PHYTONUTRIENTS OF ORGANIC TOMATOES:

SOIL FERTILITY AND/OR PLANT DEFENSE

By

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A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE IN HORTICULTURE

WASHINGTON STATE UNIVERSITY
Department of Horticulture

AUGUST 2012

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of LUKE NORDELL GUSTAFSON find it satisfactory and recommend that it be accepted.

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AKNOWLEDGEMENTS

I wish to thank some of the people who have helped make this thesis possible. I am grateful to my advisor, Preston Andrews. His guidance, mentoring, experience, perspective and encouragement have been invaluable to me. Amit Dhingra has given me valuable feedback and support throughout my time here. William Snyder has provided me advice and expertise on the project. I appreciate the technical support of Gretchen Snyder and Tadashi Takizawa in rearing, transferring and identifying aphids. Thanks to Rich Alldredge for statistical advice. Thanks to Derick Jiwan and Artemus Harper for their work on genomic aspects of the project. Taya Brown skillfully crafted the insect exclusion covers. Dan Dreesmann and Amanda Linskey provided logistical support in the greenhouse. Linda Agyen has assisted me with the lab work. My colleagues in neighboring labs, especially Marco Galli, Lei Zhang, Derick Jiwan and Chris Hendrickson helped me keep my sanity when doing repetitive bench work. Thanks to all the other graduate students in my department for their support. I am indebted to Oliver Yibing Chen for both his friendship and statistical advice. I am grateful to Tali Lee, my professor of plant physiology as an undergraduate, who helped me realize just how fascinating plants are. Thanks to my parents for sharing their love of nature with me and for helping me develop a green thumb. My wife, Ellen, has put up with endless banter on all things related to tomatoes and insects and has encouraged me throughout the writing process. I am grateful for her support, love and humor.

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Abstract

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August 2012

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There is growing evidence that organically grown crops contain higher levels of phytonutrients than their conventional counterparts. This study investigates the relative effects of organic and inorganic nutrient treatments and herbivory on tomato phytonutrient concentrations. Organically managed soils generally have higher organic nitrogen to inorganic nitrogen ratios, and research indicates that organically grown apples, strawberries and tomatoes often have higher antioxidant activity than those grown conventionally. Because organic producers have fewer available pest control options, organic crops may be exposed to more insect pests. Herbivory by insects induces production of plant defense compounds which can act as feeding deterrents to generalist insects. Many of these compounds also act as antioxidants.

In this study, tomato plants (*Solanum lycopersicum* L. 'Oregon Spring') were grown in a randomized complete-block experimental design in a greenhouse under either organic or inorganic fertility management. To determine the effect of herbivory on fruit quality, green peach aphids (*Myzus persicae*) were introduced to half of the plants within each nutrient treatment. To contain the aphids, all plants were grown in mesh exclusion covers. Fruit were harvested at varying stages of ripeness, and measurements were taken of percent soluble solids,

mass, and diameter. Frozen ripe fruit samples were analyzed for total phenolics (TP), lycopene, Trolox equivalent antioxidant capacity (TEAC) and ascorbic acid.

Red-ripe tomato fruit from organically fertilized plants had statistically higher concentrations of soluble solids, TP, lycopene, lipophilic and total TEAC and ascorbic acid. Red-ripe fruit in the organic fertility treatment had lower average mass. Concentrations of leaf TP were not affected by fertility treatment, but organic tomatoes supported lower aphid densities. Leaf tissue in the organic treatment contained higher concentrations of calcium, potassium, magnesium and sulfur, while in the conventional treatment total carbon, total nitrogen and phosphorous concentrations were higher. There were no treatment differences in red-ripe fruit mineral concentrations.

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CHAPTER ONE

INTRODUCTION

The tomato is one of the world's most important vegetables. Although botanically a fruit, tomato is culinarily classified as a vegetable due to its acidity and comparatively low sugar content. In the US it is the most consumed vegetable after potato, and it is second in acreage (USDA ERS, 2011). Because of its widespread consumption and nutrient concentrations, tomato is an important part of the US diet. Minerals in tomato, including sodium, potassium, magnesium, calcium and copper, may reduce risk of cardiovascular disease (Mertz 1982). Perhaps more importantly, tomato contains ascorbic acid, carotenoids, such as lycopene and β-carotene, as well as numerous polyphenols, including naringenin, rutin, chlorogenic acid and quercetin. These phytonutrients exhibit antioxidant properties (Caris-Veyrat *et al*., 2004 and Durazzo *et al*., 2010). While other vegetables have higher concentrations of antioxidants, their extensive use in the American diet make them the number two vegetable source of phenolic antioxidants after potato (Vinson *et al*., 1998).

Phytonutrient Quality

There is increasing consumer interest in certified organic production in the US. Between 2000 and 2008, organic cropland acreage more than doubled from 1,218,905 A to 2,643,221 A, with certified organic tomato acreage tripling from 3,063 A to 9,237 A (USDA ERS, 2010). One of the most commonly cited reasons for purchasing organic produce is the perception of higher nutritional quality compared to conventionally raised crops (Bean & Sharp, 2011).

Organic and conventional agricultural systems can differ greatly in their nutrient and pest management approaches since USDA National Organic Program (NOP) standards do not allow use of synthetic fertilizers or pesticides (USDA NOP, 2012). Nitrogen is one of the most

important plant macronutrients. In conventional systems, nitrogen – as well as other macronutrients – is applied in inorganic, water soluble forms such as ammonium, urea, nitrate and nitrite. Organic crops rely on nitrogen forms that are released more slowly such as leguminous cover crops, composts and animal byproducts. Because of this, organically managed soils generally contain higher ratios of organic to total nitrogen than those amended with inorganic forms of nutrients. Although tomato plants can uptake organic forms of nitrogen such as amino acids (Garcia *et al*., 2011), nitrate is the dominant form taken up by plants (Masclaux-Daubresse *et al*., 2010). Organic nitrogen is released to the plant more slowly than the more readily available nitrate form (Kumar *et al*., 2004; Reeve, *et al*., 2008).

Limiting nutrient availability can slow vegetative growth and shift plant metabolism towards constitutive production of secondary compounds (Stout *et al*., 1998). Plants grown under lower nitrogen availability may also have enhanced induced responses to insect herbivores (Lou & Balwin, 2004). Insect feeding deterrents such as phenolic compounds can both deter insects and contribute to human health (Vinson *et al*., 1998). Pest management in organic agriculture relies on biological controls, as well as a smaller number of chemical tools – only those that comply with NOP standards – compared to conventional agriculture (USDA NOP, 2012). As a result, organically raised crops can be subject to higher levels of insect-induced stress (Zhao *et al.*, 2009). Altered plant metabolism and these pest-induced stresses may lead to higher production of plant defensive compounds that, when consumed by humans, provide antioxidant and nutritive roles.

Soil Fertility & Phytonutrient Content

Numerous studies have investigated the influence of cultural practices on micronutrient content and fruit quality of tomatoes and other horticultural crops, many of which focus on

nitrogen differences. These studies show highly variable results; however, most studies have found at least as high or higher concentration of micronutrients under organic fertility management. In a two-year study on commercial field-grown tomatoes in Taiwan, Juroszek *et al*. (2009) found no difference in fruit quality (soluble solids, total phenolics, ascorbic acid and lycopene) between organic and conventional systems. In a similar field study on commercial conventional and organic farms in Italy, Migliori *et al.* (2010) found higher concentrations of lycopene and sugars in ripe 'Giulianova' tomato fruit, while three other cultivars showed no nutrient treatment differences for these parameters. Riahi *et al.* (2009) conducted a field study of four different cultivars of tomato under organic and conventional management in Tunisia. There were three different levels of organic treatments based on different types and amounts of compost applications and one conventional treatment based on local practices of high-yielding conventional practices. They found no management effects on lycopene or total phenolics (TP). Soluble solids were positively affected by two of the organic treatments in the cultivar 'Hypeel 108', while the cultivar 'Firenze' was negatively affected by organic management. Ordóñez-Santos *et al.* (2011) found no nutrient treatment differences for TP and lycopene concentration in 'Llado' and 'Antillas' tomatoes, while 'Antillas' under organic management had twice the concentration of ascorbic acid compared to those grown under conventional management. Raigon *et al*. (2010) conducted a two-year study on eggplant (*Solanum melongena*) phenolics under organic and conventional management in Spain. They found thirty percent higher TP concentration in year one but no difference in year two. In a ten-year study in California, Mitchell *et al.* (2007) found concentrations of three major flavonoids (quercetin, naringenin and kaemferol) to be about twice as high in tomatoes grown under organic compared to conventional management. Greenhouse-grown sweet peppers (*Capsicum annum* L.) were found to have

significantly higher levels of TP under manure-derived organic nutrient treatment compared to those under inorganic, soluble nutrient treatment (Del Amor *et al.*, 2008).

While there are trends of generally higher phytochemical content of organically grown crops, lack of information in these studies on the exact nutrient management practices or detailed soil characteristics of the different experimental sites make it difficult to directly compare these studies. Annual variation in rainfall, temperature and other environmental factors are likely causes of observed year-to-year changes in field studies. Differences in the compounds measured and methods used make direct comparisons across studies difficult. While such studies can be informative for specific areas and cultivars, a different approach is needed to eliminate confounding effects and to elucidate the mechanisms underlying the generally higher levels of phytochemicals in organically raised crops.

Insect Herbivory

Because of restrictions in practices and chemical use under organic standards, there are fewer available insect pest management tools in organically managed systems. The green peach aphid (*Myzus persicae*) is a member of the family Aphididae and is a common polyphagous herbivore insect in agricultural systems around the world (Margaritopoulos *et al*., 2009). It is an important disease vector (Blanc *et al*., 2011), but its herbivory can also cause significant reductions in yield of numerous crops (Hewson & Sagenmuller, 2000; McCarter, 1992). Aphids feed on plants using their piercing-sucking mouthparts (stylets). The stylet is inserted into the leaf tissue where it probes until puncturing a phloem sieve element (Miles, 1999). Once there, they may extract phloem sap for up to several hours (Tjallingii & Hogen Esch 1993).

Herbivory by aphids initiates a cascade of signaling within the plant that leads to induction of plant defensive compounds (Smith & Boyko, 2007) including polyphenolic

compounds and polyphenol oxidases (PPO) (Kerchev *et al*., 2012). Within plant tissues, phenolic compounds are kept from oxidation by maintaining a proper redox state through recycling of important antioxidants ascorbate and glutathione (Kuzniak, 2010). In the absence of antioxidants and in an altered redox state, such as within the aphid midgut following ingestion, phenolics can oxidize into damaging forms (Miles, 1999). The first intermediate in polyphenol oxidation is formation of quinones, which can be toxic to insects in part because of their ability to copolymerize with proteins (Bell, 1981). Over expression of PPO by tomato increased resistance to common cutworm (Mahanil, *et al.*, 2008). Aphids are able to detoxify such potentially destructive compounds from phloem sap, but the time it takes to detoxify these compounds may limit their feeding rate (Miles, 1999). Therefore, higher phenolics concentration may directly reduce aphid feeding. Plant nitrogen nutrition is known to affect oviposition and population dynamics of sucking-piercing insects (Zanic *et al*., 2011). Tobacco whitefly (*Bemisia tabaci*) populations increased more rapidly on hydroponically grown tomato plants with higher nitrate concentration in the nutrient solution and leaf tissue nitrogen content. Nitrogen form affected whitefly dynamics with lower population growth under higher ammonium-to-nitrate ratios. Leaf concentrations of phenolics were higher under organic nutrient management (Zhao *et al*., 2009).

