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The Effect of Abscisic Acid on Tomato Calcium Partitioning and Fruit Quality

Thomas Casey Barickman
University of Tennessee - Knoxville, tbarickm@utk.edu

To the Graduate Council:

I am submitting herewith a dissertation written by Thomas Casey Barickman entitled "The Effect of Abscisic Acid on Tomato Calcium Partitioning and Fruit Quality." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils, and Insects.

Carl E. Sams, Major Professor

We have read this dissertation and recommend its acceptance:

Dean A. Kopsell, Dennis Deyton, Svetlana Zivanovic

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

The Effect of Abscisic Acid on Tomato Calcium Partitioning and Fruit Quality

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ABSTRACT

Tomato (*Solanum lycopersicum*) is a widely employed plant model system for studying fruit metabolism, development and ripening. Various environmental stress factors, such as drought and high relative humidity, can cause calcium (Ca) deficiency and lead to physiological diseases such as blossom-end rot (BER) in tomato fruit. Recent studies demonstrate that abscisic acid (ABA) triggers whole-plant and fruit-specific mechanisms to increase fruit Ca uptake and prevent BER development. The objective of this study was to evaluate the effects of exogenous ABA applications during plant development on tomato carotenoid pigments, soluble sugars, organic acids, aromatic volatiles, carbohydrates, and mineral nutrient content in ripe fruit, and to assess the impacts of ABA applications on BER by evaluating how exogenous ABA will affect the distribution of Ca between the leaves and fruit. There were a series of three experiments that examined two types of tomato plants, micro tomato and a commercial tomato cultivar 'Mt. Fresh Plus'. ABA was exogenously applied to the foliar and/or root tissue. Leaves were harvested and analyzed for chlorophylls, carotenoids, and Ca concentrations. Fruit tissue was harvested at red ripe maturity and analyzed for yield, BER and fruit quality parameter, such as carotenoids, soluble sugars, organic acids and aroma volatiles. The results indicate that applications of ABA treatments to tomato plants decreased the partitioning of Ca into the leaves while increasing concentrations in the fruit tissue. ABA treatments, in combination with the Ca treatment of 180 $mg \cdot L^{-1}$ (milligram per liter), decreased the incidence of BER. Further, ABA treatments decreased BER even in the presents of low Ca in the fertilizer solution. Results indicate that ABA treatments are most effective in the early stages of plant development. This study demonstrated that

ABA is a viable treatment to significantly improve tomato fruit quality. Specifically, ABA treatments increased tomato fruit carotenoids and soluble sugar, while decreasing organic acid concentrations. However, ABA treatments had a detrimental effect on aroma volatile concentrations. ABA treatment applications in conjunction with low Ca treatments did not prove to be effective in improving tomato fruit quality. This study demonstrated that foliar spray ABA applications are more effective than root ABA applications.

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Chapter 1
Literature Review

Introduction

The United States is one of the world's leading producers of tomatoes (*Solanum lycopersicum*), second only to China. United States Department of Agriculture data indicate that fresh and processed tomatoes account for more than $2 billion in annual farm cash receipts (ERS, 2012). Over the last decade (2000-2009) in the United States an average of 119,800 acres was planted in fresh market tomatoes, with a farm value of $1,339,039,000. Greenhouse tomatoes represent an estimated 17 % of U.S. fresh market tomato supply (Cook and Calvin, 2005) and greenhouse tomato production is rapidly growing to become an important part of the United States tomato industry.

Field and greenhouse produced tomato fruit quality has been declining during the past couple of decades primarily because of harsh environmental conditions. The environmental factors affecting tomato production include temperature extremes, drought and excessive precipitation (Cook and Calvin, 2005). These environmental extremes are leading to suboptimum growing conditions for tomato production and inducing physiological disorders, such blossom-end rot (BER). In addition to adverse environmental conditions, there are high incidences of pests and diseases that can also lead to poor tomato fruit quality. Together, these factors have influenced plant breeders to tailor their research programs to growers' demands for high yielding and pest and disease resistant cultivars. The creation of these highly resistant tomato hybrids resulted in selections of tomatoes with inferior quality and poor nutritional values.

The primary objective of this study was to examine current tomato cultivars and find a treatment that can improve the nutritional qualities of the fruit in harsh environmental conditions. Recent studies have demonstrated that abscisic acid (ABA) triggers whole-plant and fruit-specific mechanisms to increase fruit Ca uptake and prevent BER development (de Freitas,

2011b). This study evaluated the effects of exogenous ABA applications during plant development on the distribution of Ca between leaves and fruit in greenhouse tomato production. This study evaluated the effects of ABA applications on carotenoid phytonutrients, soluble carbohydrates, organic acids, aroma volatiles and calcium content in ripe fruit and the impacts of ABA applications on tomato fruit yields and BER development.

The study was conducted through four experiments in the span of three years. The goal of the first experiment was to test the effects of ABA in a dwarf tomato model system on Ca partitioning between the leaves and fruit and its effect on tomato fruit quality. Dwarf tomato plants were chosen because they are genetically near identical to large field and greenhouse tomato plants but are easier to work with because of their small stature and shorter life cycle. The model system proved that ABA could be used to improve uptake of Ca into the fruit tissue while increasing fruit quality. In addition, ABA increased chlorophylls and carotenoids in the leaf tissue. The positive results from this initial experiment led to the second, third, and fourth experiments. The second experiment was a field study that lasted three years. However, only one year's data were viable for a report (appendix 8). Data collected the last two years indicated no significant differences in Ca in leaf and fruit tissue. Therefore, fruit quality parameters were not analyzed. The third and fourth experiments were conducted on full sized greenhouse tomato plants. The only difference between these two experiments was the ABA application method. Tomato plants in the third experiment were treated with foliar applications of ABA, while the tomato plants in the fourth experiment were treated with foliar and root applications of ABA. Both experiments demonstrated that applications of ABA treatments to tomato plants decreased the partitioning of Ca into the leaves while increasing concentrations in the fruit tissue. ABA treatments in addition to an optimum Ca treatment of 180 $mg \cdot L^{-1}$ (milligrams per liter) also

decreased the incidence of BER even in low Ca environments. This study demonstrated that ABA is a practical treatment to significantly improve tomato fruit quality, specifically pertaining to carotenoids, soluble sugar, organic acids and aroma volatile concentrations.

This study looked into new and novel ways of improving tomato fruit quality and its nutritional values. The hormone ABA has not been considered to have beneficial effects on tomato fruit quality. These results will help plant scientists to better understand the physiology of ABA and could affect classical physiological studies looking at stomatal conductance and signal transduction. This study used ABA as regulator of tomato flavor, aroma and firmness. Thus, exogenous application of ABA may provide growers with simple management strategies to increase yield and profitability. Potentially, this could help growers in the future by giving them an assurance that they can apply ABA onto the plant in adverse environmental conditions and still get good quality fruit by decreasing culled and increasing marketable fruit.

The following chapters report on tomato production in the United States, including the results from previous studies on Ca as an essential plant nutrient, Ca deficiencies, ABA, the impact of ABA on Ca uptake and tomato fruit quality and tomato fruit quality factors, specifically soluble sugars, organic acids, carotenoids and volatile flavor components. Chapters two and three report the results of the first experiments. Specifically, chapter two reports on the effects of ABA on Ca partitioning in dwarf tomatoes, while chapter three discusses ABA effects on carotenoids and chlorophylls in dwarf tomatoes. Chapters four and five report the results of the second group of experiment. Specifically, chapter four discusses the effects of foliar applications of ABA on distribution of Ca between leaves and fruit and the incidence of BER in greenhouse tomatoes, while chapter five reports on the effects of foliar applications of ABA on greenhouse tomato fruit quality. Chapters six, seven and eight report results of the fourth group

of experiments. Chapter six discusses the effects of foliar and root applications of ABA on the distribution of Ca between leaves and fruit and the incidence of BER in greenhouse tomatoes. Chapter seven reports on the effects of foliar and root applications of ABA on greenhouse tomato fruit quality. Chapter eight reports on the effects of foliar and root applications of ABA on greenhouse tomato fruit aroma volatiles.

Tomato Production

The United States is one of the world's leading producers of tomatoes, second only to China. United States Department of Agriculture data indicated that fresh and processed tomatoes account for more than $2 billion in annual farm cash receipts (ERS, 2012). Tomatoes, short-lived perennial plants, are cropped as annuals. Indeterminate greenhouse tomato plants can be cropped for a year or more, while freezing temperatures will kill the outdoor plants. In most cases, temperature restrictions, such as extreme summer heat, limit greenhouse production to spring and fall seasons. Spring season is March thru June, and fall season September thru December. However, many greenhouse growers to the north and south of the Mid Atlantic grow one crop continuously for about 10-11 months annually. Many side shoots terminate in a flower cluster in determinate cultivars. Fresh market growers often use shorter determinate cultivars because they are easier to stake and have a reduced harvest period.

Plasticulture production methods are commonly used for field tomatoes. These agricultural techniques make use of plastic mulch, drip irrigation and raised beds for growing vegetables. Advantages of this technique include increasing crop performance by conserving moisture and nutrients, stabilizing soil temperature, reducing some diseases, reducing or eliminating weeds and increasing early-harvest yields. Over the last decade (2000-2009) in the United States growers planted an average of 119,800 acres of fresh market tomato plants with a

farm value of $1,339,039,000. The annual U.S. exports of fresh market tomatoes over the decade in the United States averaged 357,194,000 hundredweight (*cwt),* ranging from 314,230,000 *cwt* in 2003 to 410,353,000 *cwt* in 2000. Tomato growers in Tennessee produced an average of 3,950 acres of fresh market tomatoes yielding 1,123,000 *cwt* of fruit production with a farm value of $38,306,000 (ERS, 2012).

Rapidly increasing greenhouse tomato production is an important part of the domestic tomato industry. Greenhouse tomatoes represent an estimated 17 % of fresh market tomato supply (Cook and Calvin, 2005). Although greenhouse tomatoes constitute a minor share of the fresh tomato market, their influence within niche, out of season and specialty markets continues to increase. Differential trends in greenhouse tomato markets are causing a combination of improved yields, pest and disease tolerance, resistance to environmental stresses and enhanced nutritional quality. However, issues such as the costs of growing systems, environmental inputs, fertilizers and expense skilled labor make improvements for conventional breeding and research challenging.

The appearance of greenhouse tomatoes in the market brought major changes in the quantity and composition of field tomato sales. While total retail quantity sales of all fresh market tomatoes have increased over the past decade, the volume of field tomatoes has declined. Supply and demand trends are influencing the production of greenhouse tomatoes. Greenhouse production benefits from weather-induced periods of short supplies, which causes high prices for fresh field tomatoes. For example, a short supply of field tomatoes and a record-high supply of greenhouse tomatoes in 2004 caused greenhouse tomato prices to decline. This made greenhouse tomatoes attractive to wholesale buyers for sales to foodservice establishments, retailers and consumers (Cook and Calvin, 2005).

There are many mineral nutrients in the soil or fertilizer solution that contribute to tomato fruit quality (Marschner, 1995). However, one of the most important mineral nutrients crop quality in Ca. This mineral nutrient is essential because it directly affects cell wall and membrane structure and strength (Bangerth, 1979).

Calcium: An Essential Plant Nutrient

Calcium is a large divalent cation that is readily available in most soils, and it is a macronutrient for plants. In the early nineteen-century, researcher de Saussure demonstrated that Ca is a component of plant tissue. However, Leibig was the first scientist to associate Ca as an essential element in the mid nineteenth century (Pilbeam and Morley, 2007). Early research on Ca looked at growth effects on oats (*Avena stiva)* and found that omitting it from the fertilizer had adverse growth effects (Hewitt, 1966).

Calcium plays several distinct functions within plants. Two of these functions directly affect membranes and cell walls (Bangerth, 1979). As a divalent ion, Ca can form intramolecular complexes and have the ability to link molecules (Clarkson, 1988, Pilbeam and Morley, 2007). Calcium also has dramatically positive effects on membrane stability. Ions (Ca^{2+}) maintain membrane structure by bridge phosphate and carboxylate groups of phospholipids and proteins at membrane surfaces (Legge et al., 1982). Calcium deficiency causes disintegration of membrane structures and loss of cell compartmentalization (Hecht-Buchholz, 1979, Marschner, 1995). Furthermore, Ca deficiencies lead to substrate leakage into the cytoplasm resulting in increased respiration rates (Bangerth et al., 1972). Increased respiration rates within the plant are detrimental to the overall plant metabolism causing senescence of plant tissue.

In addition to effects on membranes, Ca plays a vital role in the structure of primary cell walls. In the cell walls, cellulose microfibrils are linked together into polymers. The interlocking microfibrils are embedded in a pectin matrix. Calcium function within the cell wall is to bridge the pectate matrix in the middle lamella, which is essential for strengthening the cell wall of plant tissues (Armstrong and Kirkby, 1979, Faust and Klein, 1974). These bonds are highly resistant to degradation by cell wall enzymes, specifically polyglacturonase (Cassells and Barlass, 1976, Marschner, 1995). The proportion of Ca pectate in the cell walls affects the susceptibility of the tissue to fungal and bacterial infections and ripening of fruits. A close relationship between Ca and the functional integrity of the cell wall has been demonstrated in a number of studies (Cormack, 1955, Ho and White, 2005, Suzuki et al., 2003). Earlier studies found that lack of Ca causes softening of the tissue (Rigney and Wills, 1981) and can lead to disintegration of cell walls (Bussler, 1963). On the other hand, spraying the plant with Ca salts can lead to increases in tomato fruit firmness (Pinheiro and Almeida, 2008, Brady et al., 1985, Vaz and Richardson, 1984, Ho and Adams, 1989, Cooper and Bangerth, 1976) and delays or even prevents fruit ripening (Wills et al., 1977). In addition, postharvest Ca treatments to fruit tissues can have the same effect on fruit firmness, prevention of ripening (Akihiro et al., 2005) and fungal decay (Conway and Sams, 1983, Conway and Sams, 1987, Conway et al., 1994, Conway et al., 2002). Hao and Papadopoulos (2003) found that increasing Ca concentration to 300 mg L^{-1} and Mg at 80 mg L^{-1} had the greatest effect on growth and fruit quality in a fall greenhouse tomato crop. These concentrations improved root growth, leaf size and fruit firmness.

Recent research focused on cellular and molecular processes of Ca transport (Gilliham et al., 2011, Batistic et al., 2012). However, the plant transports Ca from tissue to tissue through

uptake and distribution. On the molecular level, Ca is transported through the symplast. On the physiological level, Ca, which originates at the root, is transported from the root cortex through the xylem tissue and distributed to vegetative and fruit tissue. The uptake of Ca is higher in apical than in basal root zones (Haussling et al., 1988, Ferguson and Clarkson, 1976). Root apical zones take up high amounts of Ca. Part of the Ca remains in the root while the rest is delivered to the shoots (Clarkson, 1984). In the apoplasm, part of the Ca is firmly bound in cellular structures and is exchangeable at the cell walls and exterior surface of the plasma membrane (Marschner, 1995).

The root is composed of many cell types specializing in a particular task. The outer cell layers of the root, the epidermis and cortex, acquire water and mineral nutrients. These cells load, unload and transport solutes in the xylem and phloem through the endodermal cells. The part of the root outside the plasma membrane forms the apoplast of the root. The apoplast consists of cell walls and the lumena of tracheary elements. However, most nutrients need to move through the intercellular spaces (symplast) from epidermal cells to the cortex and then to the stele to reach the xylem. The Casparian strip of the endodermis is a major barrier for the apoplastic movement. Calcium must enter the cytoplasm of the endodermal cell through the Ca channels in plasmalemma. Research has shown that Ca channels, Ca and hydrogen (H) antiporters and Ca-ATPase play roles in the uptake and transport of Ca from the apoplast to the symplast through all the barriers. Calcium channels discovered in root cells, include depolarization-activated Ca channels (White, 1998, White, 2000), hyperpolarization-activated Ca channels (Kiegle et al., 2000, Very and Davies, 2000), voltage-insensitive cation channels (Davenport and Tester, 2000) and secondary messenger-activated Ca channels (White, 1998).

Although Ca is a nutrient that is abundant in all but extremely acidic soils, there are numerous common Ca deficiency 'diseases' in crop plants (Kirkby and Pilbeam, 1984). Deficiency seldom arises because of a failure of Ca supply to plant roots. It is more frequently explained by problems arising from its internal distribution and its allocation in mature and growing regions of the plant. Distribution problems arise because of the special properties of the Ca ion. This divalent ion has a great ability to form inter- and intra-molecular co-ordination complexes, which link and modify structures. Calcium profoundly influences the organization of the cytoplasm and the processes that occur within. For example, Ca has a disruptive effect on the formation and stability of the mitotic spindle and microtubules (Clarkson, 1984). Gunning and Hardham (1982) estimated that cytosolic Ca activity exceeding 0.1 $mmol \cdot m^{-2}$ (micromoles per meter) inhibits polymerization of tubulin into microtubules. Chemical analysis showed that cells contain much larger concentrations of Ca in the cytoplasm (Macklon and Sim, 1981). However, it is now widely accepted that the majority of Ca in plant cells is either bound to proteins or sequestered in organelles and vesicles. Therefore, there is a small amount of free Ca in the cytoplasm available for movement from one cell to another. Such small fluxes in Ca concentrations function as secondary messengers in cellular communication (Marschner, 1995). However, the small amount of Ca in the cytoplasm is not enough to support cell growth elsewhere in the plant.

Calcium import into growth sinks takes place nearly exclusively in the xylem because of the lack of phloem mobility. In contrast, most of the total net import of potassium (K) takes place in the phloem. Magnesium (Mg) import into the phloem contributes to about 40% of the total import in the nutrient (Marschner, 1995). High Ca demands of growth sinks, particularly in vigorous crop species with a high exchange capacity in the apoplasm, require high rates of xylem

volume flow. Low rates of transpiration and xylem volume flow cause Ca deficiencies in shoot apices, young leaves and fleshy fruits. Therefore, Ca deficiency and Ca physiological disorders that accompany low rates of xylem volume flow are widespread. In addition, in organs with low transpiration rates, such as tomato fruits, a high phloem solute volume flow either strongly depresses, or even reverses direction of the xylem volume flow (Mix and Marschner, 1976). This counter-flow of water in the xylem can be substantial and can lead to the export from fruits of both Ca and organic solutes (Hamilton and Davies, 1988).

Calcium distribution in the plant can be negatively affected by environmental stresses, such as light, temperature and humidity. The negative impact on Ca distribution in the plant can cause Ca deficiencies and can have severe physiological dysfunctions that decrease tomato yield and fruit quality. Perhaps the most deleterious of these disorders is BER which is induced by Ca deficiency and plant stress.

Calcium Deficiency and Blossom End Rot in Tomato Fruit

During plant development physiological processes impact tissue, organ and cellular functions of plants. Plants grown in environments that are less stressful have physiological development that lead to healthier growth and the plants will have higher yields and better fruit quality. However, there are a number of physiological disorders that cause an aberration in normal plant physiology. Abiotic conditions and environmental and cultural factors adversely affect the function of plant growth and development causing physiological disorders. Factors implicated in the occurrence of physiological disorders include irradiance, temperature, humidity, nutrient availability, environmental contaminants and water relations. Effects of physiological disorders range from subtle symptoms not visibly apparent to severely stunted and

malformed growth. Unfortunately, physiological disorders usually are difficult to identify before the effects on the plant can be corrected.

Sufficient Ca uptake depends on the flow of water with the transpiration stream in the xylem tissue. Source-sink relationships regulate Ca movement through the plant. Calcium moves to tissues that have the lowest water potential (Marschner, 1995). In other words, Ca movement increases to tissues, such as leaves, because they are rapidly growing and have low water potential. Other parts of the plants, such as fruit tissue, have higher water potentials and lower distribution of stomata. Therefore, movement of Ca into these tissues is considerably lower. Movement of Ca into fruit tissue is greatest when cells are actively dividing and expanding in the early stages of growth. Thus, when this stage of rapid growth decreases the strength of the sink for Ca slows down its movement into the fruit tissue. This means that fruit have a limited time for critical Ca uptake for rapidly expanding fruit tissue.

Various environmental stress factors, such as drought and high relative humidity, can disrupt transpiration water movement. Disruption of acquisition can cause Ca deficiency, which leads to physiological disorders such as blossom-end rot (BER) in tomato and pepper (*Capsicum annuum*) fruit. In addition, other calcium deficiency disorders, often occurring in horticultural crops, are tipburn and brown heart in leafy vegetables and bitter pit in apples. Other physiological disorders, such as cracking in tomato, cherry and apple fruit following high humidity or excessive moisture in the growing medium, often occur in rapidly growing tissue without sufficient amounts of Ca (Shear, 1975, Simon, 1978). Slow absorption and poor distribution of Ca cause these physiological disorders.

Blossom-end rot primarily occurs because of the local deficiency of Ca in the distal end of tomato fruit (Suzuki et al., 2003; Adams and Ho, 1993). BER is generally attributed to an

inadequacy of Ca in the fruits, and it is therefore called a 'Ca-related disorder' (Shear, 1975). This disorder causes cells near the blossom end of the fruit to die, giving the tissue a water soaked appearance that can cover half of the fruit surface (Abdal and Suleiman, 2005). Although it can be caused by inadequate supply of Ca in the root zone, it frequently occurs when substrate moisture and Ca content is at adequate levels for normal plant growth. In this situation, the most likely causes of this physiological disorder are poor Ca uptake by the roots and insufficient distribution of Ca to the fruit during a period of high Ca demand. Deficiency seldom arises because of a failure of Ca supply to plant roots. The more common causes are problems arising from internal distribution of Ca and its allocation in mature and growing regions of the plant. Small amounts of free Ca in the cytoplasm are available for movement from one cell to another. Research on greenhouse tomato production has demonstrated that insufficient Ca supplied to the plants in the fertilizer solution rarely causes BER. More often, BER occurs in plants with an adequate Ca supply when grown in environmental conditions that reduce transport of Ca to rapidly growing distal fruit tissue (Saure, 2001, Ho and White, 2005). In addition, incidences of BER may occur during increased demand of distal fruit tissue for Ca in early stages of fruit development (Ho et al., 1993, Ho and White, 2005).

The main objections to a primary role for Ca in the induction of BER raised in recent years are: a) that no universal critical Ca level in the BER fruit tissue has been identified (Ho and White, 2005, Nonami et al., 1995); b) that BER can be induced by changing the concentration of mineral nutrients other than Ca in the fertilizer (Nukaya et al., 1995); and c) that there is no conclusive evidence for a role of Ca when BER is induced by various environmental stresses (Saure, 2001). However, these arguments are based on the current available literature and not on modern biochemical and molecular techniques that may be used to determine cellular and

genomic functions. Research has shown that it is possible that a local Ca deficiency for individual cells in the distal tissues might be responsible for BER (Schmitz-Eiberger et al., 2002, Suzuki et al., 2003). Other factors involved in the induction of BER are stress factors such as heat, hormones, oxidants and the effects of mineral ions other than Ca in the fertilizer. This does not necessarily exclude the involvement of Ca since changes in cytosolic Ca are likely to have a role in coordinating the cellular responses to all these stress factors (White and Broadley, 2003). These arguments contribute to the consideration of the induction of BER as a cellular occurrence.

Recent literature has suggested that plant hormonal balance may influence the occurrence of BER under certain conditions. For example, ABA is plant hormone that plays an important role in regulating plant growth and development under harsh environmental conditions. Previous research has demonstrated that foliar ABA applications decreased the incidence of BER and increased the uptake of Ca into the fruit tissue (de Freitas et al., 2011).

The Plant Hormone Abscisic Acid

Internal signals and external environmental conditions regulate plant growth and development. The apo-carotenoid plant hormone abscisic acid (ABA) is an important regulator that coordinates growth and development with responses to the environment (Inaba et al., 1976). This hormone is a metabolite known as an isoprenoid and is derived from a common five-carbon precursor, isopentenyl (IDP). ABA biosynthesis takes place in chloroplasts and other plastids in the roots and leaves via the terpenoid pathway. It is formed by the cleavage of C_{40} carotenoids derived from the non-mevalonate pathway (MEP) (Hirai et al., 1986, Kasahara et al., 2004, Milborrow and Lee, 1998). This pathway plays an essential role in creation of chloroplast

isoprenoids, such as carotenoids, phytol and terpenoids, compounds that are essential for ABA creation (Sponsel, 2002).

ABA is found in all higher plant tissues including roots, xylem tissue and sap, phloem sap, pollen, petals, fruits and seeds (Milborro, 1974). ABA concentrations in leaves of temperate crop plants vary from 50 to 500 $ng \cdot g^{-1}$ (nanograms per gram) (Wilkins, 1984). Research has demonstrated that salt stress, phosphate deficiency and ammonium nutrition enhance the percentage of re-circulated ABA (Cramer and Quarrie, 2002, Jeschke et al., 1997, Peuke et al., 1994), while nitrate deficiency, root flooding and alkaline conditions reduce ABA (Jeschke et al., 1997, Peuke et al., 1994, Wolf et al., 1990). Therefore, environmental factors regulate the metabolism and function of ABA, which in turn helps the plant to acclimate to these adverse conditions.

During stress, ABA concentrations increase dramatically in all plant tissues (Henson, 1984, Mohapatra et al., 1988, Cohen and Bray, 1990, Plant et al., 1991). ABA originates from two internal sources in plants: roots and leaves. In roots it is derived from the synthesis of root tissue carotenoids and its level increases as the soil dries out (Sauter et al., 2001). During drought conditions, ABA is synthesized in roots and transported upwards by the xylem. ABA is transported to the leaves where it closes stomatal aperture and restricts gas exchange, thereby increasing water use efficiency in the plant (Sauter et al., 2001, Hartung et al., 2002). Other responses to water status include decreased rates of photosynthesis (Chapin et al., 1988), increased photorespiration (Boyer and Bowen, 1970), accumulation of secondary messenger molecules (Rhodes, 1987), alteration in plant hormone levels (Zeevaart and Creelman, 1988) and modifications in gene expression (Bray, 1993, Bray, 1991). Other adverse environmental conditions, such as nutrient deficiencies, high light stress and temperature extremes, can cause

ABA synthesis in the leaves (Daie and Campbell, 1981). In the leaves ABA is derived from carotenoids and can be transported to the roots via phloem tissue. In the phloem tissue ABA is distributed to the tissue and re-circulated to the xylem vessels. Previous research found that ABA moves rapidly in the phloem and in the parenchymatous cells of stems and petioles. For example, Hocking et al. (1972) demonstrated that labeled 14C ABA was widely distributed inside the pea (*Pisum sativum)I* plant within 24 h. Approximately 18% of the 14C labeled ABA was found in root nodules. The movement of ABA within the plant was tracked both upwards and downwards until it reached a steam-girdled zone. Similarly, a study on ABA distribution in cotton (*Gossypium hirsutum*) seedling found that half of the hormone was transported from the leaf to the roots in 8 days. It was extracted as un-metabolized ABA from the roots (Shindy et al., 1973). Therefore, ABA as a response to adverse environmental conditions will either act on stomatal aperture or get distributed to the roots where it regulates hydraulic conductivity, (Hose et al., 2000, Thompson et al., 2007) ABA also plays an important role in regulating aquaporin density in root tissue (Wan et al., 2004). The plant responds to ABA signals by regulating ABA breakdown, transporting stress-related compounds, compartmentalizing metabolites or changing sensitivity to the environment (Zeevaart and Creelman, 1988, Addicott and Carns, 1983).

Other physiological effects of ABA in plants are tied to additional plant hormones, specifically gibberellic acid (GA). Research on ABA and GA demonstrated that ratios of the two plant hormones are critical to their physiological functions. Studies indicated that high ABA: GA ratios inhibited seed germination, regulated bud dormancy and reduced plant height (Rodrigeuz-Gacio et al., 2009, Chao et al., 2007, Weiss and Ori, 2007). For example, Schopfer et al., (1979) demonstrated that high levels of ABA inhibit the germination of *Sinapis alba* and *Brassica napus* seeds. The dose-response curve of seeds to inhibition of germination was in the

range between 16-6 µM ABA (Colorado et al., 1991). In addition, Talyor et al. (1984) examined apple bud dormancy and found a decrease in ABA and increase in GA when apples break bud dormancy. Therefore, decreasing ABA concentrations led to plant growth.

Furthermore, ABA plays a crucial role in fruit maturation and senescence by triggering ethylene synthesis and causing the fruit to ripen (Zhang et al., 2009). Besides ethylene, it can be considered as the other ripening control factor. Studies have found that ABA content is very low in unripe fruit. ABA increases during the process of fruit ripening in both climacteric (Vendrell and Buesa, 1989, Buesa et al., 1994) and non-climacteric (Inaba et al., 1976, Kojima, 1996, Kondo and Inoue, 1997, Kondo and Tomiyama, 1998) fruits. Buta and Spaulding (1994) demonstrated that the levels of ABA in tomato were high 7 days after anthesis. Thirty-three days after anthesis, ABA declined to a minimum. In addition, they found that ABA levels increased to 121 $ng \cdot g^{-1}$ fresh weight at the pink stage, then decreased significantly with ripening.

Recent studies demonstrated that ABA triggers whole-plant and fruit-specific mechanisms to increase fruit Ca uptake and prevent BER development. For example, de Freitas (2011) found that the plant hormone ABA induced lower leaf stomatal conductance and water loss, which resulted in increased Ca concentrations in the fruit and lower Ca levels in the leaves. The role of ABA as a stress hormone makes it an attractive and novel treatment to improve Ca uptake and distribution within tomato fruit, which could increase Ca concentrations in situations where Ca distribution into the fruit is low.

The Impact of ABA on Calcium Uptake

Internal and external signals regulate plant growth and development. One important regulator that coordinates these changes is the hormone ABA. ABA can trigger oscillation in the cytosolic Ca concentration, which is then perceived by Ca binding proteins to initiate a series of

signaling cascades that control many physiological processes, including adaptation to environmental stress (Guo et al., 2002). Recent studies demonstrated that ABA triggers whole-plant and fruit-specific mechanisms to increase fruit Ca uptake and prevent BER development. Research indicated that ABA impacted Ca uptake in tomato plants. de Freitas et al. (2011b) examined the effects of reduced leaf transpiration on Ca uptake in tomato plants and the incidence of BER. They found that ABA treatments prevented BER, while control treatments reached 30–45% BER fruit. In addition, ABA-treated plants had higher stem water potential, lower leaf stomatal conductance and lower whole plant water loss than water-treated plants. Furthermore, their results indicated that ABA treatment increased total tissue and apoplastic water-soluble Ca concentrations in the fruit, and decreased Ca concentrations in leaves. In ABA-treated plants, fruit had a higher number of Safranin-O-stained xylem vessels at early stages of growth and development. The results indicated that ABA prevents BER development by increasing fruit Ca uptake possibly by a combination of whole-plant and fruit-specific mechanisms.

Research on ABA and Ca has predominantly focused on examining ABA as an environmental stress signal and the impact ABA had on signal transduction on a cellular level (Chen et al., 2012, Batistic and Kudla, 2012, Batistic et al., 2012). In other words, studies looked at how endogenous ABA, increased as a result of environment stress such as drought, affected Ca levels (Du et al., 2010). For example, Guo et al. (2002) indicated that ABA triggers an oscillation in the cytosolic Ca concentrations initiating a series of signaling cascades that control physiological processes. The effects of foliar applications of ABA have not been researched extensively. Several studies found that spraying whole plants with exogenous ABA increased fruit total tissue and apoplastic Ca concentrations while reducing fruit cell membrane leakage

and the incidence of BER (de Freitas et al., 2011b, de Freitas et al., 2013). These studies suggested that ABA could regulate cellular Ca distribution, which can affect fruit's susceptibility to Ca deficiency disorders (de Freitas et al., 2011b, de Freitas et al., 2011a, de Freitas et al., 2013, Park et al., 2005). Therefore, there is evidence that exogenous applications of ABA may be a novel approach to treating Ca deficiency disorders.

In addition to influencing Ca partitioning, ABA plays a crucial role in fruit maturation and senescence (Zhang et al., 2009). Besides ethylene, it can be considered as the other ripening control factor. Bastias et al. (2011) found that as a ripening control factor ABA increases levels of sugars in tomato fruit by increasing expression of genes encoding a vacuolar invertase and a sucrose synthase. Increasing sugar levels, specifically glucose and fructose, create a higher ratio of sugar to organic acids making the fruit sweeter (Patanè et al., 2011; Tardieu et al., 1992).

Tomato Fruit Quality Factors

Taste and flavor are increasingly becoming important constituents of tomato marketability. Presently, tomatoes in the market place are bland in terms of aroma and flavor. Thus, consumers are willing to pay a premium price for what they perceive are superior, full flavored tomatoes. While tomato cultivar and postharvest practices are designed to reduce crop loss and lengthen shelf life, they have often not prioritized sweetness of the fruit, which is a large component of tomato fruit taste (Beckles, 2012). Therefore, greater emphasis is now being placed on improving traits, such as sugar content, to enrich the tomato fruit flavor.

