FRUIT LOAD REDUCTION IN *PRUNUS PERSICA* (L.): THE EFFECTS OF TIMING AND INTENSITY AND THE ROLE OF CYTOKININS

by

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(Under the Direction of Anish Malladi and Dario Chavez)

ABSTRACT

Cell division and intense competition for available resources is characteristic of early peach fruit growth and development. To better understand this period, three studies were performed. Thinning (fruit-load removal) trials were performed during the first stage of fruit development to determine the effect of its timing and intensity on fruit size, yield and quality. Early thinning increased fruit size but reduced overall yields. Hence, performing fruit thinning after the threat of freeze may be a better option for Georgia peach production. Models predicting average fruit size at harvest and the fruit development period were developed using chilling unit accumulation and early spring temperature data, among other parameters. Transcript abundance of three genes associated with cytokinin metabolism was determined during early fruit growth. Among these, LONELY GUY (LOG) expression was generally higher during initial stages of fruit growth suggesting that cytokinin activation by its gene-product regulates cell division during early growth.

INDEX WORDS: thinning, fruit growth, GDH30, prediction model, cell division, cytokinin, *IPT, LOG, CKX*

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DEDICATION

I would like to dedicate this thesis to my family, my boyfriend, and my friends for supporting me throughout the past few years. I would also like to dedicate this work to my many mentors who have guided me through this journey. Thank you all for everything.

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INTRODUCTION AND LITERATURE REVIEW

Introduction

Peach fruit growth is characterized by two stages of rapid growth divided by a lag phase. The first stage of rapid growth is critical as it is when most cell division occurs. Any limitations during this stage limits the size potential of the fruit. Fruit size is determined by the number and size of cells present; thus, the more cells created during stage I, the larger the resulting fruit can be. This stage is also marked by high levels of competition among fruit for the available resources. Peach trees also bloom before leaf-out, so during this time there are no new photosynthates coming into the system. Fruit growth is limited by the resources already stored by the tree.

Current consumer demand is for larger fruit (Lopez et al., 2007). In order to meet this demand, growers need to reduce the crop load during early fruit growth to achieve a larger size potential. Thinning is a common cultural practice used to reduce the effects of such competition through the removal of blooms or fruit. By removing a portion of the fruit load during early fruit growth, competition during the resource limited period is reduced. Reduced competition may allow maximal growth during stage I and ultimately result in larger fruit. Thinning is normally done by hand as chemical thinners are unreliable and current mechanical methods are not suitable for the training system (open-vase) used in Georgia. While current commercial recommendations suggest thinning as early as possible to maximize the use of resources, this practice deserves to be evaluated

in Georgia as the climactic conditions (warm winters, late winter/early spring freezes, etc.) are unique to Middle Georgia. While there is overlap in the cultivars grown in different states, these cultivars will behave differently in the different climates. Therefore, recommendations made in other parts of the United States are likely not applicable to Middle Georgia. Hence, the research presented herein aims to improve the recommendations made to growers about when and to what extent to thin peaches in Georgia.

Thinning practices can also affect the rate of cell division within the fruit. The amount of blooms/fruit on the tree during the early stages of growth limits the amount of cell division that can occur within a fruit. The removal of this competition allows for greater potential cell division with the total number of cells ultimately dictating the final size potential of the fruit.

Cell division is a complex process driven by several factors. Cytokinins are a well-known phytohormone involved in cell division. They are believed to play a significant role in fruit development by driving cell division. However, there has been little work done to determine their role in peach fruit growth and development, and none to determine if changes in their metabolism are associated with changes in fruit load. This work aims to determine whether the changes seen in cell division are reflected by changes in cytokinin metabolism throughout early fruit growth.

Competition is not the only thing that affects the final size potential of the fruit. Early spring temperatures are known to greatly influence the fruit development period and the final size of the fruit. It should also be noted that fruit development and fruit growth are not interchangeable terms. A fruit can be developmentally mature without its

full-size potential being realized. Higher spring temperatures increase the rate of fruit development, shortening the window for fruit growth. The growth rate also increases with higher temperatures, but not enough to compensate for the shortened growth window. This relationship between early spring temperatures and growth/developmental rates is currently being used to create models to predict fruit sizes at harvest as well as harvest dates. While such models have been created, they are location and cultivar specific as different areas will differ in climate, soil, cultivars, etc. Currently such a model does not exist for Middle Georgia peach growers based on the commonly grown cultivars in this region.

By looking at several thinning treatments, this study aimed to improve guidelines for Middle Georgia growers on when and to what extent is best to thin peaches here. Using early spring temperatures to create a model to predict final fruit sizes and harvest dates will also serve to aid the Middle Georgia peach growers in fine-tuning their thinning decisions. Determining the patterns of metabolism of specific cytokinins during stage I growth and how they are affected by thinning will deepen our understanding of early fruit growth and development process.

Literature Review

History and Economic Importance of Peaches

Peaches (*Prunus persica L.*) originated in China where they have been cultivated for over 3000 years. They spread along the Silk Roads into the Middle East and Europe. By the 17th century, peaches arrived and spread throughout the Americas. Since their arrival, peaches have grown to be an important commodity for the United States (Bassi & Monet, 2008). The United States is consistently within the top 5 producers in fresh peach production worldwide, usually behind China and Italy and ahead of Spain and Greece (FAOSTAT, 2017).

In 2018, the US fresh harvested peaches were valued at $511,226,000 with the state of Georgia contributing $23,868,000 (USDA, 2019). Georgia is currently third in the nation in fresh peach production, behind California and South Carolina. However, Georgia peach production solely contributes to the fresh market while California's is for processing (USDA, 2019). Production in South Carolina and Georgia are similar. Approximately 10,000 and 14,000 acres are dedicated to peach production in Georgia and South Carolina, respectively (USDA, 2019). While South Carolina's peach production is supported by ~200 growers, Georgia's is supported by four (Chavez, 2019).

Peach Growth and Development

Fruit growth and fruit development are often used interchangeably despite describing separate processes (Lopez & DeJong, 2007). Growth cannot occur without development, but development can proceed without the full growth potential being realized.

Peach flowers are induced and initiated on current season wood late in the summer (late June-late July) but bud break will not occur until early spring of the following year (Dennis, 2000a; Lockwood & Coston, 2005). Flower buds enter a dormant state to survive the colder winter months. To break this dormancy, peaches must accumulate chill hours (hours below 45°F)(Lockwood & Coston, 2005). Cultivars differ in the number of chill hours needed. There are some low-chill cultivars, but typical peach cultivars require 600-1000 chill hours (Basconsuelo et al., 1995; Lockwood & Coston, 2005). Failure to accumulate chill hours will negatively impact the upcoming season through poor bud break and fruit set (Lockwood & Coston, 2005; Bassi & Monet, 2008; Luedeling et al., 2013).

Chill hours may be accumulated as early as mid-January, but flower bud break will not occur until late-February-early-March. This period between chill hour accumulation and bud break is a second dormancy that is broken by a heat requirement (Lockwood & Coston, 2005; Luedeling et al., 2013). At this time, flower buds begin to swell and then break before vegetative buds (leaf-out) (Bassi & Monet, 2008). This unique period between bloom and leaf-out is characterized by high levels of competition as no new photosynthates are being synthesized, and the buds are reliant on remobilized storage reserves.

Peach trees typically set 3,000-8.000 flowers each season, but only half will set fruit (1,500-4,000) (Day & DeJong, 1998; Lockwood & Coston, 2005). Even with this initial decrease in fruit number, intense competition still exists to support of these fruit to a commercial size (Day & DeJong, 1998). The developing embryo is thought to produce hormones to prevent abscission during fruit set (Lockwood & Coston, 2005). The fruit

then develops following a double sigmoidal pattern characterized by two periods of rapid fruit growth separated by a lag phase (Conners, 1919; Day & DeJong, 1998; Lockwood & Coston, 2005). Stage I of fruit growth is the first period of rapid growth and is characterized by cell division (Conners, 1919; Lockwood & Coston, 2005). Growth during this stage determines the final size potential of the fruit. Final fruit size is determined by the number of cells present and the size of those cells (Wu et al., 2005). The greater the cell numbers generated during this initial stage, the larger the size potential of that fruit (Grossman & DeJong, 1995). Any limitations to cell division during this stage will ultimately limit the final size potential of the fruit. The second stage is a lag phase during which the pit begins to lignify, and the embryo begins to initiate primary roots, shoots, and cotyledons. The final stage is the second period of rapid growth and is characterized by cell expansion; this is when the peach appears to 'swell-up' (Lockwood & Coston, 2005).

Sizing and Grading

The size and shape of the fruit, flesh firmness, soluble solids concentration, fruit color, and ground (flesh) color can be used to determine the maturity of the fruit. The desired maturity level for harvest is dependent on the future use of the fruit (i.e. processing, fresh market, shipping) (Crisosto, 1994). In the state of Georgia, fruit should still be firm at harvest and have a yellow or cream ground color (Chavez et al., 2015). Following harvesting and processing, peaches are categorized based on their grade and size. For US processing there are four grades: US Fancy, US Extra No.1, US No.1, and US No. 2. The grades are based on the maturity, color, and shape of the fruit, as well as the amount of damage or decay on the fruit (USDA, 2004b). While size is not a set

requirement for the different grades, fruit is often divided into size categories (i.e. US Fancy 2 ¾") (USDA, 2004a). Peaches are typically packed into standard boxes/lugs/cartons. The size of the fruit determines how many fruit are needed to a box; it will take fewer large fruit to fill them than smaller fruit (Davis et al., 2004; USDA, 2004a; Lopez et al., 2007). Consumer demand has shown preference for larger fruit with larger fruit typically carrying a higher value in the market (Lopez et al., 2007).

Thinning

Fruit growth during stage I is critical in determining the final size potential of the fruit (Grossman & DeJong, 1995). The maximum growth potential is achieved under ideal environmental conditions and in the absence of competition for resources (DeJong & Grossman, 1995). Limitations during this time affect rates of cell division and, therefore, the rate of growth (DeJong & Grossman, 1995; Grossman & DeJong, 1995; Dash et al., 2013). As mentioned above, early fruit growth involves intensive competition. Growth is limited by the resources stored within the tree over the previous seasons. If maximal fruit growth potential is to be attained, steps need to be taken to minimize source competition during stage I.

Thinning is a common cultural practice that has been used for thousands of years to reduce the effects of competition during early growth (Dennis, 2000b). Thinning is the act of removing some portion of the fruit load with the hopes of attaining larger fruit. While there are differing reports on the optimal timing and magnitude of thinning, most agree thinning early during stage I results in larger fruit (DeJong et al., 1991; Day & DeJong, 1998; Costa & Vizzotto, 2000; Dennis, 2000b; Myers et al., 2002; Lockwood & Coston, 2005;Njoroge & Reighard, 2008; Malhotra & Deshmukh, 2017).

Timing

The success of thinning largely relies on the reduction of the crop load during the correct growth stage. While early studies suggested waiting until the end of stage II to thin, recent studies recommend thinning as soon as possible during stage I to maximize the fruit growth potential (Weinberger, 1941 as reviewed by Costa & Vizzotto, 2000). Thinning as early as bloom is a very lucrative option as doing so reduces competition early on and produces larger fruit than with fruit thinning (Byers et al., 2010). This early reduction in competition would ensure a more efficient use of resources through more synthesis, transport, and accumulation in the remaining fruit (Njoroge & Reighard, 2008; Malhotra & Deshmukh, 2017). However, thinning during bloom does come with risks. Bloom thinning has been shown to decrease the rates of natural fruit drop (Myers et al., 1993), but this is not always the case as fruit set is variable (Myers et al., 2002). Thinning with the expectation that all the remaining fruit will set fruit can easily lead to overthinning (Byers & Marini, 1994). It is possible that some portion of the remaining flowers will not set or the fruit will abscise later in the season. Late freezes can also damage/kill some portion of the remaining fruit load, further reducing it (Myers et al., 1993).

