ENDOREDUPLICATION AND ITS EFFECT ON FRUIT SIZE IN TOMATO AS AFFECTED BY DROUGHT STRESS

by

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(Under the Direction of MARC W. VAN IERSEL and ANISH MALLADI)

ABSTRACT

Endoreduplication in tomato fruit can result in high chromosomal DNA counts. Endoreduplication has been associated with cell expansion and may influence organ growth. These studies were conducted to understand if fruit size in tomato is affected by endoreduplication, to explore when during fruit development endoreduplication occurs, and to understand the effect that drought has on endoreduplication. Fully ripened fruit of 'Micro-Tina' tomato plants were harvested for measurements. The first study revealed that endoreduplication was affected by beginning drought stress conditions when fruit were at breaker stage. Fruit soluble solids were increased under drought compared to well-watered conditions and fruit volume was decreased by drought stress compared to well-watered conditions. There was no correlation between endoreduplication and fruit volume. The second study was designed to quantify endoreduplication rates during fruit development. Endoreduplication was not affected by drought stress, but occurred during two distinct phases; when fruit were 9-16 d old (fruit enlargement) and when fruit were 33-48 d old (fruit ripening phase). Fruit size and endoreduplication were positively correlated when fruit were young (9-16 d old). Although fruit size and endoreduplication were negatively correlated when fruit were 23-43 d old, the correlation was weak. Fruit size was reduced by drought stress beginning when fruit were young. Fruit soluble solids, titratable acidity, color, and texture were not affected by drought stress, compared to well-watered conditions. This research revealed that endoreduplication does not just occur when fruit are young, as the previous literature has reported. Instead, endoreduplication may have different roles during different stages of fruit development.

INDEX WORDS: Endoreduplication, endoreduplication factor, tomato fruit, fruit size, fruit quality, fruit age, fruit developmental stage, drought stress, sensor-based irrigation, fruit weight, Tomato ‘Micro-Tina’ *Solanum lycopersicum*, soluble solids, titratable acidity.

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DEDICATION

I would like to dedicate this thesis to my wife, Amanda, who has constantly been supportive through the entirety of graduate school and who originally suggested that I pursue furthering my education. I couldn’t have done it without your support and encouragement throughout. Thank you and I love you forever and always.

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INTRODUCTION AND LITERATURE REVIEW

Overview

Agricultural sales value in the United States is dominated by the five states with highest production: California, Nebraska, Illinois, Iowa, and Minnesota, with California ranking number one at approximately $30 billion in 2012 (USDA, 2014). With over 11 percent of the national total production value (USDA, 2014), the agricultural industry of California is very important to the U.S. agricultural economy and the food industry. The state produces a wide variety of vegetables, fruit crops, and leads US production in most nut tree crops, such as pistachios (*Pistacia vera*), almonds (*Prunus dulcis*), and walnuts (*Juglans* L.) (USDA, 2015). California is the top producer of spinach (*Spinacia oleracea*), carrots (*Daucus carota*), celery (*Apium graveolens*), and broccoli (*Brassica oleracea*) (USDA, 2015). California also leads in tomato (*Solanum lycopersicum*) production, accounting for up to 91 percent of the total U.S. production, with planted acreage ranging between 263,000 to 292,000 acres from 2013 to 2014 (USDA, 2016). Due to drought conditions and inconsistent precipitation, agricultural production regions throughout the world have begun to utilize and rely on irrigation due to reduced rainfall and groundwater availability. This can lead to areas struggling to provide adequate irrigation to crops and production areas. An example of potential threats that drought conditions can pose to crop-producing areas is the multi-year drought occurring in California. As of August of 2016, every county in California was experiencing abnormally dry or drought conditions (USDA, 2016). This included the San Joaquin Valley, where one-third of all the vegetables in California are grown (USDA, 2014). Despite drought occurring in these areas, irrigation has provided farms the ability to prevent yield of tomato fruit from dropping below previous yields (NASS, 2015), but not all farms have been successful in preventing yields from declining, based on increasing irrigation programs. Of the 25,408 farms that use surface-drip and controlled irrigation, 4,242 farms reported diminished crop yields in 2013 (USDA, 2013) from ‘irrigation interruptions’. As the drought continues, farms will have to change and adapt irrigation strategies, which was stated as a requirement in the executive order by the governor of California, B-37-16 (STATE OF CALIFORNIA, 2016). The order has been revised several times since the announcement of the state of emergency due to drought conditions in 2014, in order to further promote a water-saving lifestyle for urban water use. It also proposes that the agricultural sector be prepared to assess and propose water management plans beginning January 2017. While this requirement is only limited to farms over 10,000 acres in size, if the drought in California continues as expected (NWS, 2016), then tomatoes could be exposed to irrigation interruptions and could suffer from the negative effects of drought-conditions. The dangers of drought conditions can also be applicable to all major crop-producing regions throughout the world.

Drought Stress in Tomato

Droughts have been observed all over the world and in a range of severities. Agricultural drought can be defined as a lack of sufficient precipitation to successfully grow a crop, while often associated with elevated temperatures, low relative humidity, increased winds, and rainfall characteristics (MISHRA AND SINGH, 2010). Droughts can be economically damaging (ROSS AND LOTT, 2003), especially where agriculture is heavily dependent on irrigation for crop production. Drought stress reduced fruit size and berry weight by 40 and 50%, respectively, in olive (*Olea europaea*) (GREVEN ET AL., 2009), decreased fruit volume in olive (GIRON ET AL., 2015) and reduced fruit volume in peach (*Prunus persica*) (RAHMATI ET AL., 2014). The majority of tomatoes produced in California are grown in open-field production which requires irrigation for adequate vegetative growth and consistent fruit quality. Plant height in tomato is reduced under drought conditions (KHAN ET EL., 2015; ULLAH ET AL., 2016), at least partly as a result of reduced cell elongation (LITVIN et al., 2017). Tomato fruit yield per plant is reduced when plants are exposed to drought stress conditions (RAHMAN ET AL., 1998; ULLAH ET AL., 2016), as is fruit number per plant (MARJANOVIĆ ET AL., 2012) and fruit weight (RAHMAN ET AL., 1998). Growing tomatoes under a controlled, partial root-zone drying irrigation program can increase fruit size when compared to plants grown under fully-irrigated conditions, but this may be related to reductions in the number of fruit per plant (MARJANOVIĆ ET AL., 2012). During water deficit conditions, the tomato may abort flowers to reduce the required energy to produce more fruit. By reducing the crop load, the plant now can direct nutrients and water towards the remaining fruit, which could increase fruit size. This could mean that fruit size is not just a function of drought stress, but several complex processes and interactions that can regulate fruit size. Abscisic acid (ABA) deficiency in tomato reduces fruit and plant size (NITSCH ET AL., 2012). Under drought stress, plants synthesize ABA to close stomata to prevent water loss by transpiration (SHARP ET AL., 2000; WASILEWSKA ET AL., 2008; SUN ET AL., 2011). Another control of fruit size in tomato comes from genetic regulatory processes that can affect fruit size by up- or down-regulation of genes located on quantitative trait loci (QTL) like FW2.2, a negative regulator of cell proliferation, and *SIKLUH*, which enlarges fruit volume by increasing the cell number in the pericarp tissue (AZZI ET AL., 2015; CHARKRABARTI ET AL., 2013). Metabolic control can increase fruit size by increasing the concentration of primary and secondary metabolites (CARRARI AND FERNIE, 2006) within tomato fruit and by modulating fruit load in order to promote high source to sink potential (BOHNER AND BANGERTH, 1988; BERTIN ET AL., 2002). Another process occurring within tomato fruit is endoreduplication, which duplicates DNA within cells, without cell division. Endoreduplication levels have been positively correlated with cell size through expansion, which has a positive influence on fruit growth (BERTIN, 2005; AZZI ET AL., 2015).

Endoreduplication

Endoreduplication, or endocycle polyploidy, is a modified cell cycle in which cells duplicate their DNA content, without mitosis (TRAAS ET AL., 1998; JOUBÈS AND CHEVALIER, 2000; BAROW, 2006). It occurs in over 90% of flowering plant species (BARLOW, 1978; D’AMATO, 1989). Endoreduplication differs from the classic cell cycle in that the mother cell does not split into two daughter cells after the DNA has been duplicated. For example, DNA content of a cell within an embryo that has not undergone endoreduplication has a ploidy level of 2C (C representing a haploid cell's DNA content) (MELARAGNO ET AL., 1993). A cell that has had one round of an endoreduplication cycle would have a ploidy level of 4C, double the amount of DNA in that cell. This modified cell cycle is the largest contributor to somatic polyploidy in plants and plays a major role in the distribution and balance between cell division and cell expansion (JOUBÈS AND CHEVALIER, 2000). Current research suggests that the underlying purpose of endoreduplication is to provide enough genetic material for metabolically active tissues. For instance, chloroplasts could require higher levels of transcription for development and photosynthesis to ensure cells have adequate genetic material to respond to adverse environmental issues (GALBRAITH ET AL., 1991). Another example would be endoreduplication occurring in endosperm of maize (*Zea mays*) kernels, which increases the DNA pool to allow for increased gene expression (JOUBÈS AND CHEVALIER, 2000). According to the karyoplasmic ratio theory, cells adjust their cytoplasmic volume in response to their nuclear DNA content (SUGIMOTO-SHIRASU ET AL., 2003; CHEVALIER ET AL., 2014) and final cell size has been linked to the ploidy level of that cell (TRAAS ET AL., 1998; DOLAN AND DAVIES, 2004). Endoreduplication can be found in different tissue types and even different plant organs, across a variety of different plant species. The expression of this modified cycle has been found in endosperm tissue of maize (KOWLES AND PHILLIPS, 1988; ARTLIP ET AL., 1995), *Arabidopsis thaliana* and tomato leaves (COOKSON ET AL., 2006; MASSONNET ET AL., 2011; DERMASTIA ET AL., 2012), trichome cells in several species (BAROW, 2006), and in tomato fruit tissues (CHEVALIER ET AL., 2011; APRI ET AL., 2014; PIRRELLO ET AL., 2014). Endoreduplication appears to occur across developmental stages and processes. In tomatoes and quinoa (*Chenopodium quinoa*), endoreduplication occurs at the abscission zone of leaves as part of the cell differentiation process to promote senescence or abscission (LÓPEZ-FERNÁNDEZ ET AL., 2015; DERMASTIA ET AL., 2012). Roots of *Arabidopsis* mutants, without the ability to endoreduplicate, are shorter, lack root hairs, and have decreased DNA content in cells, which suggests that endoreduplication within roots aids in root growth and expansion (SUGIMOTO-SHIRASU ET AL., 2002; DOLAN AND DAVIES, 2004). Plant organ size is affected primarily by cell division and expansion (GILLASPY ET AL., 1993). If endoreduplication can affect the ratio between cell division and expansion, then it could affect final organ volume and size. With regards to fruit, endoreduplication can be a contributor to final fruit size and volume via its influence on cell expansion (JOUBÈS ET AL., 1999). A model system for endoreduplication was used to research the relationships between cell division, expansion, and ploidy level. The model found that the potential for an increase in endoreduplication is affected by the amount of division cycles that cells goes through (FANWOUA ET AL., 2013), but there still remains a lack of understanding about the true role of endoreduplication and of all the factors that can affect endoreduplication rates. Endoreduplication has been observed to be influenced by cyclin-dependent kinases (CDKs) and cyclins (CHEVALIER ET AL., 2011). If we can understand how endoreduplication changes during development or is affected by external stressors, then we may be able to control or even implement stressors in order to alter fruit size by affecting endoreduplication.

Tomato Fruit and Endoreduplication

Endoreduplication has been found in multiple species of fruit and with different effects on fruit development. Red pitaya (*Hylocereus undatus*), peach, apricot (*Prunus armeniaca*), cucumber (*Cucumis sativus*), and tomato are some of the fruit that have high endoreduplication rates (MENEZES ET AL., 2016; BRADLEY AND CRANE, 1955; REWERS ET AL., 2009; JOUBÈS ET AL., 1999). Observed fruit size effects of endoreduplication include increased fruit volume via enlarging parenchyma cells by 57% in apricot (BRADLEY AND CRANE, 1955), increased fruit weight and diameter in pear (*Pyrus communis* L.) (ISUZUGAWA ET AL., 2014), and large cell diameters and cortex tissues in 'Giant Gala' apples (*Malus*×*domestica* Borkh.) (MALLADI AND HIRST, 2010). Quality parameters in fruit expressing endocycles have also been affected, compared to fruit that do not have endoreduplication. Titratable acidity was increased, while fruit firmness and starch concentrations of pear were reduced and fruit were more susceptible to brown, corky flesh in fruit with higher ploidy levels (ISUZUGAWA ET AL., 2014). These changes in fruit characteristics could be attributed to the increased fruit size, but these effects can lead to decreases in fruit quality and lower value of fruit. While endoreduplication has been researched within these fruiting species, a deeper understanding of how endoreduplication affects fruit is needed. Tomatoes have been used as a model crop for analyzing the effect of endoreduplication on fruit development in multiple studies (BOURDON ET AL., 2010; FANWOUA ET AL., 2013). Tomato fruit, which can have cells with DNA contents upwards of 512C, show active endoreduplication in the pericarp tissue and in the locular cells (BOURDON ET AL., 2010; PIRRELLO ET AL., 2014; AZZI ET AL., 2015). The only tissue where endoreduplication does not appear to occur in tomato fruit is the epidermis, or skin (JOUBÈS ET AL, 1999). Tomato fruit weight increases with increasing ploidy levels, but the trend can be variety specific (CHENICLET ET AL., 2005). Fruit weight and size in tomato are influenced by many genes and QTLs (VAN DER KNAAP ET AL., 2014) and hormones (NOIR ET AL., 2013; SU ET AL., 2014), which could also be influencing endoreduplication rates. Despite understanding the process of endoreduplication and its effect on cell size, we have yet to conclusively say that the modified cell cycle directly affects fruit size (BERTIN ET AL., 2003). Due to the complex systems that can contribute to fruit development and size, endoreduplication can be an important component of fruit development because of its influence on cell size regulation. Fruit that have larger cell due to increased DNA content may have the potential to produce larger fruit. According to the USDA, firmness and size are important factors for quality standards for tomato fruit (USDA, 1991). Studies have quantified the occurrence of endoreduplication within tomato fruit, but no research has been conducted regarding drought exposure and its effect on endoreduplication rates in tomato fruit. The effect of drought exposure timing on endoreduplication during different fruit development stages has yet to be studied.

Significance and Rationale

Little is known about the effects of endoreduplication on tomato fruit growth and development when exposed to drought conditions. Endoreduplication can influence fruit shape and size, by altering the balance between cell division and expansion (GILLASPY

ET AL., 1993). If shape and size of fruit can be affected by endoreduplication in fruit tissue, then fruit quality standards for fresh market tomatoes may be affected as well.