Tomato fruit quality can be assessed by the content of chemical compounds such as soluble sugars, citric and other organic acids, volatile compounds and Ca content. The main soluble sugars in tomato fruit are glucose and fructose which make up 47% of the fruit dry matter (Petro-Turza, 1986). Organic acids in the tomato fruit are composed of citric and malic

acids and, together with acids such as carboxylic, sugar and alicydic acids, make up about 15% of the dry content of fresh tomato fruit. Further, volatile compounds, such as hexanal, trans-2-hexenal, butyl acetate and beta-ionone, are flavor and aroma enhancers that give tomato fruit their organoleptic properties (Buttery and Ling, 1993).

Soluble Sugars and Organic Acids

Balanced and high levels of sugars and organic acids are essential components of the fruit quality (Patanè et al., 2011, Tardieu et al., 1992). Research demonstrated that during ripening, concentrations of sugars, carotenoids and organic acids tend to increase (Patanè and Cosentino, 2010). Kader (2008) found that these components are responsible for the sweet and sour taste of tomato fruit and are essential to the flavor intensity. Increasing sugar levels create a higher ratio of sugar to organic acids making the fruit sweeter. Studies demonstrated that sugars, such as glucose and fructose, mainly contribute to the sweet taste of tomato (Petro-Turza, 1986). Catabolism of sucrose during the tomato fruit ripening stage creates glucose and fructose. Glucose and fructose are monosaccharaides that contribute to tomato flavor, play an important role as signaling molecules, increasing cellular carbon and energy metabolism.

In addition to high levels of sugars, tomato fruit quality depends on levels of organic acids. Organic acids come from different biological groups such as the Krebs Cycle. The Krebs Cycle generates organic acids through a series of chemical reactions mainly in mitochondria and create energy through oxidation and decarboxylation of acetate derived from carbohydrates, fats and proteins. Organic acids in tomato fruit contribute to its tart taste and balance the sweetness from sugars. The main organic acids contributing to the acid taste in tomatoes are citric acid and malic acid, which comprise over 90% of the overall organic acids in tomato fruit (Williams et al., 2009, Lin et al., 1998). Previous studies indicated that malate declines substantially during

ripening, whereas citrate has been reported to either decline (Thorne and Efiuvwevwere, 1988), remain constant (Davies, 1966) or increase slightly (Goodenough and Thomas, 1980). Further, results demonstrated that a 25% replacement of nitrate fertilizer with ammonium N significantly decreased malate and citrate acids (Dong et al., 2004). In addition, phosphates, K and free amino acids, such as glutamic acid, act as buffers to acidity and influence tomato fruit flavor (Patanè et al., 2011, Petro-Turza, 1986).

Other important compounds in ripening fruit are carotenoids. Carotenoids are powerful antioxidants linked to inhibiting cancers such as prostate (Giovannucci et al., 1995), skin (Gonzalez et al., 2003) and colon (Slattery et al., 1999). Additionally, carotenoids contribute to health factors as key flavor compounds in ripened fruit.

Carotenoids

Tomato fruit are well known for their naturally present carotenoids. Research indicated that carotenoids are linked to inhibiting cancers such as prostate (Giovannucci et al., 1995), skin (Gonzalez et al., 2003) and colon (Slattery et al., 1999). Carotenoids are also inversely associated with the occurrence of cardiovascular and heart disease (Rao and Agarwal, 2000), and can protect and maintain eye health (Semba and Dagnelie, 2003). For instance, a study concluded that healthy men consuming a low carotenoid diet every day benefited from increased consumption of carrot or tomato juice. Consumption of carrot and tomato juice led to a marked increase in β-carotene and lycopene in fecal matter and fecal water, respectively (Briviba et al., 2004).

Among carotenoids, lycopene is one of the most potent antioxidants and is a major component of tomato fruit (Miller et al., 1996). The antioxidant properties of lycopene come from its chemical structure of long congregated double bonds. Thermodynamically, the most

stable form of lycopene comes from natural plant sources existing predominantly in trans configuration (Nguyen and Schwartz, 1999). Research demonstrated that lycopene is powerful in cancer prevention and treatment. For example, Tang et al. (2005) found that naturally occurring lycopene doses of 100 and 300 $mg \cdot kg^{-1}$ (milligrams per kilograms) reduced cancerous prostate cells in mice by 50% and 78%, respectively. Furthermore, Fornelli et al. (2007) found that lycopene in concentrations ranging from 0.125 to100 µM (mirco molar) negatively influences the proliferation of MCF-7 breast cancer cells. In other words, these results indicate that lycopene could be effective in reducing the production of breast cancer cells. Prolonged exposure to lycopene for 24-72 h induced a similar response. Additionally, carotenoids contribute to health factors as important flavor compounds in ripened fruit. The carotenoids of fully ripened tomatoes are 50-80% lycopene and 2-7% β-carotene.

While carotenoids contribute to tomato fruit antioxidant capacity, aroma volatiles can enhance flavor. Aroma volatiles are derived from diverse precursors, such as amino acids, fatty acids, and carotenoids (Klee and Tieman, 2013). The main function of aroma volatiles in tomato fruit is to enhance the main flavor components, which are soluble sugars and organic acids (Klee and Tieman, 2013, Tieman et al., 2012). Therefore, aroma volatiles will enhance sweetness, acidity, or green flavor depending on the perceptions of consumer preferences.

Volatile Flavor Components

Over past decades, tomato cultivar selections from plant breeders have emphasized grower demands for yield, fruit size, firmness and resistance to biotic and abiotic diseases (Klee and Tieman, 2013). For the most part, growers are paid for pounds of product in the box with no added value for taste quality. As a result, the sensory aspects, such as flavor and aroma, of fruit quality have diminished. Consumers frequently associate newer hybrid tomato cultivars with

poor flavor. Recently, consumer, producers, and breeders have focused on improving flavor and aroma of the tomato fruit. However, flavor is also a function of aroma volatiles that enhance the flavor quality, and removing the volatiles greatly reduces flavor intensity (Baldwin et al., 2008).

Research identified approximately 400 volatile compounds in tomato fruit (Petro-Turza, 1986, Baldwin et al., 2000). A diverse set of precursors, such as amino acids, fatty acids and carotenoids, derive aroma volatiles (Klee and Tieman, 2013). The primary function of aroma volatiles in tomato fruit is to enhance the main flavor components, which are soluble sugars and organic acids (Klee and Tieman, 2013). Therefore, aroma volatiles will enhance sweetness, acidity, or green flavor depending on the perceptions of consumer preferences.

The profile of aroma volatiles across tomato cultivars is highly variable (Tieman et al., 2012). A small number of volatile compounds are more abundant than others. Previous research demonstrated that prevalent aroma volatiles in tomato fruit are hexenal, (E)-2-hexenal and 6-methyl-5-hepten-2-one (Buttery et al., 1987, Baldwin et al., 1991). In addition, research indicated that volatiles cis-3-hexenal, trans-2-hexenal, hexanal and 2-isobutylthiazole contribute to the quality of ripe tomato (Stone et al., 1975) by enhancing the fresh flavor and aroma. Carbonell-Barrachina et al. (2006) demonstrated variance among different types of tomatoes based on their flavor volatile composition. For example, the tomato cultivar De la Pera, which contained the highest content of flavor volatiles, received the highest values for odor and aroma. Likewise, not all aroma volatile compounds contribute to tomato flavor equally. A more common aroma volatile, a six carbon hexanal, is an important aroma volatile to tomato flavor, whereas geranial does not contribute as much (Klee and Tieman, 2013). The profile of aroma volatiles in any particular tomato fruit will depend on numerous environmental factors, such as temperature, light intensity, season and site variations.

Applications of plant hormones have been used to manipulate plant growth and development for many years in horticultural crops. However, research in recent years has focused on using plant hormones to improve fruit quality parameters, such as soluble sugars, fruit color, and phytonutrients (Zhang and Whiting, 2013, Buran et al., 2012, Gonzalez et al., 2012, Gu et al., 2011). For example, ABA has been used effectively to improve fruit quality in grapes (*Vitis vinifera*) (Quiroga et al., 2009, Peppi et al., 2006).

The Impact of ABA on Fruit Quality

Plant growth regulators (PGRs) are chemicals used on a wide range of horticultural crops. These exogenous chemicals, similar to endogenous plant hormones, regulate plant development and stimulate a desired growth response. In the floriculture industry PGRs are typically used for controlling plant height and promoting flower initiation or delaying flowering (Lewis et al., 2004, Blanchard and Runkle, 2007, Currey and Erwin, 2012). For example, the GA inhibitor Sumagic is used for regulating plant height and has been demonstrated to be effective in controlling plant height in tomato transplant production (Shin et al., 2009). In the nursery industry, PGRs have been used to improve crop quality by stimulating lateral branching and substituting for a cold storage requirement and controlling plant height (Latimer et al., 2003, Gibson and Whipker, 2003, Clough et al., 2001). Traditionally in the fruit industry PGRs have been used for thinning the flower blossoms of tree fruit to achieve larger fruit and improve fruit quality such as firmness and nutritional value (Jones et al., 1991, Meland et al., 2011, Greene et al., 2011). For example, the PGR CyLex plus, used in apple (*Malus domestica*) and pear (*Pyrus communis*) production, is effective in inducing flower thinning and return bloom (Stopar et al., 2009).

Research in recent years has focused on using PGRs to improve fruit quality parameters, such as soluble sugars, fruit color and phytonutrients (Zhang and Whiting, 2013, Buran et al., 2012, Gonzalez et al., 2012, Gu et al., 2011). One such PGR is ABA, which has been used effectively to improve fruit quality, especially in grape production (Quiroga et al., 2009, Peppi et al., 2006). ABA has significantly increased soluble sugars in grapes, thus improving fruit flavor. ABA also improved fruit color and nutritional values, such as anthocyanins, which increase antioxidants in the human diet (Cantin et al., 2007).

The improvement in grape fruit quality parameters and the demand for healthier fruits and vegetables have sparked additional research in other horticultural crops. These studies indicated that, in addition to PGRs, manipulating environmental factors may contribute to improving fruit quality parameters, specifically flavor and phytonutrients. For example, Barickman et al. (2013) found that manipulating mineral nutrients in soils did not negatively affect nutritional quality in *Brassica* species. They found that supplementing adequate selenium in the fertilizer maintained glucosinolate concentrations on beneficial levels for human nutrition. Therefore, manipulating environmental factors in addition to PGRs may significantly improve fruit quality.

The role of ABA in protecting the xanthophyll cycle [de-epoxidation of violaxanthin (VIO) to ZEA via antheraxanthin (ANTH)] and the photosynthetic apparatus from photooxidative stress is well documented (Du et al., 2010). For example, exogenous applications of ABA to barley (*Hordeum vulgar*) seedlings increased total and xanthophyll carotenoid concentrations by 122%, while protecting photosystem II (PSII) against photoinhibition at low temperatures (Ivanov et al., 1995). Haisel et al. (2006) found that seedlings of bean (*Phaseolus vulgaris*), tobacco (*Nicotiana tabacum*), beets (*Beta vulgaris*) and corn (*Zea mays*) pre-treated

with ABA demonstrated increased chlorophyll and carotenoid concentrations under water stress. Sorghum (*Sorghum bicolor*) seedlings supplemented with ABA and exposed to light intensities to induce photo-inhibition (2200 and 3600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (mirocmole per meter per second) photosynthetically active radiation) had better energy dissipation and much greater levels of de-epoxidation than control seedlings (Sharma et al., 2002). In addition, ABA plays an important role during fruit ripening. Research indicated that ABA associated genes were highly expressed in ripening fruit (Zhang et al., 2009) and that application of ABA accelerated ethylene biosynthesis, therefore regulating fruit ripening (Zaharah et al., 2013). The color changes from green to red as chloroplasts are transformed to chromoplasts causes the de novo synthesis of carotenoids in the tomato fruit tissue (Pek et al., 2010). ABA affects this change in color and acceleration of ethylene biosynthesis in the tomato fruit.

In addition, Bastias et al. (2011) found that as a ripening control factor, ABA increases levels of sugars in tomato fruit by increasing expression of genes encoding a vacuolar invertase and a sucrose synthase. High levels of soluble sugars are important because they are essential components of tomato fruit quality. Increasing sugar levels, specifically glucose and fructose, creates a higher ratio of sugar to organic acids making the fruit sweeter and tastier (Patanè et al., 2011, Tardieu et al., 1992).

Summary

Tomato is a widely employed plant model system to study fruit metabolism, development and ripening. Tomato fruit developments and ripening are complex processes coordinated by plant hormones critical in regulating changes (Srivastava and Handa, 2005). Ripening tomato fruit goes through changes in carotenoid concentrations, increases in sugar and organic acid content and changes in cell wall composition. Abscisic acid is an apo-carotenoid that regulates

many developmental processes in plants (Inaba et al., 1976). The role of ABA in tomato fruit ripening and senescence, investigation of antioxidant carotenoids, nutrient and carbohydrate partitioning, and key ripening enzymes in ABA and ethylene biosynthesis are needed to better understand mechanisms involved in fruit quality. Recent research has demonstrated that exogenous ABA will positively impact tomato fruit carbohydrate and lycopene concentrations. Other studies have shown that increasing ABA concentrations impact yield during late stages of fruit development. Changes in ABA concentrations, along with increasing activity of ethylene and other fruit ripening enzymes, increase tomato phytonutrients and fruit quality by increasing soluble sugars and carotenoids in the developing fruit. ABA may plant an important role in fruit quality and research is needed to examine the roles of ABA, CA and stress on BER and tomato fruit quality factors, such as soluble sugars, organic acids, carotenoids, volatiles in optimizing yield and quality.

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Chapter 2
Abscisic Acid Improves Calcium Partitioning into 'Micro' Tomato Fruit Tissue

Abstract

Abscisic acid (ABA) is known to control stomatal conductance and, therefore, transpiration. Research indicates that spray applications of ABA decrease the incidence of the physiological disorder blossom-end rot in tomato fruit tissue. This study investigated the impacts of root tissue ABA applications on tomato leaf and fruit mineral nutrient concentrations in two genotypes of genetically dwarf tomato plants. The purpose of this study was to determine the effects of ABA treatments on partitioning of mineral nutrients in 'MicroTina' and 'MicroGold' tomato (*Solanum lycopersicum)* plants. The data indicated that leaf and fruit tissues of both tomato genotypes reacted similarly to ABA treatments. Plants treated with ABA (0.5, 5.0, and 10.0 mg/L) had a significant ($P \leq 0.05$) increase in tomato fruit tissue calcium (Ca) in both 'MicroTina' and 'MicroGold' compared to the control treatment (0.0 mg/L). In 'MicroTina' tomato fruit tissue, Ca ranged from 27.45 µg/g (micrograms per gram) dry weight (DW) to 49.29 µg/g DW, and 'MicroGold' tomato fruit tissue Ca ranged from 29.26 µg/g DW to 48.38 µg/g DW. Tomato leaf Ca concentrations were also significantly affected by ABA treatments. Calcium leaf tissue concentrations were significantly lower ($P \leq 0.05$) in the the ABA treated plants. In 'MicroTina' tomato leaf tissue, Ca ranged from 25412 µg/g DW to 36280 µg/g DW, and 'MicroGold' tomato leaf tissue Ca ranged from 28160 µg/g DW to 37825 µg/g DW. Therefore, ABA affected 'Micro' tomato Ca partitioning between leaf and fruit tissue.

Keywords**:** *Lycopersicon, blossom-end rot, mineral nutrients*

Introduction

There are a number of physiological diseases that cause an aberration in normal plant physiology. For example, the diseases caused by abiotic stress of environmental and cultural factors adversely affect the functioning of plant growth and development. Effects of physiological disorders range from subtle symptoms not visibly apparent to severely stunted and

malformed growth. Unfortunately, physiological disorders usually are difficult to identify before the effects on the plant can be corrected. Calcium deficiency in fruit tissue is particularly hard to discern because it occurs early in developmental stages when cell expansion is rapid. Abiotic stress slows down Ca absorption and distribution within a plant, which in turn causes physiological disorders. The most common physiological disorders caused by Ca deficiency in horticultural crops are tip-burn and brown heart in leafy vegetables, blossom-end rot (BER) in tomatoes (*Solanum lycopersicum* L.) and melons (*Cucumis melo* L.) and bitter pit in apples (*Malus domestica* L.). BER is of particular interest due to substantial crop losses in many regions of tomato production, some reporting up to 50% (Douglas, 2010). Research has shown that there is a correlation between the occurrence of BER and Ca nutrition (Adams and Ho, 1993). Specifically in tomato fruit, BER occurs because of a local deficiency of Ca in the tissue at the distal end of tomato fruit. However, BER can occur in plants with an adequate Ca supply when grown under conditions that either: a) reduce the transport of Ca to rapidly growing distal fruit tissue; or b) increase the demand of the distal fruit tissue for Ca by accelerating fruit expansion (Ho and White, 2005). The causes of Ca distribution problems may lie in the fact that abiotic stress inhibits its transport and distribution in the plant.

One important regulator that coordinates the response to environmental stress is the hormone abscisic acid (ABA). Biosynthesis of ABA occurs from cleavage of C_{40} carotenoid compounds derived from the non-mevalonate pathway (MEP) and takes place in chloroplasts and other plastids (Milborrow and Lee, 1998). Abscisic acid has been found in all higher plant tissues which include roots, xylem tissue and sap, phloem sap, pollen, petals, fruits and seeds with concentrations in leaves of temperate crop plants varying from 50 to 500 ng/g (Wilkins, 1984). Research has demonstrated that concentrations of ABA increased dramatically in all

plant organs during nutrient deficient conditions (Vysotskaya et al., 2008), such as salt stress, phosphate deficiency, and ammonium nutrition; whereas ABA concentrations decreased with nitrate deficiency and saturated or alkaline soil conditions (Jeschke et al., 1997). For example, plants exposed to root-zone water deficits exhibited an increase in xylem ABA concentrations and a reduction in stomatal conductance (Hartung et al., 2005). Therefore, Ca has restricted uptake into vegetative tissue when ABA responses occur, and may help distribute Ca into lower transpiring tissues such as fruit tissue. Thus, the objective of this research was to examine the distribution of Ca and other mineral nutrients in the leaf and fruit tissue of two genotypes of genetically dwarf tomato plants that were subjected to increasing ABA treatment in the nutrition solution.

Materials and Methods

Plant Culture and Harvest: Seeds of 'MicroTina' and 'MicroGold' tomato (USU Crop Physiology Lab, Logan, UT) were sown into 2.5 x 2.5 cm growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark), germinated under greenhouse conditions and grown at 25°C/day and 20°C/night under supplemental light at 1,150 µmol/m^2/s. At 21 days after seeding, the plantlets were transferred to 11 L containers (Rubbermaid Inc., Wooster, OH) filled with 10 L of nutrient solution developed specifically for greenhouse tomato plants in our lab at the University of Tennessee. Elemental concentrations of the nutrient solutions were (mg/L): nitrogen (N), 180.0; phosphorus (P), 93.0; potassium (K), 203.3; calcium (Ca), 180.0; magnesium (Mg), 48.6; sulfur (S), 96.3; iron (Fe), 1.0; boron (B), 0.25; manganese (Mn), 0.25; zinc (Zn), 0.025; copper (Cu), 0.01; and molybdenum (Mo), 0.005. Experimental design consisted of four blocks containing four replications of each treatment for both varieties, with individual reservoirs representing an experimental unit. Each reservoir contained 2 plants. Treatments consisted of ABA (s-ABA,

Valent BioSciences, Libertyville, IL) applied to nutrient solutions at concentrations of 0.0, 0.5, 5.0, and 10.0 mg/L. Solutions were aerated with an air blower (Model 25E133W222; Spencer; Winsor, CT) connected to air stones in each reservoir. Complete nutrient solution and treatment changes were made every week until study conclusion. Fruit tissues were harvested 84-90 days after seeding. Ten ripe fruit for each experimental unit were juiced and prepared for elemental nutrient, sugar, and carotenoid analysis. Harvested fruit samples were stored at -20°C for no longer than 14 days prior to analysis. Leaf samples were taken from the last harvest and analyzed for mineral elements.

Elemental Nutrient Determination: The method of analysis for mineral nutrients was developed at The University of Tennessee, Department of Plant Sciences in our plant physiology and nutrition laboratory (Barickman et al., 2013) with modifications. In short, nutrient analysis was performed using a 5.0 g (gram) subsample of fresh fruit tissue, which was combined with 10 ml (milliter) of 70% HNO_3 and digested in a microwave digestion unit (Model: Ethos, Milestone Inc., Shelton, CT). The microwave temperature was ramped to 140 °C for 5 min (minute) at 1000 W (watt) and 2000 kPa (kilopascal), followed by an increase to 210 °C for 10 min at 1000 W and 3000 kPa. Furthermore, microwave temperature was held at 210 °C for 10 min at 1000 W and 4000 kPa and cooled for 10 min at 0 W and 2000 kPa. The digest was then allowed to cool to 20 °C. A 100 µl (microliter) subsample of the digest was diluted with 9900 µl of ICP-MS matrix consisting of 2% (nitric acid) and 0.5% HCl (hydrochloric acid) (v/v). Leaves were collected and triple rinsed with de-ionized water and dried for 72 h in a forced air oven at 65 °C. Dried samples were ground to homogeneity using a coffee grinder, and 0.5 g sub-samples was weighed for analysis. Samples were placed into a muffle furnace at 450 °C for 8 h to allow the sample to ash. Ashed samples were allowed to cool to room temperature then digested with 10

ml concentrated nitric acid. A 100 µl aliquot of the digested sample was diluted with a matrix containing 2% HNO_3 and 0.5% HCl for analysis. Nutrient analysis was conducted using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies, Inc., Wilmington, DE). The ICP-MS system was equipped with an octapole collision/reaction cell, Agilent 7500 ICP-MS ChemStation software, a Micromist nebulizer, a water-cooled quartz spray chamber, and a CETAC (ASX-510, CETAC Inc., Omaha, NE) autosampler. The instrument was optimized daily in terms of sensitivity with lithium (Li), yttrium (Y) and thallium (Tl), level of oxide (Ce), and doubly charged ion (Ce) using a tuning solution containing 10 µg/L of Li, Y, Tl, Ce, and Co in a 2% HNO_3/0.5% HCl (v/v) matrix. Tissue nutrient concentrations were expressed on a DW basis.

Data were analyzed using the PROC Mixed Model analysis of variance procedure of the SAS v.9.3 (SAS Institute, Cary, NC, USA). In addition, one-way analysis of variance was used to determine the differences between untreated controls and ABA treatments (Barickman et al., In Press).

Results and Discussion

In both tomato genotypes there were significant differences in Ca concentrations in the leaf tissue when comparing the control treatment to the ABA treatments in a paired-*t* test. Calcium decreased significantly ($P \leq 0.05$) in the leaf tissue when comparing the control to the ABA treatments in 'MicroTina' genotype **(Table 1.1)**, ranging from 25412 µg/g DW in the 0.5 mg ABA per L treatment to 36280 µg/g DW in the control treatment in 'MicroTina' tomato plants. In addition, Ca decreased significantly ($P \leq 0.05$) in 'MicroGold' tomato plants ranging from 28160 µg/g DW in the 10.0 mg ABA per L treatment to 37825 µg/g DW in the control treatment **(Table 1.2).** These results accounted for 30.0% and 25.5% losses of Ca in the leaf

tissue, respectively. These results support previous research, which has demonstrated that applications of ABA decrease stomatal conductance (Waterland et al., 2010). Therefore, ABA negatively regulates gas exchange and transpiration. Furthermore, research indicated that tomato plants treated with ABA had higher stem water potential and lower whole-plant water loss (de Freitas et al., 2011). Thus, Ca distribution into the leaves may decrease with the application of ABA treatments. In addition to Ca, Boron (B) levels were also affected by ABA treatments. B decreased significantly ($P \leq 0.05$) from 53.20 µg/g DW in the 10.0 mg ABA per L treatment to 68.49 µg/g DW in the control treatment in 'MicroTina' plants **(Table 1.1).** That accounts for a decrease of 24.1% in the leaf tissue. Similarly, B decreased significantly ($P \leq 0.05$) in 'MicroGold' tomato plants ranging from 53.64 µg/g DW in the 10.0 mg ABA per L treatment to 75.22 µg/g DW in the control treatment, which was a 28.7% decrease **(Table 1.2).** Boron is a mineral nutrient whose transport within the plant is similar to the transport of Ca. These results are important because ABA affects not only Ca but other nutrients that act similarly when transported into the plant as well.

Results indicate that leaf nutrient concentrations for Mn and Cu were significantly affected by ABA treatments in 'MicroTina' tomato plants as well **(Table 1.1).** Manganese in the leaf tissue treated with ABA in concentration decreased significantly ($P \leq 0.001$). Leaf tissue concentration of Mn ranged from 134.28 µg/g DW in the 10.0 mg ABA/L (abscisic acid per liter) treatment to 229.39 µg/g DW in the control treatment. There was a significant ($P \leq 0.01$) linear decrease that accounted for a 39.7% loss of Mn in the leaf tissue. Copper also decreased significantly ($P \leq 0.01$) in the leaf tissue **(Table 1.1).** Leaf tissue concentrations of Cu ranged from 13.72 µg/g DW in the 0.5 mg ABA per L treatment to 24.82 µg/g DW in the control treatment. The decrease in Cu in the leaf tissue accounted for a 44.7% loss. Similarly,

'MicroGold' tomato leaf tissue also demonstrated a significant decrease in Mn ($P \leq 0.05$) and ranged from 167.25 µg/g DW in the 10.0 mg ABA per L treatment to 279.94 µg/g DW in the control treatment **(Table 1.2).** In addition, Cu decreased significantly ($P \leq 0.05$) and ranged from 14.67 µg/g DW in the 0.5 mg ABA/L treatment to 27.68 µg/g DW in the control treatment **(Table 1.2).** These results indicate that ABA adversely affected these micro-nutrients in the leaf tissue. Previous research has demonstrated that members of the manganese-superoxide dismutase (MnSod) gene family encoding antioxidant isozymes of known function during development and oxidative stress respond differentially to ABA in developing maize (*Zea mays* L.) embryos (Lovdal et al., 2010). In a similar study, results indicated that copper-superoxide dismutase in rice (*Oryza sativa* L.) responded to external supplies of ABA (Zhang et al., 2010). The reduction of Mn and Cu may be due to the sequestration of these nutrients outside of the leaf tissue.

In this study, results indicated that Ca in the fruit tissue increased with ABA treatments. Plants treated with ABA (0.5, 5.0, and 10.0 mg/L) had a significant ($P \leq 0.05$) increase in tomato fruit tissue Ca in both 'MicroTina' and 'MicroGold' compared to the control treatment (0.0 mg/L). In 'MicroTina' tomato fruit tissue, Ca ranged from 27.45 µg/g DW to 49.29 µg/g DW **(Table 1.3).** The uptake of Ca into the fruit tissue when treated with ABA accounted for a 44.3 % increase in 'MicroTina' tomatoes. In 'MicroGold' tomato, fruit tissue Ca ranged from 29.26 µg/g DW to 48.38 µg/g DW, and this accounted for a 39.5% increase in tomato fruit Ca when plants were treated with ABA **(Table 1.4).** Thus, the application of ABA treatments to the nutrient solution increased tomato fruit tissue Ca in the dwarf plants in the current study. Research has indicated that total and apoplastic Ca concentrations increased in tomato fruit tissue when treated with ABA (de Freitas et al., 2011). This study also demonstrated that there was a

higher abundance of functional xylem vessels in the tomato fruit tissue in ABA treated plants. Together with increased concentrations of Ca in the apoplast and number of functional xylem vessels, ABA may be a viable treatment option for enhancing cell wall and membrane functions for fruit tissue that is susceptible to Ca deficiency. Thus, the results indicated that applications of ABA can increase Ca concentrations in tomato fruit tissue possibly by maintaining higher Ca uptake throughout development.

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Appendix 1: Tables

Table 1.1. Mineral nutrients of leaf tissue in 'MicroTina' tomato (*Solanum lycopersicum* L.) plants grown in a greenhouse and treated with s-ABA in the hydroponic nutrient solution.

	Concentration of mineral nutrients (µg/g) dry weight[a]										
ABA (mg/L)	B	Mg	P	S	K	Ca	Mn	Fe	Cu	Zn	Mo
0	68.49	8596	8697	14990	64980	36280	229.39	132.54	24.82	42.85	10.41
0.5	56.86	6217	8173	13320	57728	25412	149.15	120.82	13.72	39.7	12.86
5	59.92	6844	9127	14472	63468	28633	156.22	128.43	15.98	45.57	13.99
10	53.20	6707	8179	14089	54465	28042	134.28	138.85	15.07	42.42	11.78
P-Value[b]	ns	ns	ns	ns	ns	ns	***	ns	**	ns	ns
T-Test No ABA vs. ABA[b]	*	ns	ns	ns	ns	*	***	ns	**	ns	ns

[a] SE for means; B-4.42, Mg-963, P-703, S-1678, K-6121, Ca-2951, Mn-14.35, Fe-19.14, Cu-2.33, Zn-6.45, and Mo-3.47

[b] ns, *, **, and *** indicate non-significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 1.2. Mineral nutrients of leaf tissue in 'MicroGold' tomato (*Solanum lycopersicum* L.) plants grown in a greenhouse and treated with s-ABA in the hydroponic nutrient solution.

	Concentration of mineral nutrients (µg/g) dry weight[a]										
ABA (mg/L)	B	Mg	P	S	K	Ca	Mn	Fe	Cu	Zn	Mo
0	75.22	8315.25	11099	17769	60820	37825	279.94	144.68	27.68	34.88	12.8
0.5	58.36	6892	9695	15108	55100	31620	201.52	139.69	14.67	28.35	12.18
5	57.79	6628	10244	14797	58526	30401	213.61	148.82	16.35	30.1	13.36
10	53.64	6549.5	8858.75	14463	59350	28160	167.25	144.29	16.01	27.76	13.41
P-Value[b]	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
T-Test No ABA vs. ABA[b]	*	ns	ns	ns	ns	*	*	ns	*	ns	ns

[a] SE for means; B-8.81, Mg-1024, P-1687, S-2837, K-9912, Ca-3307, Mn-42.6, Fe-29.79, Cu-4.52, Zn-6.06, and Mo-3.73

[b] ns and * indicate non-significant or significant at $P \leq 0.05$.

Table 1.3. Mineral nutrients of fruit tissue in 'MicroTina' tomato (*Solanum lycopersicum* L.) plants grown in a greenhouse and treated with s-ABA in the hydroponic nutrient solution.

	Concentration of mineral nutrients (µg/g) dry weight[a]									
ABA (mg/L)	B	Mg	P	K	Ca	Mn	Fe	Cu	Zn	Mo
0	0.43	56.89	151.23	1291.98	27.45	0.66	1.68	0.42	1.29	0.13
0.5	0.52	69.56	186.68	1513.74	49.29	0.82	1.88	0.41	1.29	0.18
5	0.40	46.10	136.14	1197.72	41.25	0.50	1.28	0.31	1.17	0.13
10	0.51	65.96	188.46	1611.90	39.54	0.67	2.17	0.45	1.36	0.16
P-Value[b]	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
T-Test Control vs. ABA[b]	ns	ns	ns	ns	*	ns	ns	ns	ns	ns

[a] SE for means; B-0.07, Mg-11.58, P-29.11, K-194.84, Ca-3.00, Mn-0.14, Fe-0.32, Cu-0.06, Zn-0.20, and Mo-0.03

[b] ns and * indicate non-significant or significant at $P \leq 0.05$, respectively.

Table 1.4. Mineral nutrients of fruit tissue in 'MicroGold' tomato (*Solanum lycopersicum* L.) plants grown in a greenhouse and treated with s-ABA in the hydroponic nutrient solution.

	Concentration of mineral nutrients (µg/g) dry weight[a]									
ABA (mg/L)	B	Mg	P	K	Ca	Mn	Fe	Cu	Zn	Mo
0	0.27	50.98	127.17	1151.64	29.26	0.56	1.46	0.36	0.99	0.10
0.5	0.44	48.96	210.36	1888.44	48.38	0.77	2.26	0.47	1.35	0.19
5	0.28	48.82	129.03	1087.08	40.87	0.49	1.50	0.33	1.12	0.14
10	0.24	44.31	124.06	1057.98	39.03	0.67	1.39	0.30	1.07	0.12
P-Value[b]	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
T-Test Control vs. ABA[b]	ns	ns	ns	ns	*	ns	ns	ns	ns	ns

[a] SE for means; B-0.06, Mg-8.83, P-25.04, K-240.29, Ca-2.90, Mn-0.08, Fe-0.30, Cu-0.07, Zn-0.18, and Mo-0.03

[b] ns and * indicate non-significant or significant at $P \leq 0.05$, respectively.