Due to the added risks with thinning at bloom, waiting until post-fruit set may be the more practical option (Lockwood & Coston, 2005; Njoroge & Reighard, 2008, Malhotra & Deshmukh, 2017). Performing early fruit thinning would still allow competition to be reduced relatively early during fruit growth and allow for fruit to be removed more selectively. With fruit thinning, the largest fruit can be left on the tree while the smaller ones are removed (Weinberger, 1941 as reviewed by Costa & Vizzotto,

2000). However, fruit removed during thinning represent potential resources lost to the remaining fruit with the assimilates already distributed to the removed fruit being lost (Costa & Vizzotto, 2000). Thinning should not be delayed, however, as several reports indicate that earlier thinning results in larger fruit than later thinning dates or from unthinned trees (DeJong et al., 1991; Day & DeJong, 1998; Myers et al., 2002; Njoroge & Reighard, 2008; El-Boray et al., 2013; Deshmukh et al., 2017). These studies support the idea that competition needs to be eliminated early during fruit development to have a significant positive effect on growth (Day & DeJong, 1998: Myers et al., 2002).

Intensity

Thinning to a greater magnitude (larger spacings between fruit on a shoot) has also been shown to correlate with a larger fruit at harvest. The more fruit that is removed, the lesser the competition for remaining resources. However, there is a point where thinning to excessive magnitudes could under-utilize the available resources; excessively reducing sinks can also reduce the photosynthetic capacity of the source leaves (DeJong & Grossman, 1995; Njoroge & Reighard, 2008; Malhotra & Deshmukh, 2017). Recent studies have shown that the success of thinning doesn't necessarily rely on the spacing of fruit on the shoots but rather the amount of fruit left on the tree (Marini & Sowers, 1994; Alcobendas et al., 2012). However, thinning to a specific spacing allows for uniform thinning across the tree.

Soluble Solids Content and Titratable Acidity

Peach quality traits are typically reported as soluble solids content and titratable acidity (Byrne et al., 1991). Thinning time and intensity have been shown to affect both titratable acidity and soluble solids content. However, while studies have shown

significant differences in these two parameters as affected by thinning times and intensities, a clear relationship between fruit thinning and fruit composition has not yet been defined (Njoroge & Reighard, 2008; El-Boray et al., 2013; Malhotra & Deshmukh, 2017).

Methods

Thinning has historically been done by hand, but mechanical and chemical methods have been investigated (Costa & Vizzotto, 2000). The largest concern with chemical and mechanical methods is that they thin indiscriminately; it is difficult to control which fruit and the amount of fruit that are removed with these methods. While hand thinning incurs relatively greater time and cost investments, it is still a more reliable method (Davis et al., 2004; Costa & Vizzotto, 2000). In Georgia, hand thinning is the most commonly used practice.

Importance of Early Spring Temperatures

As mentioned above, development may proceed without the growth potential being realized. Under higher temperatures, the developmental and growth rates are increased. The increased developmental rate shortens the window for growth. While the growth rate is also increased, the hastened rate is not enough to compensate for the shorter growth window. Resources cannot be metabolized, transported, and accumulated rapidly enough to support such an increased growth rate (DeJong & Grossman, 1995; Grossman & DeJong, 1995). For this reason, higher temperatures during early fruit growth often result in undersized fruit (Day et al.,2008; Lopez & DeJong, 2007; Reighard & Rauh, 2015). As discussed earlier, stage I growth is critical in determining the size

potential of the fruit; therefore, high temperatures affecting developmental and growth rates during this stage would impact the resulting crop.

Prediction Models Using Early Spring Temperatures

Because environmental conditions can have a significant impact on the success of a crop, they should be considered when making decisions regarding the cultural practices for the current season. The need for proper chill hour accumulation remains an important indicator for crop success (Lockwood & Coston, 2005; Bassi & Monet, 2008; Luedeling et al., 2013). Similarly, weather conditions during the early stages of growth and development affect the potential success of the crop. It has been shown that early spring temperatures during the 30 days following full bloom influenced fruit growth and development (Lopez & DeJong, 2007; Day et al., 2008; Reighard & Rauh, 2015).

Temperatures during the first 30 days after bloom are being used to predict reference dates (10 days after 80% of sliced fruit have hardened pits near the peach tip), harvest dates, and final fruit size (Mimoun & DeJong, 1999, Lopez & DeJong, 2007; Day et al., 2008; Reighard & Rauh, 2015). The total number of growing degree days (GDD) or of growing degree hours (GDH) have both been used to make predictions about the fate of the crop. The GDD and GDH for those first 30 highly correlate with the length of the fruit development period (full bloom to harvest) (Mimoun & DeJong, 1999; Lopez & DeJong, 2007; Day et al., 2008). Ranges of GDD for the first 30 days have also been defined to predict final fruit sizes. If the sum of GDD is above a certain threshold value (> ~700 GDD), a significant decrease in fruit size is observed (Reighard & Rauh, 2015).

While GDH and GDD do show a strong correlation with final fruit size and season length, they are not the sole predictors of crop success. There are several other

factors that potentially affect the fate of the crop; the importance of chill hour accumulation and the detrimental effect of late freezes have been noted several times in this review. Each geographic region differs in climate and each cultivar will behave differently in these different regions; therefore, these models are likely region specific (Byers et al., 2010). Currently there are no such prediction models for peach in the state of Georgia, the "Peach State".

Phytohormones

Phytohormones are believed to play a part in every aspect of fruit growth and development. Changes in their concentrations and distributions are thought to signal the transition from one growth phase to another as well as mediate cell growth and development that occurs in each stage (Miller et al., 1987; Arnau et al., 1999). It is well documented that different concentrations and combinations of phytohormones are involved in stimulating fruit set and fruit transition from one growth stage to another (Nitsch, 1952; Crane, 1969; Ozga & Reinecke, 2003; McAtee et al., 2013; Kumar et al., 2014). Hormones may also direct transport of metabolites from other tree organs to the developing fruit (Crane, 1969).

Fruit growth and development is characterized by distinct periods of cell division and cell expansion. As described earlier, peaches progress through three main phases of development: cell division, lag, cell expansion. While cytokinins are known to play a key role in cell division, their exact role in peach fruit development remains ill-defined.

Cytokinins

Cytokinins are a large class of hormones consisting of over 20 different compounds. These compounds can be further grouped into four main categories: bases,

nucleotides, ribosides, and glucosides (Arnau et al., 1999; Kakimoto, 2003). Most natural cytokinins are adenine species with an isoprenoid or aromatic side chain substituted at N^6 (Åstot, et al., 2000). They are mainly synthesized in root tips and then translocated. The cambium, shoot apex, and immature seeds may also serve as sites of biosynthesis (Kakimoto, 2003). Two main biosynthetic pathways exist: isopentyl-adenosine-5'-monophospate(iPMP)-dependent and iPMP-independent. For both pathways, zeatin riboside (ZR) is the final product. However, many of the transitionary products are also active forms of cytokinins and can be easily converted from one form to another (Åstot et al., 2000; Kakimoto, 2003). Irreversible breakdown of these active cytokinins is largely carried out by cytokinin oxidases that are induced through the overproduction of iPMP (Åstot et al., 2000; Haberer & Kieber, 2002; Kakimoto, 2003). While most forms of cytokinins are susceptible to degradation by these oxidases, the O-glycosylated cytokinins are resistant. These cytokinins are also highly inactive suggesting they may function as storage forms (Haberer & Kieber, 2002). In this way, cytokinin levels are regulated by the balance between synthesis, catabolism, and degradation (Matsuo et al., 2012).

While the general role of cytokinins in cell division is relatively well understood, little is known about the exact activity of specific cytokinins (Schaller, et al., 2014). Most cytokinins exist as free bases along with the corresponding nucleotides and nucleosides; these forms are easily converted from one to another by various enzymes (Haberer & Kieber, 2002). The activity of these different cytokinins is dependent on the structure/conformation of the side chain. Thus, while similar in structure, functions can differ greatly. For example, trans-zeatin is one of the highly active cytokinins while cis-

zeatin, an isomer, is largely inactive (Mok & Mok, 2001; Haberer & Kieber, 2002; Schäfer et al., 2015).

Due to their involvement in cell division, cytokinins are likely present in higher quantities during stages of rapid cell division (stage I in peach). Previous studies, though few, do support this hypothesis; cytokinins in general show an increase in abundance during early stages of growth and development (Lewis et al., 1996; Arnau et al., 1999). In peach, four cytokinins have been shown to peak during stage I: dihydrozeatin riboside (DHZR), iPMP, dihydrozeatin riboside-5'-monophosphate (DHZMP), and zeatin riboside (ZR). DHZR, iPMP, DHZMP were shown to peak in the fruit flesh, while DHZMP, iPMP, and ZR peaked in the seed during early growth (Arnau et al., 1999). It has also been shown that applications of cytokinins (CPPU) promote cell division and result in increased fruit size (Lewis et al., 1996). It may be possible to genetically modify endogenous cytokinin levels to promote fruit size and quality (Lewis et al., 1996). In fact, cytokinin applications alone can induce fruit development (McAtee, 2013).

Thinning studies have shown that crop load and the thinning method used affect phytohormone accumulation (Denne, 1960; Samuolienė et al., 2016). However, the effects of thinning on cytokinin metabolism during early fruit growth, particularly in peach, has yet to be determined.

Cytokinin Biosynthesis, Activation & Degradation

Originally cytokinins were thought to be synthesized only through the breakdown of tRNA; later it was discovered this was just one of the possible pathways (Haberer & Kieber, 2002). The tRNA turnover rates were also not enough to account for the levels of cytokinins observed (Åstot et al., 2000; Haberer & Kieber, 2002) An enzyme

[isopentenyl transferase (IPT)] that could convert dimethylallyl-pyrophosphate (DMAPP) to iPMP was discovered and confirmed that the tRNA breakdown pathway was not the only way to synthesize isoprenoid cytokinins (Åstot et al., 2000; Haberer & Kieber, 2002;Kamada-Nobusada & Sakakibara, 2009). Both the tRNA and IPT driven pathways start with the precursor DMAPP (Fig 1.1). For the IPT pathway, DMAPP is largely provided through the methylerythritol phosphate (MEP) pathway, while the DMAPP for the tRNA pathway is the product of the mevolanate (MVA) pathway (Kamada-Nobusada & Sakakibara, 2009; Zhao et al., 2013).

The tRNA-driven pathway produces cis-zeatin riboside monophosphate (cZRMP) which is converted to cis-zeatin by LONELY GUY (LOG) (Fig 1.1). The IPT-driven pathway is more complex and can involve many intermediary steps and forms (Fig 1.1). Several other enzymes are involved in these intermediate steps: cytochrome P450 monooxygenases and various isomerases being two examples. Ultimately, isopentenyl adenine (iP), trans-zeatin (tZ), and dihydrozeatin (DZ) are the main active products of this pathway. LOG is responsible for the synthesis of these active cytokinins from their immediate precursors (Kamada-Nobusada & Sakakibaru, 2009). CYTOKININ OXIDASEs (CKXs) are largely responsible for the irreversible degradation of active cytokinins (Avalbaev et al., 2012). Cytokinin levels and activity are maintained through a balance of the rates of biosynthesis, activation, and degradation.