Endoreduplication has been explored as a function of gene-influenced interactions, hormonal effects, and organ-based responses. What has not been explored is whether endoreduplication within tomato fruit is influenced by drought. If it is, how is endoreduplication affected differently depending on when plants are exposed to drought? This research assessed the relationship of endoreduplication and drought severity and drought timing. Another aspect of endoreduplication that we sought to understand was at which stage during fruit development most endoreduplication occurs. Therefore, this research also analyzed how endoreduplication changes over the course of fruit development in order to create a time-course of endoreduplication rates within fruit. I conducted two experiments where tomatoes were grown under constant substrate volumetric water contents, some of which were drought treatments. The first study was to understand if endoreduplication was affected by drought and which fruit developmental stage showed the most response to drought. The second study was conducted to plot the time-course of endoreduplication rates as fruits developed under well-watered or drought conditions and to determine if there was a relationship between fruit size and endoreduplication rates.

As more is understood about the control and mechanisms that affect endoreduplication, future research may be able to utilize this adapted cell cycle in order to control polyploidy induction or even to shift the cell division to expansion ratio towards expansion, allowing for increases in cell size of desired tissues. By understanding the effect of endoreduplication on fruit growth and development, we can understand the role of endoreduplication in tomato and why it can occur at such a high rate.

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CHAPTER 2

FRUIT SIZE AND ENDOREDUPLICATION ARE AFFECTED BY DROUGHT STRESS IN TOMATO (*Solanum lycopersicum*)[1]

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Abstract

Drought threatens the production of fruits and vegetables in arid and semi-arid regions around the world, including parts of the United States. Tomato exposed to drought can have reduced quality and reduced yields. Tomato fruit cells undergo endoreduplication, a modified cell cycle that increases DNA content. To understand the relationship between drought stress and endoreduplication in fruit, tomato was grown under four different substrate volumetric water contents (VWC), 0.40, 0.25, 0.15, and 0.10 $m^3 \cdot m^{-3}$, to simulate well-watered conditions and mild, moderate, and severe drought conditions. All plants were grown under well-watered conditions before flowers were tagged on three different dates to establish three different fruit developmental stages. The three VWC drought treatments were started when these fruits were at the following stages: young fruit (YF) at 10 days post-anthesis (DPA), halfway-green (HG) at 25 DPA, and breaker stage (BR) at 40 DPA. Fruit grown under well-watered conditions had lower soluble solids than those in all three drought treatments. Fruit water content was higher in the 0.40 $m^3 \cdot m^{-3}$ treatment compared to all drought treatments. Fruit exposed to the 0.10 and 0.15 $m^3 \cdot m^{-3}$ treatments had smaller fruit compared to fruit grown at the 0.40 $m^3 \cdot m^{-3}$ treatment. Fruit that were exposed to 0.10 and 0.15 $m^3 \cdot m^{-3}$ VWC treatments starting when fruit were 40 DPA had increased endoreduplication rates compared to the 0.40 $m^3 \cdot m^{-3}$ treatment. As this study only analyzed fruit that were fully mature, future analyses should examine how endoreduplication progresses or changes as fruit develop.

Introduction

Tomato is a crop of vast economic importance in the U.S. agricultural sector with ~161,874 hectares planted in 2009 (USDA, 2010). Of those 161,874 hectares, two-thirds of the tomato crop planted in the United States is grown in California and Florida (ERS, 2016). Tomato fruit for fresh market and processed products are valued at approximately two billion dollars (ERS, 2016). Not only are tomatoes an important agricultural crop, they are high in vitamins A and C, and lycopene, an important antioxidant (VILLET ET AL., 2009; SLIMESTAD AND VERHEUL, 2009), all important for human health. Tomato can be sensitive to environmental conditions such as drought and the largest tomato-producing state, California, is experiencing region-wide drought conditions (USDA, 2016). If these conditions continue, crops could be susceptible to drought stress exposure if restrictions are placed on agricultural water use. Tomato exposed to limited irrigation can have reduced quality, lower fruit yields, below-standard seed quality, restricted aboveground growth, and reduced photosynthesis due to stomatal closure (PERVEZ ET AL., 2009; RAHMAN ET AL., 1997; CHAPIN ET AL., 1988). An integral process of tomato fruit growth and development that can be affected by water deficit is endoreduplication, which increases ploidy levels of cells within many higher plants and is associated with an increase in cell size which can lead to organ growth (SCHOLES AND PAIGE, 2015; BOURDON ET AL., 2010; CHEVALIER ET AL., 2011; DOLAN AND DAVIES, 2004). Chevalier et al. (2011) describes endoreduplication as a modified cell cycle in which the cell’s DNA is replicated multiple times within the traditional cell cycle, but with a lack of cell division. As nuclear DNA increases from endoreduplication, the cell size increases. One of the potential purposes behind this increase in cell size is to make room for additional organelles needed for the larger nucleus to function appropriately (JOUBÈS AND CHEVALIER, 2000). Endoreduplication has been observed in different tissue types. Leaves exhibit endoreduplication on both sides of the abscission zone, but during senescence, endoreduplication occurs on the proximal side of the abscission zone, which increases cell sizes to promote senescence (DERMASTIA ET AL., 2012; LÓPEZ-FERNÁNDEZ ET AL., 2015). *Arabidopsis thaliana* utilizes the endoreduplication cycle, increasing cell size within root hair cells, to elongate the hairs (DOLAN AND DAVIES, 2004). Fruits such as apricot (*Prunus armeniaca*) (BRADLEY AND CRANE, 1955), pear (*Pyrus communis* L.) (ISUZUGAWA ET AL., 2014), and cucumber (*Cucumis sativus*) (BAROW, 2006) also undergo endoreduplication in their different tissues. Tomato pericarp cells endoreduplicate, resulting in chromosomal DNA counts reaching upwards to 512C (CHENICLET ET AL., 2005; JOUBÈS AND CHEVALIER, 2000; BOURDON ET AL., 2010; PIRELLO ET AL., 2014). This process can be affected by water deficit and drought stress. *Arabidopsis* plants that were exposed to drought stress had smaller epidermal leaf area, decreased final leaf area, and reduced endoreduplication compared to plants that were well-watered (COOKSON ET AL., 2006). Initial endoreduplication activity has been reported to begin within 4 to 10 days post-anthesis in tomato pericarp tissue (BÜNGER-KIBLER and BANGERTH, 1982). Within the endosperm of maize (*Zea mays*) endoreduplication activity is most rapidly occurring between 10 and 16 days after pollination (KOWLES AND PHILLIPS, 1988). This is soon after cells have differentiated and the primary cell division stage is complete, but few studies show how endoreduplication changes or continues as the tissue ages. Since cell division occurs prior to when the majority of endoreduplication is reportedly occurring (ARTLIP ET AL., 1995), the timing of when tomato experiences drought could affect endoreduplication. Little work has been done to understand the effect of drought stress on tomato fruit, with regards to endoreduplication. The goal of this study was to determine the effect of drought severity and timing on tomato fruit growth and endoreduplication. Our hypotheses were 1) drought stress will decrease endoreduplication rates, 2) drought stress will reduce fruit volume, and 3) drought will have a greater impact on fruit size and endoreduplication if fruit are exposed to drought from an early developmental stage.

Materials and Methods

Plant Material

This experiment was conducted from February 3 until May 25 2015, in a greenhouse at the University of Georgia in Athens, GA. ‘Micro-Tina’ tomato seeds were started on February 3 in plastic 6-cell trays with soilless substrate (Fafard 1P; Sun Gro; Agawam, MA). Seedlings were irrigated on ebb and flow benches with 100 ppm N water-soluble fertilizer (15N-2.2P-12K, 15-5-15 CalMag; The Scotts Company; Marysville, OH) for 14 d before transplanting. After the third true leaf appeared, seedlings were transplanted into 15 cm pots with soilless substrate (Fafard 1P; Sun Gro), with the addition of 5-6 month controlled release fertilizer (19N-1.8P-6.6K; Harrell’s LLC; Lakeland, FL; 5-6 month release time) incorporated at a rate of 5.4 kg·m^3 of substrate.

Irrigation Control and Environmental Parameters

Irrigation was automatically controlled using an adapted design of the soil moisture sensor-based system developed by Nemali and van Iersel (2006). A greenhouse bench with 32 irrigation lines was used to irrigate the tomato plants. These irrigation lines were controlled by 32 solenoid valves (075-DV; Rain Bird; Azusa, CA) that were controlled by a datalogger (CR10X; Campbell Scientific; Logan, UT). The datalogger took substrate volumetric water content (VWC) readings from the tomato plants, using soil moisture sensors (EC-5; Decagon Devices; Pullman, WA). Six tomato plants were placed on each irrigation line, which had six pressure-compensated drip emitters 2 L/h (PCJ; Netafim; Fresno, CA), spaced approximately 20 cm apart. Drip tube rings (DR6; Dramm; Manitowoc, WI), approximately 10 cm in diameter, were attached to the drip emitters and then staked to sit on top of the substrate, around each tomato plant. Soil moisture sensors (EC-5; Decagon Devices) were then inserted into tomato pots at a 45° angle, in the center of the 15 cm pot, until the sensor tip was 6 cm deep. The 32 sensors, one per irrigation line, were connected to a multiplexer (AM16/32B; Campbell Scientific) which was connected to the datalogger (CR10X; Campbell Scientific). The datalogger took voltage output readings from the sensors and converted the voltage reading into VWC. Once the reading was taken and converted, two relay drivers (SDM-CD16AC/DC; Campbell Scientific) were used to control each irrigation line by opening or closing the solenoid irrigation valves (075-DV ¾ inch; Rain Bird) individually, based on the VWC reading. At the time of placing the plants on the irrigation system, the VWC threshold, for all 32 irrigation lines were set at 0.40 $m^3 \cdot m^{-3}$ for the first 70 d. On April 5, three other VWC treatments (0.25, 0.15, and 0.10 $m^3 \cdot m^{-3}$) were started. An experimental unit was considered six tomato plants on an irrigation line and four irrigation lines comprised a block, each irrigation line with one of the VWC treatments (0.40, 0.25, 0.15, and 0.10 $m^3 \cdot m^{-3}$) randomly assigned. VWC thresholds were approximately met 5, 10, and 15 d after changing the irrigation thresholds on April 5. If the VWC reading from a specific sensor dropped below the thresholds the relay driver provided power to the corresponding solenoid valve, irrigating for 10 s and then closing the solenoid. After the VWC thresholds were reached, the experiment ran for an additional 45 d.

Minimum, maximum, and daily average air temperature and minimum, maximum, and daily average relative humidity in the greenhouse were measured using a VP3 probe (Decagon Devices). Photosynthetic photon flux was measured with a quantum sensor (SQ-110; Apogee Instruments; Logan, UT) and daily light integral (DLI) was calculated by integrating these readings over the entire day.

Flower Tagging

After tomato plants were placed on the irrigation system, flowers on each plant were tagged on three separate days (March 4, March 25, and April 5) to ensure that fruit from different developmental stages would be exposed to the VWC treatments started on April 5. Three open flowers per plant, 18 flowers per irrigation line, were tagged on each of the three days to assure that fruit were exposed to all VWC treatments at different developmental stages: breaker (BR) starting 40 days post-anthesis (DPA), half-way green (HG) starting 25 DPA, and young fruit (YF) starting 15 DPA. These three stages were selected for their specific activities occurring at their respective stages; YF are undergoing cell division, followed by expansion, HG fruit are converting sugars to starch and preparing for ripening, and BR stage fruit are increasing in antioxidants, changing pigments, and cell walls are beginning to break down. Flowers tagged on March 4 were fruit at the BR stage of development when all VWC treatments were reached by April 15. Flowers tagged on March 25 were fruit at the halfway-green stage and flowers tagged on April 5 were young fruit when all VWC treatments were reached.

Because fruit were tagged on different days, fruit exposed to drought at different developmental stages grew under in different environmental conditions. This may have affected fruit growth and quality, making any observed differences among fruit tagged on different days difficult to interpret. Thus, the interpretation of the data focuses on comparing the effects of different VWC treatments within fruit tagged on specific dates.

Fruit Measurements

Diameter and length of three tagged fruit of each developmental stage were measured throughout the study using calipers. Fruit volume was calculated using diameter and length measurements of each of the three fruit using the following equation: calculated fruit volume = $\pi \times 4/3 \times r^3$. We used this equation with the assumption that each fruit was a perfect sphere, due to length and diameters measurements being similar.

Three different harvests of fruit, depending on when fruit were tagged, were performed. The harvest dates were two weeks after the fruit (of each respective tagging day) were at breaker stage. Fruit tagged as BR, HG, and YF were harvested, and weighed, on May 5, 11, and 18, respectively. Average fresh weight was recorded and then the fruit were put into a drying oven (Model 630; NapCo Inc.; South Haven, MI) at 80° C for 4 d. Fruit were weighed again and average dry weight was recorded. Average fruit water content was calculated from average fresh weight and average dry weight measurements.

Two fruit from each experimental unit were collected two weeks after fruit began breaking color and cut into quarters. These fruit were used for extraction of juice for soluble solids analysis. Crushed tomato tissue was placed into 50 mL tubes and centrifuged (Allegra X-22R; Beckman Coulter; Brea, CA) for 10 min at 15 °C with a rotation speed of 4500 rpm. Two mL of fluid was pipetted off of the top of the solution and passed through cheese cloth into two mL test tubes. Subsequently, 200 µL of liquid was placed onto a refractometer lens (PR-32α; Atago Co., Bellevue, WA) to measure soluble solids.

Flow Cytometry

Ripe tomato fruit were randomly sampled for flow cytometry on three separate sampling days (May 6, 14, and 20, depending on when fruit was tagged) when fruit were approximately 45 d old. On each sampling date, one tagged fruit from each irrigation line was harvested, four times throughout the day; morning, early afternoon, afternoon, and evening. At each time, two blocks were harvested, for a total of 8 fruit. The fruit were sampled at different times during the day, and by block, to account for potential circadian rhythms or sampling time effects. The preparation process uses time-sensitive stains and buffers which only allows only a few fruit to be processed at one time.

The eight fruit were harvested and brought to a lab where the tissue preparation took place. Pericarp tissue, located in between the epidermis and the locule space, was sampled for the analysis and extraction process. A nuclei extraction buffer was adapted from a previously reported buffer known as woody plant buffer (WPB), developed for problematic tissues and woody stems by Loureiro *et al.* (2007). The extraction buffer was made anew for each sampling group, to provide consistent nuclei extraction and staining. The base buffer solution was made by combining the following: Tris-HCl, EDTA, $MgCl_2$, NaCl, Triton X-100, $Na_2S_2O_5$, PVP-40, and distilled, autoclaved, and filtered H_2O. Concentrations and amount contributed to base solution can be found in Table 2.1. Items 1-9 in Table 2.1 were combined to make a 20 mL base solution. Once the 20 mL base solution was made, 9.5 mL was poured into a 15 mL tube labeled 'solution B' and propidium iodide (PI) and RNase was then added to solution B and the tube was wrapped in tin foil to prevent light from degrading the PI stain. The remaining 10.5 mL of base solution was poured into another 15 mL tube and labeled 'solution A'. Solution A was used for the nuclei extraction and solution B was added after the nuclei were extracted from the tissue to stain the nuclei for analysis by the flow cytometer. Tissue was taken from the widest part of the fruit, in the middle portion of the pericarp. Approximately, a 10 mm × 10 mm × 2 mm of pericarp tissue was placed into one mL of the extraction buffer (solution A). The tissue was then chopped with a razor blade (AccuTech Blades; Verona, VA) in a Petri dish on ice in one mL of solution A until the tissue was chopped finely and cell contents were released into the extraction buffer (solution A). Tissue particles too big for the flow cytometer to process were removed from the solution. The remaining extracted liquid was pipetted into a two mL tube. Then one mL of staining buffer (solution B) was added to the test tube and mixed to ensure the stain reached all nuclei. The completed sample was put on ice and covered to prevent light from degrading the nuclear stain. Once all eight samples were prepared, the samples were taken to the

Flow Cytometry Resource lab facility at the University of Georgia for analysis. Samples were then filtered through a 50 µm filter (Partec; Münster, Germany) into 10 mL sterile test tubes for flow cytometry. This process was done for each sample grouping every time and every buffer was made anew.