Chapter 3
Abscisic Acid Increases Carotenoid and Chlorophyll Concentrations in Leaves and Fruit of Two Tomato Genotypes

Abstract

One important regulator that coordinates response to environmental stress is the hormone abscisic acid (ABA), which is synthesized from xanthophyll pigments. In spite of the fact that there is strong evidence of increases in ABA concentrations under various environmental stresses, information concerning the effects of exogenous ABA applications on leaf pigments and fruit carotenoids in tomato (*Solanum lycopersicum*) is lacking. This study investigated the impacts of root tissue ABA applications on tomato leaf and fruit pigmentation concentrations of 'MicroTina' and 'MicroGold' tomato plants. Tomato plants were treated with increasing concentrations of ABA in the nutrient solution. Therefore, the purpose of this study was to determine dose-response effects of ABA treatment in solution culture for maximum leaf pigmentation and fruit carotenoids in two distinct genotypes of dwarf tomato. Since application of ABA to plants has resulted in increases in chlorophylls and carotenoids, we hypothesized that ABA would have a positive impact on leaf chlorophylls and carotenoids, thus increasing fruit carotenoids. The results indicated that 'MicroTina' plants treated with ABA (0.5, 5.0, and 10.0 mg·L-1) had a significant increase in β-carotene (BC; $P \leq 0.001$), lutein (LUT; $P \leq 0.001$), zeaxanthin (ZEA; $P \leq 0.05$), and neoxanthin (NEO; $P \leq 0.001$) in the leaf tissue. In 'MicroGold' tomato plants carotenoids responded similarly. For example, there were significant increases in BC ($P \leq 0.01$), LUT ($P \leq 0.001$), ZEA ($P \leq 0.05$), and NEO ($P \leq 0.001$). In 'MicroTina' tomato leaves there were significant increases in chlorophyll *a* (Chl *a*; $P \leq 0.001$) and chlorophyll *b* (Chl *b;* $P \leq 0.001$) concentrations. Furthermore, there were significant increases in Chl *a* ($P \leq 0.001$) and Chl *b* ($P \leq 0.001$) in 'MicroGold' leaf tissue. In 'MicroTina' tomato fruit tissue, the concentration increased significantly for lycopene (LYCO; $P \leq 0.01$). However, in 'MicroGold' there was no significant changes in BC and LUT concentrations. In addition, LYCO was found to be below detection limits in 'MicroGold' tomato fruit. Therefore, ABA has been shown to

positively change tomato leaf pigments in both genotypes and fruit tissue carotenoid concentrations in 'MicroTina' tomato.

Keywords**:** *β-carotene, lutein, lycopene, chlorophyll, xanthophyll*

Introduction

Plant responses to environmental stress involve a number of metabolic and physiological changes. One important regulator that coordinates response to environmental stress is the hormone ABA, which is synthesized from xanthophyll pigments (Taylor et al., 1988). Concentrations of ABA increase in plant tissues in response to environmental stress resulting in drought induced stomatal closure (Desikan et al., 2004), accumulation of secondary messenger molecules (Wang et al., 2012), and enhancement of ABA-responsive gene expression (Liu et al., 2010). One of the most important abiotic stress factors, water stress, can regulate plant growth and development thus, limiting plant production (Jiang and Zhang, 2002). However, plants will produce ABA in low levels in the absence of stress factors. Therefore, ABA is an important component in the mechanisms of resistance and adaptation to abiotic stress conditions (Berli et al., 2010).

The role of ABA in protecting the xanthophyll cycle [de-epoxidation of violaxanthin (VIO) to ZEA via antheraxanthin (ANTH)] and the photosynthetic apparatus from photooxidative stress is well documented (Du et al., 2010). For example, exogenous applications of ABA to barley (*Hordeum vulgar*) seedlings resulted in an increase in total and xanthophyll carotenoid concentrations by 122% protecting photosystem II (PSII) against photoinhibition at low temperatures (Ivanov et al., 1995). Haisel et al. (2006) found that seedlings of bean (*Phaseolus vulgaris*), tobacco (*Nicotiana tabacum*), beets (*Beta vulgaris*), and corn (*Zea mays*) pre-treated with ABA demonstrated increased chlorophyll and carotenoid concentrations under

water stress. Sorghum (*Sorghum bicolor*) seedlings supplemented with ABA and exposed to light intensities to induce photo-inhibition (2200 and 3600 µmol·m-2·s-1 photosynthetically active radiation) had better energy dissipation and much greater levels of de-epoxidation than control seedlings (Sharma et al., 2002). In addition, ABA plays an important role during fruit ripening. Research has indicated that ABA associated genes were highly expressed in ripening fruit (Zhang et al., 2009) and that application of ABA accelerated ethylene biosynthesis, therefore regulating fruit ripening (Zaharah et al., 2013). The de novo synthesis of carotenoids in the tomato fruit tissue, mainly LYCO and BC, are associated with the color changes from green to red as chloroplasts are transformed to chromoplasts (Pek et al., 2010). This change in color may be ABA's effect on the acceleration of ethylene biosynthesis in the tomato fruit. Furthermore, foliar applied ABA on grapes (*Vitis vinifera*) resulted in stimulatory effects on fruit color (Cantin et al., 2007).

In spite of the fact that there is strong evidence of increases in ABA concentrations under various environmental stresses, information regarding the effects of exogenous ABA applications on leaf pigments and fruit carotenoids in tomato is lacking. Tomato seedlings are grown in greenhouses under controlled conditions in seedling cultivation operations. However, when transplanted into the field tomato plants are exposed to a wide range of environmental conditions that can be detrimental to growth and development. Furthermore, even though the tomato plants are exposed to these adverse conditions, consumers want tomato fruit that are more nutritious. Adverse environmental conditions such as drought, excess light, and high temperature stress may negatively affect the nutritional values of tomato fruit. Among carotenoids, LYCO is one of the most potent antioxidants and is a major component of red tomato fruit (Miller et al., 1996). Research has indicated that LYCO is a powerful antioxidant

that can prevent cancers. For example, Tang et al. (2005) demonstrated that naturally occurring LYCO doses of 100 to 300 mg·kg-1 inhibited cancerous prostrate cells in mice by more than 50%. Therefore, the purpose of this study was to determine dose-response effects of ABA treatment in solution culture for maximum leaf pigmentation and fruit carotenoids in two distinct genotypes of dwarf tomato. Dwarf tomato plants were chosen for their genetic homology to large tomato plants and small size for bench top experimentation. Since application of ABA to plants has resulted in increases in chlorophylls and carotenoids, we hypothesized that ABA would have a positive impact on leaf chlorophylls and carotenoids, thus increasing fruit carotenoids.

Materials and Methods

Plant Culture and Harvest. Seeds of 'MicroTina' and 'MicroGold' tomato (Utah State University, Crop Physiology Lab, Logan, UT) were sown into 2.5 x 2.5-cm growing cubes (Grodan, Hedehusene, Denmark), germinated under greenhouse conditions and grown at 25/20 °C (day/night) under a 16-h (hour) photoperiod with supplemental light at an average of 850 µmol·m-2·sec-1. At 21 d after seeding, the plantlets were transferred to 11-L (liter) containers (Rubbermaid Inc., Wooster, OH) filled with 10-L of nutrient solution. Tomato plants were grown hydroponically with a tomato fertilizer scheme developed at the University of Tennessee. Elemental concentrations of the nutrient solutions were (mg·L-1): N (180), P (93.0), K (203.3), Ca (180), Mg (48.6), S (96.3), Fe (1.0), B (0.25), Mn (0.25), Zn (0.025), Cu (0.01), and Mo (0.005). The two genotypes were grown in separate experiments with an experimental design consisting of a randomized complete block with four replications. Each reservoir contained two plants with individual reservoirs representing an experimental unit. Treatments consisted of ABA (s-ABA; Valent BioSciences, Libertyville, IL) applied to nutrient solutions at

concentrations of 0.0, 0.5, 5.0, and 10.0 mg·L-1. Solutions were aerated with an air blower (model 25E133W222; Spencer, Winsor, CT) connected to air stones in each reservoir. Complete nutrient solution and treatment changes were made every week until study conclusion. Fruit tissues were harvested 84-90 d (days) after seeding. Ten ripe fruit for each experimental unit were juiced and prepared for carotenoid analysis. Harvested fruit samples were stored at -20 °C for no longer than 14 d prior to analysis. Leaf samples were taken upon last harvest and were frozen at -80 °C until analysis for chlorophylls and carotenoids.

Fruit Carotenoids Tissue Analysis. Carotenoids were extracted from fresh-frozen ripe fruit tissues and quantified according to the methods of Emenhiser et al. (1996) with slight modifications. Fruit was removed from -20 °C and thawed until slightly pliable. A sample of 10 whole ripe fruits from each experimental unit (treatment) was blended into a slurry. A 2.0-g subsample of the slurry was placed into a test tube (20 x 150 mm), and 5-mL of hexane and 0.8-mL of the internal standard (ethyl-β-8'-apo-carotenoate; CaroteNature GmbH, Lupsingen, Switzerland) were added. Test tubes were vortexed for 1 min before addition of 5-mL of tetrahydrofuran then vortexed for 1 min before additions of 5-mL of reverse osmosis water. After vortexing for 20 s, test tubes were stored at 4 °C for 10 min to achieve aqueous-organic separations. Tubes were then centrifuged at 500 g_n (gravity forces) for 10 min. The organic top layer was removed using a disposable Pasteur pipette and placed into a graduated conical test tube. The sample volume was reduced to dryness under a stream of nitrogen gas (N-EVAP 111; Organomation Inc., Berlin, MA). Samples were brought up to a final volume of 5-mL with acetone, and a 2-mL aliquot was filtered through a 0.2-µm polytetrafluoroethylene (PTFE) filter (Econofilter PTFE 25/20; Agilent Technologies, Santa Clara, CA) prior to high-performance liquid chromatography (HPLC) analysis.

A HPLC unit with a photodiode array detector (1200 series, Agilent Technologies) was used for pigment separation. Chromatographic separations were achieved using an analytical scale (250 x 4.6 mm (millimeter) i.d. (inner diameter)) 5-µm (micron) polymeric RP-C_{30} column (ProntoSIL; MAC-MOD Analytical Inc., Chadds Ford, PA), which allowed for effective separation of chemically similar pigment compounds. The column was equipped with a 5-µm guard cartridge (10 x 4.0 mm i.d.) and holder (ProntoSIL), and was maintained at 40 ºC using a thermostatted column compartment. All separations were achieved isocratically using a binary mobile phase of 38.00% methyl *tert*-butyl ether, 61.99% methanol, and 0.01% triethylamine (v/v/v). The flow rate was 1.0 mL·min-1, with a run time of 40 min. Eluted compounds from a 10-µL injection loop were detected at 453 nm (nanometer); and data were collected, recorded, and integrated using ChemStation Software ver. B.01.01 (Agilent Technologies). Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards of BC, LUT, and LYCO (ChromaDex Inc., Irvine, CA).

Leaf Carotenoid and Chlorophyll Analysis. The frozen tomato leaf samples were lyophilized in a programmed freeze dryer (model 6L FreeZone, LabConCo, Kansas City, MO). Freeze-dried tissues were then ground in liquid nitrogen with a mortar and pestle. Pigments were extracted and separated according to Kopsell et al. (2004), which was based on the method of Khachik et al. (1986). HPLC separation parameters and pigment quantification followed procedures of Kopsell et al. (2007). A HPLC unit with a photodiode array detector (1200 series; Agilent Technologies) was used for pigment separation.

Statistical Analysis. Data were analyzed using the PROC Mixed Model analysis of variance (ANOVA) (Garcia-Mina et al., 2013) procedure of SAS (version 9.3; SAS Institute, Cary, NC).

The fixed effect for the experiment consisted of the control (0.0 mg·L-1 ABA) and ABA (0.5, 5.0, and 10.0 mg·L-1) treatments, while the random effects were measured as replications. The mean differences among the ABA treatments (0.5, 5.0, and 10.0 mg·L-1) were not significant. Therefore, a one-way ANOVA contrast was conducted to compare the mean differences between the control treatment and the combined ABA treatments.

Results

Impact of ABA on tomato leaf carotenoids and chlorophylls. The mean separation of the ABA treatments (0.5, 5.0, and 10.0 mg·L-1) was not significant. Therefore, the ABA treatments were pooled and compared to the control treatment. The application of ABA treatments to the nutrient solution increased the accumulation of BC, LUT, ZEA, and NEO carotenoids in 'MicroTina' tomato leaf tissue when compared to the control treatment **(Table 2.1).** BC increased 49.1% in the leaf tissue. LUT increased 32.3% in all treated leaf tissue. ZEA concentrations increased 64.9% in the leaf tissue. NEO had an increase of 31.4% in the leaf tissue of ABA treated tomato plants. In 'MicroGold' tomato, leaf tissue carotenoids responded similarly when treated with ABA compared to the control **(Table 2.2).** BC increased 42.3 % in the ABA treated leaf tissue. LUT increased 25.1% in ABA treated leaf tissue. ZEA increased 35.7% in ABA treated leaf tissue. NEO increased 30.8% in the leaf tissue of ABA treated tomato plants.

In both genotypes, total carotenoids increased in plant treated with ABA in the nutrient solution when compared to the control. Total carotenoids increased significantly in 'MicroTina' tomato leaves **(Table 2.1).** Leaves of 'MicroGold' had an increase in total carotenoids in treated tomato plants **(Table 2.2).** Similarly, total leaf tissue chlorophyll pigments increased in both 'MicroTina' and 'MicroGold' tomato plants. Specifically, Chl *a* increased 40.4%; while Chl *b* increased 27.0% in 'MicroTina' treated tomato plants **(Table 2.3).** Additionally, Chl *a* and Chl *b*

in ‘MicroGold’ tomato leaves increased by 39.0 and 24.9% increase, respectively **(Table 2.4).** Therefore, the results indicate that ABA treatment applications increase carotenoid and chlorophyll pigments compared to the control treatments with no ABA.

Impact of ABA on tomato fruit carotenoids. In ‘MicroTina’ tomato fruit, there was an increase in LYCO concentrations **(Table 2.5).** The concentrations of LYCO increased by 35.5% when comparing ABA treatments concentrations to the control treatment with 0.0 mg·L-1 of ABA. In contrast, there were no significant differences in ‘MicroGold’ fruit tissue carotenoids. This may be due to the low concentrations of BC in ‘MicroGold’ tomato fruit tissue **(Table 2.6).** Additionally, LYCO concentrations in ‘MicroGold’ tomato fruit tissue were below the detection limit of the HPLC (**Table 2.6**). Thus, LYCO could not be measured accurately. There was more BC in ‘MicroTina’ tomato fruit tissue when compared to ‘MicroGold’. However, there were no significant differences in LUT concentrations when comparing the two genotypes (data not shown).

Discussion

Applications of exogenous ABA increased concentrations of tomato leaf carotenoids, such as ZEA, BC, LUT, and NEO. Thus, ABA may indirectly regulate the carotenoid pathway by increasing the activity of key enzymes, such as BC hydroxylase and phytoene synthase (PSY) (Meier et al., 2011). Under stress conditions such as drought or high salinity, ABA increases, creating a stress response in the plant. The increased activities of these enzymes require an available source of isoprenoid substrates, which leads to the production of carotenoids. Previous research has demonstrated that abiotic stress-induced ABA formation leads to the positive regulation of *PSY3* gene expression. The positive regulation increases PSY activity feeding carotenoids into the pathway for production of ABA (Welsch et al., 2008). In Arabidopsis

(*Arabidopsis thaliana)* seedlings, elevated expression of PSY resulted in increased carotenoids levels (Rodriguez-Villalon et al., 2009). In addition, Du et al. (2010) found that activity of BC hydroxylases from rice (*Oryza sativa*), which was shown to be a rate-limiting step for ABA biosynthesis, can alter the plant resistance to drought and oxidative stress by modulating the levels of xanthophylls and ABA synthesis.

This study also found that application of ABA increased Chl *a* and Chl *b* levels in the tomato leaf tissue. These finding are logical since carotenoids and chlorophylls are derived from the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. Since both carotenoids and chlorophylls are derived from the MEP pathway, ABA may act similarly by increasing the levels of both in leaf tissue. These findings are consistent with previous studies. Pospisilova et al. (1993) found that chlorophylls (*a+b*) and BC concentrations were higher in ABA-treated tobacco plantlets. In addition, applications of ABA to barley seedlings increased total carotenoids, thus protecting PSII against photoinhibition (Ivanov et al., 1995). Previous research has demonstrated that increases in carotenoid pools reduce plant sensitivity to adverse environmental stress conditions (Li et al., 2008). For example, carotenoids are considered to be the main singlet oxygen quenchers in chloroplasts and protect chlorophylls from oxidative damage. (Ramel et al., 2012a) found that the accumulation of different volatile derivatives of BC, such as β-cyclocitral, caused a photooxidative stress signal that induced changes in the expression of a large set of genes identified as singlet oxygen responsive genes. In another study, BC endoperoxide rapidly accumulated during high-light stress, which was correlated with the extent of PSII photoinhibition and the expression of various singlet oxygen marker genes (Ramel et al., 2012b).

These studies support the idea that increased levels of chlorophylls and carotenoids in the leaf tissue, induced by ABA treatments, can increase the antioxidant capacity of plants to abiotic-

induced stress. It may be possible to use ABA as a viable and novel approach to increase plant capacity to combat abiotic-induced stress by increasing leaf carotenoids, such as ZEA and BC, allowing for a better response to abiotic stress. However, the applications of ABA treatments to tomato plants may cause a reduction in growth and yield. These results pose questions regarding why the application of ABA increased some carotenoids and not others. The current study did not answer these questions. Therefore, other research may be needed to identify plausible solutions.

ABA treatments had a significant effect on LYCO concentrations in 'MicroTina' tomato fruit tissue. There was also an increase in fruit BC concentrations. Therefore, ABA demonstrated a positive impact on tomato fruit carotenoids for this genotype. However, ABA treatments had no significant impact on 'MicroGold' fruit carotenoid concentrations. The lack of influence of ABA on 'MicroGold' fruit carotenoids may be due to the very low concentrations of pigments present in the fruit tissues of this genotype. Research had demonstrated that genetic makeup of tomato cultivars determines the concentrations of metabolites in tomato. However, environmental factors also strongly affect the concentrations of metabolites (Brandt et al., 2012, Helyes et al., 2007). Thus, the impact of ABA on tomato fruit carotenoids may be from the triggering of ethylene biosynthesis that usually results from higher concentrations of ABA. Previous research from Buta and Spaulding (1994) found that the highest levels of tomato fruit ABA occurred at the pink stage (40 d), followed by a significant decline during subsequent ripening stages. They demonstrated that as the fruit ripened, ethylene concentrations increased while ABA levels decreased, which may lead to an increase in carotenoid production in the ripening fruit tissue. Other studies have also demonstrated that decreases in endogenous ABA resulted in increases of carotenoid concentrations. Decreases in ABA led to increases in

ethylene production by increasing transcription of genes related to the synthesis of ethylene during tomato fruit ripening (Sun et al., 2012). Therefore, ABA's most important function is in the pre-ripening stage of fruit tissues, when it triggers ethylene production causing an increase in carotenoid production.

This study demonstrated the positive impacts of root tissue ABA applications on tomato leaf pigmentation and fruit tissue carotenoid concentrations. The results showed that ABA increased tomato leaf chlorophylls and carotenoids, and increased tomato fruit LYCO. This means that ABA could potentially regulate carotenoid composition during ripening and may control ethylene production in tomato fruit. One of the implications of this study is that ABA has a positive effect on tomato carotenoids and chlorophylls in the leaf tissue. Increase in carotenoids and chlorophylls in the leaf tissue may improve its antioxidant capacity thus, giving protection to the photosynthetic apparatus under adverse abiotic stress conditions. In addition, the improved antioxidant capacity increases the nutritional value of the tomato fruit. However, the application of ABA treatment may cause a reduction in growth and yield. Thus the benefits of ABA as powerful tool may only be feasible by protecting the plant from oxidative stress factors and increasing nutritional values in the fruit.

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Appendix 2: Tables

Table 2.1. Carotenoid leaf tissue pigments in non-treated and abscisic acid (ABA) treated 'MicroTina' tomato grown in hydroponic nutrient solution[z].

	Concentration (mg/100 g fresh mass)[yx]						
ABA (mg·L^{-1})	**BC**	**LUT**	**ZEA**	**ANTH**	**NEO**	**VIO**	**Total CAR**
0.0	3.33	10.95	0.20	1.61	4.28	1.36	20.52
0.5	6.18	15.43	0.35	1.72	6.17	1.82	30.15
5.0	6.79	16.68	0.63	1.90	6.36	1.31	31.82
10.0	6.65	16.46	0.72	1.91	6.20	1.62	31.29
Contrast Control vs. ABA[w]	*	**	**	ns	*	ns	**

[z] Mean separation of the ABA treatments were not significant therefore ABA treatments were pooled for statistical analysis.

[y]BC = β-carotene; LUT = Lutein; ZEA = Zeaxanthin; ANTH = Antheraxanthin; NEO = Neoxanthin; VIO = Violaxanthin; Total CAR = Total carotenoids.

[x] The standard error of the mean was BC ± 0.65; LUT ± 0.84; ZEA ± 0.12; ANTH ± 0.24; NEO ± 0.44; VIO ± 0.44; Total CAR ± 1.92.

[w] ns, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 2.2. Carotenoid leaf tissue pigments in non-treated and abscisic acid (ABA) treated 'MicroGold' tomato grown in hydroponic nutrient solution[z].

	Concentration (mg/100 g fresh mass)[yx]						
ABA (mg·L^{-1})	**BC**	**LUT**	**ZEA**	**ANTH**	**NEO**	**VIO**	**Total CAR**
0.0	3.23	11.02	0.27	1.39	3.98	1.02	20.54
0.5	5.59	14.54	0.36	1.98	5.64	1.52	29.43
5.0	5.93	14.92	0.45	2.13	5.74	1.43	30.13
10.0	5.26	14.68	0.46	1.90	5.88	1.36	29.00
Contrast							
Control vs. ABA[w]	ns	**	*	ns	**	ns	**

[z] Mean separation of the ABA treatments were not significant therefore ABA treatments were pooled for statistical analysis.

[y]BC = β-carotene; LUT = Lutein; ZEA = Zeaxanthin; ANTH = Antheraxanthin; NEO = Neoxanthin; VIO = Violaxanthin; Total CAR = Total carotenoids.

[x] The standard error of the mean was BC ± 0.61; LUT ± 0.61; ZEA ± 0.08; ANTH ± 0.27; NEO ± 0.31; VIO ± 0.21; Total CAR ± 1.44.

[w] ns, *, and ** indicate nonsignificant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 2.3. Chlorophyll leaf tissue pigments in non-treated and abscisic acid (ABA) treated 'MicroTina' tomato grown in hydroponic nutrient solution[z].

	Concentration (mg/100 g fresh mass)[yx]		
ABA ($mg \cdot L^{-1}$)	**CHLA**	**CHLB**	**Total CHL**
0.0	75.71	40.78	116.49
0.5	124.81	54.21	179.03
5.0	128.04	57.22	185.27
10.0	128.21	56.24	184.45
Contrast Control vs. ABA[w]	***	***	***

[z] Mean separation of the ABA treatments were not significant therefore ABA treatments were pooled for statistical analysis.

[y] CHLA = Chlorophyll *a*; CHLB = Chlorophyll *b*; Total CHL = Total chlorophyll..

[x] The standard error of the mean was CHLA ± 11.94; CHLB ± 3.09; Total CHL ± 14.57.

[w] *** indicate significant at $P \leq 0.001$.

Table 2.4. Chlorophyll leaf tissue pigments in non-treated and abscisic acid (ABA) treated 'MicroGold' tomato grown in hydroponic nutrient solution[z].

	Concentration (mg/100 g fresh mass)[yx]		
ABA ($mg \cdot L^{-1}$)	**CHLA**	**CHLB**	**Total CHL**
0.0	86.43	43.90	130.33
0.5	142.24	57.34	199.57
5.0	144.50	58.89	203.39
10.0	138.40	59.18	197.58
Contrast Control vs. ABA[w]	***	***	***

[z] Mean separation of the ABA treatments were not significant therefore ABA treatments were pooled for statistical analysis.

[y] CHLA = Chlorophyll *a*; CHLB = Chlorophyll *b*; Total CHL = Total chlorophyll.

[x] The standard error of the mean was CHLA ± 10.26; CHLB ± 2.48; Total CHL ± 12.51.

[w] *** indicate significant at $P \leq 0.001$.

Table 2.5. Carotenoid fruit tissue pigments in non-treated and abscisic acid (ABA) treated 'MicroTina' tomato grown in hydroponic nutrient solution[z].

	Concentration (mg/100 g fresh mass)[yx]		
ABA ($mg \cdot L^{-1}$)	**BC**	**LUT**	**LYCO**
0.0	0.189	0.164	2.530
0.5	0.230	0.164	3.908
5.0	0.213	0.178	3.281
10.0	0.281	0.223	4.570
Contrast Control vs. ABA[w]	ns	ns	**

[z] Mean separation of the ABA treatments were not significant therefore ABA treatments were pooled for statistical analysis.

[y]BC = β-carotene; LUT = Lutein; LYCO = Lycopene.

[x] The standard error of the mean was BC ± 0.105; LUT ± 0.076; LYCO ± 0.631.

[w] ns and ** indicate nonsignificant or significant at $P \leq 0.01$.

Table 2.6. Carotenoid fruit tissue pigments in non-treated and abscisic acid (ABA) treated 'MicroGold' tomato grown in hydroponic nutrient solution[x].

	Concentration (mg/100 g fresh mass)[yz]		
ABA ($mg \cdot L^{-1}$)	**BC**	**LUT**	**LYCO[w]**
0.0	0.016	0.176	BDL
0.5	0.017	0.093	BDL
5.0	0.007	0.131	BDL
10.0	0.012	0.134	BDL
Contrast			
Control vs. ABA[v]	ns	ns	na

[x] Mean separation of the ABA treatments were not significant therefore ABA treatments were pooled for statistical analysis.

[y]BC = β-carotene; LUT = Lutein; LYCO = Lycopene.

[z] The standard error of the mean was BC ± 0.004; LUT ± 0.047.

[w] BDL-Below detection limit; na-Not available

[v] ns indicate nonsignificant at $P \leq 0.05$.

Chapter 4
Foliar Applications of Abscisic Acid Decrease the Incidence Blossom-end Rot in Tomato Fruit

Abstract

Various environmental stress factors, such as drought and high relative humidity, can cause calcium (Ca) deficiency and lead to physiological disorders such as blossom-end rot (BER) in tomato (*Solanum lycopersicum*) fruit. Abscisic acid (ABA) triggers whole-plant and fruit-specific mechanisms to increase fruit Ca uptake and prevent BER development. The objective of this study was to examine the effects of foliar application of ABA and hydroponic Ca treatments in fertilizer solution on localized deficiency of Ca causing BER in tomato fruit. Seeds of 'Mt. Fresh Plus' tomato were grown in the greenhouse at 25/20 °C (day/night) under a 16 h photoperiod. Plants were treated with ABA applications weekly. Ca treatments were applied at three different treatment levels of 60, 90, or 180 $mg \cdot L^{-1}$. Ca treatments were applied to the plants via irrigation lines. ABA treatments were applied as a foliar spray at concentrations of 0.0 and 500 $mg \cdot L^{-1}$. ABA spray treatments were applied each week until dripping from the foliage. Fruit tissue was harvested at red ripe maturity and evaluated for yield, BER and Ca concentrations. Leaves were harvested at time of fruit and were analyzed for Ca concentrations. The applications of 500 $mg \cdot L^{-1}$ ABA foliar spray treatments decreased the incidence of BER in tomato fruit tissue and the weight per fruit of the BER tomato fruit grown under treatments of low Ca. Furthermore, ABA treatment increased Ca concentrations in the fruit tissue. Thus, applications of 500 $mg \cdot L^{-1}$ ABA foliar spray treatments may positively regulate the partitioning of Ca into fruit tissue.

Keywords*: Calcium, Partitioning, Stress*

Introduction

Sufficient calcium (Ca) uptake depends on the flow of water with the transpiration stream in the xylem tissue. Various environmental stress factors, such as drought and high relative

humidity, can disrupt transpiration water movement. Disruption of acquisition can cause Ca deficiency, which leads to physiological disorders such as blossom end rot (BER) in tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) fruit. BER results from cell wall plasma membrane breakdown caused by insufficient levels of free Ca in the apoplast. Calcium is required for proper membrane formation due to its ability to bind to carboxylate groups of phospholipids and proteins at membrane surfaces (Suzuki et al., 2003). This disorder causes cells near the blossom end of the fruit to die, giving the tissue a water-soaked appearance that can cover half of the fruit surface (Abdal and Suleiman, 2005).

BER primarily occurs because of the local deficiency of Ca in the distal end of tomato fruit (Adams and Ho, 1993). Although it can be caused by inadequate supply of Ca in the root zone, it frequently occurs when substrate moisture and Ca content are at adequate levels for normal plant growth. In this situation, the most likely causes of this physiological disorder are poor Ca uptake by the roots and insufficient distribution of Ca to the fruit during a period of high Ca demand. Deficiency seldom arises because of a lack of Ca supply to plant roots. It is more frequently explained by problems arising from internal distribution of Ca and its allocation among mature and growing regions of the plant. There is only a very small amount of free Ca in the cytoplasm available for movement from one cell to another. These small fluxes in Ca concentration are sufficient to function as secondary messengers in cellular communication (Marschner, 1995), but are quite insufficient to provide an effective means of moving Ca amounts adequate to support cell growth elsewhere in the plant.

Plant growth and development are regulated by internal and external signals. One important regulator that coordinates these changes is the hormone abscisic acid (ABA). ABA can trigger oscillation in the cytosolic Ca concentration, which is then perceived by Ca binding

proteins to initiate a series of signaling cascades that control many physiological processes, including adaptation to environmental stress (Guo et al., 2002). Recent studies have demonstrated that ABA triggered whole-plant and fruit-specific mechanisms to increase fruit Ca uptake and prevent BER development. For example, de Freitas (2011) found that ABA induced lower leaf stomatal conductance and water loss, which resulted in increased Ca concentrations in the fruit and lower Ca levels in the leaves. The role of ABA as a stress hormone makes it an attractive and novel treatment to improve Ca uptake and distribution within tomato fruit, which could increase Ca concentrations in situations where Ca distribution into the fruit is low.

The objective of this study was to examine the effects of foliar application of ABA on localized deficiency of Ca causing blossom end rot in tomato fruit. In addition, we examined how foliar spray ABA applications at different Ca fertility levels affected the partitioning of Ca between eaves and fruit of tomato plants, especially in the distal tissue.

Materials and Methods

*Plant Culture and Harvest***.** Seeds of 'Mountain Fresh Plus' tomato (Johnny's Selected Seed, Waterville, ME) were sown into Pro-Mix BX soilless medium (Premier Tech Horticulture, Québec, Canada) and germinated in the greenhouse (Knoxville, TN; 35°N Lat.) at 25/20 °C (day/night) under a 16 h supplemental light at an average of 850 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At 30 days after seeding, the plantlets were transferred to 11-L Dutch pots (Tek Supply, Dyersville, IA) filled with Sunshine® Pro Soil Conditioner (Sungro Horticulture, Agawam, MA). Tomato plants were grown hydroponically with a tomato fertilization program developed at the University of Tennessee. Elemental concentrations of the nutrient solutions were ($mg \cdot L^{-1}$): nitrogen (N; 180), phosphorus (P; 93.0), potassium (K; 203.3), magnesium (Mg; 48.6), sulfur (S; 96.3), iron (Fe; 1.0), boron (B; 0.25), manganese (Mn; 0.25), zinc (Zn; 0.025), copper (Cu; 0.01), and

molybdenum (Mo; 0.005). Two identical experiments were conducted. The first experiment was completed in fall 2011 and the second in spring 2012. The experimental design was a randomized complete block with a 3 x 2 factorial, which consisted of six blocks and two replications of each treatment with individual pots representing an experimental unit. Calcium was applied at 60, 90, or 180 $mg \cdot L^{-1}$. Calcium treatments were applied to the plants via irrigation lines. ABA treatments were applied as a foliar spray at concentrations of 0.0 and 500 $mg \cdot L^{-1}$. ABA spray treatments were applied each week from anthesis to last fruit harvest. The spray treatments were applied until dripping from the foliage. No ABA reached the root zone. Fruit tissues were harvested 84-90 days after seeding. Subsequently, fruit were sorted by the use of USDA tomato color for red ripe (U.S.D.A., 1975) and size classification into extra-large, large, medium and small fruit (U.S.D.A., 2007). Tomato fruit with BER were categorized separately. Fruit from each treatment were separated by replication and were counted for yield. At least three fruit from three clusters for each experimental unit were separated into proximal and distal fractions in preparation for elemental nutrient analysis. Harvested fruit samples were stored at -80 °C prior to analysis. Leaf samples were taken from two clusters per plant at the last harvest for analysis of mineral elements.

