more comprehensive manner. Hata et al. (2010) outlined the common model for arbuscular symbiosis based on the phase of symbiotic development. The first stage can be separated into three major phases. The first phase or presymbiotic phase includes the establishment and induction of signal molecules. The second phase, the penetration apparatus is where AMF form hyphae once passed the epidermis layer. The third phase contains components of the common symbiosis pathway where signal molecules trigger peri- and intranuclear calcium spiking occurs inducing specific genes required to form the common pathway and transcriptome
profiling in which gene level regulation occurs in the early stages of symbiosis (Hata et al., 2010). Latter steps in the symbiotic process were less characterized beyond that of nutrient exchange (Hata et al., 2010). Increases in soil temperature appear to increase root colonization by AMF (Liu et al., 2004), but no effect of temperature on the physiological process governing colonization have yet to be elucidated. Additionally, Liu et al. (2007) determined that defense genes involved in the biotic stress response are upregulated during mycorrhizal colonization.

Host response to mycorrhizal colonization varies significantly among and within species (Devi and Reddy, 2002; Fan et al., 2008; Guo et al., 2007). Plants that are typically responsive to colonization by AMF possess poorly developed root systems whereas the root systems of “non-responsive” plants have the capacity to successfully mine the soil nutrient supply (Hata et al., 2010). Plants of the former group, like the Liliaceae family including Allium species, exhibit a positive growth response following AMF colonization. Moreover, the application of Glomus species to four frequently used crops: green pepper, parsley, carrot and tomato in Slovenia resulted in elevated carotenoids, titratable acids, chlorophyll content as well as plant and root biomass (Revgar et al., 2003). Significant increases in peroxidase and polyphenols oxidase activities following AMF inoculation have been documented (Devi and Reddy, 2002). Studies have concluded that AMF inoculation can also affect phenolic acid concentrations and flavonol glycoside concentrations, specifically quercetin and isothamnetic, in onions (Guo et al., 2006; Guo et al., 2007; Perner et al., 2008). Deguchi et al. (2007) successfully documented the up-regulation of two chalcone reductase genes
and down regulation of 4 phenylalanine ammonia-lyase genes, 3 chalcone synthase genes and 2 other chalcone reductase genes as well as other genes important to secondary metabolism and more specifically to phenylpropanoid metabolism in a model system using mycorrhizal *Japonicus lotus* roots. This may be an indication of an initial upregulation of the plant defense mechanism upon mycorrhizal contact and then a subsequent down regulation upon host recognition.
Chapter 3: Conclusions

The circulating, heated water baths used to impose root zone temperature treatments were unique to this study and were effective in maintaining relatively constant root zone temperatures with minimal maintenance. Environmental settings were held constant and experienced only a few minor disruptions with cooling systems but any effects were confined to blocks.

These results support multiple findings. First, that data indicated that the observed red onion quercetin decrease can be attributed to supra-optimal root zone temperatures while total monomeric anthocyanins increased. No studies have been uncovered that have examined the influence of root zone temperature stress on the accumulation of phenolic compounds in onion bulbs, including total anthocyanins. However, the effects of other forms of root stress such as drought stress have been documented for the changes in defense related compounds such as superoxide dismutase and catalase (Csiszár et al., 2007), yet few studies have recorded the effects of abiotic stress on the anthocyanin and quercetin concentration in onions. Further research is needed to determine if this is a common phenomenon or a genotypic function of this cultivar. Second, the data indicated that the observed anthocyanin concentration and color reflectance increase can be attributed to supra-optimal root zone temperatures while the fruit size and shape decreased. Based on this reduction in fruit fresh size and pigment concentration, we
conjecture that fruit obtained from plants of the supra-optimal root zone temperature ripen precipitately before fruit have reached developmental maturity and may infer that higher accumulations of anthocyanin concentrations may occur in plants undergoing exposure to supra-optimal root zone temperatures if allowed to further mature. Overall pigment concentrations in strawberry are higher under elevated soil temperatures compared to those of optimal soil temperatures according to one study (Wang, et al., 1998). Furthermore, data presented by Wang et al. (1998) indicate a similar trend (increasing values) for fruit surface color (external) and a slightly different trend (increasing) for fruit flesh color (internal) for cultivars ‘Northeaster’ and ‘Primetime’ compared to our results. However, results provided by Wang et al. (1998) are indicative of field grown strawberries and have considerably lower reflectance values than those found in our study as an outcome of growth chamber simulated environmental conditions. Additionally, L, a, b measurements are also a preliminary indication that the ripening times for strawberries undergoing supra-optimal root zone temperatures are shorter. These observations are different from the results found in a study performed by Kadir et al. (2006) in which strawberry fruit of ‘Chandler’ had the greatest degree of redness when grown in an air temperature of 20°C versus 30°C. Furthermore, a reduction in reproductive growth and fruit development has been documented in plants exposed to high aerial temperatures (Ledesma et al., 2008). This may indicate that there is a relationship between ripening of fruit and pigmentation development with both aerial temperature and root zone temperature. A shift in metabolic networks of the fruit may occur (Fait et al., 2008) based on changes to the growing environment and subsequent
activation of the plant defense mechanism. Further research is required to determine how the ripening mechanism is affected in developing fruit.