IPT

As mentioned earlier, IPT is involved in the first step of cytokinin biosynthesis. This step is thought to be the rate-limiting step of cytokinin production and involves the prenylation of adenosine 5’-phosphate (Sugawara et al., 2008). While IPT can use AMP,

ADP, or ATP as the prenyl acceptor, higher plants typically use AMP and ADP (Kakimoto, 2003; Sugawara et al., 2008). In all cases, DMAPP is used as the prenyl donor (Kakimoto, 2003; Sugawara et al., 2008; Kamada-Nobusada & Sakakibara, 2009). Overexpressing *IPT* through genetic modification or application has been shown to increase cytokinin levels during early development in tomato (Mao et al., 2002 Luo et al., 2005).

LOG

LOG encodes a phosphoribohydrolase that is largely responsible for converting cytokinins to their active free base form (Kamada-Nobusada & Sakakibara, 2009; Seo & Kim, 2017). There is also an alternative two-step activation pathway that involves converting nucleotides to nucleosides and then to nucleobases (Kamada-Nobusada & Sakakibara, 2009; Tokunaga et al., 2011). Due to the multiple pathways, it is difficult to determine what portion of cytokinin activation is due to LOG activity. LOG targets nucleoside 5'-monophosphates (iPMP, tZMP, DZMP, and cZMP) and converts them to the free base form (iP, tZ, DZ, cZ, respectively) by cleaving off the ribose 5-monophosphate (Kamada-Nobusada & Sakakibara, 2009). Overexpression of *LOG* increased cell division in *Arabidopsis* embryos, but not to the extent of *IPT* overexpression suggesting LOG activity is not rate-limiting (Kuroha et al., 2009). Similarly, *log* mutants show decreased cell division/cytokinin activation; LOGs act redundantly, so multiple *LOG* genes must be knocked down for a significant decrease in cell division/cytokinin activation (Tokunaga et al., 2011).

CKX

CKX is critical for cytokinin degradation. The irreversible inactivation is responsible for balancing cytokinin levels (Avalbaev et al., 2012). CKX targets cytokinin bases and nucleosides (nucleotides are not susceptible). CKX acts by cleaving the side chain; in the case of isopentenyladenine (iP), the products of this reaction are adenine and the 3-methyl-2-butenal chain (Mok & Mok, 2001; Schmülling et al., 2003; Avalbaev et al., 2012). Regions undergoing active cell division and with high levels of active IPT also show high levels of CKX expression (Avalbaev et al., 2012). Past reports show some CKXs peak during early fruit development in kiwi (Pilkington et al., 2013) and tomato (Matsuo et al., 2012).

Statement of Objectives

OBJECTIVE 1:

Quantify the effects of thinning times and intensities on peach final yield and fruit characteristics.

OBJECTIVE 2:

Develop prediction models for average fruit sizes at harvest and for the fruit development period.

OBJECTIVE 3:

Quantify cytokinin metabolism-related transcript abundance during fruit development and as affected by different thinning times.

Figures:

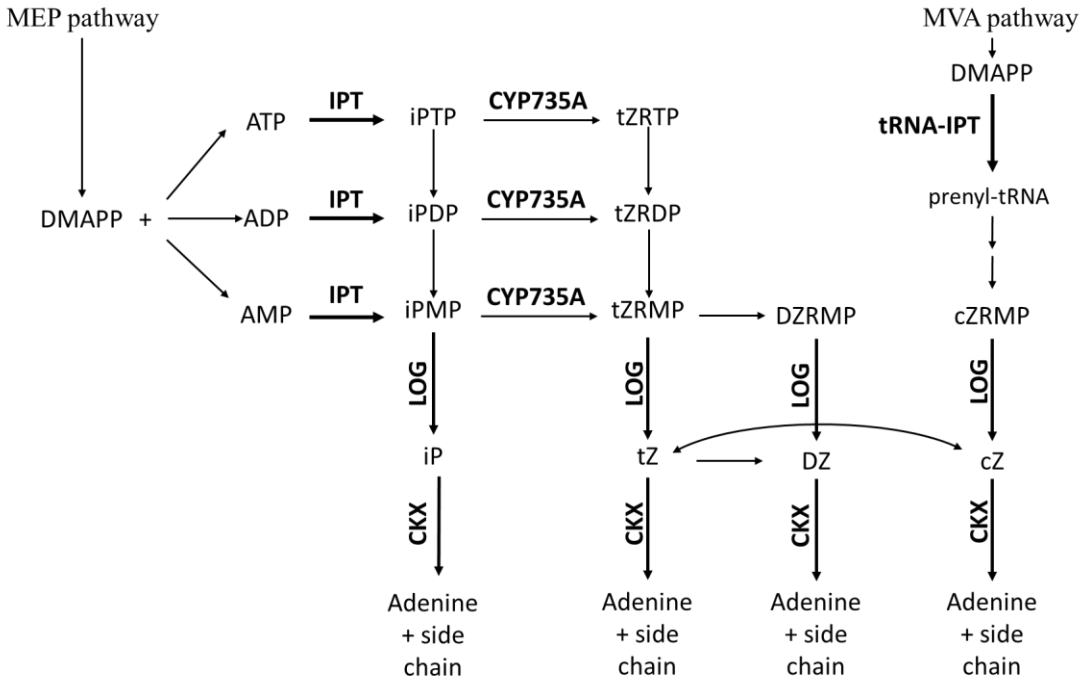

Figure 1.1) Cytokinin Biosynthesis Pathway. *Illustrates the multiple pathways that can result in the in the four basic cytokinins: isopentenyl adenine (iP), trans-zeatin (tZ), dihidrozeatin (DZ), and cis Zeatin (cZ). Isopentenyltransferase (IPT) is the rate-limiting step in biosynthesis. Lonely Guy (LOG) is responsible for converting nucleotides to their free-base form. Cytokinin oxidase (CKX) degrades active cytokinins(Schmülling et al., 2003;Kamada-Nobusada & Sakakibara, 2009).*

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CHAPTER 2

FRUIT LOAD REDUCTION IN PEACH (*PRUNUS PERSICA L.*): THE EFFECTS OF TIMING AND INTENSITY ON YIELD AND FRUIT CHARACTERISTICS ON THREE MIDDLE GEORGIA CULTIVARS

Introduction

Peach fruit growth displays a double sigmoidal curve characterized by two stages of rapid growth separated by a lag phase (Conners, 1919; Day & DeJong, 1998; Lockwood & Coston, 2005). Stage I is the first stage of rapid growth and is defined by cell division. Stage II involves the lignification of the pit (endocarp). Stage III is the final period of rapid growth and is characterized by cell expansion. The final size of the fruit is largely determined by the number of cells present and the size of those cells (Wu et al., 2005). For this reason, stage I is often considered the critical stage where limitations can result in fewer cell divisions and therefore limit future fruit growth potential (DeJong, 1995).

In the window between flower bud and vegetative bud break (leaf-out), fruit growth is largely limited by the resources stored by the tree. Peach trees also set more fruit than they can support to a commercially acceptable size (Day & DeJong, 1998; Njoroge & Reighard, 2008). The high volume of fruit on the tree in conjunction with limited resources creates competition among young fruit and limits this growth potential (DeJong & Grossman, 1995). Thinning, removal of blooms or fruit from the tree, is necessary to reduce competition among blooms/fruit and to allow them to size properly

(Njoroge & Reighard, 2008). The earlier this competition can be lessened, the lesser the negative effect on the size potential of the fruit (Day & DeJong, 1998; Costa & Vizzotto, 2000; Dennis, 2000; Lockwood & Coston, 2005).

It is essential to optimize fruit growth during stage I to prevent potential size/yield from being lost due to competition among fruit (Day & DeJong, 1998). This is often accomplished through bloom or early fruit thinning. Thinning at bloom largely negates the negative effects of competition among fruit during the source limitation periods maximizing the growth potential of the fruit. However, thinning at this time may result in over-thinning which may be more harmful than not thinning at all (DeJong et al., 1991; Byers & Marini, 1994). The remaining flowers will not all set fruit; some natural abscission will occur and further reduce the fruit load resulting in over-thinning (Byers & Marini, 1994; Myers et al., 2002). In such cases, the potential loss in crop yield could be more significant than the potential increase in fruit size (Njoroge & Reighard, 2008).

Delaying thinning will allow greater selectivity when thinning fruit, ensuring only the largest fruit are retained on the shoot. Thinning after natural abscission is less labor intensive as only excess fruit need to be thinned at this time (Costa & Vizzotto,2000). However, fruit removed at these later thinning times represent potential yield/growth that is lost to those fruit remaining on the tree. Fruit growth is still increased with later thinning treatments, but it is not enough to match those that were thinned earlier (Grossman & DeJong, 1995). Thinning too late can be just as harmful to fruit size as not thinning at all as resource limitations have already impacted growth (DeJong et al., 1990; Grossman & DeJong, 1995).

Different cultivars respond differently to thinning treatments (Costa & Vizzotto, 2000). Climactic conditions are also unique to different regions and influence response to thinning. Early spring temperatures have been shown to greatly influence the size of fruit at harvest and the length of the fruit development period (Lopez et al, 2007). Production also differs between states. Georgia production is primarily for fresh market while California's is for processing (USDA, 2019). South Carolina production is supported by ~200 growers over 14,000 acres while Georgia's is supported by four growers over 10,000 acres (Chavez, 2019; USDA, 2019). Because production needs, cultivars, and climactic conditions are unique to each region, recommendations made in one state will likely not apply to another. While there is extensive work evaluating the time and intensity of thinning, similar work has yet to be done in Middle Georgia. This study looked at the effects of timing and intensity of peach in Middle Georgia.

Materials and Methods

Plant Material and Cultural Practices

Mature, established peach trees were used for all studies. In 2017, trees located at the Fort Valley State Orchard and at Lane's Southern Orchards, both in Fort Valley, Georgia were used. Two cultivars at Fort Valley State Orchard (nine-year-old 'Springprince' and 'Juneprince') and one at Lane's Southern Orchards (seven-year-old 'CaryMac') were used in the study. In 2018 and 2019, three cultivars at Lane's Southern Orchards ('CaryMac', 'Julyprince', and 'Summerflame') were used. In 2018 these trees were eight-, seven-, eight-years-old, respectively. The aim of this study was to determine the effects of thinning treatments under standard cultural practices. The care and

maintenance of the trees was performed as per the commercial cultivation standards for Georgia.

Thinning Treatments

Thinning treatments were modified from those in Njoroge & Reighard (2008). Two spacings (15 cm and 20 cm) were applied at each of three thinning times defined as days after full bloom (DAFB). In 2017, thinning was performed at 0 DAFB, ~40 DAFB, and ~60 DAFB. The lack of chill hour accumulation and a late freeze (3/16/2017) in 2017 resulted in ~80% losses in Georgia peach production. Hence, thinning was delayed in 2017 until the damage could be assessed. In 2018 and 2019, three thinning times were evaluated: at bloom (0 DAFB), early stage I (< 25 DAFB) and late stage I (> 25 DAFB). In all three years, a control of no thinning was also included for each cultivar. In 2019, 'Julyprince' thinning at bloom could not be performed due to inclement weather. The thinning treatments are summarized in Table 2.1.

Treatments were applied in triplicate across each cultivar. Twenty-one trees were used per cultivar with seven per row used for each replication (randomized complete block design). Within each block, the row-ender tree was skipped and then one of the seven treatments randomly assigned to each tree. Each tree was treated as an experimental unit. Trees that showed obvious signs of damage or disease were also skipped over.

Yield and Fruit Quality Measurements

Fruit were harvested at commercial maturity (harvest dates are detailed in Table 2.2). At each harvest date, mature fruit were removed and placed into labeled boxes; fruit from different trees were kept separate. These boxes were then weighed on a Defender

3000 portable scale (OHAUS, NJ, USA) and the total yield recorded. When necessary, fruit were also divided into commercial (>2 ½ in diameter) and undersized (<2 ½ in diameter) categories. In such cases, commercial and non-commercial measurements were recorded separately. Around 10-12 fruit per tree were randomly selected and put into labelled bags. The bags were then placed into coolers with ice to remove field heat and transported to Athens, GA, for additional analyses. Fruit were stored in a 4 °C walk-in cooler until further analysis.