Elemental Nutrient Determination. Nutrient analysis was conducted as described by Barickman et al. (In Press; Journal of the American Society for Horticultural Science). Briefly, analysis of samples was performed using a 5.0 g subsample of fresh fruit tissue, which was combined with 10 ml of 70% HNO_3 and digested in a microwave digestion unit (Model: Ethos, Milestone Inc., Shelton, CT). The microwave temperature was ramped to 140 °C for 5 min at 1000W and 2000 kPa, followed by an increase to 210 °C for 10 min at 1000W and 3000 kPa. Furthermore, microwave temperature was held at 210 °C for 10 min at 1000W and 4000 kPa and cooled for 10

min at 0W and 2000 kPa. The digest was then allowed to cool to 20 °C. A 100 µL subsample of the digest was diluted with 9900 µL of ICP-MS matrix consisting of 2% HNO_3 and 0.5% HCl (v/v). Leaves were collected and triple rinsed with de-ionized water and dried for 48 h in a forced air oven (model large; Fisher Scientific, Atlanta, GA) at 65 °C. Dried samples were ground to homogeneity using liquid nitrogen, and 0.5 g sub-samples were weighed for analysis. Samples were microwave digested, and a 100 µL aliquot of the digested sample was diluted with 9900 µL of ICP-MS matrix for analysis. Nutrient analysis was conducted using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies, Inc., Wilmington, DE). The ICP-MS system was equipped with an octapole collision/reaction cell, Agilent 7500 ICP-MS ChemStation software, a Micromist nebulizer, a water-cooled quartz spray chamber, and a CETAC (ASX-510, CETAC Inc., Omaha, NE) autosampler. The instrument was optimized daily in terms of sensitivity (Li, Y, Tl), level of oxide (Ce), and doubly charged ion (Ce) using a tuning solution containing 10 µg l^{-1} of Li, Y, Tl, Ce, and Co in a 2% HNO_3/0.5% HCl (v/v) matrix. Tissue nutrient concentrations are expressed on a dry weight (DW) basis.

Statistical Analysis. There were no statistical differences in the data between the two experiments. Therefore, data were pooled and analyzed together for treatment means. The experimental design was a randomized complete block in a factorial arrangement. The three Ca treatment concentrations were subdivided into ABA and non-ABA treated plants. Three separate clusters of tomato fruits were collected within each factorial arrangement and measured for total tissue Ca in the proximal and distal portions. Furthermore, leaves from the three clusters were analyzed for total tissue Ca. Statistical analysis of data was performed using SAS (version 9.3; SAS Institute, Cary, NC). Data were analyzed using the PROC GLIMMIXED analysis of variance.

Results

The statistical analysis of the results indicated that there was no interaction between ABA and Ca treatments on tomato leaf tissue. Therefore, the following results are presented separately for ABA and Ca treatment effects. The application of 500 $mg \cdot L^{-1}$ ABA foliar spray treatment significantly decreased Ca in the leaf tissue (**Table 3.1**). Calcium ranged from 25.38 $mg \cdot g^{-1}$ dry mass (DM) to 28.42 $mg \cdot g^{-1}$ (milligrams per gram) DM when comparing the 500 $mg \cdot L^{-1}$ ABA foliar spray treatment to the control treatment. Calcium was 10.7% less in control leaf tissue than the tissue of ABA treated plants. Notably, Ca content decreased, from 35.01 to 21.18 $mg \cdot L^{-1}$, in the leaf tissue from the optimum Ca fertilizer concentration of 180 $mg \cdot L^{-1}$ to 60 $mg \cdot L^{-1}$ treatments, respectively (**Table 3.2**). Thus, decreasing Ca treatments from the 180 $mg \cdot L^{-1}$ to the Ca deficient treatment of 60 $mg \cdot L^{-1}$ decreased Ca concentration in the leaf tissue by 39.5%.

The statistical analysis of the results indicated that there was an interaction between ABA and Ca treatments in tomato fruit tissue (**Table 3.3**). Calcium concentrations in tomato fruit tissue were the highest with ABA treatment application and optimum Ca treatment (**Table 3.3**). The Ca concentration increased from 5.37 to 7.23 $mg \cdot g^{-1}$ fresh mass (FM) when comparing the 180 $mg \cdot L^{-1}$ Ca and 0.0 $mg \cdot L^{-1}$ ABA treatment to the combination treatment of 180 $mg \cdot L^{-1}$ Ca and 500 $mg \cdot L^{-1}$ ABA treatment. Decreasing Ca treatments of 60 and 90 $mg \cdot L^{-1}$ decreased Ca concentrations in tomato fruit tissue when compared to the 180 $mg \cdot L^{-1}$ Ca treatment (**Table 3.3**). Calcium concentrations decreased from 5.37 $mg \cdot g^{-1}$ FM in the 180 $mg \cdot L^{-1}$ Ca and 0.0 $mg \cdot L^{-1}$ ABA treatment to 3.63 and 3.30 $mg \cdot g^{-1}$ FM in 90 and 60 $mg \cdot L^{-1}$ Ca treatments and 0.0 $mg \cdot L^{-1}$ ABA treatment, respectively. Thus, the results demonstrated that the application of ABA increased Ca in the fruit tissue as Ca treatments were decreased from the optimum. The fruit Ca concentrations increased from 3.63 $mg \cdot g^{-1}$ FM in the combination 90 $mg \cdot L^{-1}$ Ca and 0.0 $mg \cdot L^{-1}$

ABA treatments to 4.85 $mg \cdot g^{-1}$ FM when adding the 500 $mg \cdot L^{-1}$ ABA treatment. Similarly, Ca concentrations increased from 3.30 $mg \cdot g^{-1}$ FM in the combination of 60 $mg \cdot L^{-1}$ Ca and 0.0 $mg \cdot L^{-1}$ ABA treatment to 3.71 $mg \cdot g^{-1}$ FM when adding the 500 $mg \cdot L^{-1}$ ABA treatment.

Calcium concentration within the fruit tissue was higher in the proximal tissue (8.05 $mg \cdot g^{-1}$ FM) than the distal tissue (4.55 $mg \cdot g^{-1}$ FM) in plants treated with 180 $mg \cdot L^{-1}$ Ca treatment. Calcium concentrations in tomato fruit proximal and distal tissue decreased with decreasing Ca treatments (**Table 3.4**). Calcium concentrations in tomato fruit proximal tissue decreased from 8.05 $mg \cdot g^{-1}$ FM in the 180 $mg \cdot L^{-1}$ Ca and 0.0 $mg \cdot L^{-1}$ ABA treatment to 5.35 and 4.65 $mg \cdot g^{-1}$ FM in 90 and 60 $mg \cdot L^{-1}$ and 0.0 $mg \cdot L^{-1}$ ABA treatment, respectively. Calcium concentrations in tomato fruit distal tissue decreased from 4.55 $mg \cdot g^{-1}$ FM in the control treatment of 180 $mg \cdot L^{-1}$ to 3.14 and 2.37 $mg \cdot g^{-1}$ FM in the 90 and 60 $mg \cdot L^{-1}$, respectively. ABA treatments had statistically significant effects on Ca concentrations in tomato fruit proximal and distal tissue (**Table 3.5**). Treatment with 500 $mg \cdot L^{-1}$ ABA significantly increased Ca from 6.48 $mg \cdot g^{-1}$ DW in tomato fruit proximal tissue compared to 5.53 $mg \cdot g^{-1}$ DW in the untreated plant tissue. Calcium concentrations also significantly increased in the tomato fruit distal tissue from 2.65 $mg \cdot g^{-1}$ DW in the control treatment to 4.05 $mg \cdot g^{-1}$ DW in the 500 $mg \cdot L^{-1}$ ABA treatment. This accounted for an increase of 14.7 % and 34.6 % in proximal and distal fruit tissue, respectively.

The statistical analysis of the results indicated that there was an interaction between ABA and Ca treatments in tomato fruit tissue. The incidence of BER in tomato fruit tissue was lowest with ABA treatments and optimum Ca treatment of 180 $mg \cdot L^{-1}$. The incidence of BER decreased by 86.2% from the combination of 180 $mg \cdot L^{-1}$ Ca and 0.0 $mg \cdot L^{-1}$ ABA treatment to the combination treatment of 180 $mg \cdot L^{-1}$ Ca and 500 $mg \cdot L^{-1}$ ABA treatment (**Table 3.6**).

Decreasing Ca treatment concentrations from the optimum 180 $mg \cdot L^{-1}$ to 90 and 60 $mg \cdot L^{-1}$ increased the incidence of BER by 62.3% and 80.1%, respectively. Thus, ABA treatments in addition to Ca treatments decreased incidence of BER by 26.6% (**Table 3.6**). The BER yield in tomato fruit tissue was the lowest with ABA treatments and optimum Ca treatment of 180 $mg \cdot L^{-1}$ (**Table 3.6**). The yield of BER fruit decreased by 92.9% from the combination of 180 $mg \cdot L^{-1}$ Ca and 0.0 $mg \cdot L^{-1}$ ABA treatment to the combination treatment of 180 $mg \cdot L^{-1}$ Ca and 500 $mg \cdot L^{-1}$ ABA treatment. Decreasing Ca treatment concentrations from 180 $mg \cdot L^{-1}$ to 90 and 60 $mg \cdot L^{-1}$ increased the yield of BER by 57.3% and 74.7%, respectively (**Table 3.6**). The statistical analysis of the results indicated there was an interaction between ABA and Ca treatment concentrations. Adding the ABA treatment to the decreased Ca treatment concentrations of 90 $mg \cdot L^{-1}$ decreased the yield of BER by 46.3% from the untreated ABA treatment. In addition, there were no statistical differences in fruit size and yield for Ca treatments (**Table 3.7**) or ABA treatments (**Table 3.8**).

Discussion

Our results demonstrated that the 500 $mg \cdot L^{-1}$ ABA foliar spray treatments reduced the total leaf Ca concentrations from 28.42 to 25.38 $mg \cdot g^{-1}$ DM. This is in accordance with previous research, which found that leaf transpiration controls the overall Ca uptake into the plant and that leaves passively accrue Ca into the leaf tissue (Ho and White, 2005). The reduction of leaf transpiration under water stress conditions is widely associated with the growth regulator ABA in horticultural crops (Agehara and Leskovar, 2012; Waterland et al., 2010). ABA can also affect evapotranspiration and leaf gas exchange by regulating stomatal conductance in tomato (Astacio and van Iersel, 2011). Previous research has demonstrated that ABA treated plants had higher stem water potential, lower leaf stomatal conductance, lower whole plant water loss, and

decreased total leaf Ca concentrations (de Freitas et al., 2011); indicating that ABA affected leaf transpiration and Ca accumulation in the leaf tissue.

Calcum uptake into the fruit tissue increased with 500 $mg \cdot L^{-1}$ ABA foliar spray treatments. These results indicate that ABA may affect Ca partitioning from the leaf tissue to the fruit tissue by regulating stomatal conductance and transpiration. Previous research demonstrated that ABA treated tomato fruit had higher total fruit Ca concentrations than water treated fruit during late stages of fruit growth and development (de Freitas et al., 2011). These findings suggest that ABA treated fruit uptake Ca when it would normally be greatly decreasing the uptake of Ca into the fruit tissue (Ho and White, 2005). de Freitas et al. (2011) also found that ABA treated plants had lower xylem and Ca flow into the leaves and increased Ca accumulation into the fruit. Our data show similar results regarding the decrease in Ca acclamation into the leaves and increased Ca concentrations in the fruit tissue when treated with ABA. In addition, decreasing Ca treatment concentrations from the optimum of 180 $mg \cdot L^{-1}$ Ca in the fertilizer solution to 60 and 90 $mg \cdot L^{-1}$ decreased Ca concentrations in the fruit tissue. The decrease in Ca accumulation with decreasing Ca in the fertilizer solutions is well documented (Evans and Troxler, 1953; Garate et al., 1991; Hao and Papadopoulos, 2003; Ortiz-Sanchez et al., 2012). Additionally, there was a significant interaction between the two main treatments of Ca and ABA. These results indicate a synergistic effect between the two treatments for the uptake and accumulation of Ca in the fruit tissue. In other words, application of ABA (500 $mg \cdot L^{-1}$) increases the uptake and accumulation of Ca into the fruit tissue for all Ca treatments, especially under limiting Ca fertility of 60 and 90 $mg \cdot L^{-1}$. Thus, the interaction of the ABA and Ca treatments improved the likelihood of a decrease in the BER incidence in tomato fruit tissue.

This study has demonstrated that ABA and Ca treatments affect the distribution of Ca from the leaves to the fruit tissue, decreasing the incidence of BER. In addition, this study looked at the differences in distribution of Ca within the fruit tissue. The proximal end had significantly higher concentration of Ca than the distal end of the fruit tissue. Previous research has indicated that the transport of Ca to the distal tissue may be reduced not only by the lower number of xylem vessels due to environmental stress conditions, but also by the longer pathway by which Ca reaches the cells within the adjacent service area via intercellular transport (Ho et al., 1993). Calcium has more binding sites to interact with through the apoplastic pathway in order to get to the distal tissue, decreasing its concentration along the way. There was also an interaction between Ca treatments in the fertilizer solution and the location of Ca in the fruit tissue. Calcium travels with water flow in the xylem tissue to the fruit, and increasing Ca concentrations in the tissue allows more total Ca to reach the fruit. Therefore, these results demonstrate that increasing Ca in the fertilizer solution increases the total Ca concentrations in both the proximal and distal tissue.

The applications of 500 $mg \cdot L^{-1}$ ABA foliar spray treatments decreased the incidence of BER in tomato fruit tissue and the weight of the BER tomato fruit grown under treatments of low Ca. Furthermore, ABA treatment increased Ca concentrations in the fruit tissue. Thus, applications of 500 $mg \cdot L^{-1}$ ABA foliar spray treatments may positively regulate the partitioning of Ca into the fruit tissue. This data indicates a possible mechanism by which ABA foliar spray treatments increase Ca concentrations in the distal tissue of tomato fruit. ABA negatively affects stomatal conductance by decreasing transpiration and inhibiting leaf expansion (Agehara and Leskovar, 2012), and may affect the partitioning of Ca into the leaves. Decreased transpiration of leaves may lead to increased Ca in the fruit tissue. ABA could indirectly be closing the

stomatas, cutting off the transpiration stream that carries Ca into the leaf tissue and thus allowing Ca to be partitioned into the fruit tissue. Therefore, the higher total Ca accumulation in the fruit tissue contributes to higher total Ca concentrations in the blossom end tissue of the tomato fruit. White and Broadley (2003), reported that higher total Ca in the tomato fruit may contribute to higher free Ca concentrations in the apoplast. The results of having a larger free Ca content in the apoplast of fruit tissue could contribute to a better cell wall and membrane structure resulting in lower membrane leakage and the decrease in BER in ABA treated tomatoes in the current study. Therefore, the 500 $mg \cdot L^{-1}$ ABA foliar spray treatments appear to indirectly inhibit the incidence of BER by increasing total Ca in tomato fruit tissue.

Although research on BER has been extensive over the last three decades, this deficiency can still be a major problem for the fresh and processed tomato industry. Increasing pressures from climate change and environmental stresses can lead to BER, decreasing fruit yield and fruit quality. In addition, treating BER is becoming more difficult during production cycles because of the timing issues of management efforts with the Ca fruit demands. The susceptibility of tomato plants to BER may be related to the inefficient uptake of Ca from the soil solution and the inability to transport it effectively into the distal tissue of the fruit. Our results demonstrate that, despite reducing Ca uptake in the leaf tissue, the 500 $mg \cdot L^{-1}$ ABA foliar spray treatments significantly increased Ca in fruit tissue and reduced the incidence of BER development in tomato fruit. Thus, ABA could be an alternative treatment to increase Ca uptake into fruit and distribution into the distal tissue of the fruit relative to leaf uptake.

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Appendix 3: Tables

Table 3.1. Calcium concentrations in leaves of 'Mt. Fresh Plus' tomato grown hydroponically in a greenhouse and treated with foliar applications of s-ABA.

ABA ($mg \cdot L^{-1}$)	Leaf Ca ($mg \cdot g^{-1}$ dry weight)[a]
0	28.42
500	25.38
P Value[b]	**

[a] The SE of the mean for Leaf Ca ± 0.71;

[b] ** indicates significance at $P \leq 0.01$ level.

Table 3.2. Calcium concentraions in leaves of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

Ca ($mg \cdot L^{-1}$)	Leaf Ca ($mg \cdot g^{-1}$ dry weight)[a]
60	21.18
90	24.44
180	35.01
P Value[b]	***

[a] The SE of the mean for Leaf Ca ± 0.87.

[b] *** indicates significance at $P \leq 0.001$.

Table 3.3. Calcium concentrations in the fruit tissue of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with a foliar spray of s-ABA and Ca in the hydroponic fertilizer solution.

Ca ($mg \cdot L^{-1}$)	ABA ($mg \cdot L^{-1}$)	Ca Fruit ($mg \cdot g^{-1}$ dry weight)[a]
60	**0**	3.30
90	**0**	3.63
180	**0**	5.37
60	**500**	3.71
90	**500**	4.85
180	**500**	7.23
	P Value[b]	*

[a] The SE of the mean for Fruit Ca ± 0.29.

[b] * indicates significance at $P \leq 0.05$.

Table 3.4. Calcium concentrations in the proximal and distal fruit tissue of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

Ca ($mg \cdot L^{-1}$)	Location	Ca Fruit ($mg \cdot g^{-1}$ dry weight)[a]
60	**Distal**	2.37
90	**Distal**	3.14
180	**Distal**	4.55
60	**Proximal**	4.65
90	**Proximal**	5.35
180	**Proximal**	8.05
	P Value[b]	*

[a] The SE of the mean for Fruit Ca ± 0.29.

[b] * indicates significance at $P \leq 0.05$.

Table 3.5. Calcium concentrations in the proximal and distal fruit tissue of 'Mt. Fresh Plus' tomato grown in a greenhouse hydroponically and treated with a foliar spray of s-ABA.

ABA ($mg \cdot L^{-1}$)	Location	Ca Fruit ($mg \cdot g^{-1}$ dry weight)[a]
500	**Proximal**	6.48 a
0	**Proximal**	5.53 b
500	**Distal**	4.05 c
0	**Distal**	2.65 d
	LSD[b]	0.65

[a] The SE of the mean for fruit Ca ± 0.23.

[b] LSD indicates significance or non-significance at $P \leq 0.05$.

Table 3.6. Blossom end-rot in the fruit tissue and yield of blossom end-rot fruit of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with a foliar spray of s-ABA and Ca in the hydroponic fertilizer solution.

		Blossom-end rot per plant[a]	
Ca ($mg \cdot L^{-1}$)	**ABA ($mg \cdot L^{-1}$)**	**% BER**	**Total BER Fruit**
60	**0**	27.41	3.56
90	**0**	14.47	2.31
180	**0**	5.45	0.86
60	**500**	7.98	0.92
90	**500**	4.00	0.47
180	**500**	0.75	0.11
	P Value[b]	**	**

[a] The SE of the mean for % BER ± 2.32Total BER Fruit ± 0.38.

[b] ** indicates significance at $P \leq 0.01$.

Table 3.7. Number of tomato fruit by classification and yield of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated Ca in the hydroponic fertilizer solution. The classification and yield do not include tomato fruit that had blossom-end rot.

	Number of fruit and yield (g) per cluster[a]							
Ca	**XL**	**XL Wt**	**Large**	**Large Wt**	**Medium**	**Medium Wt**	**Small**	**Small Wt**
60	1.91	406.54	1.25	240.50	1.20	158.25	3.26	148.94
90	1.98	401.51	1.29	202.07	1.35	157.98	3.59	158.22
180	2.05	409.71	1.52	198.48	1.37	136.75	3.27	174.67
P Value[b]	ns	ns	ns	ns	ns	ns	ns	ns

[a] The SE of the mean for XL ± 0.30; XL Wt ± 55.23; Large ± 0.21; Large Wt ± 29.75; Medium ± 0.25; Medium Wt ± 24.23; Small ± 0.62; Small Wt ± 24.15.

[b] ns indicates non-significant at $P \leq 0.05$.

Table 3.8. Number of tomato fruit by classification and yield of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with a foliar spray of s-ABA. The classification and yield do not include tomato fruit that had blossom-end rot.

	Number of fruit and yield (g) per cluster[a]							
ABA	**XL**	**XL Wt**	**Large**	**Large Wt**	**Medium**	**Medium Wt**	**Small**	**Small Wt**
0	1.90	386.29	1.26	195.26	1.35	156.35	3.81	171.48
500	2.06	425.55	1.45	232.10	1.27	145.64	2.94	149.74
P Value[b]	ns	ns	ns	ns	ns	ns	ns	ns

[a] The SE of the mean for Xl ± 0.25; XL Wt ± 47.48; Large ± 0.17; Large Wt ± 24.64; Medium ± 0.19; Medium Wt ± 22.32; Small ± 0.52; Small Wt ± 20.27.

[b] ns indicates non-significant at $P \leq 0.05$.

Chapter 5
Abscisic Acid Improve Tomato Fruit Quality by Increasing Soluble Sugar Concentrations

Abstract

Abscisic acid (ABA) plays a crucial role in fruit maturation and senescence and is considered as the other ripening control factor other than ethylene. Research also demonstrates that calcium (Ca) treatments may increase fruit quality and storability. Important components in ripening fruit are soluble sugars, which make the fruit sweeter and carotenoids, important flavor compounds in ripened fruit. The purpose of this study was to examine the effects of foliar applications of ABA and Ca fertilizer treatments (individually and in combination) on tomato (*Solanum lycopersicum*) leaf chlorophylls and carotenoids and on fruit carotenoids, and soluble sugar concentrations. Seeds of 'Mt. Fresh Plus' tomato were grown in the greenhouse at 25/20 °C (day/night) under a 16 h photoperiod. Plants were treated with ABA applications weekly. Ca treatments were applied at three different treatment levels of 60, 90, and 180 $mg \cdot L^{-1}$. Ca treatments were applied to the plants via the irrigation lines. ABA treatments were applied as a foliar spray at concentrations of 0.0 and 500 $mg \cdot L^{-1}$. ABA spray treatments were applied until dripping from the foliage once weekly. Fruit tissue was harvested at red ripe maturity and evaluated for carotenoids and soluble sugars. Leaves were harvested at time of fruit and were analyzed for chlorophylls and carotenoids. Results indicate that there were no synergistic effects between ABA and Ca treatments. Foliar spray treatment of 500 $mg \cdot L^{-1}$ ABA increased zeaxanthin (ZEA) and β-carotene (BC) in tomato leaf tissue. Increases in Ca fertilizer treatments significantly decreased tomato leaf violaxanthin (VIO), but had no effect on other carotenoids. Foliar application of ABA did not affect chlorophylls (Chl), but the higher Ca treatments did decrease Chl *a* in the leaf tissue. The application of 500 $mg \cdot L^{-1}$ ABA foliar spray treatments significantly increased glucose and fructose concentrations in tomato fruit tissue. Foliar application of ABA treatments can increase overall tomato leaf chlorophyll and carotenoid content and fruit quality.

Keywords*: Carotenoids, Chlorophylls, Fructose, Glucose, Calcium*

Introduction

One important hormone that coordinates plant growth and development in response to the environment is abscisic acid (ABA). This metabolite is an isoprenoid derived from a common five-carbon precursor, isopentenyl. ABA biosynthesis takes place in chloroplasts and other plastids in the roots and leaves via terpenoids. It is formed by the cleavage of C_{40} carotenoids derived from the non-mevalonate pathway (Hirai et al., 1986; Kasahara et al., 2004; Milborrow and Lee, 1998). This pathway plays an essential role in creation of chloroplast isoprenoids, such as carotenoids, phytol, and terpenoids, compounds that are essential for ABA creation (Sponsel, 2002).

ABA plays a crucial role in fruit maturation and senescence (Zhang et al., 2009). Besides ethylene, it can be considered as the other ripening control factor. Studies have found that while the ABA content is very low in unripe fruit, it increases during fruit ripening in both climacteric (Buesa et al., 1994; Vendrell and Buesa, 1989) and non-climacteric (Inaba et al., 1976; Kojima, 1996; Kondo and Inoue, 1997; Kondo and Tomiyama, 1998) fruits. In addition, Bastias et al. (2011) found that as a ripening control factor ABA increased levels of sugars in tomato (*Lycopersicon esculentum*) fruit by increasing expression of genes encoding a vacuolar invertase and a sucrose synthase. High levels of soluble sugars are important because they are essential components of tomato fruit quality. Increasing sugar levels, specifically glucose and fructose, create a higher ratio of sugar to organic acids making the fruit sweeter and tastier (Patanè et al., 2011; Tardieu et al., 1992). Glucose and fructose are created by catabolism of sucrose as the tomato fruit ripens. This process is conducted by activity of invertase, which is an enzyme that catalyzes the hydrolysis of sucrose.

Other important compounds in tomato fruit are carotenoids. Carotenoids are powerful antioxidants linked to inhibiting cancers such as prostate (Giovannucci et al., 1995), skin

(Gonzalez et al., 2003) and colon (Slattery et al., 1999). Additionally, carotenoids contribute to key flavor compounds in ripened fruit. The carotenoids of fully ripened tomatoes are 50-80% lycopene (LYCO) and 2-7% β-carotene (BC). In general, carotenoids are responsible for creating ABA. However, studies have shown that exogenous applications of ABA can increase chlorophylls and carotenoids in tomato leaf tissue, thus stimulating the antioxidant defense system in plants (Barickman et al., In Press; Jiang and Zhang, 2001).

In addition to being a ripening factor, ABA contributes to fruit development as a key hormone that mediates plant adaptation to environmental stresses, such as drought (Bray, 1997; Seo et al., 2012), salinity (Asensi-Fabado and Munne-Bosch, 2011) and cold stress (Nayyar et al., 2005). For example, when soil dries ABA is produced and transported from plant roots to the leaves (Sauter et al., 2001) where it helps close stomata and induce many stress-related gene products. The plant responds to ABA signals by regulating ABA breakdown, transporting stress-related compounds, compartmentalizing metabolites, or changing sensitivity to the environment (Addicott and Carns, 1983; Zeevaart and Creelman, 1988). Thus, ABA can be considered a plant stress and developmental hormone, which is involved in many different aspects of growth and development of plants in adverse environments.

Calcium (Ca) treatment application to plants has been well documented for fruit quality, especially for postharvest applications (Aghdam et al., 2013; Hernandez-Munoz et al., 2006; Luna-Guzman et al., 1999). For example, $CaCl_2$ solutions can maintain fruit firmness during storage (Sams et al., 1993). Studies have also indicated that fruit tissue infiltrated with Ca treatment mixtures contained more Ca, had prolonged storage ability, resisted fungal decay and were firmer than controls (Conway and Sams, 1983, Conway and Sams, 1987, Conway et al., 1994, Conway et al., 2002). However, Ca sprays did not affect fruit soluble sugars, titratable

acidity and starch index (Wojcik and Borowik, 2013). Therefore, research demonstrated that Ca treatments might increase fruit quality and storability.

Previous research has demonstrated that ABA positively affected the production of carotenoids in leaf and fruit tissue of tomato plants (Barickman et al., In Press). This study expands on how ABA influences the production of chlorophylls and carotenoids in leaf and fruit tissue of tomato plants. This study also examined how ABA may influence tomato fruit quality parameters, specifically soluble sugar content. In addition, studies have demonstrated that increasing concentrations of Ca treatments applied to tomato plants negatively affect carotenoid concentrations in tomato fruits (Paiva et al., 1998). Previous research has not examined the effects of ABA and Ca treatments and their combination on tomato fruit quality. Therefore, the purpose of this study was to examine the effects of foliar applications of ABA and Ca fertilizer treatments on greenhouse tomato leaf chlorophylls and carotenoids, fruit carotenoids and soluble sugar concentrations individually and in combination.

Materials and Methods

*Plant Culture and Harvest***.** Seeds of 'Mountain Fresh Plus' tomato (Johnny's Selected Seed, Waterville, ME) were sown into Pro-Mix BX soilless medium (Premier Tech Horticulture, Québec, Canada) and germinated in the greenhouse conditions (Knoxville, TN; 35°N Lat.) at 25/20 °C (day/night) under a 16 h supplemental light at an average of 925 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At 30 days after seeding, the plantlets were transferred to 11-L Dutch pots (Tek Supply, Dyersville, IA) filled with Sunshine® Pro Soil Conditioner (Sungro Horticulture, Agawam, MA). Tomato plants were grown hydroponically with a tomato fertilization program developed at the University of Tennessee. Elemental concentrations of the nutrient solutions were ($mg \cdot L^{-1}$): nitrogen (N; 180), phosphorus (P; 93.0), potassium (K; 203.3), magnesium (Mg; 48.6), sulfur (S; 96.3), iron (Fe;

1.0), boron (B; 0.25), manganese (Mn; 0.25), zinc (Zn; 0.025), copper (Cu; 0.01), and molybdenum (Mo; 0.005). There were two identical experiments conducted. The first experiment was done in fall 2011 and repeated in spring 2012. Experimental design was a randomized complete block with a 3 x 2 factorial, which consisted of six blocks and two replications of each treatment with individual pots representing an experimental unit. Ca was applied at 60, 90, and 180 $mg \cdot L^{-1}$. Ca treatments were applied to the plants via the irrigation lines. ABA treatments were applied as a foliar spray at concentrations of 0.0 and 500 $mg \cdot L^{-1}$. ABA spray treatments were applied until dripping from the foliage once weekly. Fruit tissues were harvested 84-90 days after seeding. Subsequently, fruit were sorted by the use of USDA tomato color for red ripe (U.S.D.A., 1975) and size classification into extra-large, large, medium and small (U.S.D.A., 2007). Tomato fruit with BER were categorized separately. Fruit from each treatment were separated by replication and weighed for yield. At least three fruit from two clusters for each experimental unit were juiced and prepared for soluble sugar and carotenoid analyses. Harvested fruit samples were stored at -80 °C prior to analysis. Leaf samples were taken from each of the two clusters at the last harvest for analysis of mineral elements, carotenoids, and chlorophylls.

Fruit Carotenoid Tissue Determination. Carotenoids were extracted from fresh-frozen ripe fruit tissues and quantified according to the methods of Emenhiser et al. (1996) with slight modifications from Barickman et al. (In Press). An Agilent 1200 series HPLC unit with a photodiode array detector (Agilent Technologies, Palo Alto, CA) was used for pigment separation and followed the method from Barickman et al. (In Press).

*Leaf Carotenoid and Chlorophyll Determination***.** The frozen tomato leaf samples were lyophilized in a programmed freeze dryer (Model 6L FreeZone, LabConCo, Kansas City, MO)

for 72 h, starting at -40 °C and rising 5 °C until 0 °C. Freeze-dried tissues were then ground in liquid nitrogen with a mortar and pestle. Pigments were extracted and separated according to Kopsell et al. (2004), which is based on the method of Khachik et al. (1986). HPLC separation parameters and pigment quantification followed procedures of Kopsell et al. (2007). An Agilent 1200 series HPLC unit with a photodiode array detector (Agilent Technologies) was used for pigment separation.

Soluble Sugar Analysis. Samples were ground in a bullet grinder for homogenous sub-samples. A 2.0-g sub-sample was extracted in a 15 mL test tube by adding 2 ml of RO water, vortexed, and shaken for 15 min at 200 rpm. Samples were then centrifuged at 4000 rpm for 10 min, and 1.0 mL of the supernatant was transferred into a new 15 mL test tube. After the transfer, 1.4 mL of acetonitrile was added; tubes were mixed by inversion and kept at room temperature for 30 min. Samples were then centrifuged at 4000 rpm (rounds per minute) for 10 min, and 1.0 mL of the supernatant was transferred into a new 15 mL tube and placed into a dry-bath until complete evaporation. Once dried, samples were dissolved in 0.5 mL of 75% acetonitrile and 25% reverse osmosis water. Samples were then put through a 0.2 µm syringe filter and collected in a 2 mL HPLC vial for analysis. Separation parameters and sugar quantification were carried out with authentic standards using an Agilent 1100 series HPLC with a refractive index detector (Agilent Technologies, Palo Alto, CA). Chromatographic separations were achieved using a 250 x 4.6 mm i.d., 5 µm analytical scale NH_2 carbohydrate C_{18} reverse-phase column (Agilent Technologies), which allowed for effective separation of chemically similar sugar compounds. The column was equipped with a Zorbax NH_2 4.6 x 12.5 mm i.d. guard cartridge and holder (Agilent Technologies), and was maintained at 30 ºC using a thermostatted column compartment. All separations were achieved isocratically using a binary mobile phase of 75%

acetonitrile and 25% reverse osmosis water (v/v). The flow rate was 1.0 mL/min., with a run time of 15 min, followed by a 2 min equilibration prior to the next injection. Eluted compounds from a 10 µL injection loop were detected in positive detection mode, and data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Peak assignment for individual sugars was performed by comparing retention times from the refractive index detector using external standards of fructose and glucose (Sigma-Aldrich, St. Louis, MO).

The two experiments produced results that were statistically similar. Therefore, data were pooled and analyzed together for treatment means. The experimental design was a randomized complete block in a factorial arrangement. The three Ca treatment concentrations were subdivided into ABA and non-ABA treated plants. Analysis of variance (ANOVA) was used to evaluate ABA and calcium treatments on leaf chlorophylls and carotenoids, fruit carotenoids, and soluble sugars using the PROC GLIMMIXED model. Statistical analysis of data was performed using SAS (Version 9.3 for Windows, SAS Institute, Cary, NC). LSDs ($P \leq 0.05$) was used to discern between ABA and calcium application classifications when F values were significant for main effects. Analyzed data was taken from a subsample of at least four fruit from each of the three clusters in six replications. The statistical analysis indicated there were no interactions between ABA and Ca treatments. The following results are presented individually for ABA treatment effects and Ca treatment effects on leaf chlorophylls and carotenoids and fruit tissue carotenoids and soluble sugars.