Ploidy was measured on a flow cytometer (CyAn ADP, Beckman Coulter; Hialeah, FL). The PI stain was excited by a 488 nm laser and emission was read through a filter (575/25 BP, Beckman Coulter). Fluorescence was measured in logarithmic mode. Measurements were triggered on PI and gated first on Log FSC vs. Log SSC., then on PI Pulse Width vs. Log FSC, which is a conversion and display setting the cytometer uses to display the data as a histogram. Ploidy levels were detected on a single parameter, which plots PI excitation vs cell count, in order to generate a peak histogram for analysis. Flow cytometry software (FlowJo Ver. 12; FlowJo; Ashland, OR) was used for analysis of ploidy level distribution. Samples were gated, gathered into a display output, with a protocol that excluded the first 15% of events, to exclude aggregate and non-DNA events. A leaf standard sample was included with each sampling group to gate, set the regions for ploidy levels (2C, 4C, 8C, etc...) for the appropriate ploidy peak regions. 5000 nuclei per sample were measured, allowing for a representative histogram with large peaks to determine ploidy levels and allow for accurate gating.

Endoreduplication Factor

The flow cytometer and software identified and calculated the proportion of nuclei that are distributed into the groups of chromosomal DNA (2C, 4C, 8C...512C). The “C” value represents the amount of DNA in the cell. Proportions of cells in specific

C regions, provided by the software, can be used to calculate the mean endoreduplication factor (EF), using the method proposed by Cookson *et al.* (2006). The equation used is as follows:

$$EF = (2C \times 0) + (4C \times 1) + (8C \times 2) + (16C \times 3) + (32C \times 4) + (64C \times 5) + (128C \times 6) + (256C \times 7) + (512C \times 8).$$

Values of each “C” region represents the fraction of cells, in each sample, that have a genome size of the respective levels, such as 2C, 4C, 8C, etc. The fraction is then multiplied by how many endocycles is required to achieve a DNA content, specified by the “C value”. For example, cells with a DNA content of 2C did not have an endocycle, so the proportion of cells with a 2C DNA content was multiplied by zero. The proportion of cells with a DNA content of 4C, on the other hand, went through one endocycle, so the proportion of 4C cells is multiplied by one. These values were added together and the equation followed the same rules until all chromosomal DNA counts found in the sample, were calculated into the EF value. The EF indicates the average number of cycles of endoreduplication cells in the sample went through.

Experimental Design and Statistical Analysis

VWC measurements were taken every two hours and environmental data was collected every two hours and used to calculate daily averages. Fruit volume was measured three times throughout the study, but only final fruit volume is reported. Soluble solids, fruit dry weight, water content, and endoreduplication factor were all measured once, when fruit was approximately 45 d old. Data were analyzed in JMP® (Version 12; SAS Institute Inc.; Cary, NC). The experiment consisted of a randomized complete block design with a split-plot within each block. Four VWC treatments (0.10, 0.15, 0.25, and 0.40 $m^3 \cdot m^{-3}$) were the main block effects, with a split-plot of three different fruit developmental stages (young, mid, and late exposure). An experimental unit was six plants on each irrigation line and there were 32 irrigation lines used to irrigate and maintain VWC treatments, which will be further explained in the irrigation control section (4 irrigation treatments × 8 replications). Each block was replicated 8 times, for a total of 192 plants for this study. The model used VWC treatments and fruit developmental stage as the fixed, main effects and block as a random effect. Student t-tests was used to determine differences between treatments, due to missing data because of aborted flowers and problems with the irrigation control. In case of interactive effects between fruit developmental stage and VWC, the slice option was used to look at differences among fruit of different developmental stages at the same VWC and to look at the effects of VWC within fruit developmental stages.

Results

Environmental Data

Daily average minimum and maximum temperatures were 16.9 ± 2.2 °C (±SD) and 24.5 ± 2.3 °C respectively, while the daily average temperature was 20.5 ± 1.7 °C throughout the study (Fig. 2.1A). Average daily temperature showed variation but remained relatively constant until May, when the temperatures inside the greenhouse slowly increased. Daily average minimum and maximum relative humidity (RH) readings were 37 ± 18% and 74 ± 13% respectively, while the daily average RH was 59 ± 15% over the entire study (Fig. 2.1 B). Average RH fluctuated greatly within the greenhouse, but tended to increase as the study progressed. The average daily light integral (DLI) from February 2 to May 25 was 23.1 ± 12.2 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ (Fig 2.1C). Daily light integral tended to increase over the course of the study, as the day length increased.

Average VWC readings were calculated every two hours for each irrigation treatment, from April 5 to until the final harvest day on May 25 (Fig. 2.2). The VWC thresholds for mild, moderate, and severe drought were approximately reached 5, 10, and 15 d after thresholds were changed on April 5. Substrate VWC for the 0.40 $m^3{\cdot}m^{-3}$ treatments was very stable, with a standard deviation of ± 0.005 $m^3{\cdot}m^{-3}$. The control for the 0.25 $m^3{\cdot}m^{-3}$ treatments fluctuated a little more than the well-watered treatments with a standard deviation of ± 0.008, but control was fairly stable. For the 0.15 and 0.10 $m^3{\cdot}m^{-3}$ treatments, VWC was not as stable, with substrate moistures readings fluctuating above the threshold in the 0.15 $m^3{\cdot}m^{-3}$ treatment with a standard deviation of ± 0.02 and ± 0.03. The substrate moisture readings for the 0.10 $m^3{\cdot}m^{-3}$ treatment fluctuated above and below the threshold, so it was difficult to control such a low substrate VWC threshold.

Fruit Measurements

Average final fruit volume was affected by VWC treatments (p = 0.008), regardless of fruit developmental stage at which the drought was started. Fruit grown under well-watered conditions (VWC of 0.40 $m^3{\cdot}m^{-3}$) were 18 and 25% larger than fruit grown under severe and moderate drought (0.10 and 0.15 $m^3{\cdot}m^{-3}$), respectively (Fig. 2.3). Fruit grown under the mild drought (0.25 $m^3{\cdot}m^{-3}$) were 19% larger than fruit exposed to moderate drought (0.15 $m^3{\cdot}m^{-3}$) (p = 0.015).

Soluble solids were affected by VWC treatment ($p = 0.0030$) and by fruit age at which VWC treatments were started ($p = 0.0001$), but not by their interaction. Fruit in drought treatments (0.10, 0.15, and 0.25 $m^3 \cdot m^{-3}$) had, 11, 16, and 12% higher soluble solids than the 0.40 $m^3 \cdot m^{-3}$ treatment, respectively (Fig. 2.4A). Beginning VWC treatments when fruit were 25 DPA resulted in 15% higher soluble solids compared to starting the treatments when fruit were 40 DPA and 15 DPA (Fig. 2.4B).

Fruit dry weight was affected by fruit age at which VWC treatments were started ($p = 0.0007$), but not by VWC treatments. Exposing fruit to the VWC treatments when fruit were 25 DPA resulted in 19 and 16% greater dry weight compared to exposing fruit to the VWC treatments when fruit were 40 DPA and 15 DPA, respectively (Fig. 2.5A). Increases in fruit dry weight was positively, but weakly correlated with increases in soluble solids ($R^2 = 0.13$; $P = 0.0005$).

Fruit water content was affected by VWC treatment ($p = 0.0003$) and by fruit age at which VWC treatments were started ($p < 0.0001$), but not by their interaction. Fruit grown under well-watered conditions had between 0.7 to 1.5% higher water content than fruit grown under any of the three drought treatments (Fig. 2.5B). Fruit age at which VWC treatments were started also affected water content and was lowest when the VWC treatments started when fruit were 25 DPA (Fig 2.5C). Starting the VWC treatments when fruit were 25 DPA resulted in 2.0 and 1.6% reduction in fruit water content compared to starting the VWC treatments when fruit were 40 DPA and 15 DPA, respectively.

Endoreduplication

The endoreduplication factor was affected by the interaction of fruit age at which VWC treatments were started and the VWC treatment (p = 0.015). When the VWC treatments were started when fruit were 40 DPA, fruit EF was higher in the 0.10 and 0.15 $m^3 \cdot m^{-3}$ VWC treatments than in fruit grown under a VWC of 0.25 or 0.40 $m^3 \cdot m^{-3}$ (p = 0.0019). Substrate VWC did not affect EF when the drought was imposed when fruit were 25 DPA or 15 DPA (Fig. 2.6 A).

Discussion

Endoreduplication can occur in multiple tissues and organ types across different plant species and has been associated with increased cell size, in some cases influencing organ growth (D'AMATO, 1989; BAROW, 2006; DERMASTIA ET AL., 2012). Root hair cells of *Arabidopsis* go through more endocycles to promote cell expansion and growth towards water and nutrients (DOLAN AND DAVIES, 2004). Tomato fruit can have high levels of endoreduplication, leading to a 512C nuclear DNA content (JOUBÈS ET AL., 1999; CHENICLET ET AL., 2005; CHEVALIER ET AL., 2011). In this study, we wanted to understand the relationship between endoreduplication, drought, fruit developmental stage, and fruit size.

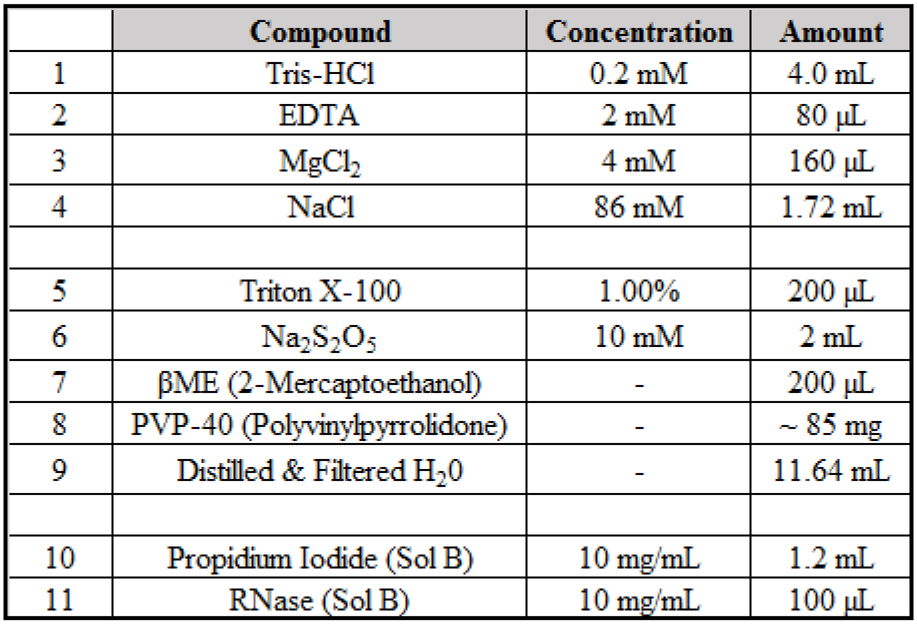
We hypothesized that drought would increase soluble solids of fruit and the soluble solids readings were indeed higher in all drought treatments compared to well-watered conditions (Fig. 2.4A). This could be attributed to fruit water content. Fruit water content was higher under well-watered conditions compared to all drought treatments (Fig 2.5B). With higher fruit water content, the soluble solids will be more diluted, resulting in lower soluble solids. This trend of decreasing soluble solids with increasing fruit water content is supported by our finding that soluble solids content was negatively correlated with fruit water content ($R = -0.71$; $P < 0.0001$). Starting VWC treatments when fruit were 25 DPA increased soluble solids compared to starting when fruit were 15 DPA and 40 DPA (Fig. 2.4B). It is important to note that the fruit that were tagged to represent different developmental stages grew under different environmental conditions. Different environmental conditions during fruit growth and development could be a confounding factor when considering differences in fruit soluble solids, water content, and dry weight, based on the effects of drought timing.

Fruit dry weight was highest when starting VWC treatments when fruit were 25 DPA (Fig. 2.5 A). Once again, differences among fruit developmental stage may be attributed to the different environmental conditions for fruit tagged at different times. Though, the increase in dry weight could be related to the increase in soluble solids, as dry weight was positively, but moderately correlated with soluble solids ($R = 0.37$; $P < 0.0005$). This greater dry weight observed when starting VWC treatments when fruit were 25 DPA has been attributed to higher increases in soluble solid levels during the HG developmental stage (YOUNG ET AL., 1993; DAVIES ET AL., 1981). What is more interesting is that exposing fruit to low VWC treatments when fruit were 40 DPA and 15 DPA, decreased the dry weight of the fruit. Accumulation of soluble solids is occurring when fruit are metabolically young and during the breaker stage (GILLASPY ET AL., 1993), but appear to be less affected by imposing VWC treatments at the BR or YF developmental stages. Again, this could be related to environmental condition differences between when the VWC treatments were exposed to the fruit developmental stages.

The severe and moderate drought treatments (0.10 and 0.15 $m^3·m^{-3}$) reduced final fruit volume when compared to fruit grown at well-watered conditions (0.40 $m^3·m^{-3}$) (Fig. 2.3). There was no correlation between fruit water content and fruit size (R^2 = 0.05; P = 0.053). Fruit age when the VWC treatments were started did not significantly affect fruit volume.