Since both nutrient and pest management strategies differ greatly between organic and conventional systems, they are likely the main drivers of enhanced phytonutrient content often seen in organic systems. Changes in plant metabolism under slow-release, higher organic nutrient ratios, as well as differences in insect-induced stress, may affect plant production of phytonutrients. This work was conducted to determine the relative contributions of these nutrient sources and herbivory by aphids on plant growth, fruit quality, and phytochemical

concentration of the determinate, fresh-market tomato cultivar, ‘Oregon Spring’ grown in a controlled environment.

CHAPTER TWO

METHODS AND MATERIALS

Tomato seeds (*Solanum lycopersicum* L. 'Oregon Spring', Johnny's Selected Seeds, Winslow, ME) were sown in August, 2010 in two cell flats of 32 cells each in LC1 Professional Growing Mix (Sun Grow Horticulture, Bellevue, WA). Flats were kept in a glasshouse and watered as needed to maintain soil moisture for seed germination and early seedling growth. Glasshouse temperatures were 21.1/18.3ºC (day/night) at a photoperiod of 14 hr/day supplemented with high-pressure sodium lamps. Three weeks after all seedlings had emerged, nutrient treatments were started. Plants in the organic nutrient treatment (ORG) were given a BioLink All Purpose Fertilizer 5-5-5 (Westbridge Agricultural Products, Vista, CA) solution at a concentration of 4 mL/L tap water, while the inorganic treatment (INORG) received a Peters 20-10-20 solution (1.02 g/L tap water). Both treatments received one liter per flat per week of their respective nutrient treatment. At week six, plants of similar size and vigor from each treatment were transferred to individual, approximately 24 liters pots (#7). The potting media for the ORG nutrient treatment consisted of a mix of LC1 Professional Growing Mix (Sun Grow Horticulture, Bellevue, WA), Whitney Farms Compost (Scotts, Marysville, OH), and sifted soil in a ratio by volume of 15:4:1. The INORG plants were potted in 100 percent LC1 Professional Growing Mix.

Upon transplanting, nutrient solution dosage was increased to 150 mL per plant, administered once per week. At week seven, one week after transplanting, 12 of the healthiest plants in each nutrient treatment were selected for the experiment and arranged in a randomized complete block design consisting of six blocks. Beginning in week eight, the plants were given 1 liter of water every other day and dosage of weekly nutrient treatments was increased to 500 mL.

All lateral shoots below the first flower cluster were removed as they appeared. At week nine, after all plants had developed flower buds, plants were sprayed with M-Pede insecticidal soap (Dow AgroSciences, Indianapolis, IN) to eliminate residual insects. All plants were then supported in standard wire tomato cages. Insect exclusion covers made of an Organza mesh fabric were placed over the cages and secured with a knot at the top and a tight elastic band below the rim of the pot. Starting in week 11, the INORG nutrient solution concentration was increased from 1.02 g/L of Peters 20-10-20 to 1.25 g/L. Beginning the following week, nutrient treatments were augmented with micronutrients. The ORG treatment was amended with BioLink Micronutrient Fertilizer (Westbridge Agricultural Products, Vista, CA) at 3.9 mL/L tap water and the INORG with calcium phosphate monobasic monohydrate at 166 mg/L tap water. Concentrations of macronutrients were equivalent in both treatments, with total nitrogen, total phosphorus and total potassium concentrations of 260 ppm, 99 ppm and 235/220 (INORG/ORG) ppm, respectively, based on laboratory analyses (Analytical Science Laboratory, University of Idaho, Moscow, ID). The forms of nitrogen varied greatly between INORG and ORG treatments, with nitrate the predominant form in INORG and organic forms (i.e. amino acids and proteins) predominating in ORG nutrient solution (Appendix A). In week 13, the air temperature in the glasshouse was increased to 23.3/20ºC (day/night) and the nutrient dosage was increased to 750 mL per plant every other day (Appendix B). In week 14 the nutrient dosage was increased to 1 liter every other day.

Clean, pathogen-free, apterous (wingless) green peach aphids (*Myzus persicae*) were reared by the Washington State University Department of Entomology on collard plants (*Brassica oleracea*) and then transferred to 'Oregon Spring' tomato plants one week prior to transfer to the experimental plants. Rearing was conducted in an isolation glasshouse that was

specifically for rearing insects. At week 14, after all plants had set fruit, tomato leaf cuttings containing approximately 100 aphids each were transferred to half of the experimental plants according to the experimental design (Appendix C). Cuttings were placed in the upper canopy on young leaves and in locations conducive to aphid growth and reproduction. Due to low initial colonization of the plants by the aphids, an additional 300 aphids per plant were introduced via collard leaf cuttings two weeks after the first transfer.

Photosynthetically active radiation (PAR) was measured using a LI-COR LI-185 Line Quantum Sensor (LI-COR, Lincoln, NE). Readings were taken at four intervals along the length of the bench at upper canopy level. They were made on three dates under full-sun, partial sun, and cloudy or at night with lights illuminated. Under these conditions, PAR averaged 176 μM photons/m^2/sec (± 24.7) (Appendix C). In week 15 the nutrient dosages were increased to 1.5 liter ever other day. In week 18, chlorosis was noticed on some of the organically treated tomatoes, indicating a nitrogen deficiency. The dosage was increased to 1.75 liters and the nutrient concentrations in both treatments were increased by 25 percent, from a nitrogen concentration of 260 ppm to 325 ppm (see Appendix B for details of nutrient applications). Fruit were harvested at red-ripe stage beginning in week 18 and continuing until a minimum of eight ripe fruit were harvested from each plant during week 23. During that week all remaining fruit, regardless of maturity stage, were harvested.

Immediately following harvest, all tomato fruit were weighed and their stages of maturity noted. A 1 cm thick slice was cut from the equatorial region of each fruit and the pericarp tissue external to each locule was sampled. The tissue was minced with a knife, one portion of which was used to measure percent soluble solids (ºBrix) using a digital refractometer (Atago PR-101, Bellevue, WA), while the remainder was frozen with liquid nitrogen, placed in 50 mL plastic

tubes, and stored in an ultra-low temperature freezer at -80ºC for later analysis. Six defect-free, mature red tomato fruit were selected from each plant by taking the maximum and minimum weights along with four more fruit at equal weight intervals in between the two extremes. The samples were ground by mortar and pestle in liquid nitrogen until finely powdered and later used for phytochemical analyses.

Three to four representative leaves were taken as sub-samples from the mid to upper canopy of all plants. The remaining above ground vegetation was oven-dried for 48 hours at 80ºC and percentage dry weight calculated. Stems and petioles were discarded and leaf tissue weighed. Analyses of total carbon and total nitrogen (via combustion) and calcium, potassium, magnesium, sodium, phosphorus and sulfur (ICP with nitric digestion) were conducted on dried leaf tissues (Analytical Science Laboratory, University of Idaho, Moscow, ID). Roots were washed and cleaned of soil and oven-dried for 48 hours at 80ºC. Aphids were quantified on the leaf sub-samples using a counter and hand lens. These leaves were then frozen in liquid nitrogen and the petiole and petiolule tissue was discarded. Leaflet tissue was then ground using a freezer mill (SPEX SamplePrep 6770, Metuchen, NJ) (3 cycles, 2 min precool, 2 min $cycle^{-1}$, 2 min cool time, 10 cycles sec^{-1}) and stored at -80ºC for later analysis.

All chemicals used for the laboratory analyses, except for 100% ethanol, were obtained from Sigma-Alrich (St. Louis, MO) and were of ACS reagent or HPLC grade. Water used in the following assays, and to make all stock solutions, was from a Barnstead Nanopure purification system (Thermo Scientific, Waltham, MA).

Total phenolic (TP) compounds were measured spectrophotometrically using a modified version of the methods outlined by Singleton *et al*. (1999). One milliliter of an 80% methanol solution was added to frozen, 350 mg samples of powdered fruit tissue or 150 mg of powdered

leaf tissue in 2 mL microcentrifuge tubes, with all samples run in triplicate. Tubes were vortexed for 30 sec until thoroughly dispersed and stored at -20ºC for 24 hr. Samples were centrifuged at 8,000 g and 4ºC for 20 min. The supernate from each tube was then poured into individual 15 mL polyethylene sample tubes, capped and stored at -20ºC. The above extraction was conducted two more times on the same tissue. The supernate was adjusted to 3 mL with 80% methanol. Then, 200 µL of extract, 1000 µL of 10% Folin-Ciocalteu (F-C) phenol reagent, 2N and 800 µL of 75 g L^{-1} Na_2CO_3 were pippetted into 2 mL microcentrifuge tubes, vortexed and stored at 20ºC for 2 hr. A second solution with each extract was made substituting 800 µL of Nanopure water for the Na_2CO_3 and stored at 20ºC for 2 hr. An ultraviolet-visible spectrophotometer (Agilent Technologies, Model HP8453, Avondale, PA) interfaced to a computer with UV-Visible ChemStation software (v. B.01.01 [21]) with tungsten bulb was blanked on 1.0 mL of 10% F-C reagent in a 1.5 mL polystyrene cuvette. One milliliter of the sample solutions was pippetted into separate cuvettes and absorbance was measured at 760 nm. Cuvettes were run in duplicate. The average difference in absorbance was compared to a standard curve for gallic acid, and the concentrations were reported as gallic acid equivalents (GAE). Because ascorbic acid is known to react with F-C reagent (Everette *et al*., 2010), a correction factor of was determined to adjust for its contribution. Known concentrations of ascorbic acid were substituted for extract and used in the F-C assay as described above. On a mass-to-mass basis, the average reactivity of ascorbic acid to GA was 1.00:0.662. Reduced ascorbic acid concentrations, determined for ripe fruit as described below, were multiplied by 0.622, the correction factor, and subtracted from the TP concentration. The correction was made for ripe fruit TP but not for leaf TP since leaf reduced ascorbic acid concentrations were not measured.