Typically, higher air temperatures determine the rate of vegetative growth in production cultivars (Kadir et al., 2006) compared to lower temperature requirements for flowering and fruit development, however the vegetative response of this study reflects the change in root zone temperature even with a lower optimum air temperature. Kadir et al. (2006) attributes these changes to higher CO₂ assimilation rates of ‘Chandler’ with the greatest degree of growth at an air temperature of 30°C which may mean enhanced tolerance to supra-optimal temperature stress. Yet, the vegetative response of this study reflects the change in root zone temperature even at a lower optimum air temperature. In this instance, thermotolerance may be a systemic response that actually promotes vegetative growth contrasting previously published information on other Fragaria species (Geater et al., 1997). Geater et al., (1997) studied the influence of high root zone temperatures on the growth and development of different wild-type Fragaria species and concluded that exposure to high root zone temperatures inhibited growth in F. chiloensis Duch., F. virginiana Duch. and F. viridis to varying degrees and associated the reduction in growth to reduced transpiration and leaf water potential.

This research may lead to greater consideration of soil micro-environments in production strategies. Current research is already demonstrating the effects of certain production strategies on soil properties. For instance, synthetic mulches can change or maintain soil and root zone temperatures in fields utilizing net solar radiation and influencing radiative flux between mulch cover and soil surface (Liakatas et al., 1986).
and specific effects of these mulches depends upon their color. Research conducted over the last several decades has proven that controlling soil temperatures using organic or synthetic mulch types can improve plant growth, quality and overall productivity and may alter additional soil properties including water relations, fertility, permeability and soil aggregation that influence plant performance (Diaz-Perez et al., 2005; Diaz-Perez and Batal, 2002; Moreno et al., 2009; Schonbeck and Evanylo, 1998; Wang et al., 1998).

Many studies have demonstrated the positive effects of mycorrhizal colonization on onion (Hata et al., 2010). However, mycorrhizal colonization was undetectable in the onion experiments. Several plausible reasons for this have been explored yet a definitive explanation would require additional research. In a study by Linderman and Davis (2003), different combinations of peat moss soil amendments and mycorrhizae inoculum resulted in variable levels of suppression and enhancement of onion mycorrhizal colonization yet some level of colonization was achieved in each instance. The plant-AMF symbiosis may require specific mycorrhizae signaling compounds to be produced by the plant in response to certain environmental cues, such as water or temperature stress. The stress level required to trigger production of signaling compounds may be predetermined by the genetics of the individual plant species or may vary with plant age. As such, colonization failure in our onion experiment might have resulted from stress levels that were below those necessary or were applied at the wrong time to elicit a plant response or may indicate the inability of ‘Rossa di Milano’ to produce signaling compounds. Hata et al. (2010) outlines the absence of specific genes, SYMRK, CASTOR, CCaMK and CYCLOPS, in plant species that are unable to form mycorrhizal
symbiosis such as the Brassica family and this onion cultivar may also lack these genes. These genes share involvement in the endosymbiotic signaling pathway, specifically with calcium spiking, which are triggered by Myc factors upon AMF hyphal contact (Hata et al., 2010).