We hypothesized that drought stress decreases endoreduplication and fruit size in tomato and that this effect would be most pronounced if the fruit is exposed to drought when young. This study revealed that the endoreduplication factor (EF) is affected by the VWC treatments only when treatments are started when fruit are 40 DPA. Exposing plants to severe and moderate drought (0.10 and 0.15 $m^3·m^{-3}$) when fruit were 40 DPA increased the EF of those fruit, compared to well-watered conditions (0.40 $m^3·m^{-3}$) and mild drought (0.25 $m^3·m^{-3}$). Beginning drought treatments when fruit are 15 DPA and 25 DPA did not affect EF of those fruit. This could be because endocycles have been found to be most active from 4-16 days after anthesis (BÜNGER-KIBLER and BANGERTH, 1982; KOWLES AND PHILLIPS, 1988), which is earlier than our fruit at HG were (25 d old). Endoreduplication reportedly occurs mostly at the cell differentiation stage (TRAAS ET AL., 1998). However, our finding that EF is affected when fruit are exposed to drought starting at the BR stage indicates that endoreduplication is still occurring at a later stage of fruit development. In chapter 3 of this thesis, I quantify endoreduplication as tomato fruit ages, and found that endoreduplication occurs in two phases of fruit development: initially when fruit are between 9-16 d old and again when fruit are 33-38 d old. The increases in EF during these two fruit developmental phases suggests that endoreduplication may have other purposes that just cell expansion. Since fruit volume was decreased with the 0.10 and 0.15 $m^3 \cdot m^{-3}$ treatments, but EF was higher in BR fruit exposed to those same treatments, the implication is that more endoreduplication did not result in larger fruit. Regardless of drought timing, fruit size was not correlated with endoreduplication factor ($R^2 = 0.022$; $P = 0.23$). Previous research has shown that cell size increases with increasing DNA content from endoreduplication (TRAAS ET AL., 1998; MASSONNET ET AL., 2011) and cell expansion can contribute to the final organ volume (MALLADI AND HIRST, 2010; OKELLO ET AL., 2015). Endoreduplication has implicated in affecting the ratio between cell division and expansion, by ending division earlier and rapidly expanding cells before the maximum potential cell number is established, which can affect final organ size (BAROW, 2006; CHEVALIER ET AL., 2014; OKELLO ET AL., 2015). We found that drought decreased fruit volume and increased EF (if fruit is exposed to drought starting at the BR stage). Tomato fruit that develop under drought had decreased volumes, but also had increased soluble solids, which is an indicator of fruit quality. Our data support the hypothesis that drought stress decreases fruit size, but do not support the hypotheses that drought decreases endoreduplication or that endoreduplication is associated with increased fruit size. Understanding the importance of drought timing and VWC can aid growers in controlling and maintaining appropriate fruit size and quality. This research allowed us to refine a second study to look at the time-course of endoreduplication rates as fruit develop over time.

Tables and Figures



	Compound	Concentration	Amount
1	Tris-HCl	0.2 mM	4.0 mL
2	EDTA	2 mM	80 µL
3	$MgCl_2$	4 mM	160 µL
4	NaCl	86 mM	1.72 mL
5	Triton X-100	1.00%	200 µL
6	$Na_2S_2O_5$	10 mM	2 mL
7	βME (2-Mercaptoethanol)	-	200 µL
8	PVP-40 (Polyvinylpyrrolidone)	-	~ 85 mg
9	Distilled & Filtered H_20	-	11.64 mL
10	Propidium Iodide (Sol B)	10 mg/mL	1.2 mL
11	RNase (Sol B)	10 mg/mL	100 µL

Table 2.1.

Adapted woody plant buffer (WPB) used in the tissue processing for flow cytometry analysis. This protocol called to add the ingredients 1-9 together, to make a 20 mL solution and to split that into 2 separate solutions (solution A and B). Approximately 9.5 mL of the base solution was poured into a separate 15 mL tube and propidium iodide and RNase were added to solution B. That tube was then wrapped in tin foil, to prevent light from degrading the stain. The concentrations and amount of propidium iodide and PVP-40 added are the adaptions made to the WPB original recipe.

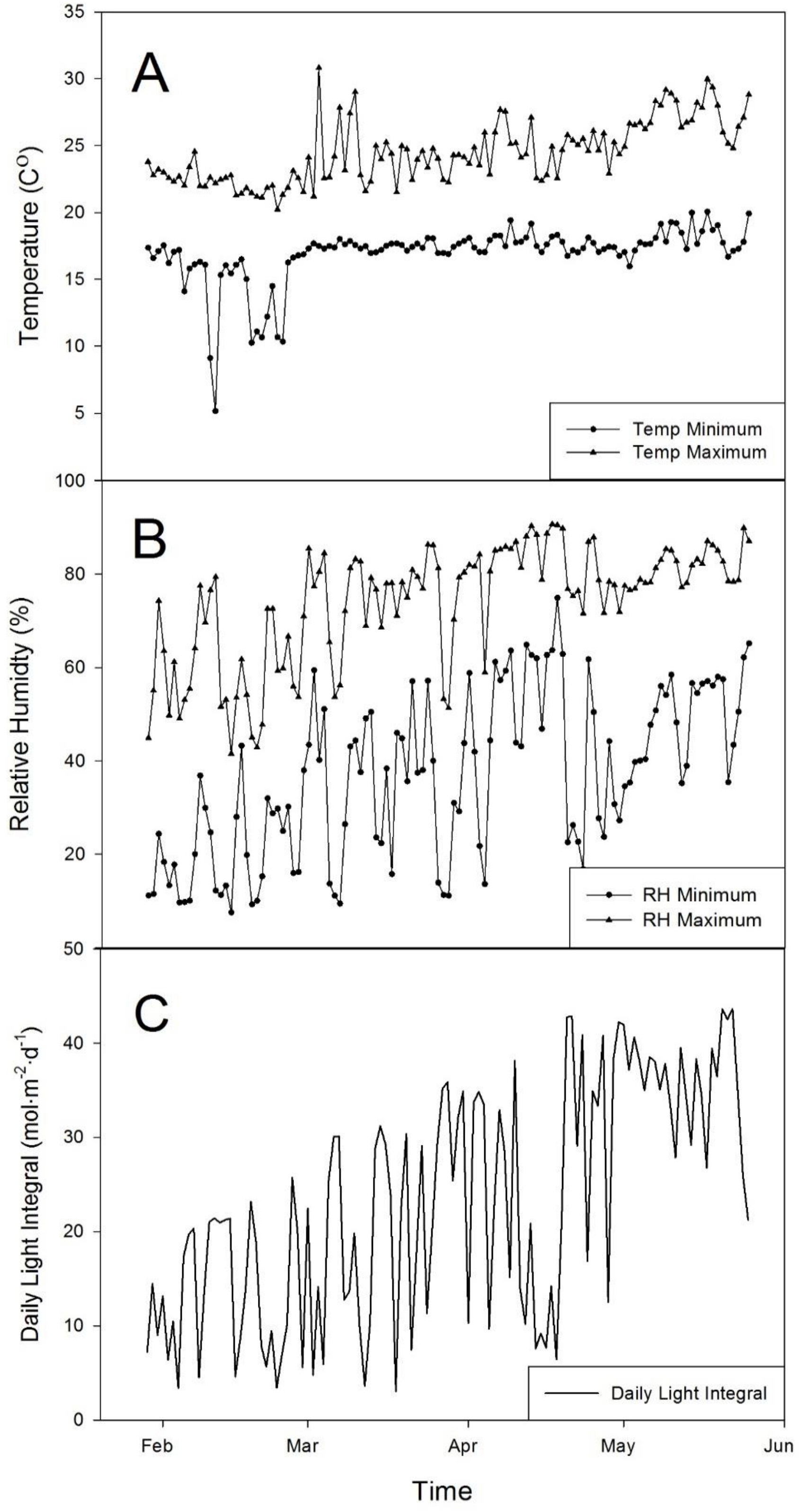

A
B
C
Temperature (C°)
Relative Humidty (%)
Daily Light Integral (mol·m-2·d-1)
35
30
25
20
15
10
5
100
80
60
40
20
50
40
30
20
10
0
Temp Minimum
Temp Maximum
RH Minimum
RH Maximum
Daily Light Integral
Feb
Mar
Apr
May
Jun
Time

Figure 2.1

Temperature (A), relative humidity (B), and daily light integral (C) inside the greenhouse from January 30 to May 25 2015.

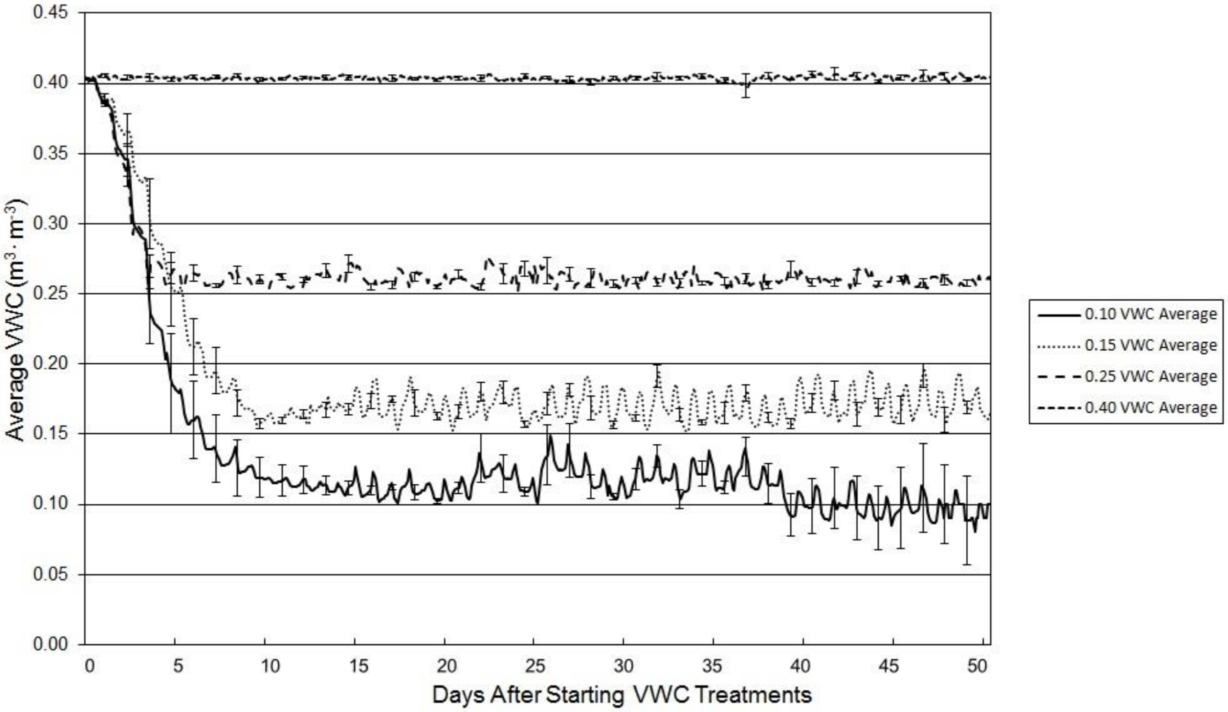

0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Average VWC (m³ · m⁻³)
0
5
10
15
20
25
30
35
40
45
50
Days After Starting VWC Treatments
0.10 VWC Average
0.15 VWC Average
0.25 VWC Average
0.40 VWC Average

Figure 2.2

Average substrate volumetric water contents (VWC) from under well-watered and mild, moderate, and severe drought conditions (VWC of 0.40, 0.25, 0.15, and 0.10 $m^3 \cdot m^{-3}$, respectively). The irrigation thresholds for mild drought, moderate drought, and severe drought were reached approximately 5, 10, and 15 d after changing the VWC thresholds. Error bars show representative standard errors (n=7).

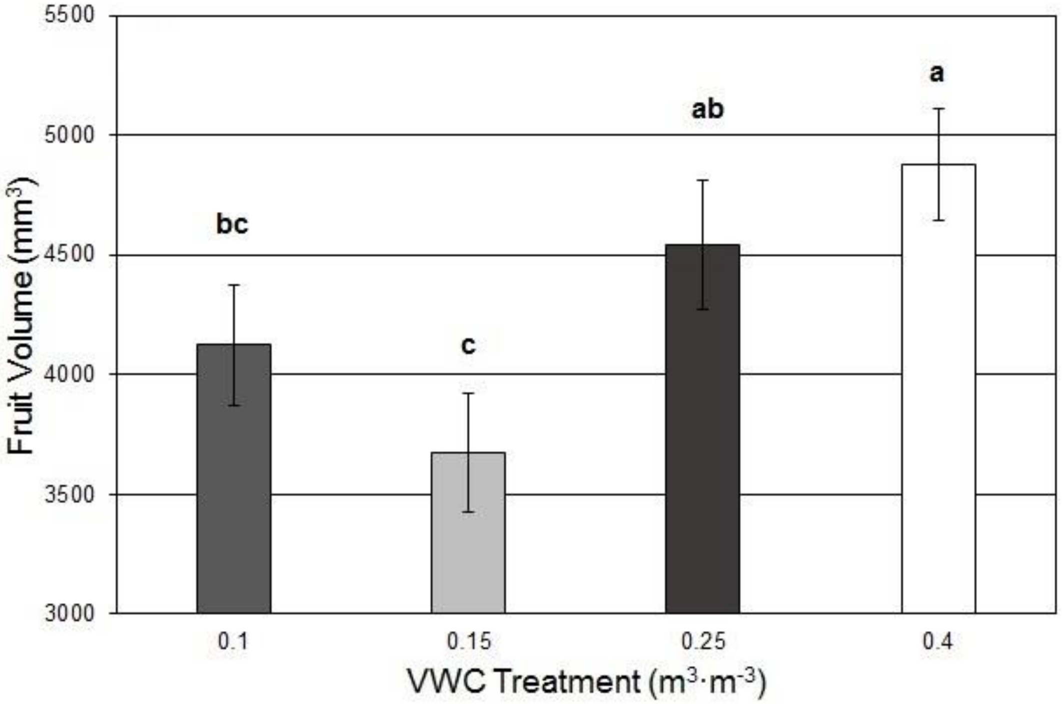

5500
5000
4500
4000
3500
3000
Fruit Volume (mm3)
bc
c
ab
a
0.1
0.15
0.25
0.4
VWC Treatment (m3·m-3)

Figure 2.3

Fruit volume at harvest for fruit grown under four VWC treatments (0.10, 0.15, 0.25, and 0.40 $m^3 \cdot m^{-3}$). The stage of fruit development at which the VWC treatments were started did not affect fruit volume, so data are averaged over the three fruit developmental stages. Means with the same letter are not significantly different according to Student’s t-test at $P = 0.05$.