Lycopene was measured spectrophotometrically using a modified version of the methods outlined by Nagata & Yamashita (1992). The following work was conducted under low light. Powdered, frozen fruit samples of 100 mg were placed in 15 mL conical polyethylene sample tubes wrapped with aluminum foil with all samples run in triplicate. To each tube, 8.33 mL of a 2:3 solution of HPLC-grade acetone and HPLC-grade hexanes at 4ºC was added. The solution was amended with 9.08 x 10^{-4} mol L^{-1} (200 mg L^{-1}) butylated hydroxytoulene (BHT) to prevent pigment oxidation. Tubes were capped and vortexed for 1 min, then stored at -20ºC for 24 hr. After storage the tubes were again vortexed for 1 min and returned to -20ºC. This was continued for a total of 96 hr of extraction. Tubes were vortexed a final time and let stand for 5 min for a complete phase separation to occur. The spectrophotometer was blanked and zeroed using a tungsten lamp and 1 mL 100% HPLC-grade hexanes in a 1.4 mL glass cuvette at 505 nm. Aliquots of 1 mL of the hexane phase were pipette into 1.4 mL glass cuvettes and absorbance was measured at 505 nm. Lycopene concentration was calculated according to the equation of Davies (1976):

$$Lycopene\ (mM) = Absorbance_{505}/3400\ mM^{-1}$$

Trolox equivalent antioxidant capacity (TEAC) was determined using an adapted version of the methods of Cano *et al*. (1998) and Arnao *et al*. (2001). Powdered, frozen tomato fruit samples of 200 mg were weighed into 2 mL microcentrifuge tubes with all samples run in duplicate. To each sample tube was added 700 µL of 50 mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer and 700 µL of 100% ethyl acetate. Samples were vortexed for 30 sec and then centrifuged at 8,000 g and 4ºC for 10 min. The organic and inorganic phases were then pippetted into separate 2 mL microcentrifuge tubes and re-centrifuged. To measure hydrophillic TEAC (HTEAC), the spectrophotometer was blanked with tungsten lamp on a 1.4 mL glass

cuvette with 1 mL 100 mM phosphate buffer (0.1M sodium phosphate monobasic plus 0.1M sodium phosphate dibasic) (pH 7.4) and zeroed at 734 nm. Forty microliters of 1 mM H_2O_2, 100 µL of 15 mM 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and 10 µL of 3.3 U μL^{-1} peroxidase from horseradish (HRP) were added to the cuvettes, and they were gently shaken for 10 sec. Then, 800 µL of 100 mM phosphate buffer (pH 7.4) was added to each cuvette and stirred with a paddle. The cuvettes were loaded into the spectrophotometer multi-cell transport and a timed kinetic assay was run at 734 nm. After two passes, 50 µL of MES extract was added and mixed with a stir paddle, and the decrease in absorbance was recorded. The assay was repeated for lipophilic TEAC (LTEAC), substituting 770 µL 100% ethanol for 800 µL 100 mM phosphate buffer and 80 µL ethyl acetate extract for 50 µL MES extract. HTEAC and LTEAC standard curves were produced by substituting standard solutions of Trolox in MES buffer and ethyl acetate, respectively, for the tissue extracts. The difference between initial and final absorbances for the samples was compared to those of the standard curves to determine TEAC.

Reduced ascorbic acid was measured according to an adapted version of the method described by Andrews *et al*. (2004). Powdered, frozen tomato fruit samples of 200 mg were weighed into 2 mL microcentrifuge tubes with all samples run in duplicate. Next, 1.5 mL of 1 M $HClO_4$ at 4ºC was added. Then, samples were vortexed for 30 sec, stored on ice for 20 min, and centrifuged at 8,000 g and 4ºC for 10 min. The supernatant (400 µL) was pipetted into a 2 mL microcentrifuge tube along with 200 µL of 100 mM N-(2-Hydroxyethyl) piperazine-N-(2-ethanesulfonic acid) (HEPES-KOH pH 7.0), and the tubes were vortexed for 30 sec. Samples were then titrated to pH 4-5 with 5M K_2CO_3 and centrifuged at 8,000 g and 4ºC for 5 min to pellet the precipitate. The spectrophotometer with deuterium lamp was blanked and zeroed at 265 nm with 500 µL of 100 mM phosphate buffer (pH 5.6) in a 0.5 mL black-walled quartz

cuvette. Next, 50 µL of the extracts along with 446 µL of 100 mM phosphate buffer (pH 5.6) were pipetted into the black-walled quartz cuvettes and mixed. The cuvettes were loaded into the multi-cell transport and a timed kinetic assay was run. After the first two measurements, 4 µL of 1 unit/µL ascorbate oxidase from *Cucurbita* sp. was stirred into each cuvette, and the decrease in absorbance was recorded. Standard curves were produced following a similar procedure as for the above measurement with stock solutions of ascorbic acid substituting for tissue extract. Changes in absorbance of the samples were compared to the standard curves to determine reduced ascorbic acid concentration.

Data were analyzed by ANOVA (mixed model and GLM) using Statistical Analysis System v. 9.1.3 (SAS Institute, Inc., Cary, NC). Fruit mass and aphid density were used as covariates. Natural log transformation or rank transformation was used when data did not meet normality and/or equal variance assumptions. Mean separations were performed by Least Squares Difference (LSD). When $P<0.05$ for fruit mass and aphid density, regression analysis was used for significant main effect treatment differences (nutrient and/or herbivory).

CHAPTER THREE

RESULTS

Aphid Density and Vegetative Growth

Because of zero aphid populations in the –aphid treatment, population densities were only analyzed for ORG and INORG in the +aphid treatment. Aphid population was four times lower and statistically notable (P = 0.0545) in the ORG treatment compared to the INORG treatment (Fig. 1). The above ground, fresh vegetative biomass was notably lower in the ORG fertility treatment than the INORG treatment, while there was no treatment difference in above ground dry biomass (Table 1). There were no treatment differences in below ground biomass, but a tendency for a greater percent root biomass fraction under ORG fertility management. There were no aphid treatment effects on plant biomass.

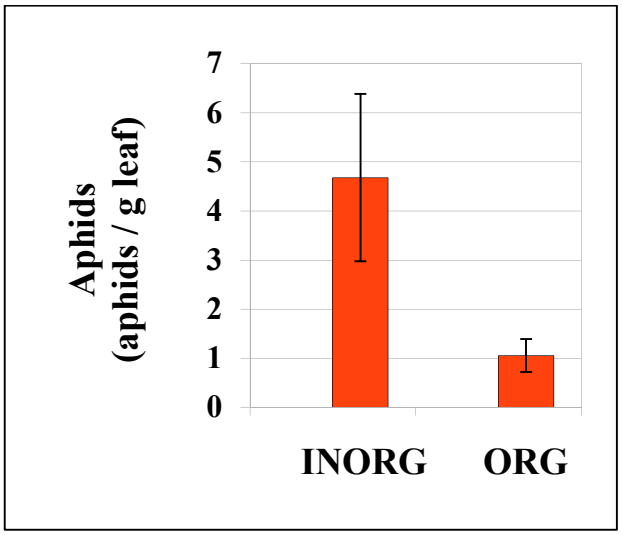


Figure 1: Aphid population density (number per gram of fresh leaf tissue) determined from three leaf subsamples taken from the mid to upper canopy under inorganic (INORG) and organic (ORG) fertility treatments of the +aphid treatment. Coefficients of variation for INORG and ORG treatments were 89.1 and 78.0, respectively. Bars represent standard error of mean. Data were analyzed using ANOVA GLM.

Table 1: Above and below ground vegetative biomass on fresh (FW) and dry weight (DW) bases, and percent root biomass fraction for inorganic (INORG) and organic (ORG) fertility treatments and with (+aphid) and without (–aphid) aphids. Data were analyzed using ANOVA GLM.

		Main effect means				Effects		
Biomass	**Unit**	**INORG**	**ORG**	**+Aphid**	**-Aphid**	**Fertility**	**Aphid**	**Fertility*Aphid**
Above Ground FW	kg	1.32	1.18	1.26	1.24	0.052	0.77	0.45
Below Ground FW	g	36.4	35.1	36.8	34.6	0.75	0.59	0.40
Above Ground DW	g	115	110	114	112	0.39	0.78	0.63
Below Ground DW	g	4.90	5.20	5.08	5.03	0.53	0.91	0.71
Root Fraction DW	%	4.19	4.71	4.49	4.40	0.1003	0.78	0.42

Fruit Yield and Quality

There were fertility treatment differences in most of the measured fruit quality parameters, but few for the herbivory treatment. ORG fertility resulted in 17% lower total fruit yields (P=0.003) than the INORG treatment due to 22% lower yields of unripe fruit (P=0.02) (Fig. 2). There were no statistically significant differences (P=0.27) in yield per plant of red-ripe fruit. Herbivory had no significant effects on ripe (P=0.71), unripe (P=0.26) or total yields (P=0.17) (data not shown). Mean mass of red-ripe fruit was 11% lower under ORG fertility than under INORG fertility treatment (P=0.0004) (Fig. 3). Herbivory had no significant effect on mean ripe fruit mass (P=0.19) (data not shown). Soluble solids of red-ripe fruit were 19% higher in the ORG fertility treatment compared to the INORG treatment, but there was no effect of herbivory (Table 2).

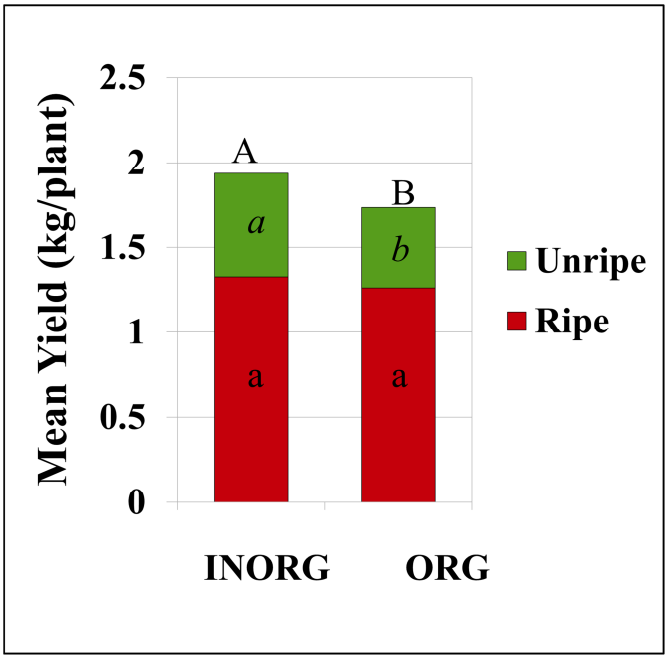


Figure 2: Mean ripe (red bars), unripe (green bars) and total fruit yield per plant under inorganic (INORG) and organic (ORG) fertility treatment. Different letters indicate statistically significant differences at the α=0.05 level. Data were analyzed using ANOVA mixed model.

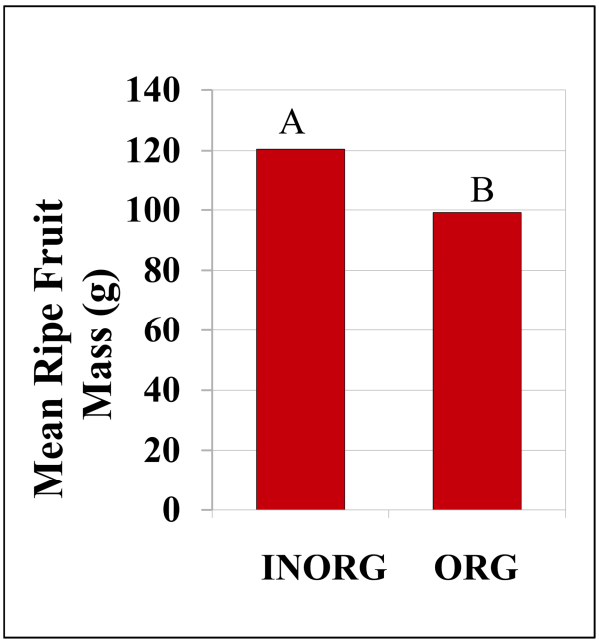


Figure 3: Mean red-ripe fruit mass under inorganic (INORG) and organic (ORG) fertility treatments. Different letters represent statistically significant differences at the α=0.05 level. Data were natural log-transformed and analyzed using ANOVA mixed model.