Ten individual fruit weights were taken with a Quintix Precision Balance (Sartorius, Germany). Two fruit diameters were also taken for each fruit using Digimatic Digital Calipers (Mitutoyo, Japan) (0-4”/100MM): a cheek-to-cheek diameter and a suture-to-opposite-side diameter. For soluble solids content (SSC) and titratable acidity (TA), three of the ten fruit were chosen randomly for juicing. The fruit were peeled, and the flesh pureed. In 2017, 40 ± 1 g of mesocarp tissue was pureed using a blender. In 2018 & 2019, 80 ± 1 g of the mesocarp tissue was pureed using a blender. The pureed flesh was strained through a double layer of cheese cloth to extract the juice. Juice was frozen at -20 °C until SSC and TA analyses could be performed.

Juice samples were thawed at room temperature for two hours prior to testing. For TA, 5 mL of juice was diluted in 45 mL of H_2O. TA was measured as percent Malic Acid and determined using a Mini Titrator (Hanna instruments, RI, USA) in 2017 and a Titrino Plus Autotitrator (Metrohm, Switzerland) in 2018 and 2019. TA values were consistent across the two titrators. With both setups, samples were titrated to ~pH 8.1 using 0.1 N NaOH. For all three years, the titrator was calibrated at the start of each day. SSC was

determined using a Palette Refractometer (Atago, WA, USA. The refractometer was cleaned with H_2O after each sample.

Statistical Analysis

All statistical analyses were performed in JMP (Version 14.1.0. SAS Institute Inc., Cary, NC, 1989-2019). Years and cultivars differed significantly across all parameters, so all years and cultivars were analyzed separately. When applicable, commercial and non-commercial measurements were also analyzed separately. Differences between thinning treatments within a cultivar were determined using ANOVA and mean separation was performed using Tukey HSD (honest significant difference).

Results

2017

In 2017, fruit were divided into commercial and undersized categories at harvest. No significant differences in average fruit weight across the treatments was observed for any of the cultivars used (Fig 2.1). Similarly, there were largely no significant differences in yield per tree across the treatments. However, for 'CaryMac', the ~ 40 DAFB, 20 cm thinning displayed significantly less undersized yield than the no thinning control (Fig. 2.2). In 2017, there was no clear correlation between thinning timing or intensity and fruit weights and total yields.

The number of fruit per tree did not differ significantly across treatments in 'CaryMac' and "Springprince'. The control treatment had significantly higher fruit

numbers than the bloom, 20cm thinning treatment and the two ~40 DAFB thinning treatments in ‘Juneprince’ (Table 2.3).

Overall, the number of fruit on the tree did show a negative correlation with average fruit weights in ‘Juneprince’ and ‘Springprince’. In ‘Juneprince’ and ‘Springprince’, 21% and 54%, respectively, of the variation in fruit weights could be explained by differences in the number of fruit on the tree. There was a slight positive correlation between the number of fruit per tree and the average fruit weight in ‘CaryMac’ but this was not significant (Fig 2.3a).

Across all cultivars, the number of fruit on the tree were significantly, positively correlated with total yield per tree. The number of fruit on the tree could account for 90%, 31%, and 94% of the variation in total yield per tree in ‘CaryMac’, ‘Juneprince’, and ‘Springprince’, respectively (Fig 2.3a).

There were no consistent trends between average fruit weight and total yield per tree. In ‘CaryMac’ and ‘Juneprince’, there was a positive correlation between fruit weight and yield with 13% and 17% of the variation in yield being accounted for by changes in average fruit weight. Conversely, there was a negative correlation between fruit weight and total yield per tree in ‘Springprince’ with the differences in average fruit weight accounting for 44% of the differences in total yield per tree (Fig 2.3b).

Across all cultivars, fruit diameter did not differ significantly across treatments. The two diameter measurements did not differ, so data for only one is presented (Table 2.3). Soluble solids content also did not differ significantly across the treatments for any of the cultivars. Titratable acidity did not differ across treatments. However, in ‘CaryMac’ the thinning at bloom, 15 cm treatment showed significantly lower titratable

acidity than the ~60 DAFB, 20 cm treatment. In the other two cultivars, these two treatments had very similar values; this suggests the differences observed in 'CaryMac' may be due to natural variation (Table 2.3). There were no consistent trends between thinning treatment and fruit diameters, soluble solids content, and titratable acidity in 2017 (Table 2.3).

2018

In 2018, average fruit weights did not differ across thinning treatments for 'Summerflame' (Fig 2.4c). For 'CaryMac', the un-thinned control did have significantly smaller average fruit weights than the 30 DAFB, 15 cm treatment (Fig 2.5a). The smaller fruit from the control trees were still of a commercial size (~2.5 inches). Similarly, in 'Julyprince', the control had significantly smaller fruit weighs than the two bloom treatments, the 14 DAFB, 20 cm, and the 33 DAFB, 20 cm treatments (Fig 2.4b). Again, the smaller fruit seen on the control tree were still of commercially marketable size. In 2018, the unthinned trees showed numerically lower fruit weights than the thinning treatments in 'CaryMac' and 'Julyprince'. There were no consistent trends among the different thinning times and intensities for average fruit weight.

For both 'CaryMac' and 'Julyprince' the control treatments had significantly higher yields than the bloom, 20 cm treatment. However, there were no differences in yield across the different thinning times and intensities (Fig 2.5a,b). None of the thinning treatments showed significantly different yields in 'Summerflame' (Fig 2.5c). While not

statistically significant, the unthinned trees consistently had numerically higher yields than the thinning treatments.

In 2018, the number of fruit per tree did not differ across treatments in ‘Summerflame’. In ‘Julyprince’ and ‘CaryMac’, the unthinned trees had the numerically highest number of fruit per tree. The control showed significantly higher fruit numbers than the thinning at bloom, 20 cm treatment in ‘CaryMac’. In ‘Julyprince’ the unthinned trees had significantly higher fruit numbers than the two thinning at bloom treatments (Table 2.4).

The number of fruit on the tree only significantly correlated with the average fruit weight in ‘Julyprince’ in 2018. The amount of fruit on the tree could account for 43% of the variation seen in average fruit weights in ‘Julyprince’ (Fig 2.6a). This is consistent with the negative correlations seen in 2017 (Fig 2.3a).

The number of fruit per tree was highly, positively correlated with the total yield per tree with the number of fruit accounting for 97%, 96%, and 99% of the variation observed in yield in ‘CaryMac’, ‘Julyprince’, and ‘Summerflame’, respectively (Fig 2.6a). These trends are consistent to those observed in 2017 (Fig 2.3a).

There were no significant correlations between average fruit weight and total yield per tree in ‘CaryMac’ and ‘Summerflame’. Average fruit weights were negatively correlated with total yields in ‘Julyprince’ with 30% of the variation seen in yields being accounted for by average fruit weight (Fig 2.6b). This is consistent with the trend seen in ‘Springprince’ in 2017.

Soluble solids content and titratable acidity did not differ significantly across thinning treatments. In ‘CaryMac’ and ‘Summerflame’ fruit diameters also did not differ

significantly. Fruit from the 14 DAFB, 20 cm fruit had significantly larger diameters than the control and the 14 DAFB, 15 cm for ‘Julyprince’ (Table 2.4). There was no consistent correlation between thinning treatments and the average fruit diameters, the soluble solids content, nor the titratable acidity.

2019

Bloom, 15 cm thinned ‘CaryMac’ trees yielded the highest fruit weights out of all the treatments in 2019. The control displayed the smallest fruit with the 19 DAFB, 20 cm thinning treatment having a comparable average fruit weight to the control. Even though they were smaller, the fruit were still of a commercial size. The other bloom and fruit thinning treatments showed average fruit weights in between the control and the 19 DAFB, 20 cm thinning treatment (Fig 2.7a). Thinning at bloom could not be completed for ‘Julyprince’; nonetheless, a similar trend to ‘CaryMac’ was seen. The control treatment yielded significantly lower fruit weights than the fruit thinning treatments; however, the smaller fruit seen on the control trees were still of commercial size (~2.5 inches). Earlier fruit thinning (21 DAFB) resulted in significantly higher fruit weights than later fruit thinning (44 DAFB) (Fig 2.7b). For both ‘CaryMac’ and ‘Julyprince’, there were no significant differences between the 15 and 20 cm spacings. Average fruit weights did not differ significantly across the thinning treatments in ‘Summerflame’ (Fig 2.7c).

Across all cultivars, total yields per tree did not differ significantly across treatments in 2019. However, ‘CaryMac’ appeared to show a similar trend to the one in 2018, but the observed differences were insignificant. The control had the numerically highest yields and the thinning at bloom treatments the numerically lowest with the fruit

thinning treatments in between the control and bloom treatments (Fig 2.8a). ‘Summerflame’ appeared to show a similar trend except for the bloom, 15 cm treatment which had comparable yields to the later fruit thinning and control treatments (Fig 2.8c). However, these differences were insignificant. Yields were comparable across the different treatments for ‘Julyprince’ (Fig 2.8b).

The total number of fruit per tree did not differ across treatments in ‘CaryMac’ and ‘Summerflame’. In ‘Julyprince’ the unthinned trees had significantly more fruit per tree than both 21 DAFB thinning treatments (Table 2.5).

In 2019, the number of fruit per tree was negatively correlated with average fruit sizes in ‘CaryMac’ and ‘Julyprince’. Differences in the amount of fruit on the tree accounted for 24% and 61% of the variation in average fruit weights for ‘CaryMac’ and “Julyprince’, respectively. There was no significant correlation between number of fruit and fruit weight in ‘Summerflame’ (Fig. 2.9a). The correlations seen in ‘CaryMac’ and ‘Julyprince’ were consistent with those noted in 2017 and 2018.

The number of fruit per tree was positively correlated with total yield per tree across all cultivars. The number of fruit per tree could account for 94%, 41%, and 98% of the variation in total yield in ‘CaryMac’, ‘Julyprince’, and ‘Summerflame’, respectively (Fig 2.9a). These observations are consistent to those made in 2018 (Fig 2.6a).

In 2019, the average fruit weight was not correlated with the total yield per tree across any cultivars (Fig 2.9b). This is consistent with the observations made in 2018 for ‘CaryMac’ and ‘Summerflame’ (Fig 2.6b).

Fruit diameters showed similar trends to the fruit weights for all cultivars. In ‘CaryMac’, the bloom and later fruit thinning (44 DAFB) had significantly larger

diameters than the control and early fruit thinning (19 DAFB) treatments. Early fruit thinning (19 DAFB) showed significantly larger fruit diameters than the later fruit thinning (44 DAFB) and both were significantly larger than the control in ‘Julyprince’. Fruit diameters were not significantly different across treatments in ‘Summerflame’. Soluble solids content did not differ across treatments for any of the cultivars. For ‘CaryMac’ and ‘Summerflame’, titratable acidity did not differ across treatments. The early fruit thinning (19 DAFB) had significantly higher titratable acidity than both the 44 DAFB thinning and control treatments in ‘Julyprince’ (Table 2.5).

Discussion

Low chill hour accumulation in 2017 likely resulted in reduced fruit set for that year (Lockwood & Coston, 2005). A hard freeze also occurred on 16 March 2017. Growers reported 80-90% losses that year due to the lack of chill and the freeze event. Under such circumstances, thinning had no effect on total commercial yield, commercial fruit weights, or fruit characteristics. Further removal of fruit from these trees did not alleviate competition among fruit. Therefore, when fruit load is severely reduced by lack of chill or late freezes, thinning may not be necessary as it will not have any significant effect on fruit quality, size, yield, or the amount of undersized fruit present.