Results

Impact of ABA on tomato leaf carotenoids and chlorophylls. Foliar spray treatment of 500 $mg \cdot L^{-1}$ ABA increased the accumulation of zeaxanthin (ZEA; $P \leq 0.01$) and BC ($P \leq 0.05$) carotenoids in tomato leaf tissue. ZEA ranged from 0.09 to 0.12 mg/100 g fresh weight (FW) when

comparing the control treatment (0.0 mg·L^{-1} ABA) to 500 mg·L^{-1} ABA foliar spray treatment (**Table 4.1**). This accounted for a 25.0% increase of ZEA in the leaf tissue. BC ranged from 2.77 mg/100 g FW in the control treatment to 3.41 mg/100 g FW in the 500 mg·L^{-1} ABA foliar spray treatment (**Table 4.1**). BC concentrations increased 18.8% in the leaf tissue. ABA treatment did not significantly affect other carotenoids, such as violaxanthin (VIO), neoxanthin (NEO), antheraxanthin (ANTH), and lutein (LUT) (**Table 4.1**). Furthermore, foliar applications of ABA did not affect chlorophyll concentrations in the leaf tissue (**Table 4.2**).

Impact of Ca on tomato leaf carotenoids and chlorophylls. Ca treatments significantly decreased VIO ($P \leq 0.05$) and chlorophyll *a* (Chl *a*; $P \leq 0.05$) concentrations in the leaf tissue. VIO concentrations ranged from 0.24 to 0.34 mg/100 g FW in the 180 mg·L^{-1} Ca treatment and 60 mg·L^{-1} Ca treatment, respectively (**Table 4.3**). Chl *a* showed a 21.9% decrease in leaf tissue concentration and ranged from 52.58 to 67.32 mg/100 g FW between 180 mg·L^{-1} and 60 mg·L^{-1} Ca treatments (**Table 4.4**). Ca treatments did not affect other carotenoids, such as NEO, ANTH, LUT, ZEA, and BC (**Table 4.3**). Ca treatments also did not influence chlorophyll *b* (Chl *b*) content in tomato leaf tissue (**Table 4.4**).

Influence of ABA on tomato soluble sugars. Two major sugars, glucose and fructose, were analyzed to see the effect of the foliar ABA treatment on their concentrations. There were significant increases in glucose ($P \leq 0.001$) and fructose ($P \leq 0.001$) with the application of 500 mg·L^{-1} ABA foliar spray treatments (**Table 4.5**). Glucose ranged from 17.74 to 21.14 mg·g^{-1} FW resulting in a 16.1% increase in concentration when comparing the control treatment to 500 mg·L^{-1} ABA foliar spray treatments. Fructose ranged from 14.43 to 16.47 mg·g^{-1} FW resulting in a 12.4% increase in concentration when comparing the control treatment to 500 mg·L^{-1} ABA foliar spray treatments.

Influence of Ca on tomato soluble sugars. The addition of Ca treatments 60, 90, and 180 $mg \cdot L^{-1}$ resulted in a significant increase in glucose ($P \leq 0.01$) and fructose ($P \leq 0.01$). Glucose increased from 18.37 to 20.61 $mg \cdot g^{-1}$ FW, and fructose increased from 14.56 to 16.39 $mg \cdot g^{-1}$ FW with increasing Ca treatment concentrations from 60 $mg \cdot L^{-1}$ to 180 $mg \cdot L^{-1}$ (**Table 4.6**).

Influence of ABA and Ca on tomato fruit carotenoids. Carotenoids of tomato fruit tissue were analyzed to determine the effects of ABA and Ca treatments. However, there were no significant differences in carotenoids in tomato fruit tissue (**Table 4.7 and 4.8**).

Discussion

This study examined the effects of foliar applied ABA and Ca treatments applied through the fertilizer solutions on tomato leaf chlorophylls, tomato leaf and fruit carotenoids, and soluble sugars. Foliar sprays of 500 $mg \cdot L^{-1}$ ABA increased two of six carotenoids measured in tomato leaf tissue. However, previous studies found that ABA increases numerous leaf carotenoids in dwarf tomato plants (Barickman et al. In Press). This disparity may be due to the difference in leaf tissue size when comparing dwarf leaf to normal size tomato leaf in this study. Normal size tomato leaves may be less responsive to ABA treatments because of their larger biomass, which could dilute the effects of ABA on carotenoid concentrations. As a result, the effects of foliar applied ABA treatments are present only on ZEA and BC. These two carotenoids are involved with the plant's antioxidant capacity under abiotic stress conditions (Ramel et al., 2012). It may be that they react to ABA because it is a stress induced plant hormone that increases in plant tissue under similar conditions. For example, ABA increases under water deficit, temperature stress, and excess light (Hartung et al., 2005).

In comparison, Ca treatments significantly decreased tomato leaf VIO when Ca was increased in the fertilizer, but it had no effect on other carotenoids measured. These results

indicate that Ca treatments in the fertilizer solution may have little to no effect on tomato leaf carotenoids. Previous research demonstrated similar results when alfalfa (*Medicago sativa*) plants were treated with calcium chloride ($CaCl_2$) (Khavari-Nejad and Chaparzadeh, 1998). Another study found that Ca treatments did not change the concentrations of carotenoids in hydroponically grown parsley (*Petroselinum crispum*) (Chondraki et al., 2012). The lack of effect on tomato leaf tissue may be due to Ca treatments not having a significant impact on the nutrient status in the tomato leaf. Calcium concentrations were not high enough to originate nutritional imbalances and the production of metabolites, such as carotenoids, in the tomato leaf tissue. The highest calcium concentration was however in the range of normal tomato calcium requirements and below Ca toxicity ranges. Thus, the levels of calcium normally required in tomato do not appear to impact carotenoid concentrations.

This study also found that while foliar application of ABA did not affect chlorophylls, increasing Ca treatments decreased Chl *a* in the leaf tissue. Previous studies have had mixed results. For example, one study demonstrated that treatment of oat (*Avena sativa*) with $CaCl_2$ had no effect on chlorophyll loss and that concentration in the leaves was similar to the control (Kaur-Sawhney and Galston, 1979). On the other hand, a study on zoysia grass (*Zoysia japonica*) found that chlorophyll content increased with $CaCl_2$ treatments when compared to the control (Xu et al., 2013). The results from this study are contradictory to previous research. Carotenoids and chlorophylls are derived from the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. Since both carotenoids and chlorophylls are derived from the MEP pathway, Ca treatments may act similarly by decreasing both. Furthermore, previous research has demonstrated that adding high concentrations of Ca in the fertilizer indirectly caused antagonistic effects on Mg uptake into the plant leading to decreases in Chl concentrations (Kopsell et al.,

2013). Therefore, increased Ca levels may be indirectly decreasing Chl concentrations because of its effect on Mg uptake.

There were increases in glucose and fructose concentrations with the application of 500 $mg \cdot L^{-1}$ ABA foliar spray treatments. These findings support previous research. For example, a study on grapes (*Vitis vinifera*) found that as ABA levels increased naturally in the ripening process, sugar accumulation also increased (Gambetta et al., 2010). A similar study demonstrated the same effects in tomato fruits (Bastias et al., 2011) where particular ABA transcription factors were activated in developing fruit. However, the current study demonstrates that foliar applications of 500 $mg \cdot L^{-1}$ ABA treatments increase soluble sugar concentrations more than normal levels of endogenous ABA found in the fruit tissue during fruit ripening conditions.

Tomato plants that were exposed to deficient Ca (60 and 90 $mg \cdot L^{-1}$) treatment concentrations had fruit tissue sugar concentration significantly less than the Ca treatment of 180 $mg \cdot L^{-1}$. The two main soluble sugars fructose and glucose decreased significantly in the fruit tissue under deficient Ca treatments. The results suggest that sufficient Ca concentrations applied to the plant may regulate sugar accumulation in tomato fruit tissue. Previous research indicated that foliar chelated Ca applications to cantaloupe (*Cucumis melo* var. cantalupensis) and honeydew (*Cucumis melo*) melon did not affect the accumulation of soluble sugars in the fruit tissue (Lester and Grusak, 2004). In a similar study, soluble sugar concentrations in honeydew melons were not affected by Ca source or rate (Lester and Grusak, 2001). Therefore, results of this study indicate that the method of applying Ca will determine its effect on soluble sugar concentrations. While foliar application of Ca did not affect soluble sugars, manipulation

of Ca treatments from the optimum level in the fertilizer may have adverse effects on soluble sugar concentrations in tomato fruit tissue.

Additionally, carotenoids in tomato fruit tissue were analyzed for ABA and Ca treatments. However, there were no significant differences in carotenoids in the tomato fruit tissue. Previous research demonstrated that tomato plants treated with ABA had a significant increase in fruit Ca concentrations (Barickman et al., In Press; de Freitas et al., 2011). Excess Ca concentration in fruit tissue can affect carotenoid levels in tomato fruit. A previous study demonstrated LYCO and carotene levels decreased with increasing Ca concentration in the first three clusters of tomato fruit (Paiva et al., 1998). This may have been due to the relationship that high concentrations of Ca can reduce K absorption in the fruit tissue. Lack of K absorption could affect the production of carotenoids in the fruit tissue. Therefore, increasing the Ca concentrations in the fruit tissue may have inhibited significant changes in carotenoid content. However, Flores et al. (2004) reported that increasing Ca in hydroponic culture, similar to the treatments in the current study, increased LYCO and BC in red pepper (*Capsicum annuum*) fruit tissue. Therefore, how Ca influences carotenoids may depend on fruit tissue type.

This study demonstrated that ABA increases carotenoids in leaf tissue, thus increasing antioxidants for stress responses to abiotic stress. ABA also increased soluble sugars, which increased fruit quality by making the fruit more flavorful (sweeter). Therefore, this data demonstrated that foliar applications of ABA treatments increase overall tomato leaf antioxidant capacity and fruit quality. In addition, this study demonstrated that Ca treatments decreased only one of six caretonoids and chlorophylls measured. Ca treatments did increase soluble sugars. However, this increase happened independently from soluble sugar increases caused by ABA treatments. Thus, combining ABA and Ca treatments did not have synergistic effect on

increasing fruit sugars. Overall, foliar applications of ABA may be a novel approach to increasing the plant's ability to fight environmental stresses while also improving tomato fruit quality. If the goal is to only improve fruit quality, then adding Ca treatments to fertilizer may present another viable option.

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Appendix 4: Tables

Table 4.1. Carotenoid leaf tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with a foliar sprays of s-ABA.

	Carotenoid concentrations (mg/100 g FM)[ab]						
ABA ($mg \cdot L^{-1}$)	**VIO**	**NEO**	**ANTH**	**LUT**	**ZEA**	**BC**	**Total CAR**
0	0.30	1.86	0.60	7.36	0.09	2.77	12.76
500	0.28	1.84	0.58	8.25	0.12	3.47	14.33
***P*-Value[c]**	ns	ns	ns	ns	**	*	ns

[a] BC-β-carotene; LUT-Lutein; ZEA-Zeaxanthin; ANTH-Antheraxanthin; NEO-Neoxanthin; VIO-Violaxanthin; Total CAR- Total carotenoids.

[b] The standard error of the mean was BC ± 0.65; LUT ± 0.84; ZEA ± 0.12; ANTH ± 0.24; NEO ± 0.44; VIO ± 0.44; Total CAR ± 1.92.

[c] ns, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 4.2. Chlorophyll leaf tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with a foliar sprays of s-ABA.

	Chlorophyll concentrations (mg/100 g FM)[ab]		
ABA ($mg \cdot L^{-1}$)	**ChlA**	**ChlB**	**Total Chl**
0	60.88	27.92	88.51
500	60.59	27.58	88.46
***P*-Value[c]**	ns	ns	ns

[a] CHLA-Chlorophyll *a*; CHLB-Chlorophyll *b*; Total CHL- Total chlorophyll.

[b] The standard error of the mean was CHLA ± 3.33; CHLB ± 4.69; Total CHL ± 4.72.

[c] ns, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 4.3. Carotenoid leaf tissue pigment in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

	Carotenoid concentrations (mg/100g) fresh weight[ab]						
Ca ($mg \cdot L^{-1}$)	**VIO**	**NEO**	**ANTH**	**LUT**	**ZEA**	**BC**	**Total CAR**
60	0.34	1.92	0.61	7.87	0.11	3.22	13.76
90	0.29	1.96	0.61	8.11	0.11	3.15	14.13
180	0.24	1.67	0.55	7.44	0.09	2.9	12.75
***P*-Value[c]**	*	ns	ns	ns	ns	ns	ns

[a] BC-β-carotene; LUT-Lutein; ZEA-Zeaxanthin; ANTH-Antheraxanthin; NEO-Neoxanthin; VIO-Violaxanthin; Total CAR- Total carotenoids.

[b] The standard error of the mean was BC ± 0.29; LUT ± 0.53; ZEA ± 0.01; ANTH ± 0.04; NEO ± 0.28; VIO ± 0.03; Total CAR ± 1.07.

[c] ns, *, **, and *** indicate nonsignificant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 4.4. Chlorophyll leaf tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

	Chlorophyll concentrations (mg/100 g FM)[ab]		
Ca ($mg \cdot L^{-1}$)	**ChlA**	**ChlB**	**Total Chl**
60	67.32	28.47	95.79
90	62.32	28.32	90.64
180	52.58	26.48	79.03
***P*-Value[c]**	*	ns	ns

[a] CHLA-Chlorophyll *a*; CHLB-Chlorophyll *b*; Total CHL- Total chlorophyll.

[b] The standard error of the mean was CHLA ± 3.99; CHLB ± 2.07; Total CHL ± 5.76.

[c] ns and * indicate nonsignificant or significant at $P \leq 0.05$.

Table 4.5. Soluble sugars in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with a foliar sprays of s-ABA.

	Concentrations ($mg \cdot g^{-1}$) fresh mass[a]	
ABA ($mg \cdot L^{-1}$)	**Glucose**	**Fructose**
0	17.74	14.43
500	21.14	16.47
P-Value[b]	***	***

[a] The SE of the mean for Glucose ± 0.48; Fructose ± 0.52.

[b] *** indicate significant at $P \leq 0.001$.

Table 4.6. Soluble sugars of fruit tissue of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

	Concentrations ($mg \cdot g^{-1}$) fresh mass[a]	
Ca ($mg \cdot L^{-1}$)	**Glucose**	**Fructose**
60	18.37	14.56
90	19.33	15.40
180	20.61	16.39
P-Value[b]	**	**

[a] The SE of the mean for Glucose ± 0.52; Fructose ± 0.52.

[b] **indicate significant at $P \leq 0.01$.

Table 4.7. Carotenoid fruit tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with a foliar sprays of s-ABA.

	Concentrations (mg/100 g FM)[ab]		
ABA ($mg \cdot L^{-1}$)	**LUT**	**BC**	**LYCO**
0	0.25	0.51	11.75
500	0.23	0.54	12.05
***P*-Value[c]**	ns	ns	ns

[a] BC-β-carotene; LUT-Lutein; LYCO-Lycopene.

[b] The standard error of the mean was LUT ± 0.02; BC ± 0.05; LYCO ± 1.26.

[c] ns indicate nonsignificant at $P \leq 0.05$.

Table 4.8. Carotenoid fruit tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

	Concentrations (mg/100 g FM)[ab]		
Ca ($mg \cdot L^{-1}$)	**LUT**	**BC**	**LYCO**
60	0.23	0.52	11.93
90	0.24	0.50	11.23
180	0.24	0.55	12.54
***P*-Value[c]**	ns	ns	ns

[a] BC-β-carotene; LUT-Lutein; LYCO-Lycopene.

[b] The standard error of the mean was LUT ± 0.02; BC ± 0.05; LYCO ± 1.26.

[c] ns indicate nonsignificant at $P \leq 0.05$.

Chapter 6
Exogenous Foliar and Root Applications of Abscisic Acid Increase the Influx of Calcium into Tomato Fruit Tissue and Decrease the Incidence of Blossom-End

Abstract

Plants encounter various environmental stress factors that can potentially impact nutritional requirements and fruit quality. Adequate levels of calcium (Ca) in tomato fruit have positive effects on fruit quality, specifically firmness. One of the results of insufficient Ca uptake and movement in tomato (*Solanum lycopersicum*) is the physiological disorder blossom-end rot (BER) in tomato, which is associated with a Ca deficiency in the distal fruit tissue. Previous research has demonstrated that foliar ABA applications decreased the incidence of BER and increased the uptake of Ca into fruit tissue. This study examined how root and foliar spray ABA applications, individually and in combination, affect the partitioning of Ca between the leaves and fruit of tomato plants, especially in the distal tissue, and how ABA affects the incidence of BER in the distal tissue of tomato fruit. Seeds of 'Mt. Fresh Plus' tomato were grown in the greenhouse at 25/20 °C (day/night) under a 16 h photoperiod. Plants were treated with different Ca concentrations in the fertilizer solution. Plants were also treated with foliar spray ABA applications weekly. Calcium was applied via the irrigation lines at 60, 90, or 180 mg $Ca \cdot L^{-1}$. ABA treatments were applied as a combination of foliar sprays and root applications. Foliar ABA applications, treatments consisted of DI water control (0.0 mg $ABA \cdot L^{-1}$) or 500 mg $ABA \cdot L^{-1}$. For ABA root applications, treatments consisted of a DI water control (0.0 mg $ABA \cdot L^{-1}$) or 50 mg $ABA \cdot L^{-1}$ applied via the irrigation lines. ABA spray treatments were applied once weekly till dripping from the foliage (tops of pots were covered to prevent spray drip into the pot), while root applications were applied four times per day through the irrigation system. Fruit tissues were harvested 84-90 days after seeding. Fruit tissue was harvested at red ripe maturity and evaluated for yield, BER and Ca concentrations. Leaves were harvested at time of fruit and were analyzed for Ca concentrations. The results indicate that a combination of the spray and root appications of ABA resulted in the greatest decrease in BER. The foliar spray

application of ABA combined with the Ca treatment of 180 $mg \cdot L^{-1}$ decreased the incidence of BER even in harsh environmental conditions. Results also demonstrate that ABA treatments are very effective in increasing fruit Ca and preventing BER in the early stages of plant development, but are less effective in preventing Ca deficiency in the later stages of growth.

Keywords: *Proximal, Distal, Leaf, Partitioning, Distribution*

Introduction

Plants encounter various environmental stresses that can potentially impact nutritional requirements and fruit quality. Environmental stresses frequently influence vegetative development by inhibiting plant growth. Studies have demonstrated that the plant hormone abscisic acid (ABA) help plants acclimate to environmental stresses such as drought, extreme temperatures and excess light (Hirayama and Shinozaki, 2007; Thompson et al., 2000). Because of influences on vegetative development, the impact of ABA can be indirectly associated with nutritional fluxes in the plant. For example, ABA can enhance potassium (K) absorption in cucumber (*Cucumis sativus*) under high temperature conditions (Du and Tachibana, 1995), and it can promote Ca uptake in tomato (*Solanum lycopersicum*) fruit (de Freitas et al., 2011; Barickman et al., unpublished manuscript-b).

Studies have demonstrated that adequate levels of Ca in tomato fruit have positive effects on fruit quality, specifically firmness (Vaz and Richardson, 1984). Cell wall integrity is maintained through the roles Ca plays in interconnections of pectinacious material (Willats et al., 2001). Research on ABA and Ca has predominantly focused on examining ABA as an environmental stress signal and its impact on signal transduction on a cellular level (Batistic et al., 2012, Chen et al., 2012). These studies looked at how endogenous ABA increased as a result of an environment stress such as drought and the effects on Ca levels (Du et al., 2010). For

example, Guo et al. (2002) indicated that ABA triggers an oscillation in the cytosolic Ca concentrations initiating a series of signaling cascades that control physiological processes, including adaptation to environmental stress. This study focused on how exogenous application of ABA could affect Ca transport and partitioning between the leaves and fruit of tomato in protected culture environments.

Calcium movement through the plant is regulated by source-sink relationships. Calcium moves to tissues that have the lowest water potential (Marschner, 1995). Calcium movement is increased to tissues such as leaves because they are rapidly growing and have low water potential due to transpiration at the stomates. Other parts of the plants, such as fruit tissue, have higher water potentials and lower distribution of stomata; therefore, movement of Ca into these tissues is considerably lower. Movement of Ca into fruit tissue is greatest when cells are actively dividing and expanding in the early stages of growth. After this stage of rapid growth, Ca movement is lessened because strength of the sink for Ca decreases. Thus, fruit have a limited time for critical Ca uptake for rapidly expanding fruit tissue. There are only a few examples in the literature of studies demonstrating that foliar application of ABA can increase Ca uptake into tomato fruit tissue (de Freitas et al., 2011), especially the distal tissue (Barickman et al., unpublished manuscript-a).

Insufficient Ca uptake and movement in tomato results in the physiological disorder blossom-end rot (BER), which is associated with a Ca deficiency in the distal fruit tissue (Ho and White, 2005). Research on greenhouse tomato production has demonstrated that insufficient Ca supplied to the plants in the fertilizer solution rarely causes BER. More often, BER occurs in plants with an adequate Ca supply when grown in environmental conditions that inhibit transport of Ca to rapidly growing distal fruit tissue (Saure, 2001). In addition, incidences of BER may

occur during increased demand of distal fruit tissue for Ca in early stages of fruit development (Ho et al., 1993). Previous research has demonstrated that foliar ABA applications decreased the incidence of BER and increased the uptake of Ca into the fruit tissue (de Freitas et al., 2011). Applications of ABA may negatively affected stomatal conductance by decreasing Ca influx into the vegetative tissue allowing more Ca to be moved into the fruit tissue.

The purpose of this study was to examine the effects of exogenous applications of ABA on Ca partitioning and distribution in tomato fruit. Exogenous application of ABA can be applied to the plant either via root or as a spray to the vegetative tissue. When applied to the root tissue, ABA has been shown to be absorbed and released to xylem tissue to travel to the vegetative tissue where it improves water use efficiency by closing the stomata and affecting plant growth (Hartung et al., 2005). Hocking et al. (1972) demonstrated that C14 labeled ABA was widely distributed within the pea (*Pisum sativum*) plant within 24 h after application to the root, while approximately 18% was found in root nodules. The movement of ABA within the plant was tracked both upwards and downwards until it reached a steam-girdled zone. Research on exogenous applications of ABA and its effects on Ca uptake and distribution mostly examined foliar applications. However, recent research has demonstrated that root applications may be effective as well. For example, Barickman et al. (In Press, Journal of the American Society for Horticultural Science) have demonstrated that ABA applied to the root tissue of tomato positively impacts Ca partitioning between 'Micro' tomato leaf and fruit tissue. Therefore, the purpose of this study was to examine how root and foliar spray ABA applications, individually and in combination, affect the partitioning of Ca between the leaves and fruit of tomato plants, especially in the distal fruit tissue. In addition, this study also examined how root and foliar

spray ABA applications, individually and in combination, affect the incidence of BER of tomato fruit.

Materials and Methods

Plant Culture and Harvest. Seeds of 'Mountain Fresh Plus' tomato (Johnny's Selected Seed, Waterville, ME) were sown into Pro-Mix BX soilless medium (Premier Tech Horticulture, Québec, Canada) and germinated in the greenhouse conditions (Knoxville, TN; 35°N Lat.) at 25/20 °C (day/night). Natural photoperiod and intensity of sunlight for tomato production in the greenhouse were supplemented with 24 individual 1000 W high pressure sodium lights under a 16 h photoperiod. The lights delivered an average of 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over the entire photoperiod. Light intensity readings were taken at 1.22-m (meters) off the ground. At 30 d after seeding, the plantlets were transferred to 11-L Dutch pots (Tek Supply, Dyersville, IA) filled with Sunshine® Pro Soil Conditioner (Sungro Horticulture, Agawam, MA). Tomato plants were grown hydroponically with a tomato fertilization program developed at the University of Tennessee (Knoxville, TN). Elemental concentrations of the nutrient solutions were ($mg \cdot L^{-1}$): nitrogen (N; 180), phosphorus (P; 93.0), potassium (K; 203.3), magnesium (Mg; 48.6), sulfur (S; 96.3), iron (Fe; 1.0), boron (B; 0.25), manganese (Mn; 0.25), zinc (Zn; 0.025), copper (Cu; 0.01), and molybdenum (Mo; 0.005). There were two identical experiments conducted. The first experiment was conducted in fall 2012 and the second in spring 2013. Experimental design was a randomized complete block with a 3 x 2 factorial arrangement of treatments that consisted of six blocks and two replications of each treatment per block, with individual plants representing an experimental unit. Calcium was applied via the irrigation lines at 60, 90, or 180 mg $Ca \cdot L^{-1}$. ABA treatments were applied as a combination of foliar sprays and root applications. For foliar ABA applications, treatments consisted of DI water control (0.0 mg $ABA \cdot L^{-1}$) or 500 mg

$ABA \cdot L^{-1}$. For ABA root applications, treatments consisted of a DI water control (0.0 mg $ABA \cdot L^{-1}$) or 50 mg $ABA \cdot L^{-1}$ applied via the irrigation lines. ABA spray treatments were applied once weekly from anthesis to final harvst. ABA sprays were applied until dripping from the foliage (tops of pots were covered to prevent spray drip into the pot), while root applications were applied four times per day through the irrigation system. Fruit tissues were harvested 84-90 days after seeding. Subsequently, fruit were sorted by the use of USDA tomato color for red ripe (USDA, 1975) and into size classification of extra-large, large, medium and small (USDA, 2007). Tomato fruit with BER were categorized separately. Fruit from each treatment were separated by replication and were weighed for biomass. At least three fruit from the second cluster for each experimental unit were separated into proximal and distal fractions and frozen pending preparation for elemental nutrient analysis. Harvested fruit samples were stored at -80 °C prior to analysis. Leaf samples for each replication were taken of the first leaf above the second cluster upon final harvest of fruit from that cluster for analysis of mineral elements.

Elemental Nutrient Determination. Nutrient analysis was conducted as described by to (Barickman et al., 2013) with slight modifications. Briefly, samples analysis was performed using a 5.0 g subsample of fresh fruit tissue, which was combined with 10 ml of 70% HNO_3 and digested in a microwave digestion unit (Model: Ethos, Milestone Inc., Shelton, CT). The microwave temperature was ramped to 140 °C for 5 min at 1000W and 2000 kPa, followed by an increase to 210 °C for 10 min at 1000W and 3000 kPa. Furthermore, microwave temperature was held at 210 °C for 10 min at 1000W and 4000 kPa and cooled for 10 min at 0W and 2000 kPa. The digest was then allowed to cool to 20 °C. A 100 µl subsample of the digest was diluted with 9900 µl of ICP-MS matrix consisting of 2% HNO_3 and 0.5% HCl (v/v). Leaves were collected and triple rinsed with de-ionized water and dried for 48 h in a forced air oven

(model large; Fisher Scientific, Atlanta, GA) at 65 °C. Dried samples were ground to homogeneity using liquid nitrogen, and 0.5 g sub-samples were weighed for analysis. Samples were microwave digested and a 100 µl aliquot of the digested sample was diluted with 9900 µl of ICP-MS matrix for analysis. Nutrient analysis was conducted using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies, Inc., Wilmington, DE). The ICP-MS system was equipped with an octapole collision/reaction cell, Agilent 7500 ICP-MS ChemStation software, a Micromist nebulizer, a water-cooled quartz spray chamber, and a CETAC (ASX-510, CETAC Inc., Omaha, NE) autosampler. The instrument was optimized daily in terms of sensitivity (Li, Y, Tl), level of oxide (Ce), and doubly charged ion (Ce) using a tuning solutions containing 10 µg l^{-1} of Li, Y, Tl, Ce, and Co in a 2% HNO_3/0.5% HCl (v/v) matrix. Tissue nutrient concentrations are expressed on a dry weight (DW) basis.

Results from the two separate experiments were statically similar. Therefore, data were pooled and analyzed together for treatment means. The three Ca treatment concentrations were subdivided into ABA and non-ABA (control) treated plants. The second cluster of tomato fruits were collected from each treated plant and measured for total tissue Ca in the proximal and distal tissue portions. Furthermore, leaves directly above the second cluster were analyzed for total tissue Ca. Statistical analysis of data was performed using SAS (version 9.3; SAS Institute, Cary, NC). Data were analyzed using the PROC GLIMMIXED analysis of variance.

Results

ABA influence on Ca concentration in leaf tissue: The statistical analysis indicated that there was no interaction between ABA and Ca treatments on tomato leaf tissue. Therefore, the following results are presented separately for ABA and Ca treatment effects. The application of ABA either as a foliar spray (500 mg·L^{-1}), root application (50 mg·L^{-1}), and/or a combination of a

foliar spray and root applications significantly decreased Ca concentrations in tomato leaf tissue (**Table 5.1**). Calcium concentrations in the leaf tissue decreased from 25.94 $mg \cdot g^{-1}$ dry weight (DW) in the non-ABA treated plants to 21.99 $mg \cdot g^{-1}$ DW in the 500 $mg \cdot L^{-1}$ ABA foliar spray plus root 50 mg $ABA \cdot L^{-1}$ treatment. This was a 15.2 % decrease in leaf tissue Ca concentrations when comparing non-ABA treated plants to the combined spray and root ABA treatment.

ABA influence on Ca concentration in fruit tissue: The statistical analysis indicated that there was no interaction between ABA and Ca treatments on Ca content in tomato fruit tissue. Therefore, the following results are presented separately for ABA and Ca effects. The application of ABA to the tomato plant significantly increased Ca uptake into the fruit tissue (**Table 5.1**). The ABA control treatment had the lowest concentration of Ca in the fruit tissue of 3.13 $mg \cdot g^{-1}$ DW. Ca concentration for fruit in the foliar spray ABA treatment was 4.02 $mg \cdot g^{-1}$ DW, and was the highest among the ABA applications. The root and the combined spray and root ABA treatment had 3.42 and 3.69 mg $Ca \cdot g^{-1}$ DW in the fruit tissue, respectively. The foliar ABA spray treatment increased Ca content in the fruit tissue by 28.4 % when comparing it to the ABA control treatment. The combination of foliar spray and root ABA treatment increased Ca content by 17.9 % when comparing to the ABA control treatment. The application of ABA to the root tissue increased Ca concentration in the fruit tissue by 9.3 % when comparing it to the ABA control treatment.

ABA influence on Ca concentration in fruit proximal and distal tissue: There was a significant difference between the foliar spray treatment in tomato fruit proximal tissue compared to the control and root treatments (**Table 5.1**). Proximal fruit tissue Ca concentrations ranged from 3.84 $mg \cdot g^{-1}$ DW in the control treatment to 4.99 $mg \cdot g^{-1}$ DW in the foliar spray treatment, which accounted increase of 23.1 %. Ca concentrations in proximal tissue increased 14.8 % more in

the foliar spray treated plants than in the root treated plants. Distal fruit tissue Ca concentration ranged from 2.43 $mg \cdot g^{-1}$ DW in the control treatment to 3.04 $mg \cdot g^{-1}$ DW foliar spray treatment, which accounted for a 20.1 % increase. Ca concentration in distal tissue significantly increased by 7.0 % in the root treatment and 17.9 % in spray and root combination treatment when compared to the control treatment. However, Ca concentration in distal tissue did not change significantly when comparing the foliar spray, root, and spray and root combination ABA treatments. In addition, Ca concentrations were significantly higher in the proximal than the distal tissue.

Ca treatment influence on Ca concentration in leaf tissue: Calcium concentrations in tomato leaf tissue decreased with decreasing Ca treatments (**Table 5.2**). Leaf tissue Ca decreased from the optimum 180 mg $Ca \cdot L^{-1}$ to 60 mg $Ca \cdot L^{-1}$ treatment. In the 180 mg $Ca \cdot L^{-1}$ leaf tissue Ca concentration was 30.55 $mg \cdot g^{-1}$ DW and decreased to 18.61 $mg \cdot g^{-1}$ DW in the 60 mg $Ca \cdot L^{-1}$ treatment. This accounted for a 39.1 % decrease in leaf tissue Ca concentrations.

Ca treatment influence on Ca concentration in fruit tissue: Calcium concentrations in the fruit tissue decreased significantly from the optimum Ca treatment concentration of 180 $mg \cdot L^{-1}$ to the lower Ca treatment concentration of 60 $mg \cdot L^{-1}$ (**Table 5.2**). Calcium concentrations decreased from 4.58 to 2.98 $mg \cdot g^{-1}$ DW when comparing the 180 $mg \cdot L^{-1}$ Ca treatment to the lower Ca treatment of 60 $mg \cdot L^{-1}$. The concentration of Ca in the fruit tissue decreased 34.9 % when comparing the optimum Ca treatment concentration of 180 $mg \cdot L^{-1}$ to the lower Ca treatment concentration of 60 $mg \cdot L^{-1}$.