Table 2: Soluble solids, total phenolics (gallic acid equivalents, GAE), lycopene, total Trolox equivalent antioxidant capacity (TTEAC), lypophilic TEAC, hydrophilic TEAC, and reduced ascorbic acid concentrations on fresh (FW) and dry weight (DW) bases (except soluble solids) under inorganic (INORG) and organic (ORG) fertility and with (+aphid) and without (-aphid) aphids. Data were analyzed using ANOVA mixed model. Failure to meet normality based on Kolmogorov-Smirnov test is indicated by asterisk (*).

		Main effect means				Effects		
Variable	**Unit**	**INORG**	**ORG**	**+Aphid**	**-Aphid**	**Fertility**	**Aphid**	**F*A**
Soluble Solids	**%**	5.13	6.10	5.57	5.46	0.0004	0.60	0.30
Total Phenolics	**mg GAE/g FW**	0.235	0.275	0.269	0.242	0.0002	0.01	0.49
	mg GAE/g DW	22.0	25.7	25.1	22.6	0.0002	0.01	0.59
Lycopene	**µg/g FW**	3.77	4.22	4.11	3.88	0.002	0.12	0.49
	µg/g DW	352	394	362	384	0.002	0.01	0.56
Total TEAC	**mmol/g FW**	2.88	3.07	2.99	2.95	0.007	0.56	0.64
	mmol/g DW	269	286	275	279	0.007	0.56	0.76
Lipophilic TEAC	**mmol/g FW**	1.48	1.61	1.54	1.56	0.005	0.73	0.19
	mmol/g DW	138	151	145	144	0.005	0.71	0.15
Hydrophilic TEAC *	**mmol/g FW**	1.27	1.45	1.34	1.38	0.0002	0.40	0.04
	mmol/g DW	1189	135	125	129	0.0002	0.41	0.04
LTEAC:TTEAC	**Ratio**	0.51	0.52	0.51	0.52	0.37	0.26	0.02
HTEAC:TTEAC	**Ratio**	0.45	0.48	0.47	0.46	0.03	0.43	0.03
Ascorbic Acid	**µg/g FW**	157	175	166	166	0.0008	0.95	0.38
	mg/g DW	14.4	16.4	15.4	15.3	0.004	0.82	0.92

Ripe Fruit Phytonutrients

Fruit TP concentration on fresh weight and dry weight bases was positively affected by both ORG fertility and herbivory, with 17% higher concentration in ORG than INORG fertility management and 11% higher with aphids present than absent (Table 2). Lycopene concentrations on both fresh and dry bases were 12% higher under ORG fertility and 6% lower on a dry weight basis when aphids were present. Lipophilic Trolox equivalent antioxidant capacity (LTEAC) and total Trolox equivalent antioxidant capacity (TTEAC) were 9 and 6% higher on both fresh and dry weight bases, respectively, under ORG fertility treatment than INORG. Reduced ascorbic acid (vitamin C) concentrations were 11 and 14% higher in ORG versus INORG treatment on fresh and dry weight bases, respectively.

There were statistically significant fertility x aphid treatment interaction effects for hydrophilic TEAC (HTEAC) on fresh and dry weight bases, as well as for ratios of LTEAC:TTEAC and HTEAC:TTEAC (Table 2). Therefore, the least significant means of these interaction effects were analyzed. Aphid herbivory had a negative effect on HTEAC in the INORG, but a positive effect in the ORG (Table 3). These differences in HTEAC concentrations were similarly reflected by the HTEAC:TTEAC ratio which was highest in the ORG treatment when aphids were present. On a dry weight basis, HTEAC was significantly higher under herbivory with ORG treatment than any other treatment combination. The ratio of LTEAC:TTEAC was higher in the ORG treatment without aphids present than both the INORG without aphids and the ORG with aphids present.

Because of the significant effect of fruit mass on soluble solids ($P=0.004$), linear regressions were conducted by fertility treatment (Fig. 4). There were no significant correlations between fruit mass and soluble solids in either organic ($P =0.46$) or inorganic ($P=0.85$) fertility

treatments. There were no significant effects of either fertility or aphid treatments on ripe fruit dry matter or mineral concentrations (Table 4).

Table 3: Treatment interactions (fertility x aphid) for hydrophilic Trolox equivalent antioxidant capacity (HTEAC) on fresh (FW) and dry weight (DW) bases, and ratios of HTEAC to total TEAC (TTEAC) and lypophilic TEAC (LTEAC) to TTEAC for red-ripe tomato fruit. Data were analyzed using ANOVA mixed model. Failure to meet normality based on Kolmogorov-Smirnov test is indicated by asterisk (*). Different letters indicate statistically significant differences at the α=0.05 level.

		INORG		ORG		
Variable	**Unit**	**+Aphid**	**-Aphid**	**+Aphid**	**-Aphid**	**F*A**
Hydrophilic TEAC *	**mmol/g FW**	1.25 a	1.30 ab	1.52 b	1.38 ab	0.04
	mmol/g DW	117 a	121 ab	142 c	129 b	0.04
HTEAC:TTEAC	**Ratio**	0.44 a	0.46 a	0.50 b	0.46 a	0.03
LTEAC:TTEAC	**Ratio**	0.52 ab	0.50 a	0.50 a	0.54 b	0.02

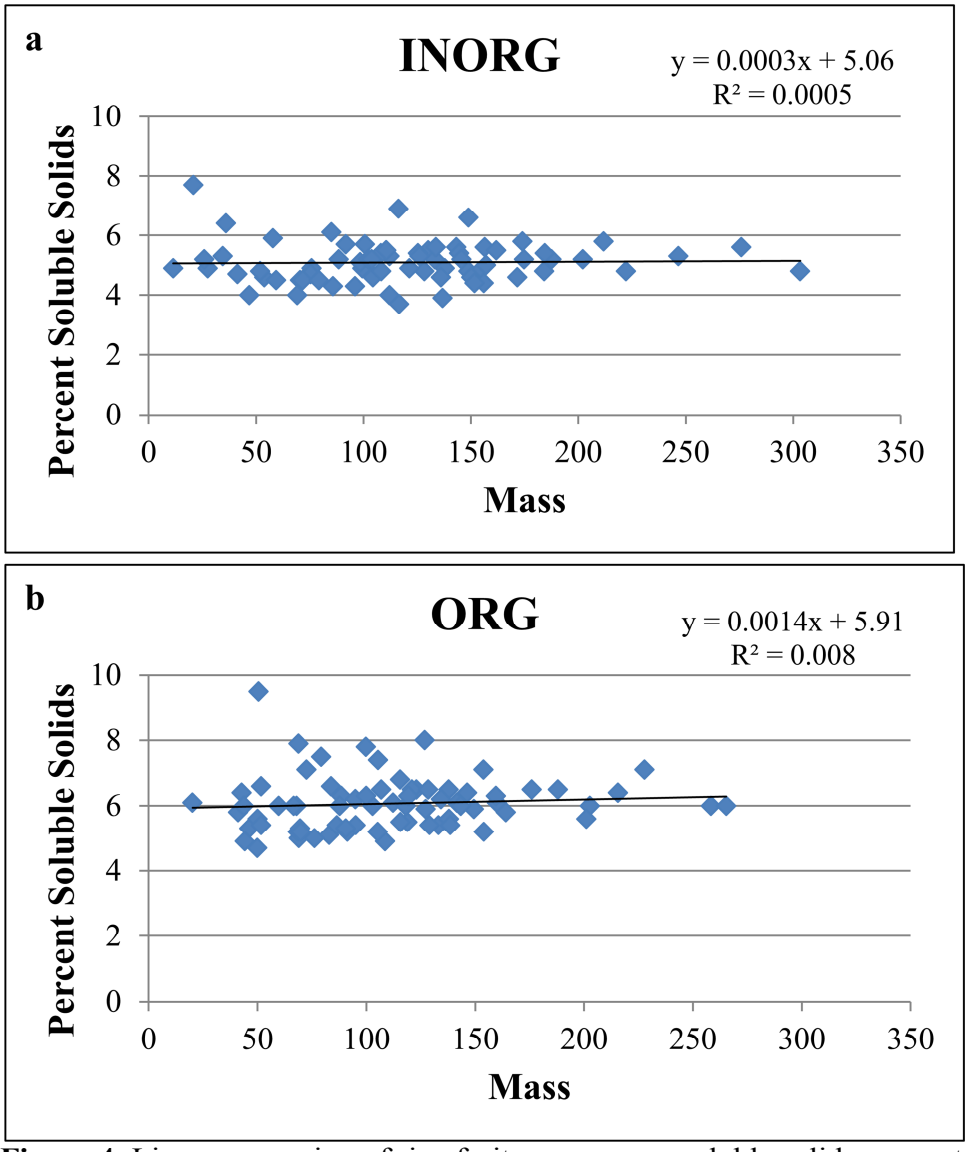


Figure 4: Linear regression of ripe fruit mass versus soluble solids concentration under a) inorganic (INORG) nutrient treatment and b) organic (ORG) fertility treatments.

Table 4: Mineral concentration of ripe red tomato fruit grown on a dry weight (DW) basis under inorganic (INORG) and organic (ORG) fertility and with (+aphid) and without (–aphid) aphids. Data were analyzed using ANOVA GLM.

		Main effect means				Effects		
Mineral	**Unit**	**INORG**	**ORG**	**+Aphid**	**-Aphid**	**Fertility**	**Aphid**	**F*A**
Dry Matter	**% DM**	6.59	6.68	6.65	6.63	0.89	0.97	0.59
$NO_3 + NO_2$	**µg/g DW**	407	550	483	473	0.54	0.97	0.39
Ca	**µg/g DW**	995	1013	1070	938	0.89	0.32	0.26
K	**µg/g DW**	48,500	46,500	48,333	46,667	0.52	0.59	0.53
Mg	**µg/g DW**	1700	1567	1617	1650	0.34	0.81	0.47
Na	**µg/g DW**	313	292	292	313	0.55	0.55	0.24
P	**µg/g DW**	6383	5933	6267	6050	0.33	0.63	0.49
S	**µg/g DW**	2433	2300	2500	2233	0.42	0.13	0.99

Leaf Mineral and Phenolics Content

Leaf total carbon (C) and total nitrogen (N) as percent of dry weight were 10 and 23% lower, respectively, in the ORG fertility treatment than in the INORG treatment (Table 5). Leaf calcium (Ca), potassium (K), magnesium (Mg) and sulfur (S) concentrations were 19, 10, 15 and 122% higher, respectively, in the ORG treatment compared to the INORG treatment. Leaf sodium (Na) and phosphorous (P) were 5 and 20% lower, respectively, in the ORG versus INORG treatment. There were no significant differences in leaf TP for fertility or aphid treatments on either a fresh or dry weight basis (Table 6).

Table 5: Mineral concentration of leaves on a dry weight (DW) basis for inorganic (INORG) and organic (ORG) fertility treatments and with (+aphid) and without (–aphid) aphids. Data were analyzed using ANOVA GLM.