Chill hour accumulation was adequate in both 2018 and 2019 (~1100 and ~950 chill hours, respectively). While there was another freeze in 2018, it was not as significant as the one in 2017 with only 10% losses reported by growers. Growing degree days for the first 30 days after bloom (GDD30) were also higher in 2019 than in 2018 (190 vs 160, respectively). Higher temperatures during early growth tend to decrease the average fruit size due to the tree’s inability to provide resources rapidly enough to

compensate for increased growth and developmental rates (Lopez et al., 2007; Lopez & DeJong, 2007). Therefore, the warmer temperatures seen during early growth in 2019 could account for the smaller fruit sizes seen in comparison to those in 2018 (Fig 2.5, 2.8).

Across 2018 and 2019 the controls had numerically higher yields and smaller fruit weights, the thinning at bloom treatments had the numerically lowest yields and largest fruit weights, and the fruit thinning treatments were in between. However, these differences were only found significant in 'CaryMac' and 'Julyprince' in 2018. This apparent trend in average fruit weights is consistent with past reports of bloom and early fruit thinning producing significantly larger fruit than later fruit thinning or no thinning (DeJong et al., 1991; Grossman & DeJong, 1994; Day & DeJong, 1998; Njoroge & Reighard, 2008; Deshmukh et al., 2017). There have been conflicting reports regarding the effects of thinning on total yield. The generally lower yields seen with bloom and early fruit thinning are consistent with those of Njoroge & Reighard (2008) and Deshmukh et al. (2017) and contradicts those of DeJong et al. (1991) and Day & DeJong (1998).

Across the three years, the number of fruit per tree were significantly, positively correlated with the total yield per tree. A relatively high percentage (31-99%) of the variation in yield could be explained by the differences observed in fruit per tree. Conversely, there was not a consistent trend between the number of fruit per tree and the average fruit weights across the three years. When significant, the correlation between fruit number and average fruit weights were negative and could only account for 24-61% of the variation seen in average fruit weights. The number of fruit per tree were more

consistently and more strongly correlated with total yields than average fruit weights. This suggests that the number of fruit on the tree is having more of an effect on yield than average fruit sizes. The relationship between average fruit weight and total yield was inconsistent across years and varieties.

The goal of thinning is to improve fruit sizes through reductions in fruit load without drastically affecting the final yield (Day & DeJong, 1998; Costa & Vizzotto, 2000; Dennis, 2000; Lockwood & Coston, 2005; Njoroge & Reighard, 2008). Therefore, it is unexpected that the number of fruit per tree appears to be more closely correlated with the total yield than average fruit weights. It is possible that with the spacings used, thinning was performed too heavily. Overthinning would result in improved fruit sizes due to the reduction in competition but would also drastically reduce yields due to the relatively low amounts of fruit left on the trees. Yields were lower simply because there was so little fruit on the tree in comparison (Byers & Marini, 1994).

Because spacing systems were studied in uniformly sized trees, the number of fruit per tree is expected to be relatively consistent across the six thinning treatments and much higher in the control treatment. There were no consistent trends in fruit number per tree. ‘Juneprince’ in 2017, ‘CaryMac’ in 2018, ‘Julyprince in 2018, and ‘Julyprince’ in 2019 did show significant differences in fruit number per tree with the control treatment consistently having the numerically highest number of fruit. ‘CaryMac’ in 2017, ‘Springprince in 2017, ‘Summerflame’ in 2018, ‘CaryMac’ in 2019, and ‘Summerflame’ in 2019 did not show any significant differences in fruit number across treatments. Altogether, this suggests the spacing system used did not result in the uniform fruit loads expected.

Overall, there were no consistent trends regarding the 15 or 20 cm spacings used. In the future, it is likely thinning will be done to obtain a certain fruit load or density rather than a certain spacing. Marini & Sowers (1994) and Alcobendas et al. (2012) report that the fruit load is more essential than spacing when thinning. To avoid overthinning at bloom, partial low-intensity thinning can be conducted at bloom with a follow-up a few weeks later (Myers et al., 2002).

It became apparent that different cultivars respond differently to thinning treatments. In 2018 and 2019, 'Summerflame' did not show any significant differences across treatments in all the parameters observed. The control treatment did, however, consistently have numerically higher yields than the thinning treatments. There was no significant correlation between the number of fruit per tree and the average fruit weight. However, the number of fruit on the tree could account for 98-99% of the variation observed in total yield in 2018 and 2019. This suggests that thinning is not necessary in low-set varieties as 'Summerflame'. A reduction in yield is the only potential effect of thinning in such varieties.

Similarly, early-season varieties, such as 'CaryMac', are more likely to suffer damage because these freezes hit near their bloom when they are vulnerable to freeze damage (Murray, 2011). In years where varieties are damaged by freezes (i.e. 2017), fruit set is relatively low, and thinning has little effect across any of the parameters observed.

Thinning responses were more apparent in 'Julyprince' which naturally sets higher fruit loads. In such cases, differences in fruit weight and total yield were observed across treatments. This suggests in the future, thinning recommendations will need to be cultivar specific or catered to early-, mid-, and late-season bloom groups.

It is difficult to know which blooms will produce sizable fruit and which will abscise naturally. The high intensities to which thinning was performed did not account for the variable nature of fruit set leading to considerably lower fruit load than desired. At earlier fruit thinning dates (< 25 DAFB), some of this uncertainty remains; determining which young fruit will produce the largest, viable fruit is still difficult. Again, the uncertainty and the high intensity to which thinning was performed resulted in the comparably lower yields and fruit numbers seen. The later thinning dates (> 25 DAFB) allowed the most selectivity when thinning. Even with the high intensity to which thinning was performed, yields at these times were comparable to the control and had fruit sizes comparable to those of the bloom thinned trees.

Conclusion

Thinning at bloom to 15 cm or 20 cm spacing does not appear to be a viable option for Middle Georgia. The uncertainty that accompanies thinning to such an extent at bloom adds risk to this process. While the higher value associated with larger fruit does offer an incentive to thin as early as possible (Davis et al., 2004), the loss in potential yield may offset the benefits of a larger fruit (Njoroge & Reighard, 2008). Therefore, waiting until after the threat of freeze and until fruit can be more selectively thinned (25-50 DAFB) may be a better option for Middle Georgia. In terms of thinning intensity, there is no clear evidence to support one spacing being superior to the other.

Acknowledgements

I would like to thank Lane's Southern Orchard and Fort Valley State Orchard for the use of their trees and for the care and maintenance of the trees.

Tables:

Table 2.1) Summary of Thinning Treatments used in 2017-2019 for peaches grown in Middle Georgia

Treatment	Timing		Spacing
	2017	2018/2019	
1	At Bloom (0 DAFB)[1]	At Bloom (0 DAFB)	15 cm
2	At Bloom (0 DAFB)	At Bloom (0 DAFB)	20 cm
3	~40 DAFB[2]	Early Fruit Thinning (<25 DAFB)	15 cm
4	~40 DAFB	Early Fruit Thinning (<25 DAFB)	20 cm
5	~60 DAFB	Late Fruit Thinning (>25 DAFB)	15 cm
6	~60 DAFB	Late Fruit Thinning (>25 DAFB)	20 cm
7	NO THINNING[3]	NO THINNING	NO THINNING

[1]Days After Full Bloom (DAFB)

[2]Thinning times differed in 2017. Poor chill accumulation and a late freeze resulted in high losses. Thinning was delayed until the full extent of the losses could be determined

[3]No thinning served as the control. No flowers/fruit were thinned from these trees.

Table 2.2) Harvest dates for 2017-2019 peaches grown in Middle Georgia

Cultivar	Harvest Dates		
	2017	2018	2019
Springprince	31 May	x[1]	x
Juneprince	8 June	X	x
CaryMac	1 June-19 June[2]	1 June-11 June	6 June-13 June
Julyprince	X	2 July-18 July	2 July- 10 July
Summerflame	X	2 July-18 July	2 July- 25 July

[1]x values indicate that cultivar was not used that year

[2]Date range indicates there were multiple harvests performed.

Table 2.3) Summary of Thinning Trials performed on peaches in Middle Georgia in 2017

Treatment	Diameter 1[2]		%	TA	Fruit per
	Commercial[3]	Undersized[3]	Brix[4]	(%MA)[5]	tree[6]
			'CaryMac'		
Bloom, 15 cm	62.05	57.72	9.57	0.89 **b**[1]	37
Bloom, 20 cm	62.74	57.91	10.35	1.06 **ab**	53
40 DAFB, 15 cm	62.46	55.59	9.88	0.95 **ab**	42
40 DAFB, 20 cm	62.06	55.43	10.47	0.98 **ab**	33
60 DAFB, 15 cm	62.10	55.39	9.98	0.98 **ab**	29
60 DAFB, 20 cm	62.89	55.29	10.83	1.12 **a**	35
No Thinning	63.20	58.00	10.15	0.95 **ab**	44
			'Juneprince'		
Bloom, 15 cm	66.54	48.82	11.02	0.83	51 **ab**
Bloom, 20 cm	66.98	49.42	10.88	0.73	32 **b**
40 DAFB, 15 cm	68.19	50.36	10.65	0.74	29 **b**
40 DAFB, 20 cm	67.05	51.23	10.63	0.73	32 **b**
60 DAFB, 15 cm	67.95	51.37	10.98	0.74	41 **ab**
60 DAFB, 20 cm	70.34	50.64	11.10	0.82	37 **ab**
No Thinning	67.55	50.38	10.08	0.80	70 **a**
			'Springprince'		
Bloom, 15 cm	59.65	43.40	10.93	0.70	96
Bloom, 20 cm	60.98	45.05	10.63	0.69	33
40 DAFB, 15 cm	59.69	43.63	10.56	0.74	52
40 DAFB, 20 cm	58.54	41.20	10.60	0.69	67
60 DAFB, 15 cm	59.16	45.73	11.33	0.80	44
60 DAFB, 20 cm	60.97	45.47	10.86	0.70	52
No Thinning	61.15	45.08	10.44	0.67	87

[1]$P \leq 0.05$. Letters of different values indicate values that are significantly different
[2]Diameter 1 refers to the cheek-to-cheek diameter
[3]Commercial fruit are those with diameters > 2 ½ in while undersized are those with diameters < 2 ½ in.
[4]% Brix is a measure of the soluble solids content
[5]TA is titratable acidity as a measure of % Malic Acid
[6]Fruit per tree was calculated using average fruit weights and the total yield.