Many studies have been conducted in which mycorrhizal inoculation has led to an increase in above ground plant growth; however AMF inoculation is typically coupled with some nutrient addition which may be more responsible for elevated growth (Castellanos-Morales et al., 2010; Sharma and Adholeya, 2004). Furthermore, even in growing systems which have limited resources, mycorrhizal plants tend to have a higher shoot biomass (Zhang et al., 2011). Perhaps while not significant, small differences in the AMF and supra-optimal treatment versus the control were large enough to reduce the overall mean. Conversely, this reduction in vegetative growth of strawberry under AMF inoculation could be an outcome of being grown in a growth chamber setting and may result differently if examined under field conditions.

Differences in anthocyanin and total phenolic compounds were not found to be significant between treatments. AMF are thought to produce a priming effect in plants where an initial elicitation inducing the plant defense system may temporarily up regulate phenylpropanoid metabolism (Bonanomi et al., 2001; Blee and Anderson, 1996). However, isolated cases have demonstrated an increase in phenylpropanoid metabolism and a higher accumulation of phenolic compounds in shoot organs (Ceccarelli et al., 2010).
AMF are thought to produce a priming effect in plants where an initial elicitation inducing the plant defense system may temporarily up regulate phenylpropanoid metabolism (Bonanomi et al., 2001; Blee and Anderson, 1996). However, isolated cases have demonstrated an increase in phenylpropanoid metabolism and a higher accumulation of phenolic compounds in shoot organs (Ceccarelli et al., 2010).

Many studies have verified that mycorrhizal inoculation can enhance the plant defense system against various forms of biotic and abiotic stress (Devi and Reddy, 2002; Fan et al., 2008; Gosling et al., 2006; Guo et al., 2007; Koide, 2000; Miransari, 2010; Perner et al., 2008; Raghothama and Karthikeyan, 2005; Revgar et al., 2003). Further research would be useful in identifying mechanisms involved in the interaction between plant and AMF under varying types of biotic and abiotic stress. While no interaction was found to be significant in this study, a closer look at the metabolic pathways in a similar study may reveal exciting, novel insight into the potential for enhancement of phytonutrients in horticulturally important crops. Further research would be useful in identifying mechanisms involved in the interaction between plant and AMF under varying types of biotic and abiotic stress. While no interaction was found to be significant in this study, a closer look at the metabolic pathways in a similar study may reveal exciting, novel insight into the potential for enhancement of phytonutrients in horticulturally important crops.

Ultimately, modifications to changes to the soil environment cause continuous alterations to root geometry, rate of root extension and respiratory activities (Smucker, 1993). The plants maximum performance is primarily based on optimum carbon
partitioning. A reduction in environmental root zone stress reduces respiration and increases plant health (Smucker, 1993). Plant defense mechanisms alleviate stress through use of secondary metabolites (phytonutrients). Additionally, upon the inability to provide adequate root structure, plants will form symbiosis with AMF which will alter the plant root architecture and provide additional root surface area in an effort to function optimally (Allen, 1991).

The novel design of this study has allowed us to further document a plant’s ability to cope with environmental stress. Increases to anthocyanins and total phenolic compounds in strawberry coupled with a change in fruit and vegetative development could have profound impacts on cultural systems that utilize mulch cover that alters the soil microclimate. Furthermore, quercetin concentrations were reduced in red onion under similar conditions. Arbuscular mycorrhizal fungi appear to also impose some changes on strawberry. However, our findings of stress alleviation by AMF are contrary to other published studies.

