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

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Abstract. **Plants encounter various environmental stress factors that can potentially impact nutritional requirements and fruit quality. Adequate levels of calcium (Ca) in tomato (*Solanum lycopersicum*) fruit have positive effects on fruit quality, specifically firmness. One of the results of insufficient Ca uptake and movement in tomato is the physiological disorder blossom-end rot (BER), which is associated with a Ca deficiency in the distal fruit tissue. Previous research has demonstrated that foliar abscisic acid (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. 'Mt. Fresh Plus' tomato were grown in the greenhouse at 25/20 °C (day/night) under a 16-hour 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 through the irrigation lines at 60, 90, or 180 mg·L^{-1}. ABA treatments were applied as a combination of foliar sprays and root applications. Foliar ABA applications, treatments consisted of deionized (DI) water control (0.0 mg ABA/L) or 500 mg ABA/L. For ABA root applications, treatments consisted of a DI water control (0.0 mg ABA/L) or 50 mg ABA/L applied through the irrigation lines. ABA spray treatments were applied once weekly until dripping from the foliage (tops of pots were covered to prevent spray drip into the pot), whereas root applications were applied four times per day through the irrigation system. Fruit tissues were harvested 84 to 90 days after seeding. Fruit tissue was harvested at red ripe maturity and evaluated for yield, BER, and Ca concentrations. Leaves were harvested at the time of fruit and were analyzed for Ca concentrations. The results indicate that a combination of the spray and root applications of ABA resulted in the greatest decrease in BER. The foliar spray application of ABA combined with the Ca treatment of 180 mg·L^{-1} decreased the incidence of BER. Results also demonstrate that ABA treatments are 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.**

Plants encounter various environmental stresses that can potentially impact nutritional requirements and fruit quality. Environmental stress factors frequently influence vegetative development by inhibiting plant growth. Studies have demonstrated that the plant hormone ABA helps plants acclimate to environmental stresses such as drought, extreme temperatures, and excess light (Hirayama and Shinozaki, 2007; Thompson et al., 2000). ABA affects gas exchange by closing stomatas, thereby decreasing photosynthesis, which has a negative effect on vegetative growth. However, ABA can have a positive effect on 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 (Barickman et al., 2014a; de Freitas et al., 2011).

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 examined how endogenous ABA increases as a result of environment stress such as drought and affects 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, which is passive and through the xylem, increases to tissues such as leaves because they are rapidly growing and have a lower water potential compared with root tissues. Other parts of the plants such as fruit tissue have higher water potentials and lower distribution of stomata than leaf tissue (Blanke, 1986). 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 (Ehret and Ho, 1986). 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., 2014b).

Insufficient Ca uptake and movement in tomato fruit tissue can result in the physiological disorder 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 such as low humidity, high light intensity, and high temperature, which 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 negatively affect stomatal conductance (g_S), which decreased Ca influx into the vegetative tissue allowing more Ca to be moved into the fruit tissue.

Exogenous ABA can be applied to the plant either through root or in the form of a spray to the vegetative tissue. When applied

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to the root tissue, ABA is released to the xylem traveling to the vegetative tissue where it improves water use efficiency by closing the stomata and reducing plant growth (Hartung et al., 2005). Hocking et al. (1972) demonstrated that C_{14}-labeled ABA was widely distributed within the pea (*Pisum sativum*) plant 24 h after application to the roots, whereas ≈18% was found in root nodules. 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. (2014a) has demonstrated that ABA applied to the root tissue of tomato plants positively increases Ca partitioning between 'Micro' tomato leaf and fruit tissue. Therefore, 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 fruit tissue. In addition, this study also examined how root and foliar spray ABA applications, individually and in combination, affect the incidence of BER in the distal tissue of tomato fruit.