Table 2.4) Summary of Thinning Trials performed on peaches in Middle Georgia in 2018

Treatment	Diameter 1[2]	% Brix[3]	TA (%MA)[4]	Fruit per tree[5]
		'CaryMac'		
Bloom, 15 cm	76.64	11.48	1.09	39 **ab**[1]
Bloom, 20 cm	77.54	12.42	1.24	25 **b**
21 DAFB, 15 cm	76.36	10.52	1.15	105 **ab**
21 DAFB, 20 cm	76.71	12.52	1.04	91 **ab**
30 DAFB, 15 cm	78.08	17.12	1.32	66 **ab**
30 DAFB, 20 cm	76.28	16.28	1.20	87 **ab**
No Thinning	75.24	15.65	0.92	142 **a**
		'Julyprince'		
Bloom, 15 cm	86.98 **ab**	12.11	0.81	153 **b**
Bloom, 20 cm	87.01 **ab**	12.90	0.80	147 **b**
14 DAFB, 15 cm	83.83 **bc**	13.04	0.86	214 **ab**
14 DAFB, 20 cm	87.30 **a**	14.10	0.85	170 **ab**
33 DAFB, 15 cm	84.55 **abc**	13.46	0.81	225 **ab**
33 DAFB, 20 cm	85.76 **ab**	14.98	0.83	265 **ab**
No Thinning	82.41 **c**	14.17	0.79	371 **a**
		'Summerflame'		
Bloom, 15 cm	80.29	12.80	1.03	15
Bloom, 20 cm	80.55	21.06	1.08	13
14 DAFB, 15 cm	81.95	14.68	1.10	18
14 DAFB, 20 cm	79.46	16.7	0.95	31
26 DAFB, 15 cm	81.27	14.57	0.99	25
26 DAFB, 20 cm	80.69	13.68	1.08	43
No Thinning	83.25	14.74	1.06	49

[1]*P≤0.05*. Letters of different values indicate values that are significantly different

[2]Diameter 1 refers to the cheek-to-cheek diameter

[3]% Brix is a measure of the soluble solids content

[4]TA is titratable acidity as a measure of % Malic Acid

[5]Fruit per tree was calculated using average fruit weights and the total yield.

Table 2.5) Summary of Thinning Trials performed on peaches in Middle Georgia in 2019

Treatment	Diameter 1 [4]	% Brix[5]	TA (%MA)[6]	Fruit per tree[7]
		'CaryMac'		
Bloom, 15 cm	71.76 **a**[1]	12.37	0.92	173
Bloom, 20 cm	68.68 **ab**	12.23	0.99	135
14 DAFB, 15 cm	67.41 **bc**	12.00	1.07	312
14 DAFB, 20 cm	64.56 **cd**	12.01	1.03	392
28 DAFB, 15 cm	68.62 **ab**	11.82	1.04	366
28 DAFB, 20 cm	68.66 **ab**	11.58	0.93	375
No Thinning	62.14 **d**	11.25	1.00	694
		'Julyprince'		
Bloom, 15 cm	**X**[3]	**X**	**X**	**X**
Bloom, 20 cm	**X**	**X**	**X**	**X**
21 DAFB, 15 cm	73.95 **a**	11.60	0.80 **a**	476 **b**
21 DAFB, 20 cm	75.05 **a**	10.68	0.70 **ab**	425 **b**
44 DAFB, 15 cm	68.98 **b**	11.47	0.63 **b**	666 **ab**
44 DAFB, 20 cm	66.49 **b**	11.13	0.67 **b**	706 **ab**
No Thinning	60.01 **c**	10.83	0.63 **b**	943 **a**
		'Summerflame'		
Bloom, 15 cm	75.23	13.20	0.86	40
Bloom, 20 cm	73.79	13.80	0.75	70
15 DAFB, 15 cm	76.30	11.83	0.75	129
15 DAFB, 20 cm	75.71	13.70	0.87	61
43 DAFB, 15 cm	74.67	13.37	0.89	92
43 DAFB, 20 cm	75.58	12.67	0.86	46
No Thinning	75.13	13.50	0.79	90

[1]$P \leq 0.05$. Letters of different values indicate values that are significantly different

[2]Treatment numbers correspond to those laid out in Table 2.1

[3]**X** refer to the 'Julyprince' bloom thinning treatments that were not completed

[4]Diameter 1 refers to the cheek-to-cheek diameter

[5]% Brix is a measure of the soluble solids content

[6]TA is titratable acidity as a measure of % Malic Acid

7Fruit per tree was calculated using average fruit weights and the total yield.

Figures:

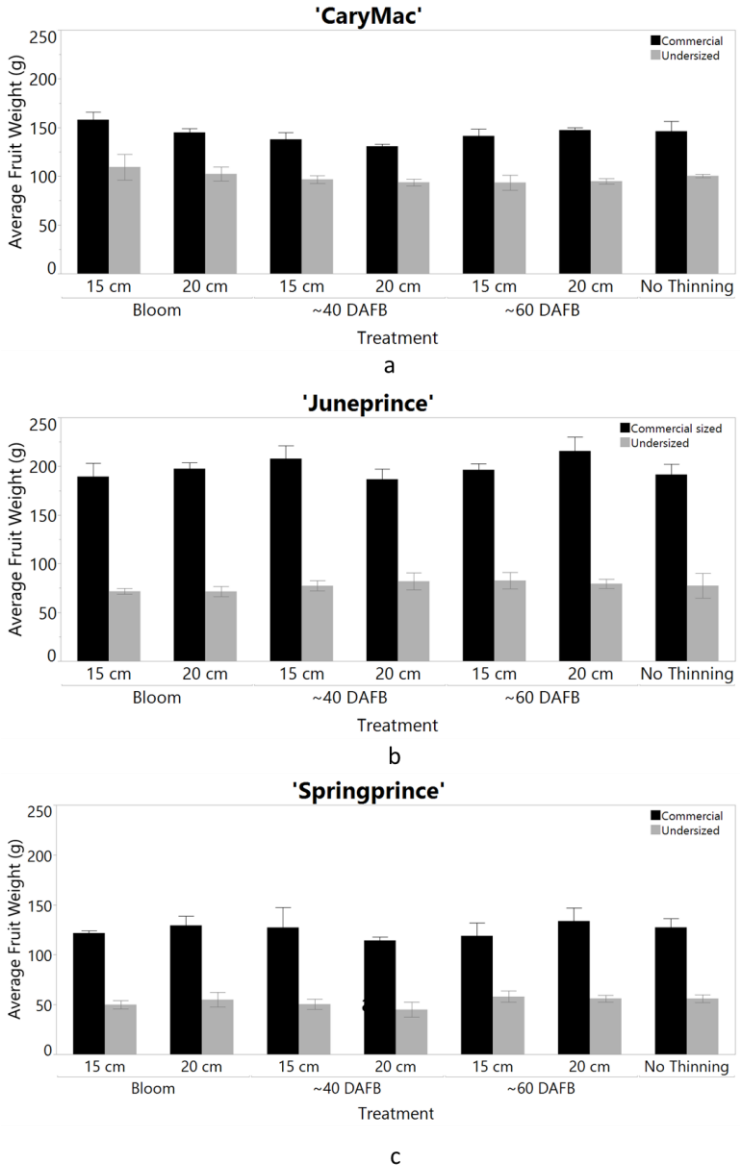


Figure 2.1) 2017 Average Commercial vs. Undersized Fruit Weights. *a) Averages for 'CaryMac' b) Averages for 'Juneprince' c) Averages for 'Springprince'. Commercial and Undersized values were analyzed separately. Different letters indicate significant differences. .$p \leq 0.05$*

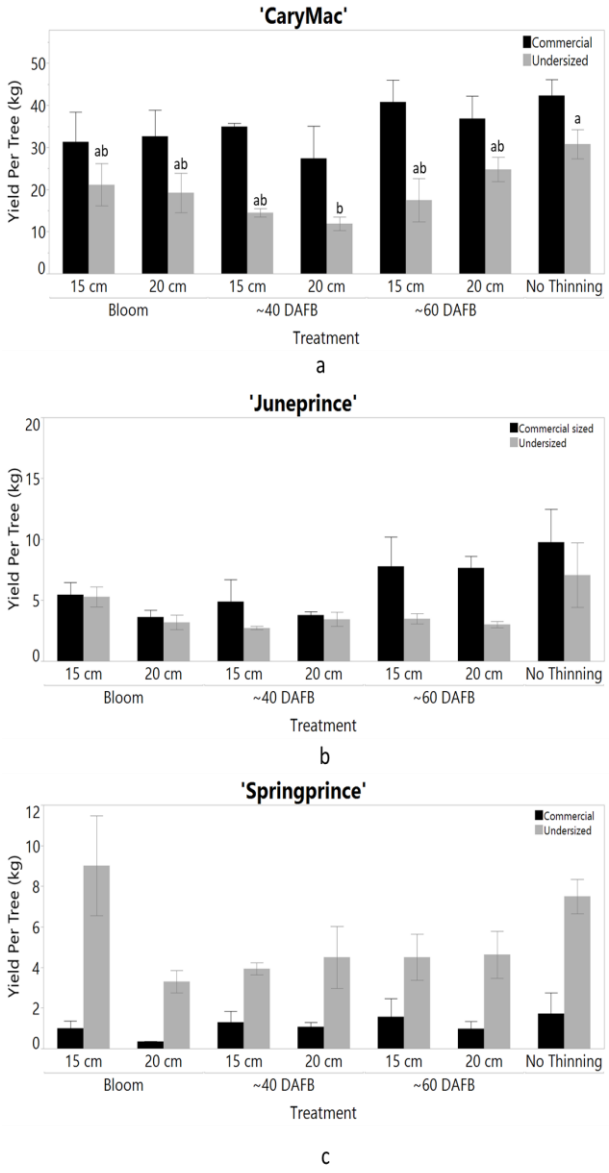
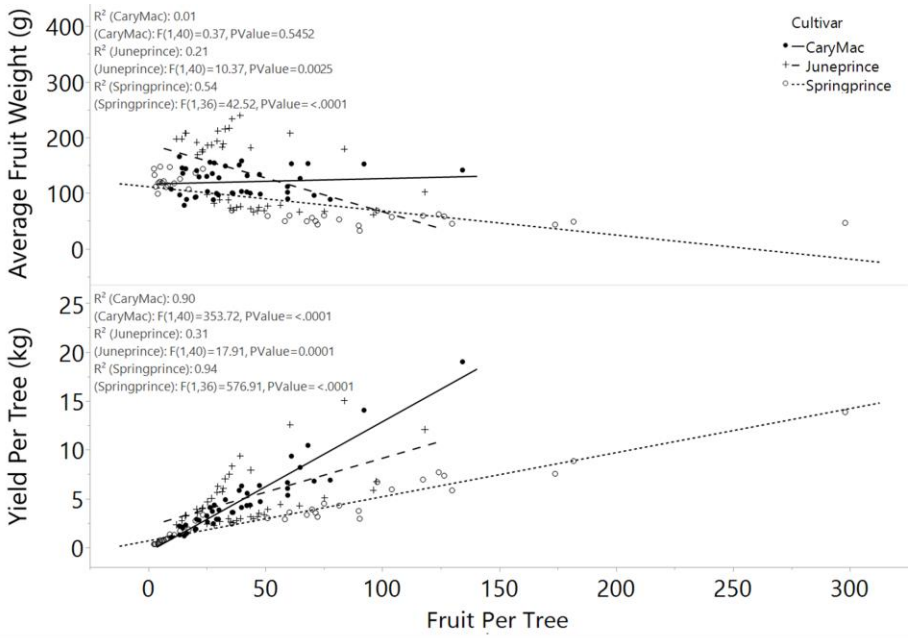


Figure 2.2) 2017 Average Commercial vs. Undersized Yield per Tree. *a) Averages for 'CaryMac' b) Averages for 'Juneprince' c) Averages for 'Springprince'. Commercial and Undersized values were analyzed separately. Different letters indicate significant differences. $p \leq 0.05$.*