		Main effect means				Effects		
Mineral	**Unit**	**INORG**	**ORG**	**+Aphid**	**-Aphid**	**Fertility**	**Aphid**	**F*A**
C	**% DW**	38.3	34.8	36.5	36.7	<0.0001	0.62	0.62
N	**% DW**	5.2	4.2	4.7	4.6	0.0003	0.43	0.91
Ca	**µg/g DW**	39,500	47,000	42,000	44500	0.001	0.11	0.11
K	**µg/g DW**	58,500	64,500	62,833	60,167	0.08	0.39	0.58
Mg	**µg/g DW**	8,467	9,750	8,833	9,383	0.02	0.22	0.19
Na	**µg/g DW**	975	930	953	952	0.80	0.99	0.54
P	**µg/g DW**	14167	11833	12,833	13,167	0.02	0.69	0.99
S	**µg/g DW**	16,167	36,000	27,000	25,167	<0.0001	0.43	0.29

Table 6: Leaf total phenolics concentration (gallic acid equivalents, GAE) on a fresh (FW) and dry weight (DW) basis under inorganic (INORG) and organic (ORG) fertility treatments and with (+aphid) and without (–aphid) aphids. Data were analyzed using ANOVA GLM.

		Main effect means				Effects		
Variable	**Unit**	**INORG**	**ORG**	**(+) Aphid**	**(-) Aphid**	**Fertility**	**Aphid**	**F*A**
Total Phenolics	**mg GAE/g FW**	0.77	0.85	0.77	0.85	0.18	0.13	0.56
	mg GAE/g DW	70.5	76.6	69.8	77.3	0.21	0.13	0.55

CHAPTER FOUR

DISCUSSION

Fruit Size

Average red-ripe fruit in this study were significantly smaller under ORG than INORG fertility management (Fig. 3). This is consistent with other studies comparing organic and conventional management. In a study on commercial field-grown strawberries, Reganold *et al*. (2010) found organically grown strawberry fruit to be about 13 percent smaller than their conventionally raised counterparts. Zuba *et al*. (2011) found a lower size distribution in 'Santa Clara' tomato grown under five different organic systems compared to conventional. Under organic management, yield of fruit classified as small was higher while yield of large size fruit was lower. In contrast, field-grown tomatoes in Tunisia showed no fertility treatment differences across four cultivars (Riahi *et al*., 2009). Further work is needed to better understand the mechanisms behind this effect.

Fruit Yield

Yield of red-ripe fruit per plant was not affected by fertility or aphid treatment (Fig. 2). Yield of immature fruit was significantly lower under ORG fertility management. As a result, the total fruit yield was lower under ORG fertility management. In this case, the yield difference is likely due to the smaller average fruit size under ORG treatment as well as lower yield of immature fruit. This same effect was seen in work by Reganold *et al.* (2010) and Zuba *et al.* (2011) as previously mentioned. While organic systems sometimes yield smaller average fruits and lower total yield, this is not always the case. Campiglia *et al*. (2011) conducted a study on tomato yield and quality on a research farm in Italy. Comparing cover crop nutrient management to conventional, inorganic nutrient management, they found higher marketable yield of tomato

under cover cropped systems. Soil nitrate levels under cover crops were higher than conventional management at key developmental stages of high plant nitrogen demand. In the current study, the majority of the nitrogen applied in the ORG nutrient solution was in organic form (Appendix A). While is it is well known that tomato roots are capable of absorbing amino acids (Garcia *et al.*, 2011), it is unknown what proportion of total nitrogen was taken up as amino acids in this study. Uptake of amino acids in two varieties of wild strawberry accounted for about 10 percent of total plant nitrogen, while in the domesticated species, *Fragaria* x *ananassa* Duch., amino acid uptake accounted for less than one percent (Reeve *et al.*, 2008). Ge *et al*. (2009) found that tomato seedlings grown in the controlled environment of sterile microcosms absorbed significant amounts of dual-labeled glycine. A total of 21 percent of supplied glycine was taken up intact by the roots. There was no other fertilizer applied, so it is uncertain how tomatoes respond in the presence of other nitrogen forms and in competition with soil microbes. A study similar to that of Reeve *et al*. has yet to be conducted on field-grown tomatoes, but such work would shed light on nitrogen nutrition under agricultural conditions. In the current study, it is likely that the organic nitrogen in the ORG treatment was less available to the tomato plants and did not supply plants with as high a quantity of readily available nitrogen as under INORG treatment. The light chlorosis observed at the early stages of fruiting in the ORG treatment was likely a result of lower available nitrogen. Nutrient solution concentrations in both treatments were subsequently increased by 25 percent in an attempt to alleviate this deficiency. After the dosage change, the signs of chlorosis diminished as the increase in nitrogen availability presumably eliminated the nutrient deficiency.

Fruit Total Soluble Solids

Under ORG fertility management, ripe fruit total soluble solids (TSS) were substantially higher compared to those raised under INORG fertility (Table 2), but fruit dry matter was not affected by soil fertility or herbivory (Table 4). There was no significant effect of fruit mass on TSS under either fertility treatment (Fig. 3). In large-fruited cultivars, fruit mass can have a negative effect on soluble solids (Balibrea *et al*., 2006; Bertin *et al*., 2009), but this effect was not measured in the medium-fruited 'Oregon Spring' used in the current study. TSS are a measure of numerous different compounds including organic acids and, primarily, sugars. Fruit sugar is derived largely from the breakdown of starch into simple carbohydrates and import of sugars such as sucrose. Both starch synthesis and accumulation occur during the immature and mature green stages of fruit development (Beckles *et al*., 2012). ADPglucose pyrophosphorylase (AGPase) is a key enzyme responsible for starch biosynthesis from sugars in fruit tissue. Under ORG fertility TSS concentration was higher, but there was no difference in fruit dry matter percentage. Organic fruits likely had higher sugar to starch ratios which would account for the similarities in percentage of dry matter between fertility treatments.

Sucrose is transported to fruit cells either symplastically through the plasmodesmata or apoplastically via cell wall invertase and hexose transporters (Beckles *et al.*, 2012). In the former, transport occurs passively as sucrose flows down a concentration gradient into the fruit cells. Conversion of sucrose and other sugars into starch decreases the osmolarity of the fruit cell, increases the concentration gradient and results in continued flow of sugars to the fruit cell. In apoplastic transport, sucrose is hydrolyzed into fructose and glucose by invertase, and the resulting sugars are then transported across the plasma membrane. It is likely that both modes of sugar accumulation are active throughout tomato fruit development (Zanor *et al*., 2009). During

the final stage of fruit maturation, sugar continues to be imported while at the same time fruit starch is broken down, resulting in high sugar accumulation (Beckles, *et al.*, 2012). In ripe tomato fruit, hexoses account for over half of the TSS (Ho, 1996).

Vacuolar invertase is one of the main determinates of mature fruit sugar composition (Ho, 1996), and high vacuolar and cell wall invertase activities are likely drivers of increased sugar accumulation in tomato fruit (Li *et al*., 2012). Mauromicale *et al*. (2011) found similar increases in high tunnel-grown tomato fruit TSS when soil was amended with organic material. Warner *et al*. (2004) found no difference in tomato fruit TSS of five cultivars under five different levels of ammonium nitrate from 0 to 250 kg ha^{-1}. Therefore, it is likely that the nutrient form affects plant partitioning of photosynthate to fruit sugars. Still, the specific mechanisms for this are not yet known.

Carbon Partitioning and Herbivory

There appears to be a significant amount of interaction between carbon partitioning pathways and plant defensive pathways as stress responses and allocation of carbon are very closely tied (Gómez *et al*., 2010). Mechanical wounding of leaf tissue can increase cell wall invertase activity (Ohyama, *et al*., 1995). However, in the current study aphid herbivory had no effect on TSS concentration or vegetative growth, likely due to the discrete piercing-sucking feeding habit of aphids compared to the more destructive habits of cutting or chewing insect mouthparts. Even though there was large variation among the treated plants, aphid population densities were not a significant co-variate for TSS concentration. Populations in both nutrient treatments were within the range previously measured on greenhouse-grown tomato plants (Boughton *et al*., 2006), but likely higher than those seen under most field conditions. All of the plants on which aphids were introduced had populations exceeding the recommended action

threshold of three to four per plant for integrated pest management of field-grown tomatoes (Webb *et al*., 2010).

Fruit Phytonutrient Concentrations

The ORG fertility treatment had a positive effect on ripe fruit TP expressed on both a fresh and dry weight basis (Table 2). The reduced ascorbic acid (vitamin C) concentration of ripe fruit was also higher under ORG fertility treatment. Ascorbic acid and phenolic compounds account for the largest portion of hydrophilic antioxidant capacity in the fruit. The same positive effect of ORG treatment was seen on hydrophilic Trolox equivalent antioxidant capacity (HTEAC). Ripe fruit phenolic concentration from rockwool-grown plants varied by truss and showed either no difference or enhanced concentration under lower nitrogen availability, while ascorbic acid concentration was 11 to 29 percent higher under low nitrogen (Bénard *et al*., 2009). The first products of oxidation of phenolic compounds are quinones, which polymerize with proteins (Bell, 1981). Such damage could occur in plant tissues given an oxidative environment. Ascorbate plays a key role in maintaining the redox state and preventing damage by oxidized phenolic compounds. Aphid feeding can decrease the pool of reduced ascorbate (Kerchev *et al*., 2012) as tissues are damaged and reactive oxygen species (ROS) are formed. In this study, aphid infestation had no effect on ripe fruit reduced ascorbic acid concentrations while ORG fertility had a slight positive effect. Ascorbate in tomato fruit can be translocated from leaves or synthesized via the D-mannose/L-galactose and D-galacturonate pathways (Badejo *et al*., 2012). Although these sugars were not measured directly, TSS concentration was significantly higher under ORG fertility. Therefore, it is possible that greater availability of substrate favored ascorbate biosynthesis under ORG fertility. In the leaves, however, phloem sap ascorbate can have both favorable and unfavorable consequences. Low leaf ascorbate hinders a plant's ability

to quench ROS, but high ascorbate can favor insect population increases. For example, Kerchev *et al*. (2012) found potato aphid colony expansion on potato plants which may be because ascorbate can act as an antioxidant in the midgut of phytophagous insects (Goggin *et al*., 2010). Plants, therefore, must perform a balancing act between managing oxidative stress and reducing suitability to herbivores (Bi & Felton, 1995).

ORG fertility treatment had a positive effect on ripe fruit lipophilic Trolox equivalent antioxidant capacity (LTEAC) and lycopene concentrations (Table 1). Lycopene is the dominant carotenoid pigment in ripe tomato fruit comprising from 65 to 85 percent of the total carotenoids in ripe fruit of red cultivars (Maršić, *et al*., 2010), and it is the dominant lipophilic antioxidant in red tomato fruit (Dumas *et al*., 2003). Light is a regulator of carotenoid biosynthesis and exposure to sunlight, including UV-C radiation, can have a positive effect on lycopene content in harvested tomato fruit (Liu *et al*., 2009). Temperature also has a direct relationship to lycopene synthesis, which is severely reduced below 12ºC and above 32ºC (Dumas *et al*., 2003). However, the temperatures in the glasshouse were maintained at 21.1/18.3ºC, which is within the ideal range for lycopene biosynthesis. Average above ground biomass under ORG treatment was notably lower ($P = 0.0518$) and these plants had visibly less dense foliage. However, with the shading provided by the insect exclusion cages, as well as the relatively cool temperatures in the glasshouse compared to field conditions, it is highly unlikely that fruit surface temperature or light exposure differences accounted for the lower lycopene content of INORG tomato fruit. Increased sulfur fertilization has shown a positive effect on tomato lycopene concentration (Zelená *et al*., 2009), possibly because of the role of cystein, a sulfur-containing amino acid, in carotenoid biosynthesis. The high foliar sulfur concentration in the ORG treatment (Table 5) may have contributed to the observed elevated levels of lycopene in ripe fruit.