Ca treatment influence on Ca concentration in proximal and distal fruit tissue: Calcium concentrations in the tomato fruit proximal and distal tissue decreased from the optimum Ca treatment of 180 $mg \cdot L^{-1}$ to a deficient Ca treatment of 60 $mg \cdot L^{-1}$ (**Table 5.2**). Concentrations of

Ca in the tomato fruit proximal tissue decreased from 5.66 $mg \cdot g^{-1}$ DW in the optimum Ca treatment of 180 $mg \cdot L^{-1}$ to 3.67 $mg \cdot g^{-1}$ DW in the Ca treatment of 60 $mg \cdot L^{-1}$. This accounted for a 35.16% decrease in the Ca concentrations in the proximal tissue. Calcium concentrations in the tomato fruit distal tissue decreased from 3.50 $mg \cdot g^{-1}$ DW in the optimum Ca treatment of 180 $mg \cdot L^{-1}$ to 2.29 $mg \cdot g^{-1}$ DW in the Ca treatment of 60 $mg \cdot L^{-1}$. This accounted for a 34.6% decrease in the Ca concentrations in the distal tissue. Furthermore, there were significant interactions between Ca treatments and proximal and distal locations in the fruit tissue (**Table 5.2**). In tomato fruit tissue, as Ca treatments increased there was an increase in Ca concentrations in the proximal and distal tissue. In the proximal and distal tissue, Ca concentrations decreased from the optimal 180 $mg \cdot L^{-1}$ to the 60 $mg \cdot L^{-1}$ Ca treatment. There was a decrease of 35.2 % and 34.6% in Ca concentrations in the proximal and distal tissue, respectively.

ABA treatment influence on the incidence and yield of fruit with BER in tomato fruit tissue: The incidence of BER in tomato fruit tissue decreased significantly with the application of ABA to the plants (**Table 5.3**). The ABA control treatment (0.0 $mg \cdot L^{-1}$) had the highest incidence of BER with 5.88 fruit/plant. The incidence of BER in the root-applied ABA treatment (50 $mg \cdot L^{-1}$) was 3.78 fruit/plant. The foliar ABA spray (500 $mg \cdot L^{-1}$) treatment and the combination of a foliar spray and root-applied ABA treatments had the lowest incidence of BER in the tomato tissue at 1.26 and 1.20 fruit/plant, respectively. The foliar ABA spray treatment decreased the incidence of BER by 78.6 % when comparing it to the ABA control treatment. The combination of foliar spray and root ABA treatment decreased the incidence of BER by 79.6 % when comparing to the ABA control treatment. The application of ABA to the root tissue decreased the incidence of BER by 35.7 % when comparing it to the ABA control treatment. The yield of

fruit with BER fruit also decreased significantly with the application of ABA to the plants (**Table 5.3**). The ABA control treatment had the highest yield of fruit with BER of 421.32 g/plant. The yield of fruit with BER in the root ABA treatment accounted for 361.98 g/plant. The foliar ABA spray treatment and the combination of a foliar spray and root ABA treatments had the lowest yield of fruit with BER in the tomato tissue of 143.64 and 124.98 g/plant, respectively. The foliar ABA spray treatment decreased the yield of fruit with BER by 65.9 % when comparing it to the ABA control treatment. The combination of foliar spray and root ABA treatment decreased the yield of fruit with BER by 70.2 % when comparing it to the ABA control treatment. The application of ABA to the root tissue decreased the yield of fruit with BER by 14.1 % when comparing it to the ABA control treatment.

Ca treatment influence on the incidence and yield of fruit with BER in tomato fruit tissue: The incidence of BER increased significantly from the optimum Ca treatment concentration of 180 $mg \cdot L^{-1}$ to lower Ca treatment concentrations of 60 and 90 $mg \cdot L^{-1}$ (**Table 5.4**). The incidence of BER increased from 1.56 to 3.72 fruit/plant when comparing the 180 $mg \cdot L^{-1}$ Ca treatment to the lower Ca treatments of 60 and 90 $mg \cdot L^{-1}$. Incidences of BER increased 58.1 % when comparing the optimum Ca treatment concentration of 180 $mg \cdot L^{-1}$ to lower Ca treatment concentrations of 60 $mg \cdot L^{-1}$ and 90 $mg \cdot L^{-1}$. The yield of fruit with BER increased significantly from the optimum Ca treatment concentration of 180 $mg \cdot L^{-1}$ to lower Ca treatment concentrations of 60 and 90 $mg \cdot L^{-1}$ (**Table 5.4**). The yield of fruit with BER was the highest in the 90 $mg \cdot L^{-1}$ Ca treatment. Therefore, yield of fruit with BER increased from 148.92 to 337.62 g/plant when comparing optimum Ca treatment concentration of 180 $mg \cdot L^{-1}$ to 90 $mg \cdot L^{-1}$ Ca treatment. Yield of fruit with BER increased 55.9 % when comparing the optimum Ca treatment concentration of 180 to 90 $mg \cdot L^{-1}$ Ca treatment.

Incidence of BER in fruit tissue by cluster: The incidence of BER significantly increased as the harvested clusters increased on the tomato plants (**Table 5.5**). Occurrence of BER ranged from 2.22 incidences of fruit with BER in the 2nd cluster to 3.60 incidences of BER in the 5th cluster. This accounted for a 38.3% increase in BER when analyzing the incidence by cluster. The yield of fruit with BER did not significantly change as the clusters increased from the 1st to the 6th cluster of the tomato plants (**Table 5.5**). Although the yield of fruit with BER ranged from 177.54 to 265.00 g/cluster, it was not significantly different among clusters.

Influence of ABA and Ca treatments on tomato fruit yield: The statistical analysis of the results indicated that there was no interaction between ABA and Ca treatments on the number and yield of tomato fruit. Therefore, the following results are presented separately for ABA and Ca effects. There were no significant differences in the number and yield of tomato fruit/plant when tomato plants were treated with Ca treatments (**Table 5.6**). In addition, there were no significant differences in the number and yield of tomato fruit/plant when tomato plants were treated with ABA treatments (**Table 5.7**).

Discussion

The purpose of this study was to examine how root and foliar ABA applications, individually and in combination, affect the partitioning of Ca between the leaves and fruit of tomato plants, especially in the distal tissue. In addition, this study also examined how foliar spray and root ABA applications, individually and in combination, affect the incidence of BER in tomato fruit. Specifically, the distal tissue of the tomato fruit is known to lack adequate concentrations of Ca resulting in frequent incidences of BER. Studies have demonstrated that a localized deficiency of Ca in the distal tissue increases the incidence of BER in tomato fruit (Adams and Ho, 1993, Ho et al., 1993). This study also examined the effects of Ca deficiency

on tomato plants. Ca treatments were given as the optimum of 180 $mg \cdot L^{-1}$ and decreased to 90 and 60 $mg \cdot L^{-1}$, respectively.

The results demonstrated that applications of ABA treatments decreased Ca concentration in the leaf tissue. The most signifficant decrease of Ca concentration occured with the combination foliar spray and root applications of ABA treatments. However, foliar spray and root ABA treatments individually were as effective inhibiting Ca uptake into the leaf tissue as the combination ABA treatment. ABA treaments may have adverse effects on Ca uptake into the leaf tissue due to its negative effect on stomatal conductance under harsh environmental condition (Waterland et al., 2010; Garcia-Mata and Lamattina, 2007; Macrobbie, 1990). When harsh environmental conditions such as drought occur, endogenous ABA triggers stomatal closure enhancing plant water use efficiency. There are only a few reports of research on the influence of ABA on Ca partitioning and these studies have demonstrated similar results. For example, de Freitas et al. (2011) found that foliar spray applications of ABA decreased total leaf Ca accumulation per plant when observed in plants 12 to 45 days after pollination. In comparison, the current study examined total Ca accumulation in tissue of tomato leaves above the second cluster. Once the second cluster of tomato was red ripe, the leaf directly above was taken for analysis of total Ca. The second cluster leaf tissue was examined because Ca deficiency is more prevelent higher in the plant (Marschner, 1995).

The results indicate that as the applications of ABA treatments decrease Ca concentrations in the leaf tissue, the Ca concentrations in the tomato fruit tissue increase. The Ca concentrations in fruit tissue may increase because of the increased xylem sap flow and Ca movement (de Freitas et al., 2013). ABA plays a crucial role in improving Ca partitioning between leaves and fruit when applied exogenously. This could be explained by negative effects

of ABA on stomatal conductance in the leaf. As the stomata close, some of the Ca that was initially directed to the leaf tissue is diverted to fruit tissue because of the decrease in the difference in the water potential between the two tissues. The Ca concentration in proximal tissue increased more than in distal tissue of ABA treated fruit. These results are logical because as Ca is taken up into the fruit tissue it binds to the first open cation exchange sites in the cell wall tissue which is the proximal tissue of the fruit (Marschner, 1995). However, the results demonstrated that ABA applications significantly increased Ca concentration in the distal tissue of the tomato fruit as well. In addition, there were no significent differences in marketable size of the tomatoes with the application of ABA treatments. The effect of ABA treatment on Ca was similar on all tomato fruit regardless of size and yield.

The foliar spray ABA treatment was most effective in the partitioning of the Ca between leaf and fruit tissue. Results were confirmed when examining the proximal and distal tissue Ca concentrations separately. Therefore, the data suggested that applying ABA as a foliar spray will have the greatest benefit for Ca partitioning between the tomato leaf and fruit tissue. The application of foliar spray ABA may be more effective than the root application because the former is applied to a larger surface area than the root tissue and reaches stomates directly. When applied to the root tissue, ABA has to change to the uncharged hydrated form (ABAH) and be partitioned into the lipid phase of the root membrane and then diffused into the cytosol of the cell (Astle and Rubery, 1980). This process may weaken the effect that ABA has on partitioning Ca into the tomato fruit tissue. It is also possible that a greater amount of ABA is needed for this application through the irrigation system in tomato production.

The results demonstrate that the application of ABA significantly decreased the incidence of BER in tomato fruit. These results support previous findings that applications of ABA

increased Ca concentrations in tomato fruit tissue (de Freitas et al., 2011; Barickman et al. Unpublished manuscript-a). There was also a significant decrease in the number and yield of fruit with BER with ABA treatments. The most dramatic decrease in the incidence of BER occurred with the combination of foliar spray and the root application of ABA treatments. However, there were also significant decreases in the incidence of BER in the foliar spray and root applications of ABA, independently. The results indicate that a combination of the foliar spray and root appications of ABA resulted in the greatest decrease in the incidence of BER. However, the foliar spray ABA treatment decreased the incidence of BER more than the root treatment independently. Thus, the foliar spray ABA treatment is almost as effective as the combination of foliar spray and root ABA treatments.

de Freitas et al. (2011; 2013) found similar results with incidences of BER when they foliarly applied ABA to tomato plants. While the researchers based their results on a single cluster of tomatoes spanning up to 45 days after pollination, this study adds to the findings by analyzing six clusters of tomato fruit over four months of growth and development repeated for two separate crops. When analyzing incidence and yield of fruit with BER separated by clusters, the results indicate that incidence and yield of fruit with BER increase as more clusters are added to the tomato plant. As the plant adds vegetative and fruit tissue, the demand for Ca is greater causing deficiencies in tomato fruit indicated by increasing incidence of fruit with BER. This means that the efficacy of ABA applications decreases as the plant matures and produces more sinks for Ca in vegetative and fruit tissue. Therefore, ABA will control Ca deficiencies in the fruit tissue early in plant growth and development, but later stages of growth may need more frequent applications, or higher concentrations, to decrease the incidence of fruit with BER. This

may be especially true for fruit on higher clusters produced later in the season on indeterminate tomato cultivars.

This study also applied Ca treatments to the tomato plants simulating Ca deficiences. Tomato plants were given an optimum amount of Ca (180 $mg \cdot L^{-1}$) in the fertilizer solution, as well as lower Ca concentrations of 90 and 60 $mg \cdot L^{-1}$. The addition of Ca treatments to tomato plants were used primarily to examine how ABA would affect the partitioning and distribution of Ca between leaf and fruit tissue under adequate and deficient Ca concentration conditions. The findings were similar to previous studies (Shear, 1975; Simon, 1978; White and Broadley, 2003; Xu et al., 2013) indicating that Ca concentrations decreased in the leaf and fruit tissue of plants treated with lower Ca treatment concentration of 60 $mg \cdot L^{-1}$. Furthermore, the incidence of fruit with BER increased in plants treated with lower Ca treatments of 60 and 90 $mg \cdot L^{-1}$. When ABA and Ca treatments were compared, the results indicate that the manipulation of Ca treatments did not significantly affect the influence of foliar spray ABA treatments on Ca uptake and partitioning. ABA had a similar impact on Ca in both leaf and fruit tissue across Ca treatments. This indicates that the ability of ABA to decrease fruit with BER is not dependent on the amount of Ca in the fertilizer solution available to the plant.

Calcium treatments alone are not a guarantee to decrease the incidence of BER. However, the application of ABA treatments in addition to an optimum Ca treatment of 180 $mg \cdot L^{-1}$ are likely to decrease the incidence of fruit with BER even in the harshest of environmental conditions, such as high light, low humidity, and temperature extremes. However, ABA is effective in decreasing BER even with less than optimal Ca concentrations in the fertilizer solution. Additionally, these results show that ABA treatments tested as most effective in the early stages of plant development, but are not enough to completely combat fruit

Ca deficiencies in the later stages of growth. Other additional treatments such as increasing the frequency of ABA applications or the treatment concentration of ABA, applying Ca spray treatments, or slowing down the rapid growth of the plants by manipulating the greenhouse environmental parameters, such as relative humidity, light, and temperature, may be needed to ensure adequate uptake and distribution of Ca throughout the harvest period.

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Appendix 5: Tables

Table 5.1. Calcium concentrations in leaf, whole fruit, and fruit proximal and distal tissue in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with exogenous applications of s-ABA.

	Concentration ($mg \cdot g^{-1}$) dry weight[a]			
ABA[b]	Leaf Ca	Fruit Ca	Proximal Tissue Ca	Distal Tissue Ca
Control	25.94	3.13	3.84	2.43
Spray	23.51	4.02	4.99	3.04
Root	22.54	3.42	4.25	2.60
Spray/Root	21.99	3.69	4.42	2.96
***P*-Value[c]**	**	***	***	***

[a] The SE of the mean for Leaf Ca ± 0.81; Fruit Ca ± 0.30; Proximal tissue ± 0.34; Distal tissue ± 0.34.

[c] ABA treatments control (0.0 $mg \cdot L^{-1}$); spray (500 $mg \cdot L^{-1}$); root (50 $mg \cdot L^{-1}$); spray/root (500 $mg \cdot L^{-1}$/50 $mg \cdot L^{-1}$).

[d] ** and *** indicate significant at $P \leq 0.01$, 0.001, respectively.

Table 5.2. Calcium in leaf, whole fruit, and fruit proximal and distal tissue in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

	Concentration ($mg \cdot g^{-1}$) dry weight[a]			
Ca ($mg \cdot L^{-1}$)	**Leaf Ca**	**Fruit Ca**	**Proximal Tissue Ca**	**Distal Tissue Ca**
60	18.61	2.98	3.67	2.29
90	21.31	3.14	3.80	2.47
180	30.55	4.58	5.66	3.50
***P*-Value[b]**	***	***	***	***

[a] The SE of the mean for Leaf Ca ± 0.71; Fruit Ca ± 0.29; Proximal tissue ± 0.32; Distal tissue ± 0.32.

[b] *** indicate significant at $P \leq 0.001$.

Table 5.3. Incidence and yield of fruit with blossom end-rot in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with exogenous applications of s-ABA.

	Blossom End-Rot[a]	
ABA[b]	**Incidence (fruit/plant)**	**Yield (g/plant)**
Control	5.88	421.32
Spray	1.26	143.64
Root	3.78	361.98
Spray/Root	1.20	124.98
***P*-Value[c]**	***	***

[a] The SE of the mean for incidence ± 0.36; weight ± 41.16.

[b] ABA treatments control (0.0 $mg \cdot L^{-1}$); spray (500 $mg \cdot L^{-1}$); root (50 $mg \cdot L^{-1}$); spray/root (500 $mg \cdot L^{-1}$/50 $mg \cdot L^{-1}$).

[c] *** indicate significant at $P \leq 0.001$.

Table 5.4. Incidence and yield of fruit with blossom-end rot in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

	Blossom-End Rot[a]	
Ca ($mg \cdot L^{-1}$)	**Incidence (fruit/plant)**	**Yield (g/plant)**
60	3.72	295.62
90	3.78	337.62
180	1.56	148.92
***P*-Value[b]**	***	**

[a] The SE of the mean for incidence ± 0.42; weight ± 39.48.

[b] ** and *** indicate significant at $P \leq 0.01$ and 0.001, respectively.

Table 5.5. Incidence and yield of fruit with blossom-end rot per cluster in 'Mt. Fresh Plus' tomato grown in a greenhouse.

	Blossom-End Rot[a]	
Tomato Cluster	**Incidence (fruit/plant)**	**Yield (g/plant)**
1	2.58	278.10
2	2.22	265.74
3	3.24	260.00
4	3.24	248.40
5	3.60	240.60
6	3.36	177.54
***P*-Value[b]**	*	ns

[a] The SE of the mean for incidence ± 0.36; weight ± 51.12.

[b] ns and * indicate non-significant or significant at $P \leq 0.05$, respectively.

Table 5.6. Number of tomato fruit by classification and yield of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

	Number of fruit and yield (g) per cluster[a]							
Ca	**XL**	**XL Wt**	**Large**	**Large Wt**	**Medium**	**Medium Wt**	**Small**	**Small Wt**
60	1.21	265.27	1.21	234.34	1.31	163.42	1.81	104.37
90	1.24	291.65	1.26	209.95	1.35	170.47	2.36	113.03
180	1.28	278.59	1.23	205.62	1.27	162.01	1.94	145.56
P Value[b]	ns	ns	ns	ns	ns	ns	ns	ns

[a] The SE of the mean for XL ± 0.0.8; XL Wt ± 19.33; Large ± 0.50; Large Wt ± 50.17; Medium ± 0.14; Medium Wt ± 17.08; Small ± 0.54; Small Wt ± 21.76.

[c] ns indicate non-significant at $P \leq 0.05$.

Table 5.7. Number of tomato fruit by classification and yield of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with exogenous applications s-ABA.

	Number of fruit and yield (g) per cluster[a]							
ABA[b]	**XL**	**XL Wt**	**Large**	**Large Wt**	**Medium**	**Medium Wt**	**Small**	**Small Wt**
Control	1.06	225.11	1.23	211.89	1.28	160.60	1.81	151.24
Spray	1.37	310.50	1.25	209.03	1.32	167.08	1.81	108.32
Root	1.28	290.30	1.23	211.89	1.35	170.16	2.67	121.08
Spray/Root	1.27	288.11	1.22	268.32	1.29	163.36	1.86	103.31
P Value[b]	ns	ns	ns	ns	ns	ns	ns	ns

[a] The SE of the mean for XL ± 0.13; XL Wt ± 15.15; Large ± 0.70 Large Wt ± 50.02; Medium ± 0.10; Medium Wt ± 11.77; Small ± 0.45; Small Wt ± 25.56.

[b] ABA treatments control (0.0 $mg \cdot L^{-1}$); spray (500 $mg \cdot L^{-1}$); root (50 $mg \cdot L^{-1}$); spray/root (500 $mg \cdot L^{-1}$/50 $mg \cdot L^{-1}$).

[c] ns indicate non-significant at $P \leq 0.05$.

Chapter 7
Abscisic Acid Impacts Tomato Fruit Quality by Increasing Soluble Sugars and Decreasing Organic Acids

Abstract

Plant growth regulators (PGRs) are chemicals used on a wide range of horticultural crops. These exogenous chemicals, similar to endogenous plant hormones, regulate plant development and stimulate a desired growth response. Research in recent years has focused on using PGRs to improve fruit quality parameters, such as soluble sugars, fruit color, and phytonutrients. One such PGR is abscisic acid (ABA), which has been used effectively to improve fruit quality, specifically flavor and phytonutrients. The purpose of this study was to examine the effects of exogenous applications of ABA on tomato (*Solanum lycopersicum*) fruit quality, such as carotenoids, soluble sugars, and organic acids. This study also examined the effects of ABA on tomato leaf chlorophylls and carotenoids. ABA treatments were applied foliarly to leaf tissue and through a soil drench to the roots since research indicated that both exogenous applications can be effective in improving fruit quality. Furthermore, this study compared how ABA and Ca treatments together affect fruit quality and whether there are added benefits to treating plants with both simultaneously. Seeds of 'Mt. Fresh Plus' tomato were grown in the greenhouse at 25/20 °C (day/night) under a 16 h photoperiod. Plants were treated with ABA applications weekly. Ca treatments were applied at three different treatment levels of 60, 90, and 180 $mg \cdot L^{-1}$. Ca treatments were applied to the plants via the irrigation lines. ABA treatments were applied as a combination of foliar sprays and root applications. For foliar ABA applications, treatments consisted of DI water control (0.0 mg $ABA \cdot L^{-1}$) or 500 mg $ABA \cdot L^{-1}$. For ABA root applications, treatments consisted of a DI water control (0.0 mg $ABA \cdot L^{-1}$) or 50 mg $ABA \cdot L^{-1}$ applied via the irrigation lines. ABA spray treatments were applied once weekly till dripping from the foliage, while root applications were applied four times per day with the irrigation cycle. Fruit tissue was harvested at red ripe maturity and evaluated for carotenoids and soluble sugars. Leaves were harvested at time of fruit and were analyzed for chlorophylls and

carotenoids. ABA applications in conjunction with low Ca treatments did not improve tomato fruit quality. Tomato plants still need an adequate concentration of Ca in the fertilizer solution in order for ABA to improve fruit. However, ABA treatments did prove effective in increasing tomato fruit soluble sugars and organic acid concentrations. This study demonstrated that ABA is a viable PGR to significantly improve tomato fruit quality, specifically pertaining to carotenoids, soluble sugar and organic acid concentrations.

Keywords: *Calcium, phytonutrients, carbohydrates, greenhouse, hydroponics*

Introduction

Plant growth regulators (PGRs) are chemicals used on a wide range of horticultural crops. These exogenous chemicals, similar to endogenous plant hormones, regulate plant development and stimulate a desired growth response. In the floriculture industry PGRs are typically used to control plant height and promote flower initiation or to delay bloom (Currey and Erwin, 2012, Lewis et al., 2004, Blanchard and Runkle, 2007). For example, the gibberellic acid (GA) inhibitor Sumagic is used to control plant height and has been demonstrated to be effective in tomato (*Solanum lycopersicum*) transplant production (Shin et al., 2009). In the nursery industry PGRs can improve crop quality by stimulating lateral branching, as a substitution for a cold storage requirement and to control plant height (Latimer et al., 2003, Gibson and Whipker, 2003, Clough et al., 2001). Traditionally, in the fruit industry PGRs have been used for thinning flower blossoms to achieve larger fruit and to improve fruit firmness and nutritional quality (Jones et al., 1991, Meland et al., 2011, Greene et al., 2011). For example, the PGR CyLex plus, used in apple (*Malus domestica*) and pear (*Pyrus communis*) production, is effective in inducing flower thinning and increasing return bloom (Stopar et al., 2009).

Applications of PGRs can manipulate plant growth and development for many years in horticultural crops. However, research in recent years focused on using PGRs to improve fruit

quality parameters, such as soluble sugars, fruit color, and phytonutrients (Zhang and Whiting, 2013, Buran et al., 2012, Gonzalez et al., 2012, Gu et al., 2011). One such PGR is abscisic acid (ABA), which has been used effectively to improve fruit quality, especially in grape (*Vitis vinifera*) production (Quiroga et al., 2009, Peppi et al., 2006). ABA has significantly increased soluble sugars in grapes, thereby improving fruit flavor. In addition, ABA also improved fruit color adding to the visual aesthetics and nutritional value. Previous research has demonstrated that ABA particularly improves anthocyanin (Cantin et al., 2007) and carotenoid (Barickman et al. In Press. Journal of the American Sociey for Horticultural Sciences), which increase antioxidants in the human diet.

The improvement in grape fruit quality parameters and the demand for healthier fruits and vegetables have sparked additional research in other horticultural crops. These studies indicated that in addition to PGRs, manipulating environmental factors might contribute to improving fruit quality parameters, specifically flavor and phytonutrients. For example, Barickman et al. (2013) observed that manipulating mineral nutrients found in soils affected nutritional quality in *Brassica* species. They found that supplementing adequate selenium in fertilizer solutions maintained glucosinolate concentrations at beneficial levels for human nutrition. Therefore, manipulating environmental factors in addition to PGRs may significantly improve crop quality.

The purpose of this study was to examine the effects of exogenous applications of ABA on tomato fruit quality parameters, such as carotenoids, soluble sugars, and organic acids. This study also examined the effects of ABA on tomato leaf chlorophylls and carotenoids. ABA treatments were applied foliarly and through the root because research indicated that both exogenous applications can be effective in improving fruit quality (Barickman et al., In Press,

Barickman et al., Unpublished manuscript). In addition, Ca fertilizer concentrations were manipulated because Ca treatments can affect tomato fruit quality parameters (Barickman et al., Unpublished manuscript, de Freitas et al., 2013). Furthermore, this study compared how ABA and Ca treatments together affect fruit quality and whether there are added benefits to treating plants with both simultaneously.

Methods and Materials

Plant Culture and Harvest. Seeds of 'Mountain Fresh Plus' tomato (Johnny's Selected Seed, Waterville, ME) were sown into Pro-Mix BX soilless medium (Premier Tech Horticulture, Québec, Canada) and germinated in greenhouse conditions (Knoxville, TN; 35°N Lat.) at 25/20 °C (day/night). Natural photoperiod and intensity of sunlight for tomato production in the greenhouse were supplemented with 24 individual 1000 W high pressure sodium lights under a 16 h photoperiod. The lights delivered an average of 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over the entire photoperiod. Light intensity readings were taken at 1.22-m off the ground. At 30 days after seeding, the plantlets were transferred to 11-L Dutch pots (Tek Supply, Dyersville, IA) filled with Sunshine® Pro Soil Conditioner (Sungro Horticulture, Agawam, MA). Tomato plants were grown hydroponically with a tomato fertilization program developed at the University of Tennessee. Elemental concentrations of the nutrient solutions were ($mg \cdot L^{-1}$): nitrogen (N; 180), phosphorus (P; 93.0), potassium (K; 203.3), magnesium (Mg; 48.6), sulfur (S; 96.3), iron (Fe; 1.0), boron (B; 0.25), manganese (Mn; 0.25), zinc (Zn; 0.025), copper (Cu; 0.01), and molybdenum (Mo; 0.005). There were two identical experiments conducted. The first experiment was done in fall 2012 and replicated in spring 2013. Experimental design was a randomized complete block with a 3 x 2 factorial which consisted of six blocks and two replications of each treatment with individual pots representing an experimental unit. Ca was

applied at three different treatment levels of 60, 90, and 180 mg·L^{-1}. Ca treatments were applied to the plants via the irrigation lines. ABA treatments were applied as a combination of foliar sprays and root applications. For foliar ABA applications, treatments consisted of DI water control (0.0 mg ABA·L^{-1}) or 500 mg ABA·L^{-1}. For ABA root applications, treatments consisted of a DI water control (0.0 mg ABA·L^{-1}) or 50 mg ABA·L^{-1} applied via the irrigation lines. ABA spray treatments were applied once weekly till dripping from the foliage, while root applications were applied four times per day with the irrigation cycle. Fruit tissues were harvested 84-90 days after seeding. Subsequently, fruit were sorted by the use of USDA tomato color for red ripe (U.S.D.A., 1975) and size classification into extra-large, large, medium and small (U.S.D.A., 2007). Tomato fruit with BER were categorized separately. Fruit from each treatment were separated by replication and weighed for biomass. At least three fruit from two clusters for each experimental unit were frozen and prepared for elemental nutrient, water soluble carbohydrate, and carotenoid analyses. Harvested fruit samples were stored at -80 °C prior to analysis. Leaf samples were taken from each of the two clusters at the last harvest for analysis of mineral elements, carotenoids, and chlorophylls.

Fruit Carotenoid Tissue Determination. Carotenoids were extracted from fresh-frozen ripe fruit tissues and quantified according to the methods of Emenhiser et al. (1996) with slight modifications from Barickman et al. (In Press). An Agilent 1200 series HPLC unit with a photodiode array detector (Agilent Technologies, Palo Alto, CA) was used for pigment separation and followed the method from (Barickman et al., In Press Journal of the American Society for Horticultural Science).

Leaf Carotenoid and Chlorophyll Determination. The frozen tomato leaf samples were lyophilized in a programmed freeze dryer (Model 6L FreeZone, LabConCo, Kansas City, MO)

starting at -40 °C for 72 h, rising 5 °C until 0 °C. Freeze-dried tissues were then ground in liquid nitrogen with a mortar and pestle. Pigments were extracted and separated according to Kopsell et al. (2004), which is based on the method of Khachik et al. (1986). HPLC separation parameters and pigment quantification followed procedures of Kopsell et al. (2007). An Agilent 1200 series HPLC unit with a photodiode array detector (Agilent Technologies) was used for pigment separation.

Soluble Sugar Analysis. Procedure was taken from Barickman et al. (Unpublished manuscript). Briefly, samples were ground in a bullet grinder for homogenous sub-samples. A 2.0 g sub-sample was extracted in a 15 mL test tube by adding 2 ml of RO water, vortexed, and shaken for 15 min at 200 rpm. Samples were then centrifuged at 4000 rpm for 10 min and 1.0 mL of the supernatant was transferred into a new 15 mL test tube. After the transfer, 1.4 mL of acetonitrile was added; tubes were mixed by inversion and kept at room temperature for 30 min. Samples were then centrifuged at 4000 rpm for 10 min, and 1.0 mL of the supernatant was transferred into a new 15 mL tube and placed into a dry-bath until complete evaporation. Once dried, samples were dissolved in 0.5 mL of 75% acetonitrile and 25% RO water. Samples were then put through a 0.2 µm syringe filter and collected in a 2 mL HPLC vial for analysis. Separation parameters and sugar quantification were carried out with authentic standards using an Agilent 1100 series HPLC with a refractive index detector (Agilent Technologies). Chromatographic separations were achieved using a 250 x 4.6 mm i.d., 5 μm analytical scale NH_2 (amino) carbohydrate C_{18} reverse-phase column (Agilent Technologies), which allowed for effective separation of chemically similar sugar compounds. The column was equipped with a Zorbax NH_2 4.6 x 12.5 mm i.d. guard cartridge and holder (Agilent Technologies), and was maintained at 30 ºC using a thermostatted column compartment. All separations were achieved isocratically

using a binary mobile phase of 75% acetonitrile and 25% RO (reverse osmosis) water (v/v). The flow rate was 1.0 mL min^{-1}, with a run time of 15 min, followed by a 2 min equilibration prior to the next injection. Eluted compounds from a 10 µL injection loop were detected in positive detection mode, and data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Peak assignment for individual sugars was performed by comparing retention times from the refractive index detector using external standards of fructose and glucose (Sigma-Aldrich, St. Louis, MO).

Organic Acid Analysis. A 20 g sub-sample of three fresh tomato fruit were frozen at -80 °C freezer until processing for organic acid extraction. Tomato fruit were then homogenized with mortar and pestle, and a 1.0 g sample was taken for extraction and followed the procedure of Suarez et al. (2008) with some modifications. Briefly, the 1.0 g sub-samples was placed into a 15 mL polyproplylene centrifuge tube and mixed with 2 mL of 80% ethanol. Afterwards, the tubes were placed in an ultrasonic bath for 5 min. The tubes were then centrifuged for 5 min at 1090 x g-force. The supernatant was decanted, and the pellet was extracted again as stated above. The combined supernatant was then concentrated with a nitrogen stream until dryness. Once dried, samples were dissolved in 5.0 mL of ultra-pure water. Samples were then put through a 0.45 µm nylon syringe filter and collected in a 2 mL HPLC vial for analysis.

Separation parameters and organic acid quantification were carried out with authentic standards using an Agilent 1200 series HPLC with a refractive index detector. Chromatographic separations were achieved using a 300 x 7.7 mm i.d., 8 μm analytical scale Hi-Plex H column (Agilent Technologies), which allowed for effective separation of organic acid compounds. The column was equipped with a Zorbax NH_2 4.6 x 12.5 mm i.d. guard cartridge and holder (Agilent Technologies), and was maintained at 50 ºC using a thermostatted column compartment. All

separations were achieved isocratically using a mobile phase of 100% 0.1 M H_2SO_4 (sulfuric acid). The flow rate was 0.6 mL min^{-1}, with a run time of 15 min, followed by a 2 min equilibration prior to the next injection. Eluted compounds from a 10 µL injection loop were detected in positive detection mode, and data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Peak assignment for individual organic acids was performed by comparing retention times from the refractive index detector using external standards of malic and citric acids (Sigma-Aldrich, St. Louis, MO).