Materials and Methods

Plant culture and harvest. Seeds of 'Mountain Fresh Plus' tomato (Johnny's Selected Seed, Winslow, ME) were sown into Pro-Mix BX soilless medium (Premier Tech Horticulture, Québec, Canada) and germinated in greenhouse conditions (Knoxville, TN; lat. 35° N) 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 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). Stock solutions of the nutrient solution were diluted 1:100 by Dosatron® (Model D25RE2; Clearwater, FL) injectors. Tomato plants were fertigated four times daily for 10 min each irrigation cycle. Elemental concentrations of the nutrient solutions were ($mg \cdot L^{-1}$): nitrogen (N) (180), phosphorus (93.0), K (203.3), magnesium (48.6), sulfur (96.3), iron (1.0), boron (0.25), manganese (0.25), zinc (0.025), copper (0.01), and molybdenum (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 three × four factorial arrangement of treatments that consisted of six blocks and two replications of each treatment per block with individual plants representing an experimental unit. Treatments of Ca were applied through the irrigation lines at 60, 90, or 180 $mg \cdot L^{-1}$. The application of 180 $mg \cdot L^{-1}$ of Ca was considered the optimum Ca concentration for greenhouse tomato plants. The application of 60 and 90 $mg \cdot L^{-1}$ of Ca was considered deficient Ca concentrations to simulate a low Ca environment. Ammonium nitrate was added to the 60 and 90 $mg \cdot L^{-1}$ of Ca-treated plants to balance N requirements. The ABA treatments were applied as foliar sprays, root applications, or a combination of foliar and root application. For foliar ABA applications, treatments consisted of DI water control (0.0 mg ABA/L) or 500 $mg \cdot L^{-1}$ ABA. ABA root treatment applications consisted of a 50 $mg \cdot L^{-1}$ ABA applied through the irrigation lines. The combination treatment consisted of a foliar ABA spray of 500 $mg \cdot L^{-1}$ ABA and a root application of 50 $mg \cdot L^{-1}$ ABA. ABA spray treatments were applied once weekly from anthesis to final harvest. ABA sprays were applied until dripping from the foliage (tops of pots were covered to prevent spray drip into the pot), whereas root applications were applied four times per day through the irrigation system. Tomato plants did not experience water stress conditions for the time of the entire experiments. Fruit tissues were harvested 84 to 90 d after seeding. Subsequently, fruit were sorted by the use of USDA tomato color for red ripe (USDA Agricultural Marketing Service, 1975) and size classification into extralarge, large, medium, and small (USDA Agricultural Marketing Service, 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. Proximal tissue was the top third of the tomato fruit. Distal tissue was the bottom third of the tomato fruit. Harvested fruit samples were stored at –80 °C before analysis. Leaf samples for each replication were taken of the first leaf above the second cluster on final harvest of fruit from that cluster for analysis.

Elemental nutrient determination. Nutrient analysis was conducted according 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 (Ethos; Milestone Inc., Shelton, CT). Leaves were collected and triple-rinsed with DI water and dried for 48 h in a forced-air oven (large; Fisher Scientific, Atlanta, GA) at 65 °C. Dried samples were ground to homogeneity using liquid N, and 0.5-g subsamples were weighed 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 (Agilent Technologies, Inc.), 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 [lithium (Li), yttrium (Y), thallium (Tl)], level of oxide [cerium (Ce)], and doubly charged ion (Ce) using a tuning solutions containing 10 $\mu g \cdot L^{-1}$ of Li, Y, Tl, Ce, and cobalt in a 2% HNO_3/0.5% HCl (v/v) matrix. Tissue nutrient concentrations are expressed on a dry weight (DW) basis.

Statistical analysis. Results from the two separate experiments were statistically similar. Therefore, data were pooled and analyzed together for treatment means. The fixed effects for the experiment consisted of three Ca treatment concentrations designed into a factorial arrangement with ABA and non-ABA (control) -treated plants, whereas blocks were analyzed as random effects. 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 followed by mean separation. Analysis of variance was used to evaluate ABA and Ca treatments. Duncan's multiple range test ($P \leq 0.05$) was used to differentiate between ABA and Ca application classifications when F values were significant for main effects.

Results

ABA influence on Ca concentration in leaf tissue. The statistical analysis of the results indicated that there was no interaction between ABA and Ca treatments on tomato leaf tissue. Therefore, the results are presented separately for ABA and Ca treatment effects. The application of ABA either as a foliar spray (500 $mg \cdot L^{-1}$), root application (50 $mg \cdot L^{-1}$), and/or a combination of a foliar spray and root applications significantly decreased Ca concentrations in tomato leaf tissue (Table 1). The Ca concentrations in the leaf tissue decreased from 25.94 $mg \cdot g^{-1}$ DW in the control treatment to 21.99 $mg \cdot g^{-1}$ DW in the ABA foliar spray and root combination treatment. This was a 15.2% decrease in leaf

Table 1. Calcium in leaf, whole fruit, and fruit proximal and distal tissue in 'Mt. Fresh Plus' tomato (*Solanum lycopersicum*) grown in a greenhouse and treated with exogenous applications of ABA.