Additional studies exploring similar environmental conditions in a field setting may further exemplify the full range of effects imposed on both strawberry and red onion that may be limited by growth in growth chambers. With the current direction of food and agricultural science towards enhanced focus on health promoting compounds along with crop quality, it will be of even greater importance that information pertaining to growing systems and biotic and abiotic conditions be made available to growers.
References


Castellanos-Morales, V., J. Villegas, S. Wendelin, H. Vierheilig, R. Eder and R. Cardenas-Navarro. 2010. Root Colonization by the Arbuscular Mycorrhizal Fungus Glomus intraradices Alters the Quality of Strawberry Fruits (Fragaria x ananassa)


Appendix A: Water Bath Design

The water baths (Figure A.1) were constructed using 85.17 L Sterilite® storage containers (Wal-Mart, Bentonville, AR) measuring 87.2 cm x 41.875 cm x 32.5 cm. Holes were cut in both the lid and bottom of container to align with and accommodate eight 35 cm tree pots from Stuewe and Sons, (Hummert International, Earth City, MO). The narrow end of the tree pots were inserted into the bottom of the unit and sealed well with Homax Professional Welder glue (Lowe’s, Mooresville, NC). The lids fit over the top of the tree pots but were not sealed in an effort to simplify maintenance the units. Two 2.5 cm holes were made in both ends of the lids for placement of two Sunleaves Heatwave Stainless Steel 200 W reservoir heaters (Growco Indoor Garden Supply, Grand Rapids, MI). Two additional holes were drilled in either end of the lid for tubing to be connected to a two outlet ActiveAir air pump (The Big Tomato, Aurora, CO) and for checking water levels in the unit. Additionally, each water bath contained two air stones to promote water circulation. Each water bath was covered with 1.25 cm thick insulation board (Lowe’s) around the sides (Figure A.2).

The growth chamber settings were maintained within constant environmental parameters: air temperature 18°C (Serce and Hancock, 2005), relative humidity 30%, photoperiod 11 hours (Serce and Hancock, 2005), PAR 600-610 μmol*m⁻¹*s⁻¹ using high pressure sodium and halogen lamps and irrigation to container capacity. Soil
temperature was controlled by using the circulating, heated water baths. Temperature for the control units was maintained by the aerial temperature of 18°C (Serce and Hancock, 2005) and temperature for the stress treatment units was controlled using the reservoir heaters and set for 28°C. Soil temperatures were collected twice daily for the duration of the experiment. Water baths for the strawberry experiment were constructed in a similar manner to the units for the onion experiment with a couple simple modifications. Units in this experiment were constructed using five tree pots (Figure A.3) rather than eight. Additionally, the water baths were placed on specially made benches to raise them off the floor (Figure A.4).

Figure A.1. Circulating, heated water bath.
Figure A.2. Water baths with insulation board.

Figure A.3. Water baths modified to accommodate 5 tree pots.
Figure A.4. Water bath units raised up from floor.
Appendix B: Phenolic Compound Extractions

Using 3 g tissue, phenolic acid extractions were carried out using a 40 mL of 1% HCl ethanol solvent based on a modified procedure of Proteggente et al. (2002) and Kajdzanoska et al. (2011). The extraction was allowed to take place for 60 minutes and then filtered using a Buchner funnel and number 1 Whatman filter paper. A second extraction was completed with an additional 40 mL of solvent and an extraction period of 30 minutes. Filtration was repeated as before and then the tissue was rinsed with approximately 10 mL H2O. Following extraction, 600 μL of 10% KOH was added dropwise in order to neutralize the HCL. The total volume of extract was poured off into two 25x150 glass screw top test tubes and placed in an N-Evap 111 unit (Organomation Associates, Inc., Berlin, MA) for evaporation of the solvent. The temperature on the N-Evap111 unit was set for 30-40°C and extractions were allowed to dry to an approximate final volume of 7 mL. The two extract fractions were recombined and H2O2 was added to the extracts to bring to an approximate total volume of 20 mL. The extracts were then placed into 50 mL test tubes and centrifuged for 15 minutes at 7500 rpm. Clarified extractions were placed into four 15 mL falcon tubes, topped with N2 and placed in -20°C for storage. Prior to use of each extract, a pH correction was performed to bring the pH of 9 mL extract at room temperature to a pH of 3.0 ± 0.005 by adding 10M, 1.0 M and 0.01 M NaOH dropwise until the target pH was achieved. Enough H2O was added to make a
volume of 10 mL. Then a total volume was achieved of 25 mL using a volumetric flask of which would be used for spectrophotometric assays.
Appendix C: Ferric Reducing Antioxidant Power (FRAP) Method