Statistical Analysis. The two experiments were statistically similar. Therefore, data were pooled and analyzed together for treatment means. The experimental design was a randomized complete block in a factorial arrangement. The three Ca treatment concentrations were subdivided into ABA and non-ABA treated plants. Analysis of variance (ANOVA) was used to evaluate ABA and calcium treatments on leaf chlorophylls and carotenoids, fruit carotenoids and soluble sugars using the PROC GLIMMIXED model. Statistical analysis of data was performed using SAS (Version 9.3 for Windows, SAS Institute, Cary, NC). Duncan's multiple range test ($P \leq 0.05$) was used to differentiate between ABA and calcium application classifications when F values were significant for main effects. Data are the average of four fruit and six replications per treatment application. Statistical analyses indicated there were no interactions between ABA and Ca treatments. The following results are presented individually for ABA treatment effects and Ca treatment effects on leaf chlorophylls and carotenoids and fruit tissue carotenoids, soluble sugars and organic acids.

Results

Impact of ABA on tomato leaf carotenoids and chlorophylls. Root ABA treatments significantly decreased leaf lutein (LUT) concentrations in tomato plants (**Table 6.1**). LUT concentrations

decreased from 9.23 to 7.91 mg/100 g FW when comparing the ABA control treatment to the ABA root treatment. This accounted for a 14.3% decrease in LUT concentration in the tomato leaf tissue. Foliar spray ABA treatment significantly increased zeaxanthin (ZEA) in the leaf tissue (**Table 6.1**). ZEA changed from 0.05 to 0.10 mg/100 g FW when comparing the ABA control treatment to the foliar spray and root combination ABA treatment. This accounted for an increase of 50.0% in tomato leaf tissue.

ABA also had a significant, but negative impact on tomato leaf chlorophyll concentrations. Root ABA treatment decreased chlorophyll *a* (ChlA) and chlorophyll *b* (ChlB) significantly in tomato leaf tissue (**Table 6.2**). ChlA decreased from 84.21 to 69.51 mg/100 g FW, and ChlB decreased from 31.55 to 26.14 mg/100 g FW when comparing the ABA control treatment to the ABA root treatment. This accounted for a 17.46 % and a 17.14 % decrease in ChlA and ChlB, respectively.

Impact of Ca on tomato leaf carotenoids and chlorophylls. Ca treatment deficiencies of 60 and 90 $mg \cdot L^{-1}$ significantly increased ChlA concentrations in tomato leaf tissue (**Table 6.3**). ChlA ranged from 68.69 mg/100 g FW in the optimum 180 $mg \cdot L^{-1}$ Ca treatment to 77.71 mg/100 g FW in 60 $mg \cdot L^{-1}$ Ca treatment and accounted for an 11.61% increase in concentration in tomato leaf tissue. However, Ca treatments did not affect ChlB concentrations in the leaf tissue (**Table 6.3**). In addition, Ca treatments did not affect the concentration of tomato leaf carotenoids (**Table 6.4**).

Influence of ABA and Ca on tomato fruit carotenoids. The application of ABA had a significant impact on tomato fruit carotenoids. There were significant differences in LUT, BC, and lycopene (LYCO) (**Table 5.5**). LUT increased from 0.11 to 0.15 mg/100 g FW when comparing the ABA control treatment to the ABA root treatment. This accounted for a 26.7% increase in

LUT concentrations in the tomato fruit tissue. The applications of foliar spray ABA treatments increased BC concentrations in tomato fruit tissue. BC concentrations increased from 0.29 to 0.36 mg/100 g FW when comparing the ABA control treatment to the foliar spray ABA treatment. This accounted for a 19.4% increase in BC concentrations in tomato fruit tissue. Root ABA treatments significantly decreased LYCO concentrations in the tomato fruit tissue. LYCO concentrations decreased from 6.07 to 4.91 mg/100 g FW when comparing the foliar spray ABA treatment to the foliar spray and root combination ABA treatment. This accounted for a 19.1% decrease in LYCO concentrations in the fruit tissue. Ca treatments did not have a significant impact on tomato fruit carotenoid concentrations (**Table 6.6**).

Influence of ABA and Ca on tomato soluble sugars. The application of ABA had a significant positive impact on tomato fruit soluble sugar concentrations (**Table 6.7**). Glucose incresed from 13.39 to 19.19 mg/100 g FW when comparing the ABA control to the ABA root treatment. Glucose increased 30.2% in the tomato fruit tissue with ABA treatment. Fructose increased from 13.72 to 19.24 mg/100 g FW when comparing the ABA control to the ABA root treatment. Fructose increased 28.7% in the tomato fruit tissue with ABA treatment. Additionally, Ca treatments had a significant positive impact on tomato fruit soluble sugar concentrations (**Table 6.8**). Glucose increased from 15.70 to 19.29 mg/100 g FW when comparing the 60 and 180 $mg \cdot L^{-1}$ Ca treatment. Glucose increased 18.6% in the tomato fruit tissue. Fructose increased from 16.29 to 18.62 mg/100 g FW when comparing the 60 and 180 $mg \cdot L^{-1}$ Ca treatment. Fructose increased 12.5% in the tomato fruit tissue.

Influence of ABA and Ca on tomato fruit organic acids. The application of ABA had a significant negative impact on tomato fruit soluble sugar concentrations (**Table 6.9**). Malic acid decreased from 2.06 to 0.74 mg/100 g FW when comparing the ABA control to the ABA root

treatment. Malic acid decreased 64.71% in the tomato fruit tissue with ABA treatment. Citric acid decreased from 4.07 to 1.34 mg/100 g FW when comparing the ABA control to the ABA root treatment. Citric acid decreased 67.08% in the tomato fruit tissue. Ca treatments did not have a significant impact on tomato fruit organic acid concentrations (**Table 6.10**).

Discussion

This study examined the effects of exogenously applied ABA and Ca treatments on tomato fruit quality. Specifically, the study examined the effect on tomato soluble sugars, organic acids and carotenoids in the fruit tissue. In addition, this study looked at the effects of treatments on tomato leaf chlorophylls and carotenoids.

The applications of ABA significantly increased ZEA in tomato leaf tissue. This confirms a previous report that foliar applications of ABA increased ZEA (Barickman et al., Unpublished manuscript, Barickman et al., In Press Journal of the American Society for Horticultural Science), which increases under environmental stress, especially light induced stress (Depka et al., 1998, Havaux and Niyogi, 1999). This study also demonstrated that no matter the application process of ABA, ZEA will increase in the leaf tissue. Foliar spray applications are just as effective as root and the combination of foliar spray and root applications of ABA. This may be due to ABA's effectiveness in creating a stress response no matter how it was applied. Thus, even small amounts of ABA may increase the induction of gene expression leading to the increased production of ZEA. For example, previous research demonstrated that induction of carotenoid biosynthetic genes by ABA could alter the plant's resistance to drought and oxidative stress by modulating levels of xanthophylls, such as ZEA and LUT (Du et al., 2010).

Additionally, this study found root ABA applications significantly decreased LUT in the leaf tissue. Previous research found mixed results in relation to carotenoid concentrations in leaf tissue. For example, Li et al. (2010) and Barickman et al. (In Press Journal of the American Society for Horticultural Science) found that exogenous applications of ABA directly to the root tissue in the hydroponic solution increased carotenoid concentrations in the leaf tissue of lettuce (*Lactuce sativa*) and 'Micro' tomato, respectively. On the other hand, Barickman et al. (Unpublished manuscript) demonstrated that applying ABA exogenously as a foliar spray treatment had no significant impact on LUT concentrations in tomato leaf tissue. Furthermore, Baldermann et al. (2013) demonstrated that exogenously applied ABA decreased total carotenoids in tea (*Camellia sinensis*) plant flowers. Therefore, the mixed results merit an investigation on the process of how ABA is applied to plants and the response of carotenoids in different plant tissues. Further research is required to determine the best application method and concentration to positively affect carotenoid concentrations in leaf verses fruit tissue.

In the current study the root ABA treatments decreased ChlA and ChlB in tomato plant leaf tissue. This may be due to the fact that ABA links environmental stress perception with the reduction of plant growth and photosynthetic capacity (Saibo et al., 2009). Thus, ABA carries the stress signal to the stomata and acts to close them, affecting plant growth and photosynthetic capacity. Apart from restricting gas exchange by stomatal closure as a short-term effect of enhanced ABA levels, long-term ABA effects on photosynthesis include the inhibition of thylakoid formation, chlorophyll biosynthesis and Rubisco activity (Khokhlova et al., 1978, Kusnetsov et al., 1998, Lichtenthaler and Becker, 1970). Research demonstrated that exogenous applications of ABA reduce chlorophyll content and repress transcription of chloroplast genes leading to reduction in chloroplast-localized proteins that impact photosynthesis (Yamburenko et

al., 2013, Kusnetsov et al., 1994, Wang et al., 2010). Thus, ABA negatively impacts the production and function of proteins in the photosynthetic process. Exogenous application of ABA in the current study may indirectly affect expression of chloroplast genes and regulate their activity leading to decreases in chlorophyll concentrations in tomato leaves. Therefore, the effect of ABA may lead to an indirect down-stream signal, such as transcription factors, that regulate chloroplast genes function and, to a larger extent, photosynthesis as a response to abiotic stress.

This study found that decreasing Ca treatments increased chlorophyll concentrations while not affecting leaf carotenoid concentrations. Tomato leaf chlorophyll concentrations increased when plants were treated with the optimum Ca treatment of 180 $mg \cdot L^{-1}$. In addition, leaf carotenoid concentrations did not change from the optimum Ca treatment of 180 $mg \cdot L^{-1}$ to the deficient treatments of 60 and 90 $mg \cdot L^{-1}$. The effect of varying Ca supply on leaf chlorophyll and carotenoid concentrations has been investigated with mixed results. The differences in results may be due to plant species, stage of growth and environmental conditions. For example, varying Ca treatment concentrations in *Arabidopsis* did not impact the chlorophyll and carotenoid content in the leaves (Kaddour et al., 2012). In leaves of *Cyclocarya paliurus* seedlings, decreasing the Ca treatment concentration from 18 to 12 mM increased the chlorophyll concentrations (Yao and Wang, 2012). Like the current study, Xu et al. (2013) and Kaddour et al. (2012) recorded results for plants that were not under any environment stress. On the other hand, Ca treatments alleviated photoinhibition of the photosystem II by positively impacting the xanthophyll cycle pigment concentrations in peanut (*Arachis hypogaea*), a calciphilous plant species, during heat stress and high light conditions (Yang et al., 2013). In addition, research has demonstrated that applications of $CaCl_2$ treatments resulted in higher concentrations of ChlA and ChlB in *Zoysia japonica* under drought conditions (Xu et al., 2013).

These studies demonstrated that Ca treatments may have a bigger impact on chlorophyll and carotenoid concentrations under environmental stress conditions. Previous research indicated that Ca has a central role in the plants' defense mechanisms that are induced by environmental stress, and Ca signaling is required for plants' tolerance to this stress (Cousson, 2009, Cousson, 2007). In addition, Kopsell et al. (2013) demonstrated that Ca:Mg ratios significantly affect the mineral nutrient uptake and carotenoid concentraitons in kale (*Brassica oleracea* var. Acephala). Therefore, increasing Ca treatments may be able to positively regulate chloroplast genes that help to increase leaf chlorophyll and carotenoid concentrations under environmental stress.

The application of ABA affected the concentration of different carotenoids in tomato fruit tissue in a different way. LUT increased in the fruit tissue when ABA was applied in the foliar spray or root treatments when compared to the ABA control treatment. BC increased in the fruit tissue when ABA was applied in the foliar spray treatment when compared to the ABA control treatment. However, LYCO concentrations in the foliar spray treatment did not differ from the ABA control treatment. LYCO did decrease when ABA was applied to the root tissue. Thus, the foliar spray applications of ABA had the greatest effect on carotenoids in tomato fruit tissue. Previous research supported the current study's findings. Research demonstrated that ABA plays an essential role in fruit ripening. For example, ABA controls ethylene production in tomato fruit (Zhang et al., 2009), leading to increases in pigmentation and carotenoid levels. Furthermore, data also indicated that ABA positively regulated the degree of pigmentation and carotenoid composition during tomato fruit ripening by acting on gene functions (Sun et al., 2012). The application of exogenous ABA to tomato plants could be a novel approach to increasing carotenoid concentrations in the fruit tissue, leading to a more nutritious tomato fruit.

The application of Ca treatments to tomato plants did not affect the carotenoid content in the fruit tissue. However, research demonstrated that excess Ca concentrations in the fruit tissue could affect the carotenoid concentrations. For example, Paiva et al. (1998) demonstrated that an increase in Ca concentration in the nutrient solution results in a decrease in LYCO content due to the antagonism between Ca and potassium (K). Increases in Ca content in plant tissue have an antagonistic effect on K and can reduce its absorption. Lack of K absorption into the fruit tissue can have a negative effect on the production of carotenoids. Research demonstrated that increases in K improve the quality of tomato fruit by positively influencing carotenoid biosynthesis (Ramirez et al., 2009). Therefore, in the current study, increasing the Ca treatment concentrations to the optimum level of 180 $mg \cdot L^{-1}$ does not affect the K absorption into tomato fruit and negatively influence carotenoid concentration.

This study demonstrated that exogenous applications of ABA positively influence soluble sugar concentrations in the fruit tissue. Previous research found similar results. Bastias et al. (2011) found that over expressing key ABA regulated genes increases soluble sugar concentrations in tomato fruit. Thus, under stressful conditions ABA increases, which in turn increases sugar accumulation by activating signals associated with stress responses (Saito et al., 2008). Therefore, not only will ABA increase soluble sugar accumulation under normal ripening conditions, it will also stimulate soluble sugar accumulation under stress conditions.

This study found decreases in soluble sugar concentrations in tomato fruit tissue when Ca treatments were decreased from the optimum of 180 $mg \cdot L^{-1}$ to Ca deficiency levels of 60 and 90 $mg \cdot L^{-1}$. Thus, plants treated with optimum Ca concentration treatments had the highest levels of soluble sugars in the tomato fruit tissue. These findings correspond to previous research, which indicated that the addition of Ca as a pre-harvest treatment increased total soluble solids, total

soluble sugars, and Ca in pear (*Pyrus communis*) fruit tissue (Omaima et al., 2010). However, in the current study there were no interactions when analyzing ABA and optimum Ca treatments together. The increased levels of soluble sugars in the fruit tissue treated by ABA and Ca were similar, indicating that applications of either ABA or Ca alone would yield the same results. Applying ABA and Ca treatments together did not result in greater soluble sugar concentrations because, at low Ca treatments, ABA did not increase the soluble sugar concentrations in tomato fruit tissue.

This study found that tomato plants treated with exogenous applications of ABA had decreased organic acid concentrations in the fruit tissue. Specifically, malic and citric acid decreased in tomato fruit tissue in all treatment applications of ABA when compared to the ABA control treatment. Previous research supported data that exogenous applications of ABA accelerate ethylene production leading to fruit quality changes in mango (*Mangifera indica*) fruit (Zaharah and Singh, 2012). In addition, Zaharah et al. (2013) found that exogenous applications of ABA promoted the activities of ethylene biosynthesis enzymes leading to fruit softening, increases in soluble sugars, and degradation of total organic acid. These experiments support the current study, which demonstrated that exogenous applications of ABA lead to an increase in carotenoids and soluble sugars and decreases in organic acids. This data indicateed that ABA can improve tomato fruit quality by positively influencing the sugar to acid ratio, thereby improving the flavor and increasing the nutritional value by increasing carotenoid concentrations.

This study demonstrated that ABA can improve tomato fruit quality, specifically pertaining to carotenoids, soluble sugar, and organic acid concentrations. However, ABA applications in conjunction with low Ca treatments did not prove to be more effective in

improving tomato fruit quality. Tomato plants still need the adequate concentration of Ca in the fertilizer solution in order for ABA to improve fruit carotenoids, soluble sugars, and decrease organic acid concentrations. The application of ABA could be a novel application of a PGR to improve overall fruit quality with adequate fertilization of the plant. The efficacy of ABA may be more an issue of application process to improve tomato fruit quality. Thus, this study indicates that foliar spray applications of ABA are a more viable choice to improve carotenoid and soluble sugar and decrease the organic acid concentrations in tomato fruit. However, further research is needed to investigate the optimum concentrations and frequency of ABA applications to the root and leaf to fine tune the impact on fruit quality.

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Appendix 6: Tables

Table 6.1. Carotenoid leaf tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with exogenous applications of ABA.

	Mean (mg/100 g FM)[ab]					
ABA[c]	**VIO**	**NEO**	**ANTH**	**LUT**	**ZEA**	**BC**
Control	0.46	2.46	1.23	9.23	0.05	3.56
Spray	0.42	2.26	0.99	8.76	0.09	3.44
Root	0.42	2.04	1.06	7.91	0.09	2.98
Spray/Root	0.44	2.19	1.05	9.01	0.10	3.41
P-Value[d]	ns	ns	ns	**	*	ns

[a] BC-β-carotene; LUT-Lutein; ZEA-Zeaxanthin; ANTH-Antheraxanthin; NEO-Neoxanthin; VIO-Violaxanthin; Total CAR- Total carotenoids.

[b] The standard error of the mean was VIO ± 0.07; NEO ± 0.12; ANTH ± 0.14; LUT ± 0.31; ZEA ± 0.01; BC ± 0.19.

[c] ABA treatments control (0.0 $mg \cdot L^{-1}$); spray (500 $mg \cdot L^{-1}$); root (50 $mg \cdot L^{-1}$); spray/root (500 $mg \cdot L^{-1}$/50 $mg \cdot L^{-1}$).

[d] ns, *, and **indicate nonsignificant or significant at $P \leq 0.05$, 0.01, respectively.

Table 6.2. Chlorophyll leaf tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with exogenous applications of ABA.

	Concentraiton (mg/100 g FM)[ab]	
ABA[c]	**CHLA**	**CHLB**
Control	84.21	31.55
Spray	74.31	28.99
Root	69.51	26.14
Spray/Root	70.22	29.05
P-Value[d]	*	**

[a] CHLA-Chlorophyll *a*; CHLB-Chlorophyll *b*; Total CHL- Total chlorophyll.

[b] The standard error of the mean was CHLA ± 4.01; CHLB ± 1.08.

[c] ABA treatments control (0.0 $mg \cdot L^{-1}$); spray (500 $mg \cdot L^{-1}$); root (50 $mg \cdot L^{-1}$); spray/root (500 $mg \cdot L^{-1}$/50 $mg \cdot L^{-1}$).

[d] * and ** indicate significant at $P \leq 0.05$, 0.01, respectively.

Table 6.3. Chlorophyll leaf tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

	Concentration (mg/100 g FM)[ab]	
Ca ($mg \cdot L^{-1}$)	**ChlA**	**ChlB**
60	77.71	29.36
90	77.29	28.91
180	68.69	28.53
P-Value[c]	*	ns

[a] CHLA-Chlorophyll *a*; CHLB-Chlorophyll *b*; Total CHL- Total chlorophyll.

[b] The standard error of the mean was CHLA ± 3.57; CHLB ± 0.97; Total CHL ± 4.34.

[c] ns and * indicate nonsignificant or significant at $P \leq 0.05$, respectively.

Table 6.4. Carotenoid leaf tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

	Concentrations (mg/100 g FM)[ab]					
Ca ($mg \cdot L^{-1}$)	**VIO**	**NEO**	**ANTH**	**LUT**	**ZEA**	**BC**
60	0.40	2.34	1.10	8.83	0.09	3.54
90	0.41	2.21	1.04	8.68	0.08	3.21
180	0.50	2.17	1.11	8.68	0.08	3.30
P-Value[c]	ns	ns	ns	ns	ns	ns

[a] BC-β-carotene; LUT-Lutein; ZEA-Zeaxanthin; ANTH-Antheraxanthin; NEO-Neoxanthin; VIO-Violaxanthin; Total CAR- Total carotenoids.

[b] The standard error of the mean was VIO ± 0.07; NEO ± 0.11; ANTH ± 0.14; LUT ± 0.27; ZEA ± 0.01; BC ± 0.17; Total CAR ± 0.52.

[c] ns indicate nonsignificant at $P \leq 0.05$.

Table 6.5. Carotenoid fruit tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with exogenous applications of ABA.

	Concentrations (mg/100 g FM)[ab]		
ABA[c]	**LUT**	**BC**	**LYCO**
Control	0.11	0.29	5.97
Spray	0.13	0.36	6.07
Roots	0.15	0.31	5.31
Spray/Roots	0.12	0.30	4.91
P-Value[d]	**	*	*

[a] BC-β-carotene; LUT-Lutein; LYCO-Lycopene.

[b] The standard error of the mean was LUT ± 0.01; BC ± 0.04; LYCO ± 0.72.

[c] ABA treatments control (0.0 mg·L^{-1}); spray (500 mg·L^{-1}); root (50 mg·L^{-1}); spray/root (500 mg·L^{-1}/50 mg·L^{-1}).

[d] *, **, and *** indicate significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 6.6. Carotenoid fruit tissue pigments in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

	Concentrations (mg/100 g FM)[ab]		
Ca ($mg \cdot L^{-1}$)	**LUT**	**BC**	**LYCO**
60	0.13	0.31	5.49
90	0.13	0.34	5.38
180	0.13	0.31	5.84
P-Value[c]	ns	ns	ns

[a] BC-β-carotene; LUT-Lutein; LYCO-Lycopene.

[b] The standard error of the mean was LUT ± 0.01; BC ± 0.02; LYCO ± 0.70.

[c] ns indicate nonsignificant at $P \leq 0.05$.

Table 6.7. Soluble sugars in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with exogenous applications of ABA.

	Concentration ($mg \cdot g^{-1}$) fresh mass[a]	
ABA[b]	**Glucose**	**Fructose**
Control	13.39	13.72
Spray	18.74	18.32
Root	19.19	19.24
Spray/Root	18.15	17.76
P-Value[c]	***	***

[a] The SE of the mean for Glucose ± 1.24; Fructose ± 1.04.

[b] ABA treatments control (0.0 $mg \cdot L^{-1}$); spray (500 $mg \cdot L^{-1}$); root (50 $mg \cdot L^{-1}$); spray/root (500 $mg \cdot L^{-1}$/50 $mg \cdot L^{-1}$).

[c] *** indicate significant at $P \leq 0.001$.

Table 6.8. Soluble sugars of fruit tissue in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

	Concentration ($mg \cdot g^{-1}$) fresh mass[a]	
Ca ($mg \cdot L^{-1}$)	**Glucose**	**Fructose**
60	15.70	16.29
90	17.10	16.88
180	19.29	18.62
P-Value[b]	***	**

[a] The SE of the mean for Glucose ± 1.25; Fructose ± 1.01.

[b] ** and *** indicate significant at $P \leq 0.01$, 0.001, respectively.

Table 6.9. Organic acids in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with exogenous applications of ABA.

	Concentration ($mg \cdot g^{-1}$) fresh mass[a]	
ABA[b]	**Malic Acid**	**Citric Acid**
Control	2.06	4.07
Spray	0.81	1.59
Root	0.74	1.34
Spray/Root	0.92	1.83
P-Value[c]	***	***

[a] The SE of the mean for Malic acid ± 1.24; Citric acid ± 0.24.

[b] ABA treatments control (0.0 $mg \cdot L^{-1}$); spray (500 $mg \cdot L^{-1}$); root (50 $mg \cdot L^{-1}$); spray/root (500 $mg \cdot L^{-1}$/50 $mg \cdot L^{-1}$).

[c] *** indicate significant at $P \leq 0.001$.

Table 6.10. Organic acids of fruit tissue in 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

	Concentration ($mg \cdot g^{-1}$) fresh mass[a]	
Ca ($mg \cdot L^{-1}$)	**Malic Acid**	**Citric Acid**
60	1.09	2.21
90	1.03	1.87
180	1.28	2.54
P-Value[b]	ns	ns

[a] The SE of the mean for Malic acid ± 0.16; Citric acid ± 0.21.

[b] ns indicate non-significant at $P \leq 0.05$.

Chapter 8
Effects of Abscisic Acid on Tomato Fruit Aroma Volatiles

Abstract

Aroma volatiles are derived from a diverse set of precursors, such as amino acids, fatty acids and carotenoids in tomato fruit. Many of these volatiles enhance the main flavor components in the fruit, particularly soluble sugars and organic acids. There are no published reports on the effect of ABA on aroma volatiles in tomato (*Solanum lycopersicum*) fruit. ABA is derived from the carotenoid pathway and there may be an indirect connection to flavor volatiles through this pathway. Therefore, the purpose of this study was to examine the influence of ABA on tomato fruit aroma volatiles. Seeds of 'Mt. Fresh Plus' tomato were grown in the greenhouse at 25/20 °C (day/night) under a 16 h photoperiod. Plants were treated with ABA applications weekly. Ca treatments were applied at three different treatment levels of 60, 90, and 180 $mg \cdot L^{-1}$. Ca treatments were applied to the plants via the irrigation lines. ABA treatments were applied as a combination of foliar sprays and root applications. For foliar ABA applications, treatments consisted of DI water control (0.0 mg $ABA \cdot L^{-1}$) or 500 mg $ABA \cdot L^{-1}$. For ABA root applications, treatments consisted of a DI water control (0.0 mg $ABA \cdot L^{-1}$) or 50 mg $ABA \cdot L^{-1}$ applied via the irrigation lines. ABA spray treatments were applied once weekly till dripping from the foliage, while root applications were applied four times per day with the irrigation cycle. This study identified five flavor volatile compounds that were consistently present in 'Mt. Fresh Plus' tomato fruit tissue. They were 2-methyl furan, (E)-2-hexeanl, 1-hexanol, hexenal, and 6-methyl-5-hepten-2-one. ABA treatments did not have an effect on aroma volatile concentrations in 'Mt. Fresh Plus' tomato fruit. Majority of the volatiles identified did not differ between the ABA treated plants and the ABA control plants. However, ABA treatments did significantly decrease (E)-2-hexenal. These results indicated that while ABA treatments are beneficial for increasing quality by influencing soluble sugar and organic acid content, the treatments did not have a major effect on the aroma volatile profile of the fruit.

Keywords: *Mt. Fresh Plus, Calcium, flavor, hexenol, hexanal*

Introduction

Tomato (*Solanum lycopersicum*) cultivar selections from plant breeders have emphasized grower demands for yield, fruit size, firmness and resistance to biotic and abiotic diseases (Maul et al., 2000). For the most part, growers get paid for pounds of product in the box and not for taste quality. As a result, the sensory aspects of fruit quality, such as flavor and aroma, have diminished. Even more troubling is that consumers frequently associate newer hybrid tomato cultivars with poor flavor and aroma (Klee and Tieman, 2013). In recent years there has been a reconnection between the consumer, producers and breeders to bring to the forefront the flavor and aroma of the tomato fruit. Flavor is a function of aroma volatiles that enhance the flavor quality and removing them greatly reduces flavor intensity (Baldwin et al., 2008).

The chemicals that contribute to the flavor of tomato fruit have been well documented. Approximately 400 volatile compounds have been identified in tomato fruit (Petro-Turza, 1986, Baldwin et al., 2000). Aroma volatiles are derived from a diverse set of precursors, such as amino acids, fatty acids and carotenoids (Klee and Tieman, 2013). The main function of aroma volatiles in tomato fruit is to enhance the main flavor components, which are soluble sugars and organic acids (Klee and Tieman, 2013, Tieman et al., 2012). Therefore, aroma volatiles will enhance sweetness, acidity, or green flavor depending on the perceptions of consumer preferences.

There is a high variation in the profile of aroma volatiles across tomato cultivars (Tieman et al., 2012). Previous research has demonstrated that the more prevalent aroma volatiles in tomato fruit are hexenal, (E)-2-hexenal and 6-methyl-5-hepten-2-one (Buttery and Ling, 1993, Baldwin et al., 1991b). In addition, research demonstrated that volatiles cis-3-hexenal, trans-2-hexenal, hexanal and 2-isobutylthiazole contribute to the quality of ripe tomato fruit by

enhancing the fresh flavor and aroma (Stone et al., 1975). Carbonell-Barrachina et al. (2006) demonstrated a difference between different types of tomatoes based on their flavor volatile composition. The tomato cultivar De la Pera, which contained the highest content of flavor volatiles, received the highest values of odor and aroma. Additionally, not all aroma volatile compounds contribute to tomato flavor equally. For example, a more common aroma volatile, a six carbon hexanal, contributes to tomato flavor more than some other aroma volatile, such as geranial (Klee and Tieman, 2013). The profile of aroma volatiles in any particular tomato fruit will depend on numerous environmental factors, such as temperature, light, seasonal differences and site variations (Tieman et al., 2012).

There are no published reports on the effect of ABA on aroma volatiles. However, ABA is derived from the carotenoid pathway and there may be an indirect connection to flavor volatiles through this pathway. Therefore, the purpose of this study was to examine the influence of ABA on tomato fruit quality, specifically aroma volatiles. Previous research demonstrated that ABA positively influences tomato fruit quality by increasing soluble sugars and decreasing organic acids (Barickman et al., Unpublished manuscript-a; Barickman et al., Unpublished manuscript-b). Since aroma volatiles are derived from a diverse set of precursors the assumption is that ABA may positively affect them as well. In addition, aroma volatiles in newer tomato cultivars have not been studied extensively. Previous research studied the carotenoid, soluble sugar and organic acid content of 'Mt. Fresh Plus' tomato (Barickman et al., Unpublished manuscript-a; Barickman et al., Unpublished manuscript-b). Therefore, this study further examined this tomato cultivar's fruit quality by identifying its aroma volatiles.

Methods and Materials

Plant Culture and Harvest. Seeds of 'Mountain Fresh Plus' tomato (Johnny's Selected Seed, Waterville, ME) were sown into Pro-Mix BX soilless medium (Premier Tech Horticulture, Québec, Canada) and germinated in the greenhouse conditions (Knoxville, TN; 35°N Lat.) at 25/20 °C (day/night) under a 16 h supplemental light at an average of 925 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At 30 days after seeding, the plantlets were transferred to 11-L Dutch pots (Tek Supply, Dyersville, IA) filled with Sunshine® Pro Soil Conditioner (Sungro Horticulture, Agawam, MA). Tomato plants were grown hydroponically with a tomato fertilization program developed at the University of Tennessee (Knoxville, TN). Elemental concentrations of the nutrient solutions were ($mg \cdot L^{-1}$): nitrogen (N; 180), phosphorus (P; 93.0), potassium (K; 203.3), magnesium (Mg; 48.6), sulfur (S; 96.3), iron (Fe; 1.0), boron (B; 0.25), manganese (Mn; 0.25), zinc (Zn; 0.025), copper (Cu; 0.01), and molybdenum (Mo; 0.005). Experimental design was a randomized complete block with a 3 x 2 factorial arrangement of treatments, which consisted of six blocks and two replications of each treatment, with individual pots representing an experimental unit. Calcium was applied via the irrigation lines at 60, 90, or 180 mg $Ca \cdot L^{-1}$. ABA treatments were applied as a combination of foliar sprays and root applications. For foliar ABA applications, treatments consisted of DI water control (0.0 mg $ABA \cdot L^{-1}$) or 500 mg $ABA \cdot L^{-1}$. For ABA root applications, treatments consisted of a DI water control (0.0 mg $ABA \cdot L^{-1}$) or 50 mg $ABA \cdot L^{-1}$ applied via the irrigation lines. ABA spray treatments were applied once weekly till dripping from the foliage, while root applications were applied four times per day with the irrigation cycle. Fruit tissues were harvested 84-90 days after seeding. Subsequently, fruit were sorted by the use of USDA tomato color for red ripe (USDA, 1975) and size classification into extra-large, large, medium and small (USDA, 2007). Tomato fruit with BER were categorized separately. Fruit from each treatment

were separated by replication and were weighed for biomass. At least three fruit from the second clusters for each experimental unit were subsampled, combined, and frozen in liquid nitrogen. Harvested fruit samples were stored at -80 °C prior to analysis.

Tomato Aroma Volatiles. Tomato fruit aroma volatiles were extracted and measured from fresh-frozen red ripe fruit tissues and quantified according to Baldwin et al. (1991a) with slight modifications. Fruit was thawed until slightly friable. A sample of red ripe fruit from each treatment/replication was blended to a slurry. A 5 mL subsample of the slurry was placed into a 20 mL headspace vial and 2 mL of a saturated $CaCl_2$ solution was added. Sample vials were vortexed for 1 min then allowed to set for 30 min. Samples were placed on a static headspace analyzer (Agilent Technologies, Palo Alto, CA) prior to gas chromatography mass spectroscopy (GC-MS) analysis.

An Agilent 6890 series GC unit with a 5873 mass spectrometer detector (Agilent Technologies) was used to identify tomato aroma volatiles. Chromatographic separations were achieved using an analytical scale (0.25 mm i.d. x 30 m) 0.25 μm DB-wax column (JW; Agilent Technologies), which allowed for effective separation of chemically similar volatile compounds. The peak assignment for individual volatiles was performed by comparing retention times and mass spectra (NIST, 2002) using external standards (hexenal, (E)-2-hexenal, 1-hexanol, 2-methyl furan, and 6-methyl-5-hepten-2-one, (Z)-3-hexenal, isobutyl-2-heptenone, dimethylulfide, 1-penten-3-one; (Acros Organics, Fisher Scientific, Pittsburg, PA).