	Concn ($mg \cdot g^{-1}$) dry wt[z]			
ABA[y]	Leaf Ca	Fruit Ca	Proximal tissue Ca	Distal tissue Ca
Control	25.94 a	3.13 c	3.84 b	2.43 c
Spray	23.51 b	4.02 a	4.99 a	3.04 a
Root	22.54 b	3.42 bc	4.25 b	2.60 ab
Spray/root	21.99 b	3.69 b	4.42 ab	2.96 ab
P value[x]	**	***	***	***

[z]The SE of the mean for Leaf Ca ± 0.81; Fruit Ca ± 0.30; Proximal tissue ± 0.34; Distal tissue ± 0.34. Proximal tissue: top third of tomato fruit; Distal tissue: bottom third of tomato fruit.
[y]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 $mg \cdot L^{-1}$).
[x]**, ***Significant at $P \leq 0.01$ and 0.001, respectively.
ABA = abscisic acid; Ca = calcium.

tissue Ca concentrations when comparing the control treatment with the foliar ABA treatment application.

ABA influence on Ca concentration in fruit tissue. 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 to the tomato plant significantly increased Ca uptake into the fruit tissue (Table 1). The ABA control treatment had the lowest concentration of Ca in the fruit tissue of 3.13 mg·g^{-1} DW. The Ca concentration for fruit in the foliar spray ABA treatment was 4.02 mg·g^{-1} DW and was the highest among the ABA applications. The root and the combination of spray and root ABA treatment applications accounted for 3.42 and 3.69 mg·g^{-1} Ca on a DW basis in the fruit tissue, respectively. The foliar ABA spray treatment increased Ca content in the fruit tissue by 28.4% when comparing it with the ABA control treatment. The combination of foliar spray and root ABA treatment increased Ca content by 17.9% when compared with 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 with the ABA control treatment.

ABA influence on Ca concentration in fruit proximal and distal tissue. The results indicate there were significant differences between the foliar spray treatment for tomato fruit proximal tissue Ca compared with the control and root treatments (Table 1). Proximal fruit tissue Ca concentrations ranged from 3.84 mg·g^{-1} DW in the control treatment to 4.99 mg·g^{-1} DW in the foliar spray treatment, which accounted for an increase of 23.1%. The 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·g^{-1} DW in the control treatment to 3.04 mg·g^{-1} DW foliar spray treatment, which accounted for a 20.1% increase. The Ca concentration in distal tissue significantly increased by 7.0% in the root treatment and 17.9% in the spray and root combination treatment when compared with 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. Leaf tissue Ca decreased from the 180 mg·L^{-1} to 60 mg·L^{-1} Ca treatment (Table 2). In the 180-mg·L^{-1} Ca treatment, leaf tissue Ca concentration was 30.55 mg·g^{-1} DW and decreased to 18.61 mg·g^{-1} DW in the 60-mg·L^{-1} Ca 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 Ca treatment concentration of 180 mg·L^{-1} to the lower Ca treatment concentration of 60 mg·L^{-1} (Table 2). Calcium concentrations decreased from 4.58 to 2.98 mg·g^{-1} DW when comparing the 180-mg·L^{-1} Ca treatment with the lower Ca treatment of 60 mg·L^{-1}. The concentration of Ca in the fruit tissue decreased 34.9% when comparing the Ca treatment concentration of 180 mg·L^{-1} with the lower Ca treatment concentration of 60 mg·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 Ca treatment of 180 mg·L^{-1} to a deficient Ca treatment of 60 mg·L^{-1} (Table 2). Concentrations of Ca in the tomato fruit proximal tissue decreased from 5.66 mg·g^{-1} DW in the Ca treatment of 180 mg·L^{-1} to 3.67 mg·g^{-1} DW in the Ca treatment of 60 mg·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·g^{-1} DW in the Ca treatment of 180 mg·L^{-1} to 2.29 mg·g^{-1} DW in the Ca treatment of 60 mg·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 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 180 mg·L^{-1} to the 60-mg·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 3). The ABA control treatment (0.0 mg·L^{-1}) had the highest incidence of BER with 5.88 fruit per plant. The incidence of BER in the root-applied ABA treatment (50 mg·L^{-1}) was 3.78 fruit per plant. The foliar ABA spray (500 mg·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 per plant, respectively. The foliar ABA spray treatment decreased the incidence of BER by 78.6% when comparing it with the ABA control treatment. The combination of foliar spray and root ABA treatment decreased the incidence of BER by 79.6% when compared with the ABA control treatment. The application of ABA to the root tissue decreased the incidence of BER by 35.7% when comparing it with the ABA control treatment. The yield of fruit with BER fruit also decreased significantly with the application of ABA to the plants (Table 3). The ABA control treatment had the highest yield of fruit with BER of 421.32 g per plant. The yield of fruit with BER in the root ABA treatment accounted for 361.98 g per 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 per plant, respectively. The foliar ABA spray treatment decreased the yield of fruit with BER by 65.9% when comparing it with 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 with 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 with 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·L^{-1} to lower Ca treatment concentrations of 60 and 90 mg·L^{-1} (Table 4). The incidence of BER increased from 1.56 to 3.72 fruit per plant when comparing the 180 mg·L^{-1} Ca treatment with the lower Ca treatments of 60 and 90 mg·L^{-1}. Incidences of BER increased 58.1% when comparing the optimum Ca treatment concentration of 180 mg·L^{-1} with lower Ca treatment concentrations of 60 mg·L^{-1} and 90 mg·L^{-1}. The yield of fruit with BER increased significantly from the Ca treatment concentration of 180 mg·L^{-1} to lower Ca treatment concentrations of 60 and 90 mg·L^{-1} (Table 4). The yield of fruit with BER was the highest in the 90-mg·L^{-1} Ca treatment. Therefore, yield of fruit with BER increased from 148.92 to 337.62 g per plant when comparing Ca treatment concentration of 180 mg·L^{-1} with 90-mg·L^{-1} Ca treatment. Yield of fruit with BER increased 126.7% when comparing the Ca treatment concentration of 180 with 90-mg·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). Occurrence of BER ranged from 2.22 incidences of fruit with BER in the second cluster to 3.60 incidences of BER in the fifth cluster. This accounted for