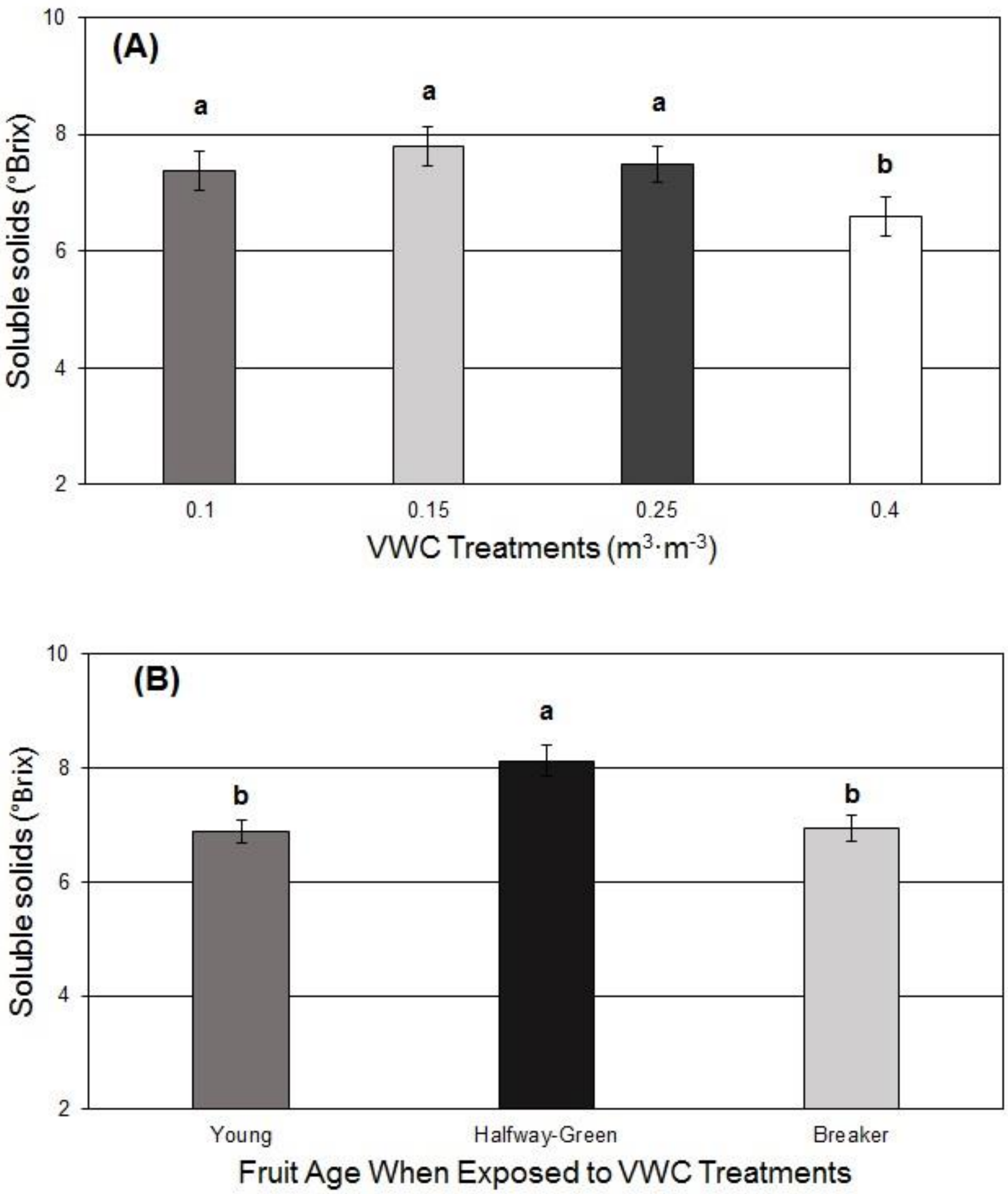

(A)
Soluble solids (°Brix)
10
8
6
4
2
a
a
a
b
0.1
0.15
0.25
0.4
VWC Treatments (m3·m-3)
(B)
Soluble solids (°Brix)
10
8
6
4
2
b
a
b
Young
Halfway-Green
Breaker
Fruit Age When Exposed to VWC Treatments

Figure 2.4

Average soluble solids measurements from all fruit across each VWC treatment (A) and fruit age at VWC exposure (B). No significant effect was observed for the interaction between VWC treatment and fruit age when exposed to the VWC treatments. Means with the same letter are not significantly different from each other according to Student's t-test with $P = 0.05$.

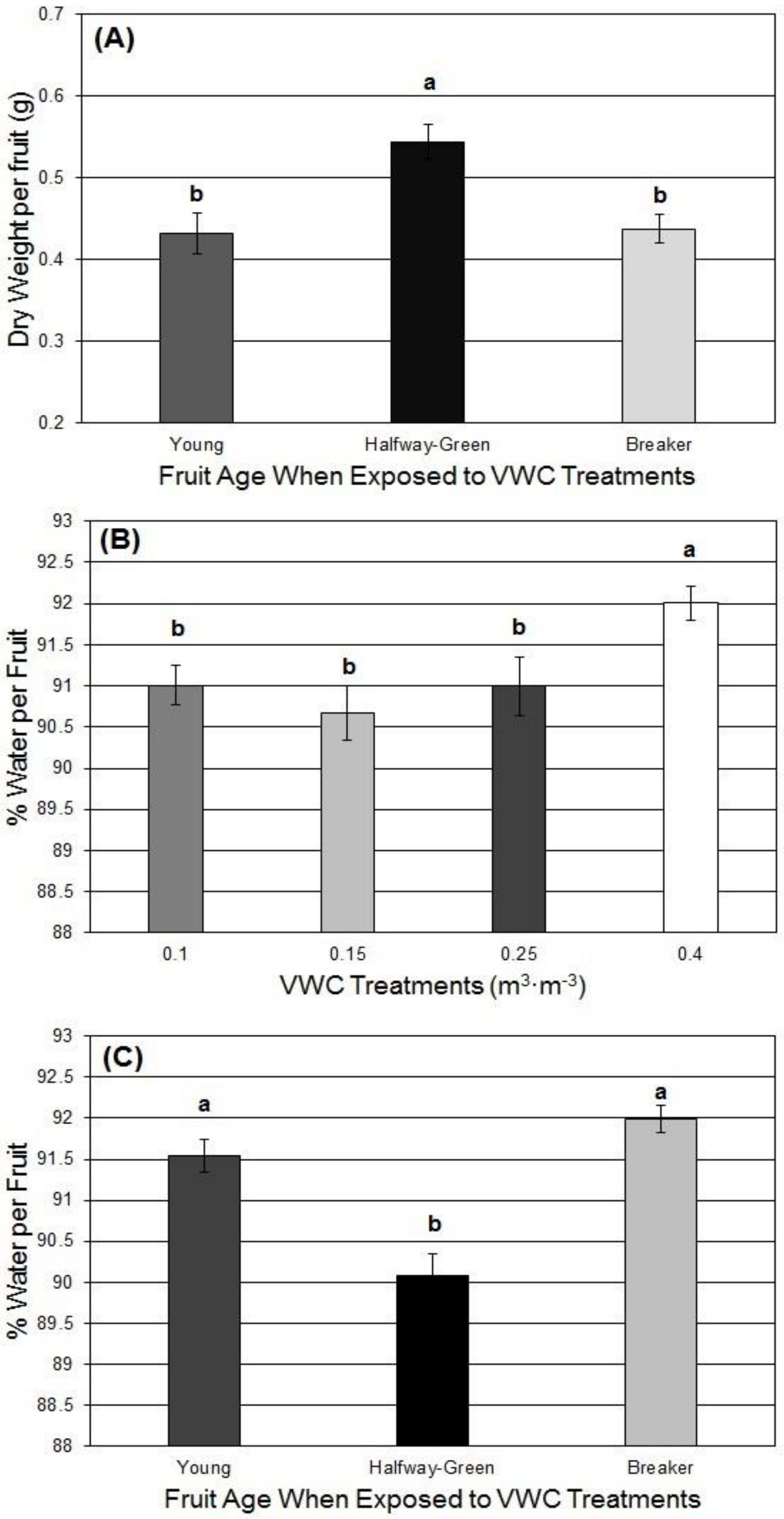
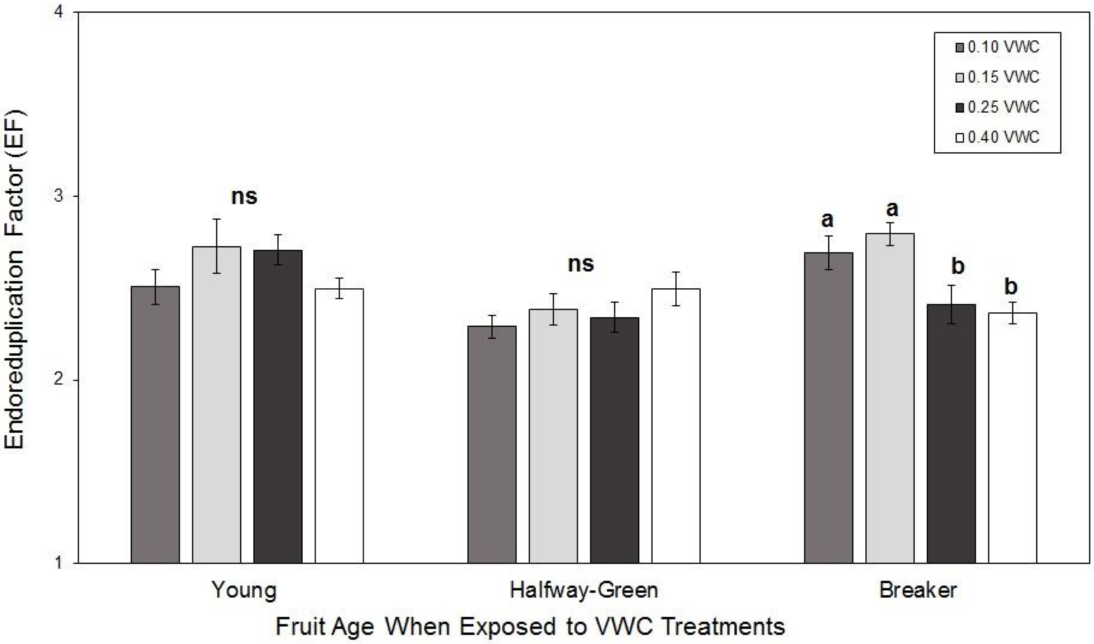

(A)
Dry Weight per fruit (g)
0.7
0.6
0.5
0.4
0.3
0.2
b
a
b
Young
Halfway-Green
Breaker
Fruit Age When Exposed to VWC Treatments
(B)
% Water per Fruit
93
92.5
92
91.5
91
90.5
90
89.5
89
88.5
88
b
b
b
a
0.1
0.15
0.25
0.4
VWC Treatments (m³·m⁻³)
(C)
% Water per Fruit
93
92.5
92
91.5
91
90.5
90
89.5
89
88.5
88
a
b
a
Young
Halfway-Green
Breaker
Fruit Age When Exposed to VWC Treatments

Figure 2.5

Fruit dry weight as a function of fruit developmental stage when exposed to the VWC treatments (A), fruit water content as a function of VWC treatments (B) and fruit age when exposed to the treatments (C). Means with the same letter are not significantly different according to Student's t-test at *P* = 0.05.



Endoreduplication Factor (EF)
4
3
2
1
0.10 VWC
0.15 VWC
0.25 VWC
0.40 VWC
ns
ns
a
a
b
b
Young
Halfway-Green
Breaker
Fruit Age When Exposed to VWC Treatments

Figure 2.6

Endoreduplication factor (EF) of pericarp of ripe tomato fruit (45 d old) as affected by VWC and the fruit developmental stage when VWC treatments started. Means with the same letter are not significantly different according to Student’s t-test at *P* = 0.05.

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CHAPTER 3

DROUGHT EFFECTS ON FRUIT GROWTH AND ENDOREDUPLICATION IN TOMATO (*Solanum lycopersicum*)[1]

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[1] Gianino, D., van Iersel, M., Malladi, A. To be submitted to *Journal of the American Society for Horticultural Science* for publication.

Abstract

Endoreduplication is often associated with cell expansion, which influences organ growth. Tomato fruit carry out this modified cell cycle in which DNA is replicated without cell division. Endoreduplication occurs at different developmental stages, generally associated with cell expansion in organ growth or in response to organ senescence. This study had two objectives: to determine when during tomato fruit development endoreduplication occurs and to determine how endoreduplication is affected by drought. To explore these objectives, tomato was grown under three different substrate volumetric water content (VWC) treatments, representing drought exposure beginning when fruit are young (2 d old), drought exposure beginning when fruit are at breaker stage (31 d old), and fruit grown under well-watered conditions throughout the study. The two drought treatments began when fruit were at their respective developmental stage, and continued until final harvest. Beginning the drought treatment when fruit were young resulted in a 19% reduction in fruit size and an 18% reduction in fruit weight compared to fruit that were well-watered throughout. The endoreduplication factor (EF), an index of how many endoreduplication events occurred, was not affected by drought, but increased when fruit were young and again when fruit entered the breaker stage of development. Fruit volume and fresh weight both increased from day 9 to 23, but did not increase after 23 d. Fruit volume showed an 82% increase from day 9 to 23 day and fruit weight increased by 84% from day 9 to 23 as well. Considering fruit from all harvests, EF was weakly correlated with fruit volume (R = -0.36). Fruit soluble solids, titratable acidity, texture parameters, and fruit color were not affected by VWC treatments. Endoreduplication occurred mainly when tomato fruit are between 9 and 16 d

old (fruit enlargement), and then again from 38 to 43 d (ripening). Endoreduplication has been associated with cell expansion, but it also may be involved with metabolic activity during fruit ripening.

Introduction

Endoreduplication, or endocycle, is an altered cell cycle that promotes the replication of chromosomal DNA, without the usual cell division stage (D’AMATO, 1979; BERGERVOET ET AL., 1996; BOURDON ET AL., 2010). The traditional cell cycle allows for DNA duplication in the mother cell, followed by division into two daughter cells (JOUBÉS AND CHEVALIER, 2000; BOURDON ET AL., 2010). Cells performing endoreduplication replicate their DNA but do not divide, thereby doubling their DNA content with each cycle. This increase in DNA content and an often-related increase in nuclear size have been associated with increases in cell size (DOLAN AND DAVIES, 2004; BAROW, 2006; BERTIN ET AL., 2007) and in some cases, increases in organ size. Leaf size of *Arabidopsis thaliana* increases with increasing rates of endoreduplication (MELARAGNO ET AL., 1993; COOKSON ET AL., 2006). Endoreduplication also increases root hair cell size, influencing root hair length (DOLAN AND DAVIES, 2004). Final fruit volume in tomato is affected by cell expansion, which can be affected by endoreduplication (CHENICLET ET AL., 2005; CHEVALIER ET AL., 2014; AZZI ET AL., 2015). The endoreduplication cycle within plant cells responds to hormone signaling, developmental signals and potentially abiotic factors as well. Environmental factors such as drought, light levels, and temperature can promote or reduce endoreduplication activity in different species of plants (BAROW, 2006). In some cases, environmental stress may not be directly affecting the endoreduplication cycle. Drought can decrease cell expansion and fresh weight (both effects have been associated with endoreduplication) of *Zea mays* kernels during early development (5 days after pollination) through the production of abscisic acid (ABA) (MEYERS ET AL., 1990). Gibberellic acid accumulation increased the rate of endoreduplication of epidermal cell growth in *Ornithogalum umbellatum* (KWIATKOWSKA ET AL., 2007). Fruit growth can by modified by targeting genes involved in either the mitosis stage of the cell cycle or endoreduplication. A suite of endocycle controlling genes (*SICCS52A*, *SIWEE1*, and *SI-IAA17*) can be downregulated or overexpressed to promote or repress endocycles, affecting fruit growth in tomato (AZZI ET AL., 2015). Ploidy levels of tomato pericarp cells were repressed by the expression of *SI-IAA17*, an auxin transcriptional repressor that acts to suppress cell size, fruit volume, and fruit weight in tomato. When *SI-IAA17* is suppressed, ploidy levels increase via endoreduplication, increasing cell size, fruit volume, and fruit weight (SU ET AL., 2014). The overexpression of the anaphase promoting complex, *SlCCS52A*, led to a reduction in endoreduplication-driven cell expansion when fruits were young (MATHIEU-RIVET ET AL., 2010), inferring that endoreduplication may be influenced or affected more at specific fruit developmental stages. Overexpression of the cyclin-dependent protein kinase gene *CDKB*, which impacts the cell division stage of fruit development, led to a reduction in endoreduplication (CZEREDNIK ET AL., 2012).