The total antioxidant capacity was measured using the ferric reducing antioxidant power (FRAP) method (Benzie and Strain, 1996). Stock solutions were prepared in advance of performing the assay. Solution A consisted of 2.4 g of sodium acetate in 14.5 mL glacial acetic acid adjusted to a final pH of 3.6 and brought to a final volume of 1 L. Solution B consisted of 324 mg FeCL₃ and 80 mL of H₂O. The solution was diluted with H₂O to a final volume of 100 mL and stored in an amber bottle. Solution C consists of 312 mg 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) (Sigma-Aldrich, St. Louis, MO), 80 mL H₂O and 0.33 concentrated HCl. The solution was brought to a final volume of 100 mL with H₂O. A working solution was then prepared using a ration of 10:1:1 of each stock solution respectively. An addition of 2.98 mL of working solution and 40 μL of methanolic extract was added to a test tube and allowed to stand for 1 hour for development. The absorbance of each sample was measured at 593 nm. A standard curve was prepared utilizing concentrations of 0, 0.02, 0.04, 0.06, 0.08 and 0.10 μM 6-hydroxy-2,5,7,8-tetramethylchroman-22-carboxylic acid (trolox) (Sigma-Aldrich, St. Louis, MO) equivalents. Results are expressed as μM trolox equivalents per g FW sample.
Appendix D: Folin-Ciocalteu (FC) Reagent Method

Total phenolics were measured using the Folin-Ciocalteu (FC) reagent method (Singleton et al., 1999; Singleton and Rossi, 1965; Lee et al., 2003). The assay was performed by mixing 1 mL of methanolic extract, 1 mL FC and 23 mL H₂O and letting stand for 8 minutes. Then 10 mL of 7% sodium carbonate solution and 20 mL H₂O were added. The solution was allowed to develop for 2 hours. The absorbance was measured at 750 nm. A standard curve was completed using concentrations of 0, 100, 200, 300, 400 and 500 mg/L gallic acid in H₂O. Results were expressed as mg gallic acid equivalents (GAE) per g tissue FW.
Appendix E: Total Monomeric Anthocyanin

Total monomeric anthocyanins were measured using a modified pH-differential method (Giusti and Wrolstad, 2001). Two dilutions of the sample extract were made using a 0.025M potassium chloride buffer, pH 1.0 and a 0.4M sodium acetate buffer, pH 4.5 which were allowed to equilibrate for 30 minutes. The absorbance of each dilution was measured at $\lambda_{\text{vis-max}}$, 520 nm for red onion and 500 nm for strawberry, and at 700 nm. The absorbance of the diluted sample was calculated and expressed as total mg of anthocyanins per 100g of FW tissue. The formula used to calculate anthocyanin concentration is $C=\frac{\text{ABS}}{\varepsilon} \times L$. The $\varepsilon$ used for red onion based on cyanidin-3-glucoside (Sigma-Aldrich, St. Louis, MO) is 26900 (Giusti et al., 1999) and for strawberry based on pelargonidin-3-glucoside (Sigma-Aldrich, St. Louis, MO) is 27300 (Giusti et al., 1999). In order to proper calculate concentration, moles anthocyanin per liter of extract had to be converted to mg anthocyanin per g FW of tissue.
Appendix F: Total Quercetin in Red Onion

Total quercetin in red onion was determined using a modified procedure by Lombard et al. (2002). Five grams of cryomilled onion tissue was extracted using 10 mL of methanol acidified with 1% HCl. The mixture was agitated every 10 minutes for 1 hour and then centrifuged at 7500 rpm for 20 minutes. The extract mixture was filtered using a Buchner funnel, suction filtration flask and Whatman No. 1 filter paper. The residue was rinsed with 1-2 mL of methanol acidified with 1% HCl. The tissue remaining on the filter paper was then scraped back into a falcon tube and 10 mL of extraction solvent was added. This mixture was allowed to stand for 30 minutes with agitation every 10 minutes. Then the extraction was repeated 3 more times using only 5 mL of methanol acidified with 1% HCl. The entire extract was refiltered a previously described and the placed in a 50 mL volumetric flask. The suction filtration flask was rinsed with solvent and poured off into the volumetric flask. The sample was brought to a final volume of 50 mL using solvent. If the extract exceeded 50 mL, it would be condensed using nitrogen gas. A 1:10 dilution was prepared for each sample and read using spectrophotometer at a fixed wavelength of 362 nm.
Appendix G: Quantification of Flavonoids in Red Onion using HPLC