Table 2. Calcium in leaf, whole fruit, and fruit proximal and distal tissue in 'Mt. Fresh Plus' tomato (*Solanum lycopersicum*) grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

	Concn (mg·g^{-1}) dry wt[z]			
Ca (mg·L^{-1})	Leaf Ca	Fruit Ca	Proximal tissue Ca	Distal tissue Ca
60	18.61 c	2.98 b	3.67 b	2.29 b
90	21.31 b	3.14 b	3.80 b	2.47 b
180	30.55 a	4.58 a	5.66 a	3.50 a
P value[y]	***	***	***	***

[z]The SE of the mean for Leaf Ca ± 0.71; Fruit Ca ± 0.29; Proximal tissue ± 0.32; Distal tissue ± 0.32. Proximal tissue: top third of tomato fruit; Distal tissue: bottom third of tomato fruit.
[y]***Significant at $P \leq 0.001$.
Ca = calcium.

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 first to the sixth cluster of the tomato plants (Table 5). Although the yield of fruit with BER ranged from 177.54 to 278.10 g per 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 without BER per plant when tomato plants were treated with Ca treatments (Table 6). In addition, there were no significant differences in the number and yield of tomato fruit without BER/plant when tomato plants were treated with ABA treatments (Table 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 in Ca deficiency on tomato plants. Ca treatments were given as the optimum of 180 mg·L^{-1} and decreased to 90 and 60 mg·L^{-1}, respectively.

The results demonstrated that applications of ABA treatments decreased Ca concentration in the leaf tissue. The most significant decrease of Ca concentration occurred 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. The reason ABA may have adverse effects on Ca uptake into the leaf tissue is that it has a negative effect on g_S under drought condition (Garcia-Mata and Lamattina, 2007; Macrobbie, 1990; Waterland et al., 2010). 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 d after pollination. In comparison, the current study examined total Ca accumulation in tissue of tomato leaves above the second cluster. The second cluster leaf tissue was examined because Ca deficiency is more prevalent higher in the plant (Marschner, 1995).

The results indicated 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., 2014). ABA plays a crucial role in allocating more Ca into the fruit tissue when applied exogenously. This could be explained by negative effects of ABA on g_S 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 significant 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 allocating Ca to the 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 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 demonstrated 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 (Barickman et al. 2014b; de Freitas et al., 2011). There was also a significant decrease in the number and

Table 3. Incidence and yield of fruit with blossom-end rot in 'Mt. Fresh Plus' tomato (*Solanum lycopersicum*) grown in a greenhouse and treated with exogenous applications of ABA.