Endoreduplication is a process that occurs after cell division and is potentially one of the drivers for cell expansion in tomato fruit (ARTLIP ET AL., 1995), which is one of the primary drivers for fruit growth. If endoreduplication occurs mainly at specific developmental stages fruit could be vulnerable to reduced growth if the fruit experiences stressors during times of high endoreduplication activity. In tomato fruit, cell expansion begins after most of the cell division has ended, around 7 to 10 DAP (CHEVALIER ET AL., 2011). It has been reported that final fruit size, in tomato, has been achieved at the mature green stage of fruit development (CHEVALIER, 2007), but it is unknown whether endoreduplication continues until fruit ripening, or if endoreduplication occurs in specific phases of fruit development.

The goal of this study was to quantify endoreduplication over the course of fruit development and to determine how this is affected by drought stress starting when fruit are young or at breaker stage. I also wanted to look at the relationship between endoreduplication and fruit growth and quality. The hypotheses for this study were 1) most endoreduplication will occur during the first few weeks of fruit growth, 2) drought stress will decrease endoreduplication rates in tomato fruit, 3) fruit will be smaller when exposed to drought when fruit are young, and 4) fruit quality will be impacted by drought stress.

Materials and Methods

Plant Material

This experiment was conducted from March 29 to June 22 2016, in a greenhouse at the University of Georgia's greenhouse complex in Athens, GA. 'Micro-Tina' tomato seeds were planted on March 29 in plastic 36-cell trays with soilless substrate (Fafard 1P; Sun Gro; Agawam, MA). Trays were placed on ebb and flow benches and irrigated with 100 ppm N water soluble fertilizer (15N-2.2P-12K, 15-5-15 CalMag; The Scotts

Company; Marysville, OH) for 30 d before transplanting. When seedlings were 30 d old, they were transplanted into 15 cm pots filled with soilless substrate (Fafard 1P; Sun Gro; Agawom, MA) with a controlled release fertilizer (19N-1.8P-6.6K; Harrell's LLC; Lakeland, FL; 5-6 month release time) incorporated at a rate of 5.4 $kg \cdot m^{-3}$, then placed back onto ebb and flow benches for 4 d, after which plants were placed onto an automated irrigation system.

Irrigation Control and Environmental Parameters

Irrigation was automatically controlled using an adapted design of the soil moisture sensor-based system developed by Nemali and van Iersel (2006). An irrigation system with 32 irrigation lines and solenoid valves (075-DV; Rain Bird; Azusa, CA) was used to irrigate the tomato plants. Each irrigation line had six pressure-compensated drip emitters, rated for 2 L/h (PCJ; Netafim USA; Fresno, CA) and spaced approximately 20 cm apart. Drip tube rings (DR6; Dramm; Manitowoc, WI), approximately 10 cm in diameter, were attached to the drip emitters and then staked to sit on top of the substrate, around the tomato plant. Six tomato plants were placed on each irrigation line, representing one experimental unit. Thirty-two soil moisture sensors (EC-5; Decagon Devices; Pullman, WA), one per irrigation line) were connected to the datalogger (CR10X; Campbell Scientific, Logan UT) using a multiplexer (AM16/32B; Campbell Scientific) to take substrate volumetric water content (VWC) readings. The soil moisture sensors were inserted into tomato pots at a 45° angle, in the center of the pot, until the sensor tip was 6 cm deep. The datalogger took voltage output readings from the sensors and converted the voltage reading into a VWC reading. Once the VWC reading was taken, the datalogger compared the measured VWC to the VWC threshold for each irrigation line. If the measured VWC was below the threshold, the datalogger powered the solenoid valve for 10 s using two relay drivers (SDM-CD16AC/DC; Campbell Scientific). The experiment lasted 60 d from placing the tomato plants onto the automated irrigation system until final data collection. After transplanting and placing the plants on the irrigation system, all 32 VWC thresholds were set at 0.40 $m^3 \cdot m^{-3}$. After 7 d on the irrigation system (May 10), three flowers per plant were tagged, with six plants per irrigation line for a total of 18 tagged flowers per irrigation line. The first drought treatment (VWC threshold of 0.15 $m^3 \cdot m^{-3}$ starting at young fruit stage) was initiated in 8 experimental units, on May 10 and it took ~2 d for the VWC readings to reach the new, lower threshold. Plants in this treatment were 41 d old and fruit were ~2 d old when the threshold was reached and stable. The second drought treatment (VWC threshold of 0.15 $m^3 \cdot m^{-3}$ starting at breaker stage) was initiated, in another 8 experimental units, on June 3. Plants were 67 d old and fruit were 31 d old. All thresholds for the 0.15 $m^3 \cdot m^{-3}$ at breaker stage treatment were reached on June 6 (Fig 3.2). The remaining 8 experimental units remained at 0.40 $m^3 \cdot m^{-3}$ for the entirety of the study.

Air temperature and relative humidity in the greenhouse were measured using a VP3 probe (Decagon Devices). Vapor pressure deficit (VPD) was calculated by the datalogger (CR10X; Campbell Scientific) using the temperature and relative humidity values. Photosynthetic photon flux was measured with a quantum sensor (SQ-110; Apogee Instruments; Logan, UT) and daily light integral (DLI) was calculated by integrating these readings over the entire day.

Flow Cytometry and Fruit Size

Fruit were sampled at 9, 16, 23, 28, 33, 38, and 43 d after the tagging date to determine fruit endoreduplication rates, fresh weight, and volume measurements. At each sampling time, one tagged fruit from each irrigation line was harvested, weighed, and fruit volume was measured before being processed for flow cytometry. The sampling occurred in 3 harvest groupings throughout the day; morning, afternoon, and evening. The first two groups (morning and afternoon) consisted of 9 fruit (three replications) sampled and 6 fruit (two replications) were sampled in the evening group. The fruit were sampled in groups because the preparation process uses time-sensitive stains and buffers, which allows only a few fruit to be processed at one time.

The harvested fruit were weighed with a scale (Model AB57; Mettler Toledo; Columbus, OH) and diameter and length each fruit were measured with calipers. Fruit volume was calculated using: $\pi \times 4/3 \times r^3$. We used this equation under the assumption that each fruit was a perfect sphere, because diameter and length measurements were similar. Although this assumption may not be exactly true, it likely introduces the same error in all treatments.

Once the other measurements were taken, the tissue preparation for flow cytometry took place. Pericarp tissue (~10 mm × 10 mm × 2 mm) from the middle portion of the widest part of the fruit, located in between the epidermis and the locule region, was sampled. A nuclei extraction buffer was adapted from a previously successful buffer known as woody plant buffer (WPB), which was developed for problematic tissues and woody stems by Loureiro *et al.* (2007). The extraction buffer was made anew for each sampling group, to provide consistent nuclei extraction and staining. The base buffer solution was made by combining the following: Tris-HCl, EDTA, $MgCl_2$, NaCl, Triton X-100, $Na_2S_2O_5$, PVP-40, and distilled, autoclaved, and filtered H_2O. Concentrations and amounts contributed to the base solution can be found in Table 2.1. Items 1-9 in Table 2.1 were combined to make a 20 mL base solution. Once the 20 mL base solution was made, 9.5 mL was poured into a 15 mL tube labeled ‘solution B’ and propidium iodide (PI) and RNase was then added to solution B and the tube was wrapped in tin foil to prevent light from degrading the PI nuclei stain. The remaining 10.5 mL of base solution was poured into another 15 mL tube and labeled ‘solution A’.

The tissue was chopped with a razor blade (AccuTech Blades; Verona, VA) in a Petri dish on ice in 1 mL of solution A until the tissue was chopped finely and cell contents were released into the extraction buffer (solution A). Tissue particles too big for the flow cytometer to process were separated from the solution. The remaining solution was pipetted into a 2 mL tube. Then 1 mL of staining buffer (solution B) was added to the test tube and mixed to ensure the stain reached all nuclei. The completed sample was put on ice and covered to prevent light from degrading the nuclear stain. Once all samples were prepared, the samples were taken to the Flow Cytometry Resource lab facility at the University of Georgia for analysis. Samples were then filtered through a 50 µm filter (Partec; Münster, Germany) into 10 mL sterile test tubes for flow cytometry.

Ploidy was measured on a flow cytometer (CyAn ADP, Beckman Coulter; Hialeah, FL). The PI stain was excited by a 488 nm laser and emission was read through a filter (575/25 BP, Beckman Coulter). Fluorescence was measured in logarithmic mode. Ploidy levels were detected on a single parameter, which plots PI excitation vs cell count, in order to generate a peak histogram for analysis. Flow cytometry software

(FlowJo Ver. 12; FlowJo; Ashland, OR) was used for analysis of ploidy level distribution. Samples were gated, gathered into a histogram display, with a protocol that excluded the first 15% of events, to exclude aggregate and non-DNA events. A leaf standard sample was included with each sampling group to gate, set the regions for ploidy levels (2C, 4C, 8C, etc...) for the appropriate ploidy peak regions. 10,000 nuclei per sample were measured, allowing for a representative histogram with large peaks to determine ploidy levels and allow for accurate gating.

Endoreduplication Factor

The flow cytometer and software identified and calculated the proportion of nuclei that are distributed into the groups of chromosomal DNA (2C, 4C, 8C...512C). The "C" value represents the amount of DNA in the cell. Proportions of cells in specific C regions, provided by the software, can be used to calculate the mean endoreduplication factor (EF), using the method proposed by Cookson *et al.* (2006). The equation used is as follows:

$$\mathrm{EF} = (2C \times 0) + (4C \times 1) + (8C \times 2) + (16C \times 3) + (32C \times 4) + (64C \times 5) + (128C \times 6) + (256C \times 7) + (512C \times 8).$$

Values of each "C" region represents the fraction of cells, in each sample, that have a genome size of the respective levels, such as 2C, 4C, 8C, etc. The fraction is then multiplied by how many endocycles is required to achieve a DNA content, specified by the "C value". For example, cells with a DNA content of 2C did not have an endocycle, so the proportion of cells with a 2C DNA content was multiplied by zero. The proportion of cells with a DNA content of 4C, on the other hand, went through one endocycle, so the proportion of 4C cells is multiplied by one. These values were added together and the equation followed the same rules until all chromosomal DNA counts found in the sample, were calculated into the EF value. The EF indicates the average number of cycles of endoreduplication cells in the sample went through.

Fruit Quality Parameters

Two weeks after fruit began turning red (24 June 2016), three fruit per experimental unit were harvested and used for quality parameter analysis. First, the fruit were analyzed for color using a handheld colorimeter (NR1100; 3nh Technology Co.; Shenzhen, China). Three measurements per fruit were taken. The colorimeter provides measurements that represent three aspects of color, L* for lightness, a* for values between green and red/magenta, and b* for values between blue and yellow.

The fruit were then measured on a fruit texture analyzer (GS-15; Güss Instruments; Strand, South Africa) to test compression and puncture resistance. Each fruit was compressed on one plane and then the fruit was turned 90° and punctured on another plane. Compression was carried out using a 15 mm compression plate and the puncture force was measured using a probe 1 mm in diameter and 20 mm in length, both of which attached to the fruit texture analyzer. Compression was performed using a forward speed of 6 mm/s, measure speed of 5 mm/s, with a compression distance of 1 mm; puncture was performed using a forward speed of 10 mm/s, measure speed of 5 mm/s, with a puncture distance of 3.0 mm.

The three fruit were then quartered and squeezed in cheesecloth to extract juice from the tomato for soluble solids and titratable acidity analysis. The extracted tomato juice was filtered through another layer of cheesecloth and then poured into two 50 mL tubes. The juice for soluble solids was centrifuged (Allegra X-22R; Beckman Coulter; Brea, CA) for 10 min at 15°C with a rotation speed of 4500 rpm. Two mL of fluid was pipetted off of the top of the solution and passed through a cheese cloth into a 2 mL test tube. 200 µL of liquid was placed onto a refractometer lens (PR-32α; Atago Co.; Bellevue, WA) to measure soluble solids.

Titratable acidity was analyzed using a fruit juice titrator (HI-84532; Hanna Instruments Inc., Woonsocket, RI). The titrator used 5 mL of a pump calibration solution (HI84532-55; Hanna Instruments Inc.) with 45 mL of de-ionized water (DI H_2O) and measured how much low range titrant (HI84532-50; Hanna Instruments Inc.) was used to perform the titration of the tomato sample consisting of 5 mL of the extracted juice and 45 mL of DI H_2O. Sample pH was determined using a pH meter (Accumet AB15; Fisher Scientific International Inc.; Pittsburgh, PA). Titratable acidity readings were measured as grams of acid per 100 mL of solution. In tomato fruit, the dominant acid is citric acid, and so the acidity is expressed as a % of citric acid equivalents.

Experimental Design and Statistical Analysis

The experimental design was a randomized complete block with three irrigation treatments and eight replications. An experimental unit was a group of six plants on one irrigation line. Data were analyzed in JMP® (Version 12; SAS Institute Inc.; Cary, NC) with block treated as a random effect. The block by VWC interaction was used as the error term to test for VWC treatment effects. For endoreduplication factor, fruit size, and fruit weight, which were measured multiple times throughout the study, fruit age and its interaction with VWC treatment were included in the model as well and the interaction between fruit age and block number within VWC treatments was used as the error term to test for significance of these effects. In case of significant effects, Tukey's HSD was used for mean separation.

Results

Environmental Conditions and Irrigation Control

Average daily minimum and maximum temperatures were 19.7 ± 2.6°C (±SD) and 27.3 ± 2.8°C, respectively, while the average temperature was 23.0 ± 1.9°C throughout the study. Temperature readings increased slightly from the beginning of the study until the final harvest time (Fig. 3.1A). The daily minimum temperature tended to increase as the study progressed. Average daily minimum and maximum relative humidity (RH) were 52 ± 16% and 85 ± 6%, respectively, while the daily average RH within the greenhouse was 73 ± 10% over the whole study (Fig. 3.1B). Average RH fluctuated within the greenhouse on a day-to-day basis, but did not show a long-term trend. The DLI from April 29 to June 22 averaged 34.1 ± 10.3 $mol \cdot m^{-2} \cdot d^{-1}$ and was low (< 15 $mol \cdot m^{-2} \cdot d^{-1}$) on cloudy days (Fig. 3.1C).

Irrigation control was much more accurate for this study with only two VWC thresholds, compared to the study where we had four different VWC thresholds. Average VWC readings were maintained slightly above the target thresholds, with little variation (Fig 3.2). The VWC threshold for the first drought treatment was changed from 0.40 $m^3 \cdot m^{-3}$ to 0.15 $m^3 \cdot m^{-3}$ and was reached when the tagged fruit of those 8 experimental units were ~2 d old. The VWC threshold for the second drought treatment was changed to 0.15 $m^3 \cdot m^{-3}$ and was reached when the tagged fruit of those 8 experimental units were ~31 d old.