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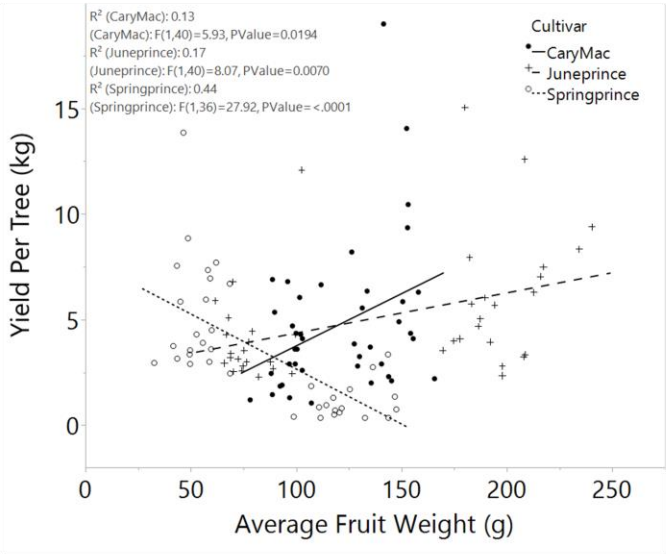


b

Figure 2.3) 2017 Correlations between total yield per tree, average fruit weights, and the number of fruit per tree. *Cultivars are combined for these analyses.* ***a)*** *Upper graph looks at the relationship between fruit per tree and total yield per tree. Lower graph looks at the correlation between fruit per tree and average fruit weight.* ***b)*** *Correlation between average fruit weight and total yield per tree. $p \leq 0.05$.*

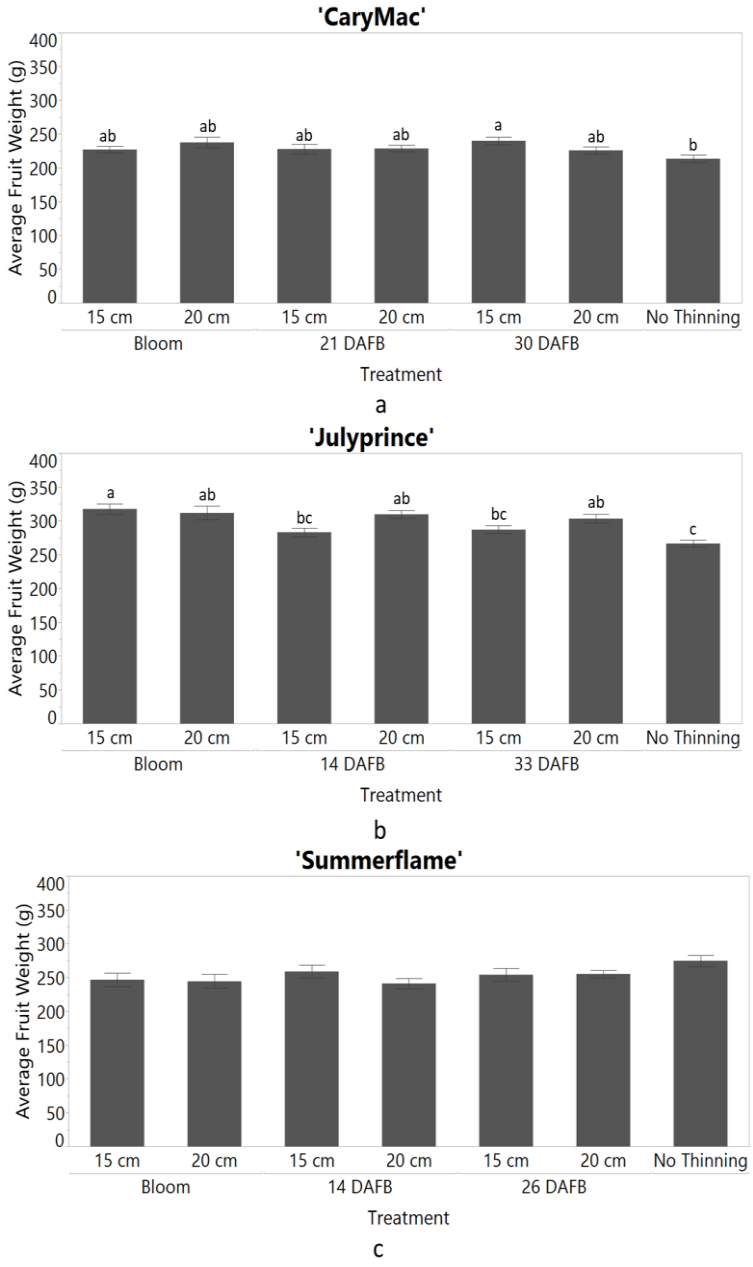


Figure 2.4) 2018 Average Fruit Weights. *a) Averages for 'CaryMac' b) Averages for 'Julyprince' c) Averages for 'Summerflame'. Values with different letters are significantly different. $p<0.05$.*

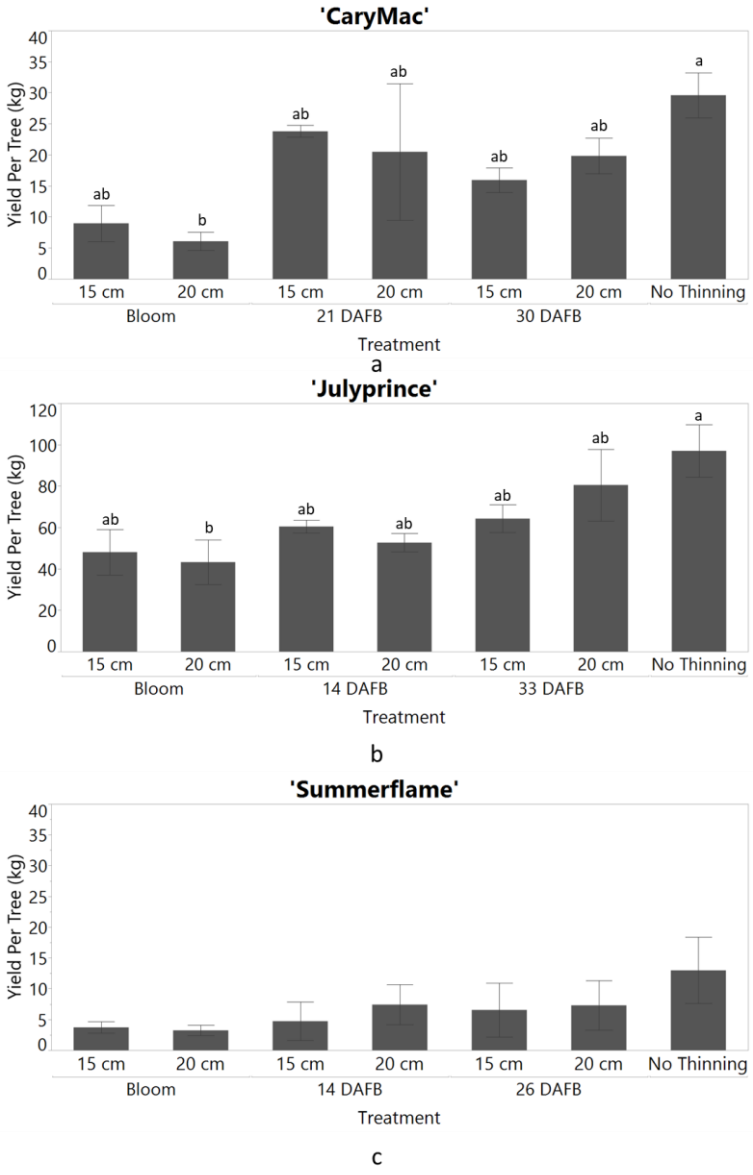
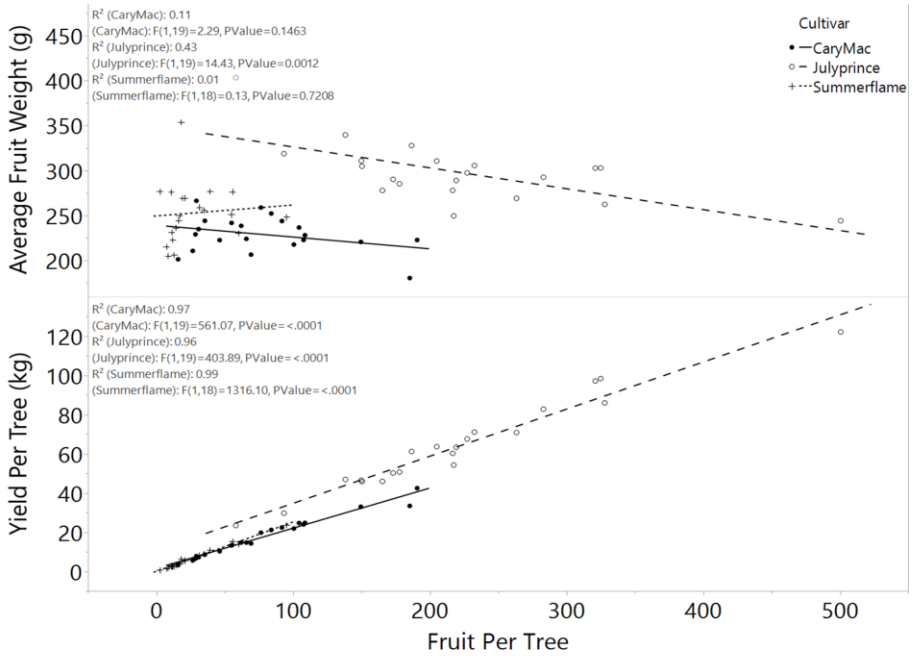


Figure 2.5) 2018 Average Total Yield Per Tree*. a) Averages for 'CaryMac' b) Averages for 'Julyprince' c) Averages for 'Summerflame'. Values with different letters are significantly different. p<0.05.*



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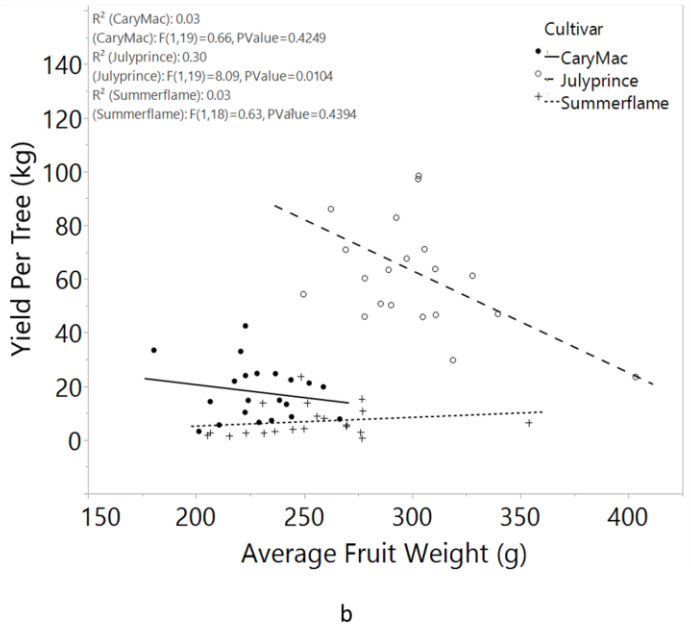

b

Figure 2.6) 2018 Correlations between total yield per tree, average fruit weights, and the number of fruit per tree. *Cultivars are combined for these analyses.* ***a)*** *Upper graph looks at the relationship between fruit per tree and total yield per tree. Lower graph looks at the correlation between fruit per tree and average fruit.* ***b)*** *Correlation between average fruit weight and the total yield per tree. $p \leq 0.05$.*