Methanolic extracts were made as previously described. The procedure for further extraction of flavonoids was adapted from Maatta-Riihinen et al. (2004). A solution of 10 mL methanolic extract, 10 mL H₂O, 20 mL sodium acetate (0.2M at pH7.0) and 10 mL ethyl acetate were added to a separatory funnel. The mixture was shaken and allowed to separate. The ethyl acetate fraction was removed and placed into a screw top test tube. The process was repeated adding in an additional 10 mL and 5 mL ethyl acetate for a total of 25 mL ethyl acetate fraction until the ethyl acetate fraction was nearly colorless. The ethyl acetate fractions were collected in a clean test tube and then placed on a N-evap 111 system to dry. Flavonoid quantification was performed by redissolving the material in 2% acetic acid and 98% methanol.

Analytical chromatography was performed utilizing high performance liquid chromatography (HPLC). Solvents, 0.2% H₂O acidified with acetic acid (A) and 100% acetonitrile (B), were prepared and degassed. A 3 mL disposable luer-lock syringe modified with a 0.45 μm Fisher Brand nylon filter (Barrington, Illinois) was used for each sample. Each extract, to a maximum of 1mL, was added into the barrel of the syringe using a glass transfer pipette. The extracts were then filtered through the syringe into small test tubes. Using a 12.5 cm glass transfer pipette, the filtered extracts were transferred to 100 μL insert and placed into a chromatographic sample vial topped with a
septum cap. The vials were placed into the autosampler tray. The samples were chromatographed using a Phenomenex Gemini (EUC6-phenyl) column (250 X 4.6 mm) held at 30°C. A Beckman System Gold 126p instrument with a 168 DAD and procedures/software controlled the chromatograph process. The solvent program that was used which delivers solvent at 0.7mL per minute is: hold at 91% A and 9% B from 0 to 5 minutes; transition to 78% A and 22% B from 5 to 15 minutes; transition to 70% A and 30% B from 15 to 20 minutes; transition to 40% A and 60% B from 20 to 30 minutes; hold 40% A and 60% B from 30 to 35 minutes; transition to 91% A and 9% B from 35 to 40 minutes; hold at 91% A and 9% B from 40 to 42 minutes. The program injection volume was 10 μL and 30 μL for the ethyl acetate extractions.
Appendix H: Quantification of Arbuscular Mycorrhizal Fungi (AMF)

The presence and quantification of arbuscular mycorrhizal fungi (AMF) in red onion and strawberry roots were documented using a modified procedure based on the work of Grace and Stribley (1991) and Barrow and Aaltonen (2001). Root segments 2 cm in length stored in 4% isopropanol were selected for analysis. Samples were cleared by placing root segments submerged in 10% KOH in glass mason jars into a heated water bath and kept at 90°C for 1 hour. Once samples were removed and allowed to cool for several minutes, the samples were rinsed with H₂O and then placed in 1% HCL for 5 minutes to neutralize them. Samples were again rinsed with water and then placed in mason jars along with a stock solution of Trypan Blue. The solution contains 0.5 g Trypan Blue, 500 mL glycerol, 450 mL H₂O and 50 mL HCl (Barrow and Aaltonen, 2001). Once stained, the root samples were placed in pure ethanol for destaining for 5 to 7 days. Following destaining, the samples were mounted on slides and preserved with polyvinyl alcohol resin (PVA) lactophenol. Slides were then analyzed using a light microscope with a magnification of 20x-40x. Quantification was done based on the visual method as described by Giovannetti and Mosse (1980) and Rajapaske and Miller, Jr. (1992).