Endoreduplication Factor

The endoreduplication factor (EF) was affected by fruit age ($p < 0.0001$), but not by the VWC treatments or by the interaction between fruit age and VWC. The EF increased in two distinct stages of fruit development; early in fruit development, from day 9 to 16 (fruit enlargement), remained relatively constant from day 16 to 33, and then increased again from day 33 to 38 (fruit ripening) (Fig. 3.3A). Endoreduplication factor and fruit volume were positively, but weakly correlated ($R^2 = 0.13$; $P < 0.0001$) (Fig. 3.4A) The EF in young fruit (9-16 d old) had a reasonable, positive correlation with fruit volume ($R^2 = 0.47$; $P < 0.0001$) (Fig. 3.4B). The EF older fruit (23-43 d old) were negatively, although weakly, correlated to fruit volume ($R^2 = 0.12$; $P < 0.0001$) (Fig. 3.4C). Although EF did not increase significantly from day 16 to 33, a small fraction of cells with very high DNA content (128C to 512C) appeared (Table 3.2). This indicates that the proportions of DNA content shifts from primarily 2C to 32C to having more cells with higher DNA counts of 128C to 512C.

Fruit volume and fresh weight were affected by fruit age ($p < 0.0001$) and by VWC treatments ($p = 0.017$), but not by their interaction. Fruit volume and weight increased rapidly from day 9 to 23, with no significant increases later during fruit development (Fig. 3.3A). Fruit volume and weight were lower when exposed to the 0.15 $m^3 \cdot m^{-3}$ VWC treatment starting when fruit were young, as compared to fruit grown at the 0.40 $m^3 \cdot m^{-3}$ VWC (Fig. 3.3B & C).

Fruit Quality

Fruit soluble solids, titratable acidity, fruit color, and fruit texture were all measured two weeks after the fruit breaker stage (fully ripened fruit) when fruit were 45 d old. None of the fruit quality parameters were affected by VWC treatment (Table 3.1). Soluble solids of fruit averaged 5.02 ± 0.12 (±SE) °Brix, while titratable acidity averaged 0.33 ± 0.01 g/100 mL. Fruit compression and puncture values averaged 0.98 ± 0.03 kg and 0.46 ± 0.02 kg, respectively, but neither were affected by the VWC treatments. Fruit color measurements (L*, a*, and b*) also were not affected by the VWC treatments.

Discussion

Endoreduplication can be tissue specific, with most activity in tomato fruit reportedly occurring soon after the cell division stage (4-10 days post anthesis) and continuing through the cell expansion phase of fruit development (BÜNGER-KIBLER AND BANGERTH, 1982; CHENICLET ET AL., 2005; BOURDON ET AL., 2010; CHEVALIER ET AL., 2014). Our data show that there are two endoreduplication phases in tomato fruit; one when fruit are between 9-16 d old (fruit enlargement) and a second phase when fruit is 33-43 d old (fruit ripening) (Fig. 3.3A). It should be noted that by the first sample date the fruit were approximately 9 d old. At this time, EF ranged from 1.51 – 1.93, indicating that many of the cells had already undergone endoreduplication. Because the pericarp tissue was not discernable in very young fruit, we were not able to quantify endoreduplication before day 9. Although we did not sample fruit younger than 9 d old, endoreduplication clearly occurs during the first 9 d post-anthesis. We did not continue to take measurements beyond 43 d old fruit, because the fruit were fully ripe, so we do not know if endoreduplication continues to occur beyond 43 d old fruit. Tomato fruit can reach high chromosomal DNA counts, upwards of 512 C (BERGERVOET ET AL., 1996; CHEVALIER ET AL., 2011; AZZI ET AL., 2015), which was consistent with our observations, but cells with a DNA content of 512C were only present in fruit that were 28 d old or older and only in small proportions of the sample total (0.23 to 0.35% of total cells in sample) (Table 3.2). Cells that have high levels of endoreduplication activity tend to be larger in volume compared to cells that do not carry out endoreduplication (D’AMATO, 1989; TRAAS ET AL., 1998; CHENICLET ET AL., 2005). While there is no hard evidence for a direct effect of endoreduplication on fruit size via cell expansion alone, endoreduplication acts as a limiting factor of cell growth (CHEVALIER ET AL., 2014), which aids in determining the final fruit volume (BOURDON ET AL., 2010; CHEVALIER ET AL., 2011).

We hypothesized that drought would decrease endoreduplication rates in tomato fruit, but the EF was not affected by the VWC treatments in this study. Our data did not support the hypothesis that endoreduplication was affected by drought but do show that the EF was affected by fruit age ($p < 0.0001$). We hypothesized that most endoreduplication activity occurs when fruit is young, but we saw that endoreduplication occurred during two distinct phases, fruit enlargement (9 to 16 d old) and fruit ripening (33 to 43 d old) (Fig. 3.3A). This suggests that endoreduplication has more functions than just increasing DNA to support cell expansion. We speculate that one such function could be to increase DNA content necessary to support metabolism that occurs during fruit ripening.

Fruit volume and fresh weight both increased during the first 23 d of fruit development, and then were stable for the remainder of fruit development (Fig. 3.3A). Unlike the EF, fruit volume and fresh weight both were affected by the VWC treatments ($p = 0.017$ and p = 0.008). Exposing fruit to drought conditions starting when the fruit were young, reduced fruit volume and fresh weight, compared to the well-watered treatment (Fig. 3.3B,C), which does support our hypothesis that fruit volume is most affected by drought when exposed from a young age. Fruit volume and fresh weight were not affected by the drought treatment starting when fruit were at the breaker stage compared to the well-watered fruit, likely because the fruit had already reached its final size after 23 d, before the drought was started. This agrees with Chevalier (2007), who found that tomato reach their maximum size by the mature green stage of fruit development. We hypothesized that fruit volume would be a function of the EF and there was a correlation between fruit volume and EF ($R^2 = 0.13$; $P < 0.0001$) (Fig 3.4). We believe the reason the correlation was present was the result of the simultaneous increase in both EF and volume as the fruit grows. For example, younger fruit will have a smaller volume, and young fruit will have a small EF. So we analyzed the EF in young fruit (9-16 d old) and found that it had a positive correlation with fruit volume ($R^2 = 0.47$; $P < 0.0001$). But when we analyzed fruit that had reached maximum volume (23-43 d old) the fruit volume was weakly and negatively correlated with EF ($R^2 = 0.12$; $P < 0.0001$). This leads us to believe that endoreduplication may not influence fruit size, as it has been observed to do in other organs (DOLAN AND DAVIES, 2004; COOKSON ET AL., 2006).

Finally, we hypothesized that fruit quality parameters would be affected by the drought treatments. None of the fruit quality parameters (fruit soluble solids, titratable acidity, texture analysis, or fruit color) were affected by the drought treatment during this study (Table 3.1), which differs from the increase in soluble solids observed in Chapter 2 when fruit was exposed to different levels of drought. In the previous study, fruit were tagged at different times and thus grew under different environmental conditions. The results from this study do not support the notion that drought affects tomato fruit quality (DODDS ET AL., 1997; MINGCHI AND DIANKUI, 2002; PERVEZ ET AL., 2009). The tomato cultivar used, ‘Micro-Tina’, may be a confounding factor in this study, due to its genetics and phenotype. The ‘Micro-Tina’ tomato was bred to be a small, compact plant with small fruit that could grow efficiently in small spaces. Other cultivars, with a more typical growth habit, may respond differently to drought. Further research should explore if other tomato cultivars respond similarly.

Endoreduplication rates in this study were not affected by the VWC treatment, unlike the first study where we saw an increase in the EF when starting VWC treatments at the breaker stage of fruit development (Fig. 2.6). The results from the 2016 study indicate that EF was not sensitive to drought stress. Fruit volume and weight, on the other hand, were affected by VWC treatment. Our data support the hypothesis that fruit volume is decreased when fruit are exposed to drought starting at an early stage. Our data do not support the hypothesis that drought decreases endoreduplication.

Tables and Figures

VWC treatment	Soluble Solids (°Brix)	Titratable Acidity (g/100 mL)	Compression (kg of force)	Puncture (kg of force)	L*	a*	b*
0.15 Young	5.03 ± 0.15	0.35 ± 0.01	0.92 ± 0.06	0.43 ± 0.02	39.5 ± 1.1	23.9 ± 0.8	25.2 ± 1.6
0.15 Breaker	5.03 ± 0.27	0.32 ± 0.03	1.04 ± 0.05	0.50 ± 0.02	39.4 ± 0.9	24.1 ± 0.6	24.8 ± 1.4
0.40	5.00 ± 0.16	0.34 ± 0.02	0.98 ± 0.04	0.43 ± 0.04	38.8 ± 0.6	24.6 ± 0.5	24.2 ± 1.0
P-value	0.99	0.52	0.13	0.18	0.79	0.81	0.84

Table 3.1

Average fruit quality parameters of fruit harvested when they were 45 d old, for all three VWC treatments (drought beginning when fruit are young (0.15 $m^3 \cdot m^{-3}$ young), drought beginning when fruit are at breaker stage (0.15 $m^3 \cdot m^{-3}$ breaker), and well-watered conditions (0.40 $m^3 \cdot m^{-3}$)), of soluble solids, titratable acidity, fruit compression and puncture force, and fruit color index values (L, a, and b). Data are mean ± standard error (n=8).

Fruit Age (d)	**2C AVG**	**4C AVG**	**8C AVG**	**16C AVG**	**32C AVG**	**64C AVG**	**128C AVG**	**256C AVG**	**512C AVG**
9	15.70	35.74	25.40	14.04	7.52	1.49	0.11	0.01	0.00
16	13.56	29.10	21.12	17.14	12.46	6.09	0.53	0.00	0.00
23	13.46	29.36	18.67	15.85	13.72	7.66	1.28	0.00	0.00
28	17.32	30.08	18.32	13.39	10.83	6.05	2.62	1.15	0.23
33	15.85	26.44	16.96	15.00	14.12	6.82	3.27	1.18	0.35
38	12.57	21.85	16.48	15.23	16.76	12.49	3.44	0.90	0.28
43	16.45	19.41	15.65	15.30	15.07	12.07	4.03	1.72	0.30

Table 3.2

Average percentage of cells in the tissue sample analyzed for flow cytometry by each fruit age of sample fruit. The two phases where the endoreduplication factor (EF) was increasing are between 9 to 16 day old fruit and 38 to 43 day old fruit.

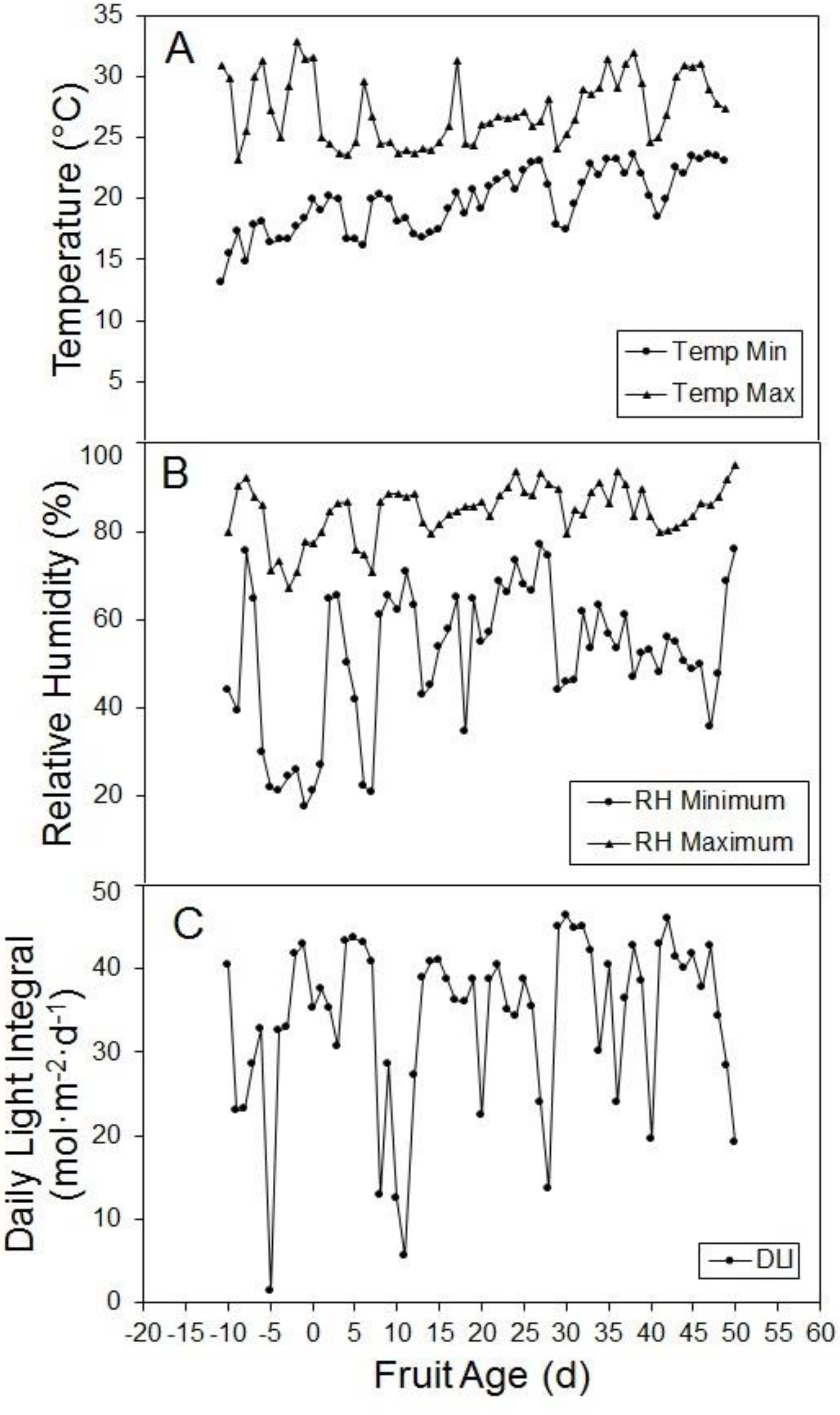
A
Temperature (°C)
35
30
25
20
15
10
5
Temp Min
Temp Max
B
Relative Humidity (%)
100
80
60
40
20
RH Minimum
RH Maximum
C
Daily Light Integral
(mol·m-2·d-1)
50
40
30
20
10
0
DLI
-20 -15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60
Fruit Age (d)

Figure 3.1

Temperature (A), relative humidity (B), and daily light integral (C) measurements taken inside the greenhouse from April 29 to June 28 2016.