Leaf Minerals and Plant Defense

Foliar nitrogen concentration under ORG treatment was within the sufficiency range of 3.5-5.0% for greenhouse tomato production while under INORG treatment nitrogen slightly exceeded that range (NCDA & CS, 2011). Magnesium concentrations in both fertility treatments were within sufficiency range. Foliar concentrations of calcium, potassium, phosphorous and sulfur exceeded sufficiency ranges in both fertility treatments. Sulfur concentration in the ORG treatment was more than twice as high as under INORG management (Table 5). Although the total concentrations of measured minerals in the nutrient solutions were equivalent, sulfur was not measured in the fertilizer analyses. Therefore, it is unclear whether there was a significant difference in total sulfur applied via the nutrient treatments or if foliar differences stem from differences in sulfur availability between nutrient treatments. Nitrogen and sulfur assimilation are directly related, and sulfur deficiency can lead to a decrease in nitrate reductase activity (De Bona *et al*., 2011) likely because sulfur is part of the structure of the enzyme. However, in this study, foliar nitrogen concentration was higher under the INORG treatment, so it is unlikely that those plants suffered from decreased nitrate reductase activity. Glutathione is a sulfur-containing tripeptide that, along with ascorbate, is an important plant antioxidant that regulates redox state (Kuzniak, 2010). Synthesis of cystein and glutathione can increase in response to jasmonic acid (JA) signaling under disease and aphid-induced stresses (Kruse *et al.*, 2007). It is likely that decreased nitrogen availability under ORG nutrient enhanced sulfur metabolism via synthesis of sulfur-containing amino acids

Aphid Populations and Nitrogen Nutrition

Aphid populations were notably lower on plants under ORG nutrient treatment (Fig. 5), but due to the small sample size and high variance, the less than four-fold difference was not

quite significant at the $\alpha = 0.05$ level ($P = 0.0545$). Nitrogen is a limiting nutrient in diets of most phytophagous insects (Mattson, 1980). Foliar nitrogen content is known to positively affect aphid population growth on plants (Sauge *et al*., 2010). The ORG treated plants had significantly lower foliar nitrogen content compared to INORG (Table 4).

Aphids and Plant Defense

Tomato plants employ a variety of defenses against phytophagous insects, including phenolic compounds (Steinbrenner *et al*., 2011), polyphenol oxidase (Mahanil *et al*., 2008), trichomes (Simmons *et al*., 2003; Peiffer *et al.*, 2009) and glycoalkaloids such as tomatine (Duffey & Stout, 1996). In this study, aphid herbivory did not induce production of phenolic compounds in leaves (Fig. 4), but did induce them in ripe fruit (Table 1). Fertility treatment did not have a significant effect on leaf phenolics on either a fresh or dry weight basis. A larger sample size may have yielded statistical differences since there were only six plants per fertility/aphid treatment combination. While phenolics were the only anti-feedant compounds measured, it is likely that there were other factors that affected aphid populations between the ORG and INORG treatments. Aphids use their long, slender stylets to feed on the sap of phloem sieve elements. Around the stylet, a proteinaceous sheath is secreted which may decrease leaf tissue damage (Tjallingii & Hogen Esch, 1993) and decrease plant perception of aphid feeding. Additionally, aphid watery saliva contains pectinases, oxidases and cellulases that aid in feeding, and detoxification of plant defensive compounds (Goggin 2007). Herbivory by third instar beet army worm caterpillars (*Spodoptera exigua*) induced greater accumulation of plant defensive compounds in tomato compared to feeding by potato aphid (Rodriguez-Saona, *et al*., 2010).

Ethylene, JA and salicylic acid (SA) signaling pathways are involved in tomato responses to herbivory by potato aphid (*Macrosiphum euphorbiae*) (Mantelin *et al*., 2009) and green peach aphid (De Ilaryduya *et al*., 2003). Exogenously applied methyl jasomnate (MeJA) has been found to induce biochemical changes in tomato plants and increase mortality of potato aphid (Cooper & Goggin, 2005). Boughton *et al*. (2006) measured decreased green peach aphid fecundity on plants treated with MeJA or benzothiadiazole (BTH), a SA mimic, but no difference when treated with ethephon, an ethylene-releasing agent. MeJA and BTH increased aphid populations, but MeJA had the greatest effect of these elicitors and was the only one to induce polyphenol oxidase (PPO) activity. Population growth of green peach aphids was severely reduced on *Arabidopsis* mutants with constitutively activated JA signaling (Ellis *et al.*, 2002).

Nitrogen availability affects both constitutive and induced levels of tomato plant defense (Stout *et al*., 1998). Lou & Baldwin (2004) found that low nitrogen availability positively affects induction of phenolics following application of exogenous MeJA or mechanical wounding. On field-grown cabbage, Staley *et al.* (2010) found lower populations of green peach aphid under organic compared to conventional management. They also found lower foliar nitrogen concentration and higher concentrations of plant defensive compounds, including glucosinolates, under organically managed cabbage plants. This is consistent with the findings of the current study in which plants grown under organic fertility management had lower foliar nitrogen and aphid populations. It is likely that lower foliar nitrogen, higher foliar sulfur and possible differences in yet unmeasured plant defense compounds were responsible for decreased aphid populations on organically fertilized tomato plants.

CHAPTER FIVE

CONCLUSIONS AND FUTURE DIRECTIONS

Aphid infestation was markedly lower under ORG fertility. Based on the findings of lower foliar nitrogen and higher foliar sulfur levels, organically fertilized plants may be less nutritious to insects and better defended than those under inorganic fertility management. In this study, fertility treatment had a greater effect than herbivory on phytonutrients and growth of tomato fruit. These results suggest that plant nutrition is a more important determinate of fruit quality than herbivory, at least in controlled environments. However, piercing-sucking insects, such as aphids, do not elicit the same effects on host plants as insects of other feeding strategies (Rodriguez-Saona *et al*., 2010). Future work should examine the effects and interactions of different insect guilds on field-grown tomatoes under different soil fertility treatments.

Under ORG fertility treatment, ripe fruit had significantly higher TSS concentration. TSS are positively associated with consumer preference. High TSS is a trait sought after by the processing tomato industry since higher sugar content translates into less energy input to produce paste and concentrates while reducing the need for costly added sugars. Under ORG fertility, plants exhibited altered carbon allocation and increased solute concentration in the fruit. It is possible that this is due to increased activity of cell wall invertase and AGPase in fruit tissue. Cultivars of *S. lycopersicon* and wild tomato species differ in their ability to accumulate fruit sugars. Future studies that include multiple tomato cultivars and species could shed more light on the genotypic factors affecting fruit quality under different soil fertility levels. Breeding selection in agricultural systems, particularly during the last half century, has focused largely on high yield under the relatively high soil nitrate conditions that exist in modern agriculture. Wild species of domesticated plants can have greater ability to uptake amino acids (Reeve *et al.*,

2008). Cultivars of wheat bred for organic systems generally showed higher ability to uptake amino acids compared to those bred in and for conventional systems (Murphy *et al*., 2007). Additionally, fruit quality attributes, including TSS and lycopene, have been selected traits in breeding programs and vary widely among cultivars. It is important to breed for and identify cultivars with horticultural and fruit quality traits adapted to organic systems (Van Bueren *et al*., 2011).

Phytonutrient concentration as measured by TP, lycopene, TTEAC, LTEAC, HTEAC and ascorbic acid were also higher under ORG fertility, while aphid treatment had a positive effect only on ripe fruit TP. Leaf TP were unaffected by either fertility or aphid treatments. This may indicate that the plants preferentially allocated defense compounds to their reproductive structures. Lower aphid populations in the ORG fertility treatment were likely due to lower foliar nitrogen since plant nitrogen can be a limiting factor for phytophagous insects (Mattson, 1980). In future work, it would be useful to measure individual phenolic compounds to gain a deeper understanding of the effects of soil fertility and herbivory on specific phenolic compounds.

TP concentration of leaf tissue was not affected by soil fertility or herbivory. In the future, differences may be detected with a larger sample size. Polyphenol oxidases (PPO) play an important role in defense mechanisms by phenolic compounds since they catalyze oxidation into reactive quinones. The latter are anti-feedants due to their ability to bind to proteins. In future work, measuring PPO activity may provide a more accurate view of the defensive ability of the plant phenolics. ORG fertility negatively affected C, N and P, but positively affected Ca, K, Mg and S. Of those, S showed the largest fertility treatment effect. Sulfur-containing glutathione, along with ascorbate, is an important antioxidant in plant tissues and helps to

maintain a favorable redox state (Kerchev *et al*., 2012). As such, sulfur is a vital mediator of plant response to herbivory. Therefore, future work should also include measurement of foliar and fruit glutathione concentrations as well as sulfur-containing amino acids.

Functional genomic analyses will broaden the scope of this work and shed more light on the observed physiological treatment effects. While ORG fertility had a significant, positive effect on fruit TSS, the mechanisms behind this are not apparent. Notably lower allocation to vegetative growth was observed under ORG fertility. Differential gene expression involved in sucrose synthesis and carbon partitioning may explain those effects. Signaling pathways including JA, SA and ethylene are involved in plant responses to herbivory, but the work described in this study did not directly measure activity of these pathways. Activation of genes involved in those pathways would provide more information on the relative effects of herbivory between fertility treatments. Finally, a better understanding of gene expression in relation to sulfur metabolism will likely clarify the relationship between foliar sulfur concentration and plant defense. These genomic analyses will help to further elucidate the contributions of soil fertility and herbivory to tomato fruit phytonutrients and plant defense.

BIBLIOGRAPHY

Badejo, A.A., Wada, K., Gao, Y., Maruta, T., Sawa, Y., Shigeoka, S., Ishikawa, T. 2012. Translocation and the alternative D-galacturonate pathway contribute to increasing the ascorbate level in ripening tomato fruits together with the D-mannose/L-galactose pathway. J. Exp. Botany (63): 229-239.

Balibrea, M.E., Martinez-Andujar, C., Cuartero, J., Bolarin, M.C., Perez-Alfocea, F. 2006. The high fruit soluble sugar content in wild *Lycopersicon* species and their hybrids with cultivars depends on sucrose import during ripening rather than on sucrose metabolism. Funct. Plant Biol. (33): 279-288.

Bean, M., Sharp, J.S. 2011. Profiling alternative food system supporters: The personal and social of local and organic food support. Renewable Ag. Food Sys. (26): 243-254.

Bell, A.A. 1981. Biochemical mechanisms of disease resistance. Ann. Rev. Plant Physiol. (32):21-81.

Bénard, C., Gautier, H., Bourgaud, F., Grasselly, D., Navez, B., Caris-Veyrat, C., Weiss, M., Génard, M. 2009. Effects of low nitrogen supply on tomato (*Solanum lycopersicon*) fruit yield with special emphasis on sugars, acids, ascorbate, carotenoids, and phenolic compounds. J. Agric. Food Chem. (57): 4112-4123.