	Blossom-end rot[z]	
ABA[y]	Incidence (fruit per plant)	Yield (grams per plant)
Control	5.88 a	421.32 a
Spray	1.26 c	143.64 b
Root	3.78 b	361.98 a
Spray/root	1.20 c	124.98 b
P value[x]	***	***

[z]The SE of the mean for incidence ± 0.36; weight ± 41.16.
[y]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}).
[x]***Significant at $P \leq 0.001$.
ABA = abscisic acid.

Table 4. Incidence and yield of fruit with blossom-end rot in 'Mt. Fresh Plus' tomato (*Solanum lycopersicum*) grown in a greenhouse and treated with different concentrations of Ca in the hydroponic fertilizer solution.

	Blossom-end rot[z]	
Ca (mg·L^{-1})	Incidence (fruit per plant)	Yield (grams per plant)
60	3.72 a	295.62 a
90	3.78 a	337.62 a
180	1.56 b	148.92 b
P value[y]	***	**

[z]The SE of the mean for incidence ± 0.42; weight ± 39.48.
[y]**, ***Significant at $P \leq 0.01$ and 0.001, respectively.
Ca = calcium.

Table 5. Incidence and yield of fruit with blossom-end rot per cluster in 'Mt. Fresh Plus' tomato (*Solanum lycopersicum*) grown in a greenhouse.

	Blossom-end rot	
Tomato cluster	Incidence (fruit per plant)	Yield (grams per plant)
1	2.58 ab	278.10 ab
2	2.22 b	265.74 b
3	3.24 a	260.00 a
4	3.24 a	248.40 b
5	3.60 a	240.60 ab
6	3.36 ab	177.54 ab
P value[z]	*	NS

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

Table 6. Number of tomato fruit by classification and yield of 'Mt. Fresh Plus' tomato (*Solanum lycopersicum*) grown in a greenhouse and treated with Ca in the hydroponic fertilizer solution.

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

[z]NS = nonsignificant at $P \leq 0.05$.
Ca = calcium.

Table 7. Number of tomato fruit by classification and yield of 'Mt. Fresh Plus' tomato (*Solanum lycopersicum*) grown in a greenhouse and treated with exogenous applications ABA.

	Number of fruit and yield (grams) per cluster							
ABA[z]	XL	XL wt	Large	Large wt	Medium	Medium wt	Small	Small wt
Control	1.06 b	225.11 b	1.23 a	211.89 a	1.28 a	160.60 a	1.81 a	151.24 a
Spray	1.37 a	310.50 a	1.25 a	209.03 a	1.32 a	167.08 a	1.81 a	108.32 a
Root	1.28 ab	290.30 ab	1.23 a	211.89 a	1.35 a	170.16 a	2.67 a	121.08 a
Spray/root	1.27 ab	288.11 ab	1.22 a	268.32 a	1.29 a	163.36 a	1.86 a	103.31 a
P value[y]	NS	NS	NS	NS	NS	NS	NS	NS

[z]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}).
[y]NS = nonsignificant at $P \leq 0.05$.
ABA = abscisic acid.

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 indicated that a combination of the foliar spray and root applications 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. Furthermore, despite differences in BER, there were no differences in marketable fruit and yields of marketable fruit. These data suggest that tomato plants treated with ABA produced as much fruit and the yield of the fruit was comparable to control treatments.

de Freitas et al. (2011, 2014) found similar results with incidences of BER when they foliarly applied ABA to tomato plants. Although the researchers based their results on a single cluster of tomatoes spanning up to 45 d after pollination, this study adds to the findings by analyzing six clusters of tomato fruit over 4 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 BER increased 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 deficiencies. Tomato plants were given an optimum amount of Ca (180 mg·L^{-1}) in the fertilizer solution as well as lower Ca concentrations of 90 and 60 mg·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. Furthermore, the incidence of fruit with BER increased in plants treated with lower Ca. 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. Application of ABA treatments in addition to an optimum Ca treatment of 180 mg·L^{-1} are likely to decrease the incidence of fruit with BER. However, ABA is effective in decreasing BER even with less than optimal Ca concentrations in the fertilizer solution. These results demonstrate that ABA could be a potential novel treatment to combat BER issues when environmental conditions restrict the uptake and unequal distribution of Ca in the plant. Additionally, results indicated that ABA treatments were 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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