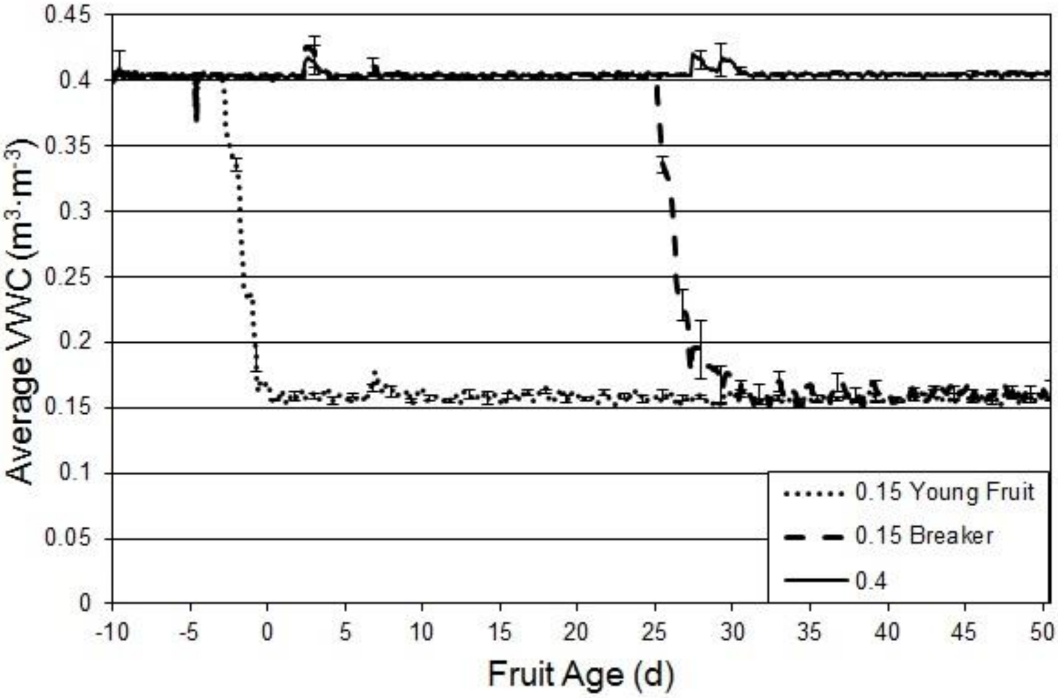
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Average VWC ($m^3 \cdot m^{-3}$)
-10
-5
0
5
10
15
20
25
30
35
40
45
50
Fruit Age (d)
0.15 Young Fruit
0.15 Breaker
0.4

Figure 3.2

Average soil volumetric water contents (VWC) in the three VWC treatments; 0.15 $m^3 \cdot m^{-3}$ beginning when fruits are young, 0.15 $m^3 \cdot m^{-3}$ beginning when fruits are at breaker stage, and well-watered control throughout whole study. The 0.15 $m^3 \cdot m^{-3}$ VWC threshold average for all irrigation lines, beginning drought exposure when fruit are young, was approximately reached when fruit were approximately 2 d old. The second 0.15 $m^3 \cdot m^{-3}$ VWC threshold average for all irrigation lines, for drought exposure at breaker stage, was reached approximately on June 6, 2016. Standard errors were calculated every 30 h to display the limited variation in the VWC values (n = 8).

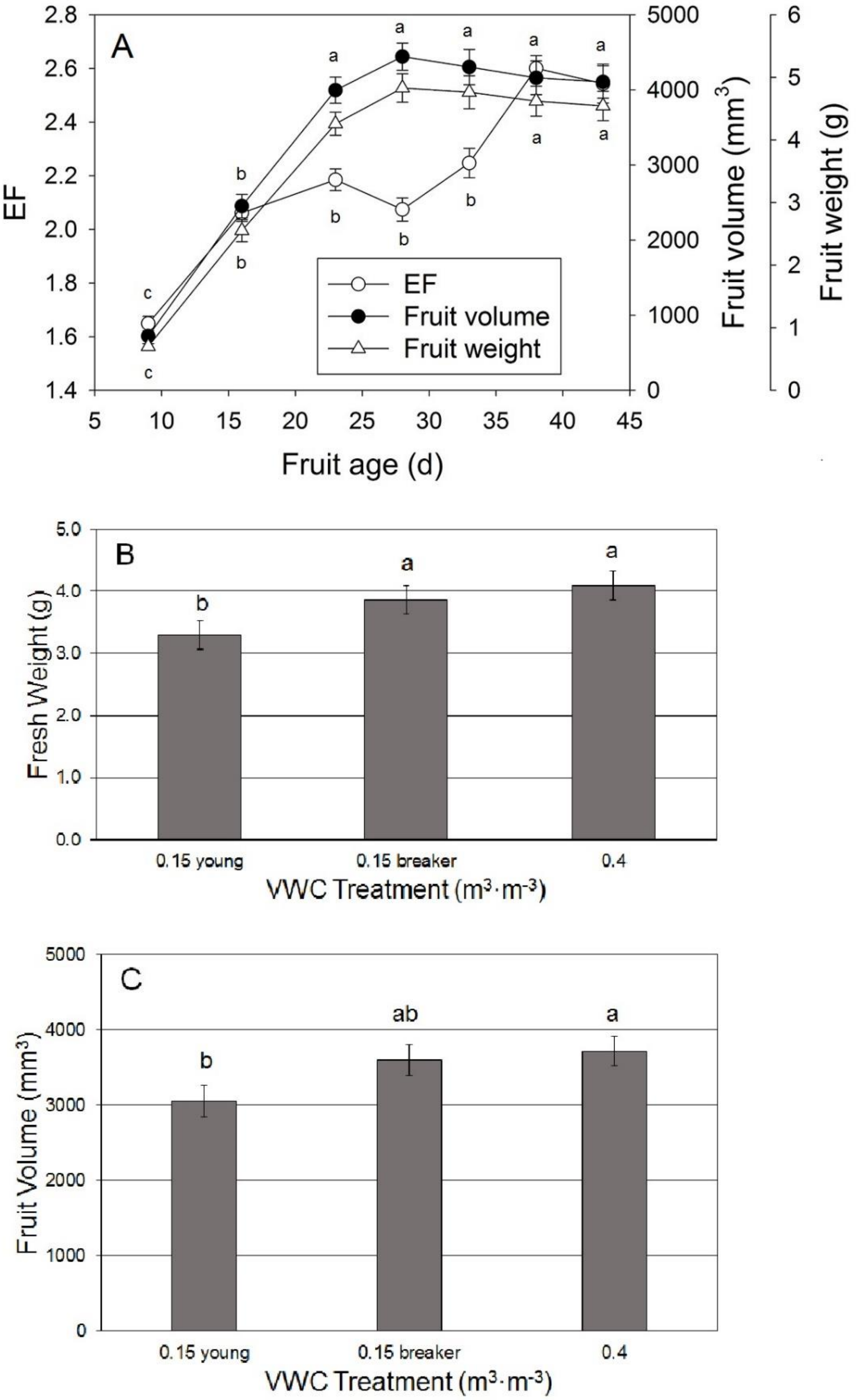
A
EF
Fruit volume (mm^3)
Fruit weight (g)
Fruit age (d)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
5000
4000
3000
2000
1000
0
6
5
4
3
2
1
0
5
10
15
20
25
30
35
40
45
EF
Fruit volume
Fruit weight
a
b
c
B
Fresh Weight (g)
5.0
4.0
3.0
2.0
1.0
0.0
b
a
a
0.15 young
0.15 breaker
0.4
VWC Treatment (m^3·m^{-3})
C
Fruit Volume (mm^3)
5000
4000
3000
2000
1000
0
b
ab
a
0.15 young
0.15 breaker
0.4
VWC Treatment (m^3·m^{-3})

Figure 3.3

Endoreduplication factor (EF), fruit volume, and fruit weight of tomato harvested throughout study as a function of fruit age (A). Fruit volume (B) and fresh weight as a function of VWC treatments (C). Fruit age is how old the fruit is after the tagging date. Means with the same letter are not significantly different according to Tukey’s HSD at *P* = 0.05. Letters above plotted data in figure A represent differences among fruit of different ages for both fruit volume and weight, while letters below plotted data represent EF differences among fruit of different ages.

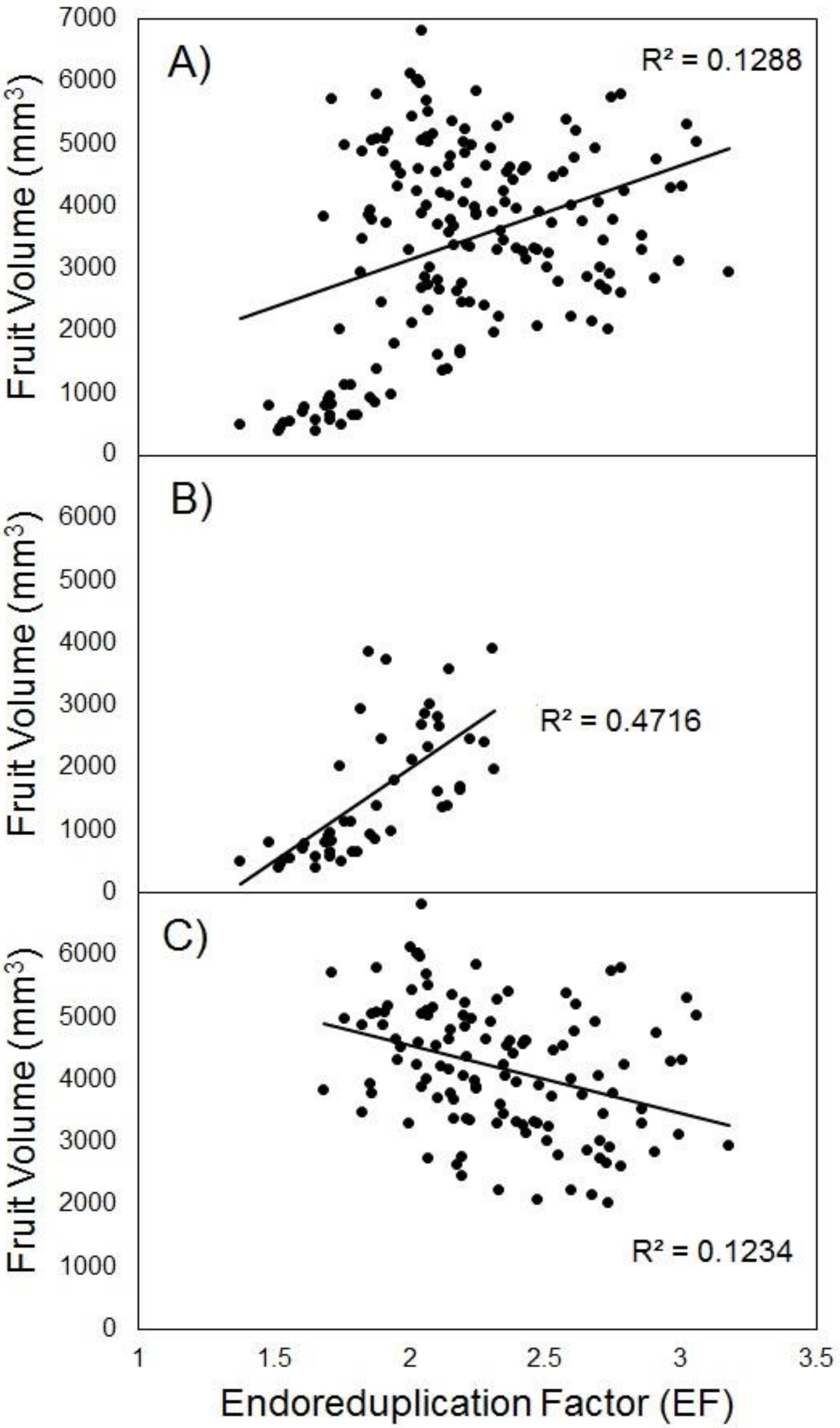
A)
R² = 0.1288
B)
R² = 0.4716
C)
R² = 0.1234
Fruit Volume (mm^3)
7000
6000
5000
4000
3000
2000
1000
0
1
1.5
2
2.5
3
3.5
Endoreduplication Factor (EF)

Figure 3.4

Fruit volume as a function of endoreduplication factor (EF), for all fruit measured throughout the study (A), for young fruit aged 9 to 16 d old (B), and for fruit aged 23-43 d old (C). Considering all fruit harvest, correlation between the two is positive, but weak, showing that as EF increases, fruit volume tends to increase (A). The positive correlation is stronger when fruit are 9-16 d old (B) and becomes negatively correlated with a weak correlation that fruit volume decreases at higher EF values, when fruit 23-43 d old (C).

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CHAPTER 4

CONCLUSIONS

Tomato fruit size is regulated by complex interactions among hormone signaling and gene expression. Environmental stressors, such as elevated temperature and drought stress, decrease tomato plant growth, reducing plant size and photosynthesis. The goal of this research was to determine the effects of drought stress on fruit size, quality, and endoreduplication. Results from the 2015 and 2016 study differed, but similarities do exist between the two studies. In 2015, tomato fruit size was reduced when exposed to moderate drought [substrate volumetric water content (VWC) of 0.15 $m^3 \cdot m^{-3}$] conditions, compared to well-watered (VWC of 0.40 $m^3 \cdot m^{-3}$) conditions. In the 2016 study, tomato fruit size was reduced when drought was started while the fruit were young, compared to well-watered conditions, but fruit size was not affected by drought started when fruit was close to the breaker stage, compared to well-watered conditions. When fruit were 23 days old, around the mature green stage, the fruit had reached their final size. Any drought occurring beyond this stage would likely not have detrimental effects on fruit size because the maximum size has been attained.

In the 2015 study, endoreduplication, which impacts cell expansion rates, was affected when fruit in the breaker stage of development were exposed to drought conditions. We believe the differences in endoreduplication based on different fruit developmental stage were because flowers were tagged on different days, roughly one month apart, which could have exposed each developmental group of tagged fruit to different environmental conditions. Previous reports suggested that endoreduplication in tomato fruit occurs early during fruit growth, so the effect on endoreduplication on fruit exposed to drought starting at the breaker stage was unexpected. Therefore, the second study determined when during fruit development endoreduplication occurs. Endoreduplication occurred mainly during two phases: in 9 to 16 day old fruit, and in 33 to 38 days old fruit, contradicting previous reports suggesting that most endoreduplication occurs early during fruit development. In 2016, endoreduplication rates did not respond to drought treatments. The relationship between fruit volume and endoreduplication factor was weak. The finding that there is a second period of endoreduplication during fruit ripening, helps explain the drought effect on endoreduplication in fruit exposed to drought at the breaker stage.

Beginning drought when fruit were young and at breaker stage did reduce soluble solids, but we attribute the differences in soluble solids between the fruit development stages to differences in environmental conditions during fruit growth and development for fruits tagged at different times. In 2015, fruit that were grown under drought had a higher soluble solids concentration, than those grown under well-watered conditions. This was correlated with fruit water content. The relationship between water content and soluble solids content of fruit is the result of dilution of soluble solids by water. Interestingly, none of the fruit quality parameters measured (soluble solids, titratable acidity, fruit texture, or fruit color) were affected by drought in the 2016 study. We do not have an explanation for this lack of effect, but the environmental conditions of the greenhouse in 2016 were different than in 2015, simply because the 2016 study began later in the growing season. We also believe that drought effects may not have had a large impact on fruit quality because of the cultivar of tomato used for both studies. These studies were limited by the fact that only one cultivar of tomato fruit was used, ‘Micro-Tina’, which was bred to be extremely small, with small fruit. Results might have been different if a tomato with larger fruit had been used. Further research with different cultivars should be done to see if fruit quality remains unaffected by drought. Additional studies should analyze different cultivars of tomato with varying phenotypes and fruit growth habits, to further understand the implications of endoreduplication on tomato fruit growth. As endoreduplication is better understood, our ability to manipulate fruit size by controlling factors involved in this process may increase.