Bertin, N., Causse, M., Brunel, B., Tricon, D., Genard, N. 2009. Identification of growth processes involved in QTLs for tomato fruit size and composition. J.Exp. Bot. (60): 237-248.

Beckles, D.M., Hong, N., Stamova, L., Luengwilai, K. 2012. Biochemical factors contributing to tomato fruit sugar content: a review. Fruits (67) 49-64.

Bi, J.L., Felton, G.W. 1995. Foliar oxidative stress and insect herbivory – primary compounds, secondary metabolites, and reactive oxygen species as components on induced resistance. J. Chem. Ecol. (21): 1511-1530.

Blanc, S., Uzest, M., Drucker, M. 2011. New research horizons in vector-transmission of plant viruses. Curr. Opin. Microbiol. (14): 483-491.

Boughton, A.J., Hoover, K., Felton, G.W. 2006. Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. Entomologia Experimentalis et Applicata (120): 175-188.

Campiglia, E., Mancinelli, R., Radicetti, E. 2011. Influence of no-tillage and organic mulching on tomato (*Solanum lycopersicon* L.) production and nitrogen use in the mediterranean environment of central Italy. Scientia Horticulturae (130): 588-598.

Caris-Veyrat, C., Amiot, M.J., Tyssandier, V., Grasselly, D., Buret, M., Mikolajczak, M., Guilland, J.C., Bouteloup-Demange, C., Borel, P. 2004. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. J. Agric. Food Chem. (52): 6503-6509.

Cooper, W.R., Goggin, F.L. 2005. Effects of jasmonate-induced defenses in tomato on the potato aphid, *Macrosiphum eurphorbiae*. Entomologia Experimentalis et Applicata (115): 107-115.

De Bona, F.D., Fedoseyenko, D., von Wirén, N., Montiero, F.A. 2011. Nitrogen utilization by sulfur-deficient barley plants depends on the nitrogen form. Environmental Experimental Biol. (74): 237-244

De Ilarduya, O.M., Xie, Q., Kaloshian, I. 2003. Aphid-induced defense responses in *Mi-1*-mediated compatible and incompatible tomato interactions. Mol. Plant Microbe Interact. (16): 699-708.

Del Amor, F.M., Serrano-Martinez, A., Fortea, I., Núñez-Delicado, E. 2008. Differential effect of organic cultivation on the levels of phenolics, peroxidase and capsidiol in sweet peppers. J. Sci. Food Agric. (88): 770-777.

Duffey, S.S., Stout, M.J. 1996. Antinutritive and toxic components of plant defense against insects. Arch. Insect Biochem. Physiol. (32): 3-37.

Dumas, Y., Dadomo, M., Di Lucca, G., Grolier, P. 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J. Sci. Food Agric. (83): 369-382.

Ellis, C., Karafylldis, I., Turner, J.G. 2002. Constitutive activation of jasmonate signaling in an *Arabidopsis* mutant correlates with enhanced resistance to *Erysiphe cichoracearum, Pseudomonas syringe,* and *Myzus persicae.* Molecular Plant-Microbe Interact. (15): 1025-1030.

Everette, J.D., Bryant, Q.M., Green, A.M., Abbey, Y.A., Wangila, G.W., Walker, R.B. 2010. Thorough study of reactivity of various compounds towards the Folin-Ciocalteu reagent. J. Agric. Food Chem. (58): 8139-8144.

Garcia, A.L., Madrid, R., Gimeno, V., Rodriguez-Ortega, W.M., Nicholas, N., Garcia-Sanchez, F. 2011. The effects of amino acids fertilization incorporated to the nutrient solution on mineral composition and growth in tomato seedlings. Spanish J. Agric. Research (9): 852-861.

Ge, T., Song, S., Roberts, P., Jones, D.L., Huang, D., Iwasaki, K. 2009. Amino acids as a nitrogen source for tomato seedlings: the use of dual-labeled (13C, 15N) glycine to test for direct uptake by tomato seedlings. Environmental Experimental Bot. (66): 357-361.

Goggin, F.L. 2007. Plant-aphid interactions: molecular and ecological perspectives. Curr. Opin. Plant Biol. (10): 399-408.

Goggin, F.L., Avila, C.A., Lorence, A. 2010. Vitamin C content in plants is modified by insects and influences susceptibility to herbivory. Bioessays (32): 777-790.

Gómez, S., Ferrieri, R.A., Orians, C.M. 2010. Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. New Phytologist (188): 835-844.
Hewson, R.T., Sagenmuller, A. 2000. Some contributions to integrated crop management in Europe. Pest Managmt.Sci. (56): 954-956.

Ho, L.C. 1996. The mechanism of assimilate partitioning and carbohydrate compartmentalization in fruit in relation to the quality and yield of tomato. J. Exp. Biol. (47): 1239-1243.

Juroszek, P., Lumpkin, H.M., Yang, R.Y., Ledesma, D.R., Ma, C.H. 2009. Fruit quality and bioactive compounds with antioxidant activity of tomatoes grown on-farm: comparison of organic and conventional management systems. J. Ag Food Chem. (57): 1188-1194.

Kerchev, P.I., Fenton, B., Foyer, C.H., Hancock, R.D. 2012. Infestation of potato (*Solanum tuberosum* L.) by the peach-potato aphid (*Myzus persicae* Sulzer) alters cellular redox status and is influenced by ascorbate. Plant Cell Environ. (35): 430-440.

Kruse, C., Jost, R., Lipschis, M., Kopp, B., Hartmann, M., Hell, R. 2007. Sulfur-enhanced defense: effects of sulfur metabolism, nitrogen supply, and pathogen lifestyle. Plant Biol. (9): 608-619.

Kuzniak, E. 2010. The ascorbate-glutathione cycle and related redox signals in plant-pathogen interations, in: Anjum, N.A, Chan, M.T., Umar, S. (Eds.), Ascorbate-Glutathione Pathway and Stress Tolerance in Plants. Springer Publishing, The Netherlands, pp. 115-136.

Li, Z., Palmer, W.M., Martin, A.P., Wang, R., Rainsford, F., Jin, Y., Patrick, J.W., Yang, Y., Ruan, Y. 2012. High invertase activity in tomato reproductive organs correlates with enhanced sucrose import into, and heat tolerance of, young fruit. J. Exp. Bot. (63): 1155-1166.

Liu, L.H., Zabaras, D., Bennett, L.E., Aguas, P., Wooton, B.W. 2009. Effects of UV-C, red light and sun light on the carotenoid content and physical qualities of tomatoes during post-harvest storage. Food Chem. (115): 495-500.

Lou, Y., Baldwin, I.T. 2004. Nitrogen supply influences herbivore-induced direct and indirect defenses and transcriptional responses in *Nicotiana attenuata.* Plant Physiol. (135): 496-506.

Mahanil, S., Attajarusit, J., Stout, M.J., Thipyapong, P. 2008. Over expression of tomato polyphenol oxidase increases resistance to common cutworm. Plant Sci. (174):456-466.

Mantelin, S., Bhattarai, K.K., Kaloshian, I. 2009. Ethylene contributes to potato aphid suseptability in a compatible tomato host. New Phytologist (183): 444-456.

Margaritopoulos, J.T., Kasprowicz, L., Malloch, G.L. & Fenton, B. 2009. Tracking the global dispersal of a cosmopolitan insect pest, the peach potato aphid. BMC Ecology (9):13.

Maršić, N.K., Sircelj, H., Kastelec, D. 2010. Lipophilic antioxidants and some carpometric characteristics of fruits of ten processing tomato varieties, grown in different climatic conditions. J. Agric. Food Chem. (58): 390-397.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzkia, A. 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann. Bot. (105): 1141-1157.

Mattson, W.J. 1980. Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Systematics (11): 119-161.

Mauromicale, G., Longo, A.M.G., Lo Monaco, A. 2011. The effect of organic supplementation of solarized soil on the fruit quality of tomato fruit. Scientia Horticulturae (129): 189-196.

McCarter, S.M. 1992. Effects of bactericide treatments on bacterial spot severity and yield of different pepper genotypes and on populations of certain insects. Plant Disease (76):1042-1045.

Mertz, W., 1982. Trace minerals and atherosclerosis. J. Federation Proceed. (41): 2807-2812.

Miles, P. 1999. Aphid saliva. Biol. Rev. (74):41-85.

Mitchell, A., Hong, Y.J., Koh, E., Barrett, D.M., Bryant, D.E., Denison, R.F., Kaffka, S. 2007. Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavinoids in tomatoes. J. Agric. Food Chem. (55): 6154-6159.

Migliori, C., Di Cesare, L.F., Campanelli, G., Ferrari, V. 2010. Coltivazione biologica: qualità nutraceutica-alimentare di alcune orticole. Progress in Nutrition (12): 246-260.

Murphy, K.M., Campbell, K.G., Lyon, S.R., Jones, S.S. 2007. Evidence of varietal adaptation to organic farming systems. Field Crops Research (102): 172-177.

NCDA & CS, 2011. Tissue sampling and analysis for greenhouse tomatoes. Retrieved June, 12, 2012 from http://www.ncagr.gov/agronomi/pdffiles/gtomato.pdf

Ohyama, A., Hidekazu, I., Sato, T., Nishimura, S., Imai, T., Hirai, M. 1995. Suppression of acid invertase activity by antisense RNA modifies the sugar composition of tomato fruit. Plant Cell Physiol. (36): 369-376.

Ordoñez-Santos, L.E., Vázquez-Odériz, M.L., Romero-Rodriguez, M.A. 2011. Micronutrient contents in organic and conventional tomatoes (*Solanum lycopersicon* L.). Int. J. of Food Sci. and Tech. (46): 1561-1568.

Peiffer, M., Tooker, J.F., Luthe, D.S., Felton, G.W. 2009. Plants on early alert: glandular trichomes as sensors for insect herbivores. New Phytologist (184): 644-656.

Reeve, J.R., Smith, J.L., Carpenter-Boggs, L., Reganold, J.P. 2008. Soil-based cycling and differential uptake of amino acids by three species of strawberry (*Fragaria* spp.) plants. Soil Biol. Biochem. (40): 25-47-2552.

Reganold, J.P., Andrews, P.K., Reeve, J.R., Carpenter-Boggs, L., Schadt, C.W., Alldredge, J.R., Ross, C.F., Davies, N.M., Zhou, J. 2010. Fruit and soil quality of organic and conventional strawberry systems. PLoS ONE (9): e12346.

Riahi, A., Hdider, C., Sanaa, M., Tarchoun, N., Ben Kheder, M., Guezal, I. 2009. Effect of conventional and organic production systems on the yield and quality of field tomato cultivars grown in Tunisia. J. Sci. Food Agric. (89): 2275-2282.

Rodriguez-Saona, C.R., Musser, R.O., Vogel, H., Hum-Musser, S.M., Thaler, J.S. 2010. Molecular, biochemical, and organismal analyses of tomato plants simultaneously attacked by herbivores from two feeding guilds. J. Chem. Ecol. (36): 1043-1057.

Sauge, M., Grenchi, I., Poëssel, J. 2010. Nitrogen fertilization effects on *Myzus persicae* aphid dynamics on peach: vegetative growth allocation or chemical defense? Entomologia Experimentalis et Applicata (136): 123-133.