Results

This study found five aroma volatile compounds that were consistently identified in ‘Mt. Fresh Plus’ tomato fruit tissue produced in the ABA and Ca treatments. These aroma volatiles were 2-methyl furan, (E)-2-hexeanl, 1-hexanol, hexenal, and 6-methyl-5-hepten-2-one (**Table**

7.1; Table 7.2). Several flavor volatiles were identified in tomato fruit but were not present at detectable levels consistently enough to analyze statistically. These compounds were acetone, (Z)-3-hexenal, isobutyl-2-heptenone, dimethylulfide, 1-penten-3-one, dimethyl disulfide and 2-nonynoic acid (Data not shown).

The statistical analysis of the results indicated that there was no interaction between ABA and Ca treatments on tomato fruit tissue. Therefore, the following results are presented separately for ABA and Ca effects. The application of ABA either as a foliar spray (500 $mg \cdot L^{-1}$), root application (50 $mg \cdot L^{-1}$), or a foliar spray and root combination application did not significantly affect tomato aroma volatile concentrations of most compounds in tomato fruit tissue overall (**Table 7.1**). However, the application of ABA did significantly decrease (E)-2-hexenal (**Table 7.1**). ABA applications decreased (E)-2-hexenal by 53.8% when comparing the ABA control treatment to the combination of foliar spray and root treatment combination. Ca treatments did not have a significant effect on tomato fruit volatile concentrations (**Table 7.2**).

Discussion

This study examined the effects of ABA and Ca treatments on tomato fruit aroma volatiles. Five aroma volatiles that were identified in tomato fruit correspond to results reported in other studies (Buttery and Ling, 1993, Baldwin et al., 2000, Baldwin et al., 2008). Previous published research has not profiled the fruit aroma volatiles of 'Mt. Fresh Plus' tomato cultivar. However, Baldwin et al. (1991b) studied six tomato cultivars and identified a similar profile of aroma volatiles as the current study. One of the most prevalent groups of volatiles identified was aldehyde. Hexenal, one of the major aldehydes in tomato fruit, is considered to be important for fresh tomato flavor (Petro-Turza, 1986) and it significantly contributes to tomato fruit flavor

(Buttery and Ling, 1993). Therefore, despite differences in tomato cultivars there are several aroma volatiles that are key components contributing to distinct tomato flavor.

This study found that ABA treatments did not affect most of the aroma volatile concentrations in 'Mt. Fresh Plus' fruit. The majority of the volatile concentrations did not differ from the ABA control treatment. However, ABA treatments did significantly decrease (E)-2-hexenal. These results indicated that while ABA treatments are beneficial for increasing flavor by influencing soluble sugars and organic acids (Barickman et al., Unpublished manuscript-a; Barickman et al., Unpublished manuscript-b), the treatments may not be conducive for positively affecting the aroma volatile profile of the fruit. In fact, these results indicated that ABA treatments could negatively affect certain aroma volatiles, but do not have a significant overall impact on most aroma volatiles. This is the first report on the effect of ABA on tomato fruit aroma volatiles in any tomato cultivar, and the first report on the aroma volatile profile of the 'Mt. Fresh Plus'. Different tomato cultivars have varying aroma volatile profiles. Therefore, future research on ABA treatments should compare the 'Mt. Fresh Plus' to other tomato cultivars to gain a better understanding of ABA's effects on tomato aroma volatiles.

Additionally, results of this study indicated that decreasing Ca concentration did not affect tomato aroma volatiles. This may indicate that tomato aroma is not affected in Ca deficient environments. However, previous research demonstrated that lack of Ca in the hydroponic fertilizer solution decreased other key tomato fruit flavor components, such as soluble sugars and organic acids, while it did not affect carotenoid concentrations (Barickman et al., Unpublished manuscript-a; Barickman et al., Unpublished manuscript-b). Therefore, despite the fact that Ca deficiencies did not affect on tomato fruit aroma volatiles and carotenoids,

overall tomato flavor is still negatively affected since soluble sugars and organic acid are the main components that add flavor to the fruit.

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Appendix 7: Tables

Table 7.1. Tomato fruit aroma volatiles in fruit of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

	Concentration (µmol·g^{-1}) fresh weight[a]				
ABA	**2-Methyl Furan**	**(E)-2-Hexenal**	**6-Methyl-5-Hepten-2-one**	**1-Hexanol**	**Hexenal**
Control	1.17	462.28	212.86	2.31	133.37
Spray	0.99	381.64	145.54	1.52	125.47
Root	0.97	259.12	155.03	1.40	109.07
Spray/Root	1.03	213.49	210.04	1.13	93.31
***P*-Value**	ns	**	ns	ns	ns

[a] The SE of the mean for 2-Methyl Furan ± 0.36; (E)-2-Hexenal ± 81.93; 6-Methyl-5-Hepten-2-one ± 54.32; 1-Hexanol ± 0.81; Hexanol ± 19.46.

[b] ABA treatments control (0.0 mg·L^{-1}); spray (500 mg·L^{-1}); root (50 mg·L^{-1}); spray/root (500 mg·L^{-1}/50 mg·L^{-1}).

[c] ns and ** indicate non-significant or significant at $P \leq 0.01$.

Table 7.2. Tomato fruit aroma volatiles of 'Mt. Fresh Plus' tomato grown in a greenhouse and treated with and treated Ca in the hydroponic fertilizer solution.

	Concentration (µmol·g^{-1}) fresh weight[a]				
Ca (mg·L^{-1})	**2-Methyl Furan**	**(E)-2-Hexenal**	**6-Methyl-5-Hepten-2-one**	**1-Hexanol**	**Hexenal**
60	0.95	295.63	156.46	1.21	112.15
90	1.41	335.43	190.00	2.41	127.98
180	0.76	356.34	196.14	1.13	105.79
***P*-Value**	ns	ns	ns	ns	ns

[a] The SE of the mean for 2-Methyl Furan ± 0.34; (E)-2-Hexenal ± 77.60; 6-Methyl-5-Hepten-2-one ± 49.98; 1-Hexanol ± 0.69; Hexanol ± 17.45.

[b] ns indicate non-significant at $P \leq 0.05$.

Appendices

Appendix 8

The purpose of this study was to determine if exogenous abscisic Acid (ABA), applied during the growth and development of tomato fruit, will improve fruit quality in fresh market tomatoes. This study was conducted using field grown tomatoes.

Objectives

- Determine the effects of exogenous ABA on field grown tomato plants for stress responses to disease pressure.
- Evaluate the effects of exogenous ABA on field grown tomato fruit for yield and quality.
- Results of these studies will demonstrate the effectiveness of exogenous ABA applied to tomato plants during growth on tomato fruit quality.

Material and Methods:

Tomato plants of 'Mt. Fresh Plus' were grown 16 in apart within the row and 60 in on center. Abscisic Acid was applied with a CO_2 back-pack sprayer at a pressure of 10 psi to field tomatoes just before anthesis. Tomato plants were sprayed with 4 concentrations of ABA and a control (DI water) until drip. Abscisic Acid was applied in concentrations of 0.0 (Control), 250, 500, 1000, 2000 mg $ABA \cdot L^{-1}$ weekly until harvest for a total of five applications. Red ripe fruits were harvested, graded and analyzed for yield. A sub-sample of three fruit were taken and processed into quarters and frozen for chemical analysis. Fruit mineral nutrients and soluble sugars were analyzed at The University of Tennessee, Department of Plant Science.

Nutrient Analysis: Eight representative leaves and red ripe fruit were collected from clusters two through four from each plot, triple rinsed with de-ionized water and dried for five days in a forced air oven at 65 °C. Dried samples were ground to homogeneity using a coffee grinder and 0.5 g sub-samples were weighed for analysis. Samples were placed into a muffle furnace at 450

°C for 6 hrs to allow the sample to ash. Ashed sample were allowed to cool to room temperature then digested with 10 ml concentrated nitric acid. A 100 µl aliquot of the digested sample was diluted with a matrix containing 2% nitric acid and 0.5% hydrochloric acid for analysis. Nutrient analysis was conducted using an inductively coupled plasma mass spectrometer (ICP-MS) at The University of Tennessee, Department of Plant Science.

Results

Plant Growth. Throughout the course of this study there was bi-weekly rating of plant health on disease pressure. Ratings were marked on a scale of 1 to 10, with 10 being a complete loss due to disease (Table 5). Once the onset of disease leaf samples were taken to confirm the type of disease. There were two types of disease, *Alternaria solani* and a bacterial leaf spot, which inhabited the tomato plants. Disease ratings were significant across all dates with the control treatment increasing to a mean rating of 7.67. All ABA treatments increased in disease rate to October 22, and then we observed a decrease of disease rating on October 27. Within each date the disease rating were highly significant and had linear and quadratic trends. On October 22 there were significant disease ratings however, the margins became closer between each ABA treatment and the control.

Yield. Field tomatoes were harvested over four weeks and sorted into fresh market grades. Tomato fruit yield was significantly affected by increasing ABA foliar concentrations (Table 7). In addition, there were significant ABA treatments by data interactions therefore, dates were analyzed separate. As would be expected ABA treatments concentrations when sprayed foliar affected the health of the plant with the occurrence of disease and stunted growth. Consequently, the amount of cull tomatoes due to ABA related stress increased significantly except for the final harvest date on October 27th. Increasing ABA treatment concentrations resulted in a significant increase in large and extra-large fruit yield per plant on the first and last harvest dates,

respectively. However there was no difference in yield on harvest dates October 11th, 14th, and 22nd across all grades. Therefore, in this study applications of ABA treatments resulted in a delay of harvestable fruit. Total yield was significantly different when analyzed by date (Table 8). The first harvest date on October 22nd total fruit yield and number of fruit was highly significant ($P \leq 0.001$) with total number of fruit ranging from 1.39 to 14.87 fruit per plant and total weight ranging from 0.88 to 6.56 lbs per fruit. As the season progress the total number and weight of fruit did not have significant differences and may have been caused by ABA treatment stress or the unusually high early fall temperatures. The last harvest date, ripening fruit were harvested and the plants were stripped of remaining fruit for potential fruit number and yield. The number and yield of immature fruit were not significantly different however; we did see a significant quadratic trend. Therefore, ABA may cause a delay in fruit development after the effects of the spray treatments ceased at the beginning of the harvest.

Nutrient Analysis. Tomato fruit nutrient concentrations were highly significant ($P \leq 0.001$) for sulfur with decreasing concentrations at ABA treatments increased (Table 9). Sulfur had a decrease in concentration by over 1000 $mg \cdot g^{-1}$ DW with increasing concentrations of ABA. Magnesium and P had significant linear trends with increasing concentrations in the fruit as ABA treatments increased. Tomato leaf nutrient concentrations were significant ($P < 0.05$) for Mg, P, S, K and Cu (Table 10). Generally, there were increasing in concentrations as ABA treatment concentrations increased. However, Cu had a significant decrease in concentrations as ABA treatments increased. Nutrients Mg, P, S, Mn, Fe, Cu, and Zn had significant linear trends with increasing concentrations of ABA. Nutrients B and Ca had significant quadratic trends as ABA concentrations increased. Overall, the macro-nutrients increased in concentrations in the leaf tissue and had no significant change in fruit concentrations.

Table 8.1 Number and yield of tomato fruit harvested at different dates in summer of 2010.

Date: 9/22/10	Number of tomato fruit and yield (lbs·plant^{-1})[a]									
ABA (mg·L^{-1})	Jumbo	Jumbo Wt.	X-Large	X-Large Wt.	Large	Large Wt.	Medium	Medium Wt.	% Cull	% Cull Wt.
0	1.54 ± 0.53	1.04 ± 0.0.37	3.19 ± 0.62	1.66 ± 0.31	2.59 ± 0.30	0.85 ± 0.09	0.75 ± 0.22	0.28 ± 0.06	8.57 ± 1.68	8.42 ± 1.65
250	0.33 ± 1.05	0.26 ± 0.74	1.56 ± 0.68	0.92 ± 0.34	1.15 ± 0.30	0.36 ± 0.09	0.37 ± 0.34	0.07 ± 0.08	11.59 ± 1.68	11.27 ± 1.65
500	NA	NA	0.33 ± 1.52	0.19 ± 0.76	0.56 ± 0.30	0.20 ± 0.09	0.50 ± 0.48	0.11 ± 0.11	13.51 ± 1.68	13.63 ± 1.65
1000	NA	NA	0.48 ± 1.07	0.23 ± 0.54	0.98 ± 0.37	0.32 ± 0.11	0.45 ± 0.34	0.10 ± 0.08	14.16 ± 1.68	14.14 ± 1.65
2000	NA	NA	NA	NA	0.25 ± 0.52	0.08 ± 0.16	0.46 ± 0.24	0.07 ± 0.06	12.43 ± 1.68	13.58 ± 1.65
P Value[b]	ns	ns	ns	ns	**	***	ns	ns	ns	ns
Contrast										
Linear	ns	ns	ns	ns	*	**	ns	ns	ns	*
Quadratic	ns	ns	ns	ns	ns	*	ns	ns	*	*

Date: 10/11/10	Number of tomato fruit and yield (lbs·plant^{-1})[a]									
ABA (mg·L^{-1})	Jumbo	Jumbo Wt.	X-Large	X-Large Wt.	Large	Large Wt.	Medium	Medium Wt.	% Cull	% Cull Wt.
0	NA	NA	0.52 ± 0.18	0.26 ± 0.09	1.86 ± 0.61	0.51 ± 0.12	NA	NA	7.73 ± 1.81	8.78 ± 1.72
250	NA	NA	0.17 ± 0.31	0.08 ± 0.16	1.61 ± 0.80	0.16 ± 0.27	NA	NA	16.59 ± 1.81	16.89 ± 1.72
500	NA	NA	0.17 ± 0.44	0.12 ± 0.23	1.28 ± 0.70	0.08 ± 0.19	NA	NA	16.67 ± 2.01	16.68 ± 1.92
1000	NA	NA	0.25 ± 0.44	0.11 ± 0.23	NA	0.06 ± 0.19	NA	NA	13.32 ± 1.81	12.21 ± 1.72
2000	NA	NA	NA	NA	NA	NA	NA	NA	17.64 ± 3.99	16.89 ± 3.84
P Value[b]	ns	ns	ns	ns	ns	ns	ns	ns	*	*
Contrast										
Linear	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Quadratic	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 8.1 (Continued) Number and yield of tomato fruit harvested at different dates in summer of 2010.

Date: 10/11/10	Number of tomato fruit and yield (lbs·plant^{-1})[a]									
ABA (mg·L^{-1})	Jumbo	Jumbo Wt.	X-Large	X-Large Wt.	Large	Large Wt.	Medium	Medium Wt.	% Cull	% Cull Wt.
0	NA	NA	0.81 ± 0.18	0.35 ± 0.08	1.86 ± 0.70	0.55 ± 0.20	0.13 ± 0.07	0.09 ± 0.26	7.60 ± 1.97	7.98 ± 1.93
250	NA	NA	0.34 ± 0.26	0.15 ± 0.11	0.36 ± 0.86	0.10 ± 0.24	0.06 ± 0.12	0.04 ± 0.26	9.13 ± 2.16	9.04 ± 2.11
500	NA	NA	0.17 ± 0.32	0.09 ± 0.13	0.14 ± 1.72	0.03 ± 0.48	0.03 ± 0.12	0.01 ± 0.26	12.23 ± 2.41	11.97 ± 2.37
1000	NA	NA	0.17 ± 0.45	0.07 ± 0.18	NA	NA	NA	NA	17.56 ± 2.41	17.56 ± 2.37
2000	NA	NA	NA	NA	NA	NA	NA	NA	11.12 ± 3.41	11.22 ± 3.35
P Value[b]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Contrast										
Linear	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Quadratic	ns	ns	ns	ns	ns	ns	ns	ns	*	*

Date 10/22/10	Number of tomato fruit and yield (lbs·plant^{-1})[a]									
ABA (mg·L-1)	Jumbo	Jumbo Wt.	X-Large	X-Large Wt.	Large	Large Wt.	Medium	Medium Wt.	% Cull	% Cull Wt.
0	NA	NA	0.53 ± 0.74	0.17 ± 0.28	1.14 ± 0.34	0.39 ± 0.11	0.54 ± 0.46	0.19 ± 0.12	10.91 ± 0.85	10.75 ± 1.04
250	NA	NA	1.32 ± 0.60	0.64 ± 0.28	1.57 ± 0.38	0.55 ± 0.12	1.28 ± 0.41	0.29 ± 0.11	8.18 ± 0.85	8.37 ± 1.04
500	NA	NA	0.25 ± 0.74	0.13 ± 0.34	0.33 ± 0.77	0.13 ± 0.25	NA	NA	14.44 ± 0.95	14.06 ± 1.16
1000	NA	NA	NA	NA	0.17 ± 0.77	0.06 ± 0.25	NA	NA	16.67 ± 1.90	16.67 ± 2.32
2000	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F-test	ns	ns	ns	ns	ns	ns	ns	ns	**	*
Contrast										
Linear	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Quadratic	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 8.1 (Continued) Number and yield of tomato fruit harvested at different dates in summer of 2010.

Date 10/22/10	Number of tomato fruit and yield (lbs·plant^{-1})[a]									
ABA (mg·L^{-1})	Jumbo	Jumbo Wt.	X-Large	X-Large Wt.	Large	Large Wt.	Medium	Medium Wt.	% Cull	% Cull Wt.
0	NA	NA	0.68 ± 0.23	0.28 ± 0.11	1.02 ± 0.44	0.31 ± 0.12	1.10 ± 0.23	0.19 ± 0.04	5.91 ± 1.20	1.51 ± 0.42
250	NA	NA	0.68 ± 0.21	0.33 ± 0.10	1.63 ± 0.40	0.48 ± 0.11	0.68 ± 0.23	0.12 ± 0.04	3.11 ± 1.10	0.54 ± 0.38
500	NA	NA	0.84 ± 0.37	0.41 ± 0.17	0.77 ± 0.43	0.22 ± 0.12	1.17 ± 0.36	0.19 ± 0.06	2.05 ± 1.20	0.25 ± 0.42
1000	NA	NA	4.5 ± 0.52	2.26 ± 0.24	1.69 ± 0.55	0.45 ± 0.15	NA	NA	3.20 ± 1.20	0.47 ± 0.42
2000	NA	NA	NA	NA	NA	NA	0.17 ± 0.51	0.03 ± 0.09	2.06 ± 1.87	0.38 ± 0.65
F-test	ns	ns	**	**	ns	ns	ns	ns	ns	ns
Contrast										
Linear	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Quadratic	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a] NA= Not available

[b] ns, *, **, and *** indicate non-significant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 8.2 Mean values for mineral nutrient concentrations in leaf tissue of 'Mt. Fresh Plus' tomato plants grown in the field and treated with a foliar spray of s-ABA.

	Concentrations of mineral nutrients ($\mu g \cdot g^{-1}$)[a]										
ABA ($mg \cdot g^{-1}$)	**B**	**Mg**	**P**	**S**	**K**	**Ca**	**Mn**	**Fe**	**Cu**	**Zn**	**Mo**
0	45.70	7274	3533	3223	33333	27915	125.71	106.51	17.12	25.23	0.17
250	46.44	6499	3501	3464	40073	24894	118.51	108.77	15.00	23.06	0.17
500	44.55	7002	3506	3567	40197	22993	123.08	110.10	14.11	28.10	0.16
1000	38.09	7104	3541	4407	38147	23565	130.68	109.01	10.55	26.36	0.16
2000	46.87	8868	4433	6079	39283	28457	164.71	124.87	13.12	30.56	0.14
P Value[b]	ns	*	**	***	*	ns	ns	ns	**	ns	ns
Contrast											
Linear	ns	**	**	***	ns	ns	*	*	**	*	ns
Quadratic	*	ns	ns	ns	ns	*	ns	ns	**	ns	ns

[a] Standard Error (SE) - B ± 2.22; Mg ± 546; P ± 184; S ± 339; K ± 1613; Ca ± 2259; Mn ± 16.88; Fe ± 6.33; Cu ± 1.41; Zn ± 1.89; Mo ± 0.02.

[b] ns, *, **, and *** indicate non-significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 8.3 Mean values for mineral nutrient concentrations in fruit tissue of 'Mt. Fresh Plus' tomato plants grown in the field and treated with a foliar spray of s-ABA.

	Concentrations of mineral nutrients ($\mu g \cdot g^{-1}$)										
ABA	**B**	**Mg**	**P**	**S**	**K**	**Ca**	**Mn**	**Fe**	**Cu**	**Zn**	**Mo**
0	3.91	710	1243	2453	13319	477	8.05	23.29	3.85	11.79	0.01
250	3.58	800	1624	2099	14217	431	9.57	38.15	3.46	12.22	0.02
500	3.52	772	1386	1799	13387	442	7.36	24.45	3.17	10.34	0.01
1000	4.19	856	1787	1462	16661	372	8.74	64.44	5.11	11.92	0.03
2000	4.66	1053	2121	1416	17943	607	8.14	31.46	4.36	12.92	0.04
F-Test	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	ns
Contrast											
Linear	ns	*	*	***	ns	ns	ns	ns	ns	ns	ns
Quadratic	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	ns

[a] Standard Error (SE) - B ± 0.54; Mg ± 119; P ± 259; S ± 65; K ± 2042; Ca ± 118; Mn ± 1.64; Fe ± 11.74; Cu ± 0.72; Zn ± 1.44; Mo ± 0.02.

[b] ns, *, **, and *** indicate non-significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Appendix 9

SAS Output Statements

Field Study Fruit ICP SAS Statement

```
data one;
input Trt Rep  Dilution B Mg P S K Ca Mn Fe Cu Zn Mo;
datalines;
;
%include 'c:\danda.sas';
%orthpoly (0 250 500 1000 2000);
%mmaov (one, B      Mg     P      S      K      Ca     Mn     Fe     Cu     Zn     Mo,
class=trt rep, fixed= trt, random= rep, contrast=%str(
 Contrast 'Linear'                Trt
                  -3       -2       -1        1        5 ;
Contrast 'Quadratic'              Trt
                 15.5       1     -9.5     -18.5     11.5 ;));
```

Field Study Leaf ICP SAS Statement

```
data one;
input Trt Rep   Dilution B Mg P S K Ca Mn Fe Cu Zn Mo;
datalines;
;
%include 'c:\danda.sas';
%orthpoly (0 250 500 1000 2000);
%mmaov (one, B       Mg      P       S       K       Ca      Mn      Fe      Cu      Zn      Mo,
class=trt rep, fixed= trt, random= rep, contrast=%str(
Contrast 'Linear'                   Trt
                    -3          -2          -1          1           5 ;
Contrast 'Quadratic'                Trt
                  15.5          1         -9.5        -18.5        11.5 ;));
```

Field Study Fruit Sugars SAS Statement

```
data one;
input trt rep fructose glucose sucrose total fruit weight;
datalines;
;
%include 'c:\danda.sas';
%mmaov (one, fructose glucose sucrose total fruit weight, class=trt rep, fixed= trt, random=
rep,sort=yes);
```

Field Study Fruit Carotenoids SAS Statement

```
OPTIONS PAGESIZE=60;
DATA ONE;
INPUT TRT REP BC LUT LYC;
TRT2=TRT**2;
TRT3=TRT**3;
CARDS;
;
PROC GLM;
CLASS TRT REP;
MODEL BC LUT LYC = TRT;
TEST H=TRT E=TRT*REP;
MEANS TRT;
MEANS TRT / DUNCAN;
RUN;

PROC REG DATA=ONE;
MODEL BC LUT LYC=TRT;
MODEL BC LUT LYC=TRT TRT2;
RUN;
```

Field Study Fruit Carotenoids SAS Statement

```
data one;
input Trt Rep DisRate1 DisRate2 DisRate3 DisRate4 DisRate5 DisRate6;
datalines;
;
%include 'c:\danda.sas';
%orthpoly (0 250 500 1000 2000)
%mmaov (one, DisRate1 DisRate2 DisRate3 DisRate4 DisRate5 DisRate6 ,
class= trt rep, fixed=trt, random= rep,contrasts=%str(

Contrast 'Linear' trt
                -3 -2 -1 1 5 ;


Contrast 'Quadratic' trt
                15.5 1 -9.5 -18.5 11.5 ;));
```

Mini Tomato Leaf ICP SAS Statement

```
Data one;
input cul $ trt  rep B    Mg P    S        K        Ca       Mn       Fe       Cu       Zn       Mo;
datalines;
;
%include 'c:\danda.sas';
%orthpoly (0 .5 5 10);
%mmaov (one,B        Mg       P        S        K        Ca       Mn       Fe       Cu       Zn       Mo,
class= trt rep, fixed=trt , random= rep,contrasts=%str(
Contrast 'Linear'                  Trt
                  -3.44          -3          1       5.44 ;
Contrast 'Quadratic'               Trt
                  3.26           1       -8.42       4.16;));
```

Mini Tomato Leaf Carotenoids SAS Statement

```
Data one;
input cul $      Trt      Rep     BC      LUT     ZEA     ANTH NEO     VIO     TCAR CHLA CHLB
        TCHL  ZAV    ZAZAV          AB      CARCHL;
datalines;
;
%include 'c:\danda.sas';
%orthpoly (0 .5 5 10);
%mmaov (one,BC      LUT     ZEA     ANTH NEO     VIO     TCAR CHLA CHLB TCHL  ZAV
        ZAZAV          AB      CARCHL, class= cul trt rep, fixed=cul trt cul*trt, random=
rep,contrasts=%str(
Contrast 'Linear'                Trt
                -3.44          -3           1       5.44 ;
Contrast 'Quadratic'               Trt
                3.26           1       -8.42       4.16;));
```

Mini Tomato Fruit Carotenoids SAS Statement

```
Data one;
input cul $       Trt       Rep      BC       LUT LYCO;
datalines;
;
%include 'c:\danda.sas';
%mmaov (one, BC      LUT     LYCO, class=trt rep, fixed=trt, random= rep,contrasts=%str(
 contrast 'control vs. ABA' trt 3 -1 -1 -1));
```

Mini Tomato Fruit ICP SAS Statement

```
Data one;
input cul $ trt  rep B    Mg P    S        K        Ca       Mn       Fe       Cu       Zn       Mo;
datalines;
;
%include 'c:\danda.sas';
%orthpoly (0 .5 5 10);
%mmaov (one,B        Mg       P        S        K        Ca       Mn       Fe       Cu       Zn       Mo,
class= trt rep, fixed= trt, random= rep, contrasts=%str(
Contrast 'Linear'                  Trt
                -3.44          -3          1       5.44 ;
Contrast 'Quadratic'               Trt
                3.26           1      -8.42      4.16;));
```

GH Spray ABA Yield SAS Statement

```
data one;
input CaTrt ABA Rep Cluster Jumbo JumboWt XXL XXLWt XL XLWt
      Large LargeWt Medium MediumWt Small SmallWt BER
      BERWt total totalwt;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,Jumbo JumboWt XXL XXLWt XL XLWt Large LargeWt
      Medium MediumWt Small SmallWt BER BERWt total totalwt,
class=CaTrt ABA rep cluster, fixed=CaTrt|ABA, random=rep rep*ABA*CaTrt
cluster(rep*ABA*CaTrt), option=sort);
```

GH Spray ABA Leaf ICP SAS Statement

```
data one;
input Sample CaTrt ABA Rep Cluster B Na Mg P S K Ca
Mn Fe Cu Zn Mo;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one, B Na Mg P S K Ca Mn Fe Cu Zn Mo, class=CaTrt ABA rep cluster,
fixed=CaTrt|ABA|cluster, random=rep rep*ABA*CaTrt*cluster, options=sort);
```

GH Spray ABA Leaf Carotenoids and Chlorophylls SAS Statement

```
data one;
input CaTrt ABA Rep Cluster VIO NEO ANTH ChlB LUT ZEA ChlA      BC  TotalCAR
TotalChl;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one, VIO NEO ANTH       ChlB LUT ZEA ChlA BC TotalCAR TotalChl, class= rep
CaTrt ABA cluster, fixed=CaTrt|ABA, random=rep rep*ABA*CaTrt cluster(rep*ABA*CaTrt),
options=sort);
```

GH Spray ABA Fruit ICP SAS Statement

```
data one;
input CaTrt ABA Rep Cluster Loc $ B Na Mg P K Ca Mn Fe Cu Zn Mo;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one, B Na Mg P K Ca Mn Fe Cu Zn Mo, class=rep CaTrt ABA cluster Loc,
fixed=CaTrt|ABA|Loc|cluster, random=rep rep*ABA*CaTrt*loc*cluster, options=sort);
```

GH Spray ABA Fruit Carotenoids SAS Statement

```
data one;
input CaTrt ABA Rep Cluster Loc $ LUT BC LYCO;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one, LUT BC LYCO, class= CaTrt ABA Rep cluster Loc, fixed=CaTrt|ABA|Loc,
random=rep rep*ABA*CaTrt*loc cluster(rep*ABA*CaTrt), options=sort);
```

GH Spray ABA Fruit Sugars SAS Statement

```
data one;
input CaTrt ABA Rep Cluster Loc $ glucose fructose;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one, glucose fructose, class= CaTrt ABA Rep cluster Loc, fixed=CaTrt|ABA|Loc,
random=rep rep*ABA*CaTrt cluster(rep*ABA*CaTrt), options=sort);
```

GH Spray/Root ABA Yield SAS Statement

```
data one;
input block rep CaTrt ABA $ Cluster Jumbo JumboWt XXL XXLWt XL XLWt
      Large LargeWt Medium MediumWt Small SmallWt BER
      BERWt ;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,Jumbo JumboWt XXL XXLWt XL XLWt Large LargeWt
      Medium MediumWt Small SmallWt BER BERWt, class=block rep
CaTrt ABA cluster, fixed=CaTrt|ABA|cluster, random=block rep block*rep
block*rep*ABA*CaTrt*cluster , option=sort);
```

GH Spray/Root ABA Leaf ICP SAS Statement

```
data one;
input block rep CaTrt ABA $  B       Na       Mg      P       S       K       Ca       Mn      Fe
        Cu      Zn      Mo;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,B       Na      Mg      P       S       K       Ca      Mn      Fe      Cu      Zn
        Mo, class=block rep CaTrt ABA, fixed=CaTrt|ABA, random=block rep block*rep
block*rep*ABA*CaTrt, option=sort);
```

GH Spray/Root ABA Leaf Carotenoids and Chlorophylls SAS Statement

```
data one;
input block rep CaTrt ABA $  VIO    NEO    ANTH CHLB LUT    ZEA    CHLA BC;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,VIO     NEO    ANTH CHLB LUT    ZEA    CHLA BC      , class=block rep
CaTrt ABA, fixed=CaTrt|ABA, random=block rep block*rep block*rep*ABA*CaTrt,
option=sort);
```

GH Spray/Root ABA Fruit ICP SAS Statement

```
data one;
input block rep CaTrt ABA $  loc $ B        Na      Mg      P       S       K       Ca      Mn
        Fe      Cu      Zn      Mo;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,B        Na      Mg      P       S       K       Ca      Mn      Fe      Cu      Zn
        Mo, class=block rep CaTrt ABA loc, fixed=CaTrt|ABA|loc, random=block rep block*rep
block*rep*ABA*CaTrt, option=sort);
```

GH Spray/Root ABA Fruit Carotenoids SAS Statement

```
data one;
input block rep CaTrt ABA $  loc $ LUT      BC      LYCO;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,LUT     BC      LYCO, class=block rep CaTrt ABA loc, fixed=CaTrt|ABA|loc,
random=block rep block*rep block*rep*ABA*CaTrt, option=sort);
```

GH Spray/Root ABA Fruit Sugars SAS Statement

```
data one;
input block rep CaTrt ABA $ Loc $   fructose glucose ;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,fructose glucose, class=block rep CaTrt ABA loc, fixed=CaTrt|ABA|loc,
random=block rep block*rep*ABA*CaTrt, option=sort);
```

GH Spray/Root ABA Fruit Organic Acids SAS Statement

```
data one;
input block rep CaTrt ABA $ Loc $   malic citric ;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,malic citric, class=block rep CaTrt ABA loc, fixed=CaTrt|ABA|loc,
random=block rep block*rep*ABA*CaTrt, option=sort);
```

GH Spray/Root ABA Fruit Aroma Volatiles SAS Statement

```
data one;
input block rep CaTrt ABA $  Furan  twoHexenal    Methylhepten  oneHexanol    Hexenal;
datalines;
;
%include 'c:\danda.sas';
%mmaov(one,Furan  twoHexenal    Methylhepten  oneHexanol    Hexenal, class=block rep
CaTrt ABA, fixed=CaTrt|ABA, random=block rep block*rep block*rep*ABA*CaTrt,
option=sort);
```

Vita

Thomas Casey Barickman was born in Quincy, IL on June 23, 1978. He was raised in Canton, MO until moving to New Sharon, IA in 1992, where he graduated from North Mahaska High School in 1997. He went on to graduate college from Iowa State University of Science and Technology with a B.S. degree in Horticultural Science and Plant Health and Protection in the spring 2005. He has since graduated with a M.S. in Plant Science with a concentration in horticulture from the University of Tennessee and will earn his Ph.D. in May 2014. Currently, he is a Research Associate II at The University of Tennessee, Knoxville, were he manages a horticultural crop physiology and biochemistry laboratory while pursuing a Ph.D. in Plant, Soil, and Insects from the Plant Sciences Department. His research interest includes plant physiology and applied horticulture specializing in fruit and vegetable production and management.