Simmons, A.T., Gurr, G.M., McGrath, D., Nicol, H.I., Martin, P.M. 2003. Trichomes of *Lycopersicon* spp. and their effect on *Myzus persicae* (Sulzer) (Hemiptera: Aphidae. Australian J. Entom. (42): 373-378.

Smith, C.M., Boyko, E.V. 2007. The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomologia Experimentalis et Applicata (122):1-6.

Staley, J.T., Stewart-Jones, A., Pope, T.W., Wright, D.J., Leather, S.R., Hadley, P., Rossiter, J.T., Van Emden, H.F., Poppy, G.M. 2010. Varying responses of insect herbivores to altered plant chemistry under organic and conventional treatments. Proc. R. Soc. B (277): 779-786.

Steinbrenner, A.D., Gomez, S., Osorio, S., Fernie, A.R., Orians, C.M. 2011. Herbivore-induced changes in tomato (*Solanum lycopersicum*) primary metabolism: a whole plant perspective. J. Chem. Ecol. (37): 1294-1303.

Stout, M.J., Brovont, R.A., Duffey, S.S. 1998. Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato, *Lycopersicon esculentum*. J. Chem. Ecol. (24): 945-963.

Tjallingii, W.F., Hogen, Esch, T. 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiological Entomology (18): 317-328.

USDA ERS, 2011. Vegetables and melons yearbook. Retrieved May, 5, 2012 from http://www.ers.usda.gov/Data/Vegetables/

USDA ERS, 2010. Organic production data sets: certified organic and total U.S. acreage, selected crops and livestock. Retrieved Mar 21, 2012 from http://www.ers.usda.gov/Data/Organic/
USDA NOP, 2012. Organic Production and Handling Requirements. USC 205. Retrieved May, 10, 2012 from http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=645cc7eefa64695c8efbefeaaef620fd&rgn=div6&view=text&node=7:3.1.1.9.32.3&idno=7

Van Bueren, E.T.L., Jones, S.S., Tamm, L., Murphy, K.M., Myers, J.R., Leifert, C., Messmer, M.M. 2011. The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: a review. NJAS-Wageningen J. Life Sciences (58): 193-205.

Vinson, J.A., Hao, Y., Su, X., Zubik, L. 1998. Phenol antioxidant quantity and quality in foods: vegetables. J. Agric. Food Chem. (46): 3630-3634.

Warner, J., Zhang, T.Q., Xao, X. 2004. Effects of nitrogen fertilization on fruit yield and quality of processing tomatoes. Canadian J. Plant Sci. (84): 865-871.

Webb, S.E., Stansly, P.A., Schuster, D.J., Funderburk, J.E. 2010. Insect management for tomatoes, peppers and eggplant. University of Florida Extension ENY-461.

Zanic, K., Dumicic, G., Skaljac, M., Goreta Ban, S., Urlic. 2011. Effects of nitrogen rate and the ratio of $N0_3^-$: NH_4^+ on *Bemisia tabaci* populations in hydroponic tomato crops. Crop Protection (30): 228-233.

Zanor, M.I., Osorio, S., Nunes-Nesi, A., Carrari, F., Lohse, M., Usadel, B., Kuhn, C., Bleiss, W., Giavalisco, P., Willmitzer, L., Sulpice, R., Zhou, Y.H., Fernie, A.R. 2009. RHA interference of LIN5 in tomato confirms its role in controlling Brix content, uncovers the influence of sugars on the levels of fruit hormones, and demonstrates the importance of sucrose cleavage for normal fruit development and fertility. Plant Physiol. (150): 1204-1218.

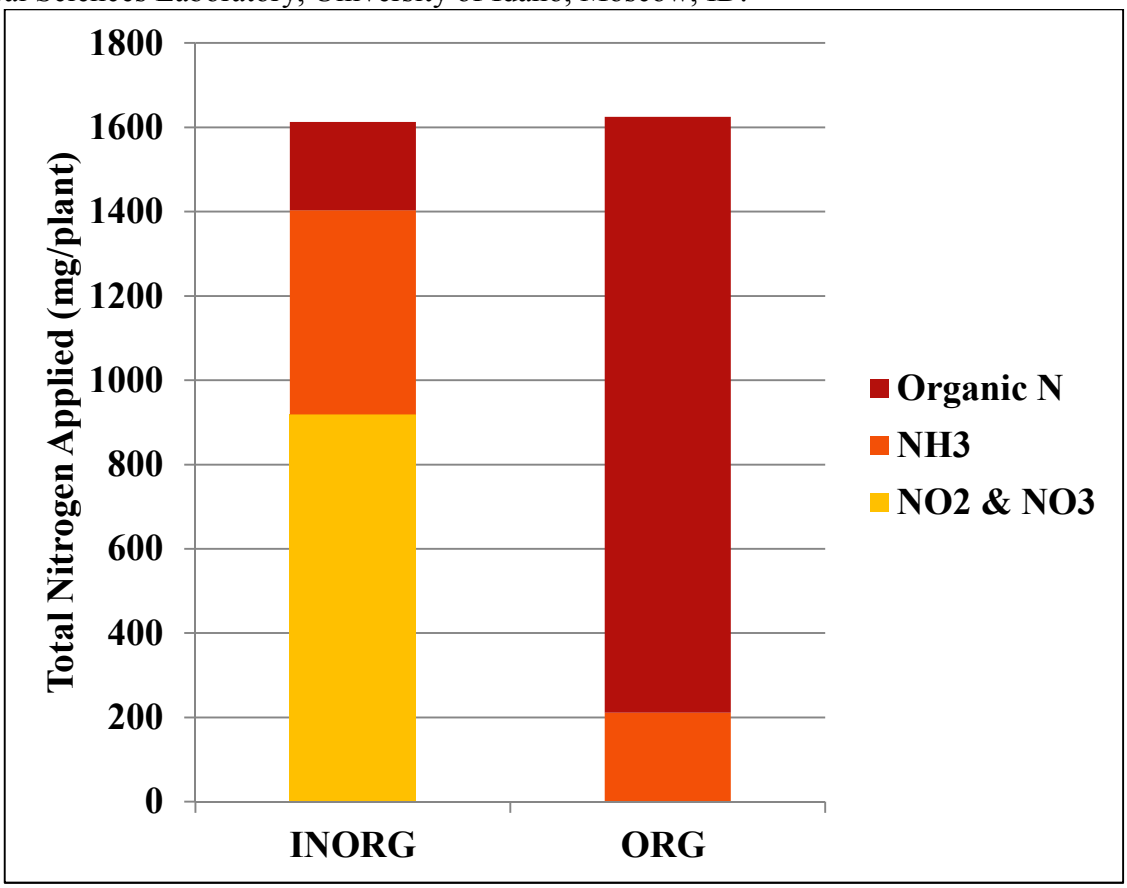
Zelená, E., Holasová, M., Zeleneý, F., Fiedlerová, V., Novotoná, P., Landfeld, A., Houška, M. 2009. Czech J. Food Sci. (27):S80-S84.

Zhao, X., Nechols, J.R., Williams, K.A., Wang, W., Carey, E.E. 2009. Comparison of phenolic acids in organically and conventionally grown pac choi (*Brassica rapa* L. *chinensis*). J. Sci. Food Agric. (89):940-946.

Zuba, S.N., Nogueira, W.C., Fernandes, L.A., Sampaio, R.A., da Costa, C.A. 2011. Yield and nutrition of tomato using different nutrient sources. Horticultura Brasileira (29): 50-56.

APPENDIX

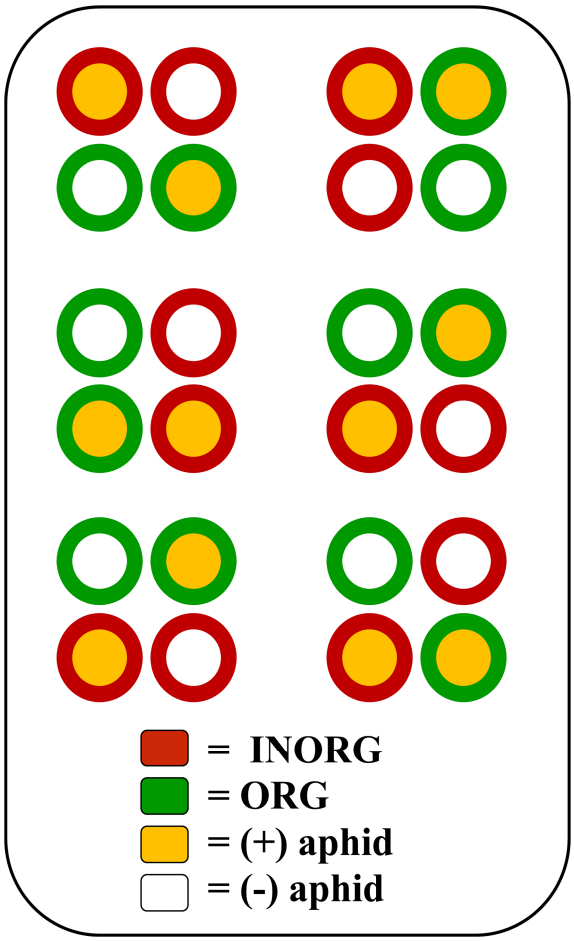
Appendix A: Total nitrogen applied per plant by nitrogen form during the experiment. Calculations are based on analysis for mineral content of the applied nutrient solutions at the Analytical Sciences Laboratory, University of Idaho, Moscow, ID.



Appendix B: Nutrient treatments application schedule starting the third week after seedling emergence. Listed are the concentrations, volumes and frequencies of fertilizer applications in the two treatments throughout the study. Tap water was used in all fertilizer dilutions.

	Organic Treatment		**Inorganic Treatment**			
Week	**BioLink 5-5-5 (ml L^{-1} H_20)**	**BioLink Micro (ml L^{-1} H_20)**	**Peters 20-10-20 (g L^{-1} H_20)**	**$Ca(H_2PO_4)_2.H_2O$ (mg L^{-1} H_20)**	**Volume (mL/plant)**	**Frequency (times/week)**
3	4	0	1.01	0	31	1
4	4	0	1.01	0	31	1
5	4	0	1.01	0	31	1
6	4	0	1.01	0	150	1
7	4	0	1.01	0	500	1
8	4	0	1.01	0	500	2
9	4	0	1.01	0	500	2
10	4	3.9	1.25	166	500	2
11	4	3.9	1.25	166	500	2
12	4	3.9	1.25	166	500	2
13	4	3.9	1.25	166	750	3.5
14	4	3.9	1.25	166	1000	3.5
15	4	3.9	1.25	166	1500	3.5
16	4	3.9	1.25	166	1750	3.5
17	4	3.9	1.25	166	1750	3.5
18	5	4.9	1.56	207.5	1750	3.5
19	5	4.9	1.56	207.5	1750	3.5
20	5	4.9	1.56	207.5	1750	3.5
21	5	4.9	1.56	207.5	1750	3.5
22	5	4.9	1.56	207.5	1750	3.5

Appendix C: Experimental design layout of randomized complete blocks. Blocks were arranged in a randomized complete block design of six blocks on two separate benches in a glasshouse.



Appendix D: Photosynthetically active radiation (PAR) in the experimental glasshouse. PAR averaged 176 µM photons/m^2/sec (standard deviation ±24.7). Measurements were taken with a LiCor LI-185 Line Quantum Sensor under full-sun, partial sun, and cloudy or at night with lights illuminated.