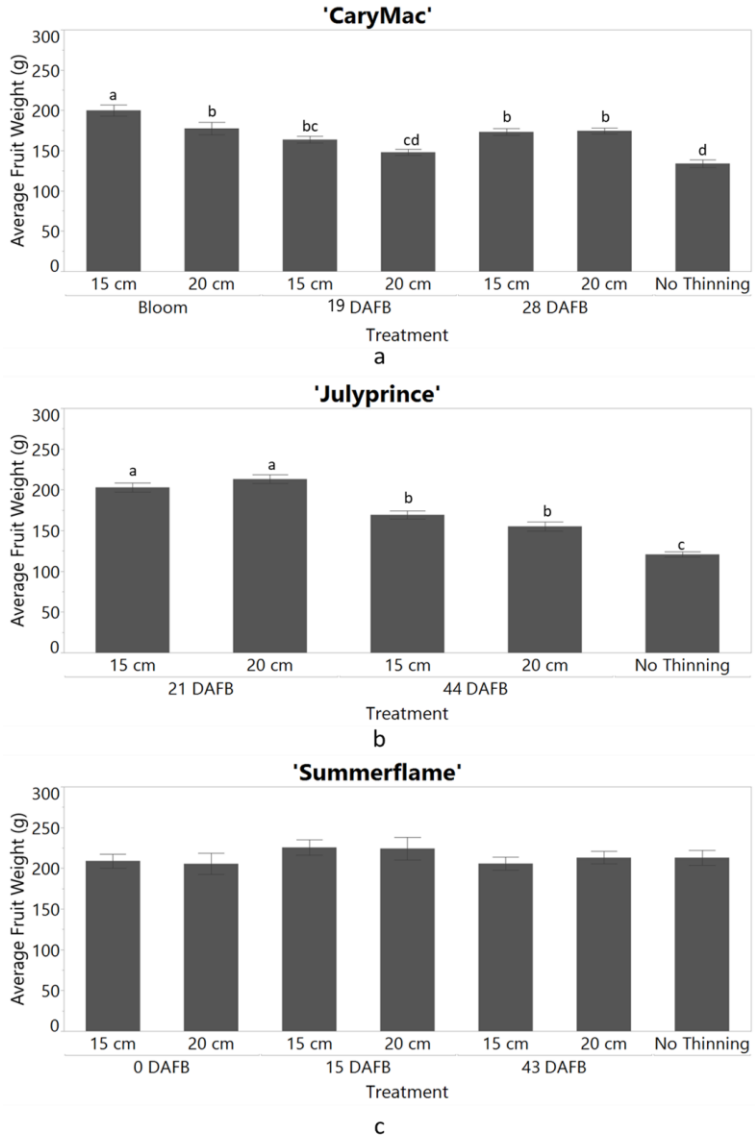

Figure 2.7) 2019 Average Fruit Weights. *a) Averages for 'CaryMac' b) Averages for 'Julyprince' c) Averages for 'Summerflame'. Values with different letters are significantly different. $p<0.05$.*

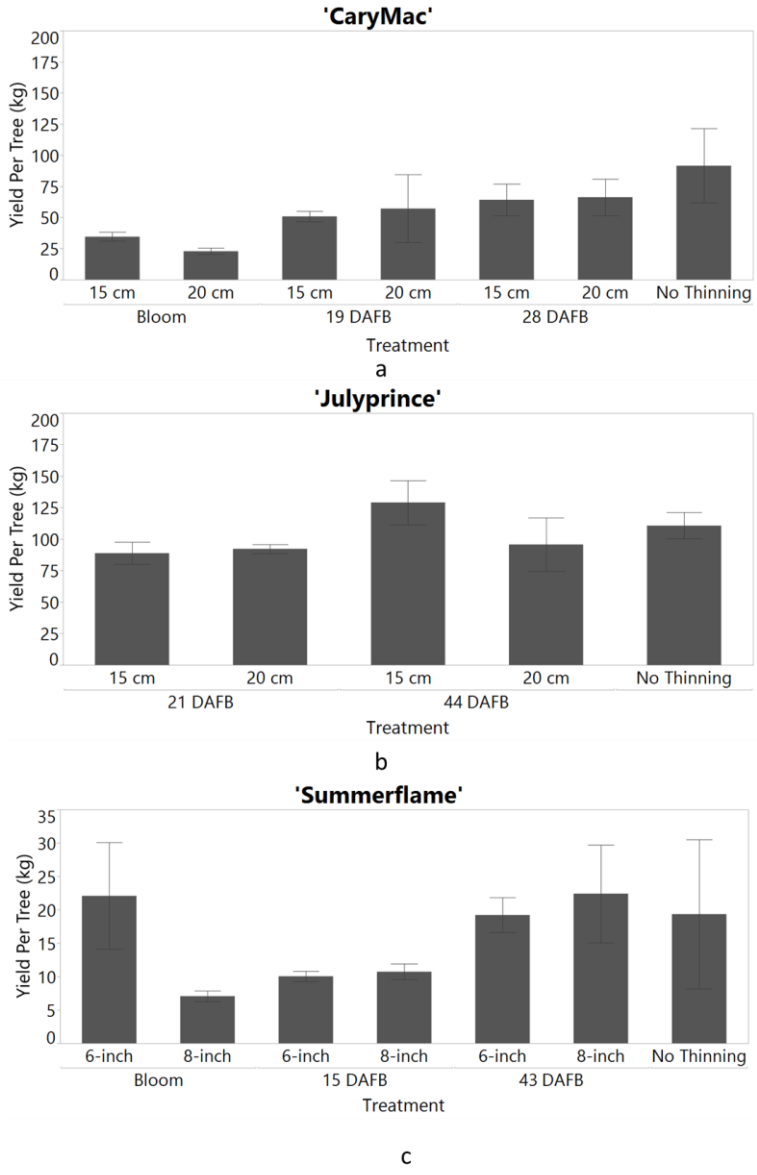

Figure 2.8) 2019 Average Total Yield Per Tree*. a) Averages for 'CaryMac' b) Averages for 'Julyprince' c) Averages for 'Summerflame'. Values with different letters are significantly different. p<0.05.*

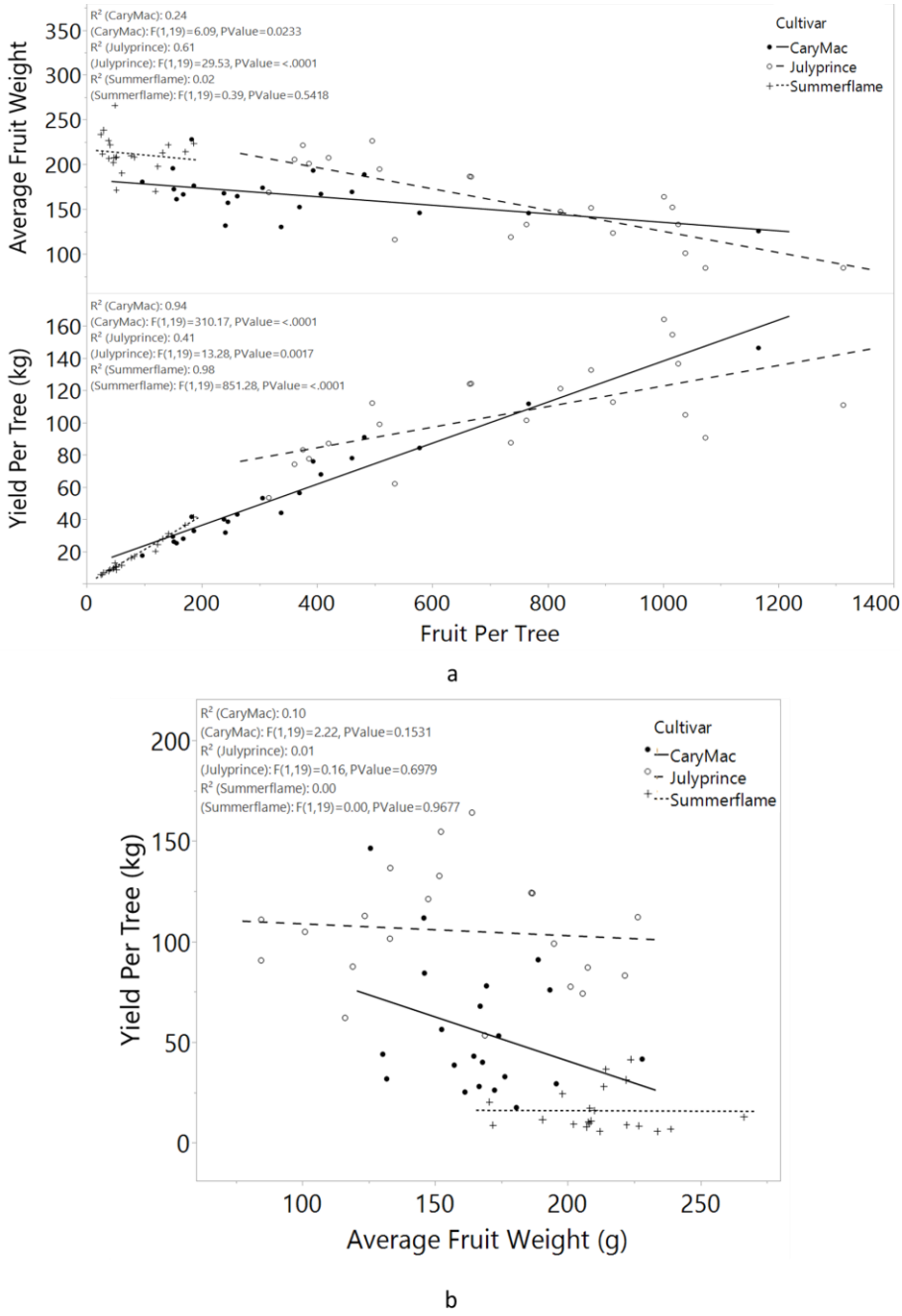

Figure 2.9) 2019 Correlations between total yield per tree, average fruit weights, and the number of fruit per tree. *Cultivars are combined for these analyses.* ***a)*** *Upper graph looks at the relationship between fruit per tree and total yield per tree. Lower graph looks at the correlation between fruit per tree and average fruit* ***b)*** *Correlation between the average fruit weight and total yield per tree. P≤0.05.*

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CHAPTER 3

USING EARLY SPRING TEMPERATURES AND CHILL HOUR ACCUMULATION TO PREDICT AVERAGE FRUIT SIZES AT HARVEST AND THE LENGTH OF FRUIT DEVELOPMENT

Introduction

Peach fruit growth follows a double sigmoidal pattern (Fig 3.1). The first stage of rapid growth is characterized by cell division, the second is a lag phase involving pit hardening, and the third is another period of rapid growth due to cell expansion (Conners, 1919; Lockwood & Coston, 2005). Growth during stage I is critical as it ultimately determines the size of the peach. Peach size is a measure of the number of cells present and the size of those cells (Wu et al., 2005). Factors that affect growth during this stage can also affect final fruit size.

Fruit growth and fruit development describe separate, though related, processes. Growth cannot proceed without development while development may proceed without the growth potential being fully realized (Lopez & DeJong, 2007). Temperatures during the early season can influence both developmental and growth rates. However, the increased developmental rates shorten the window for growth. While growth rates are also increased, it is not sufficient to compensate for the shorter growth window. Resources cannot be made available quickly enough to support the demand associated with the higher growth rates (DeJong & Grossman, 1995; Grossman & DeJong, 1995). For this reason, higher temperatures affecting developmental and growth rates during

stage I growth often result in undersized fruit (Day et al.,2008; Lopez & DeJong, 2007; Reighard & Rauh, 2015).

Currently, models that use temperatures during the first 30 days after full bloom (DAFB) to predict reference dates, harvest dates, and final fruit size have been developed. Growing degree days (GDD) and growing degree hours (GDH) have both been demonstrated to correlate with the length of the season (Mimoun & DeJong, 1999; Lopez & DeJong, 2007; Day et al., 2008). GDD are calculated by subtracting a base temperature (7°C for peach) from the daily mean temperature (Reighard & Rauh, 2015). GDH are calculated similarly except using hourly mean temperatures. GDH have also been used to predict the final size of the fruit; increased temperatures during the first 30 days correlate with decreased fruit sizes (Marra et al., 2002; Reighard & Rauh, 2015). These models are likely region specific, so recommendations made in one region likely may not be applicable elsewhere. Currently such a model does not exist for peach production in Georgia.

In addition to GDD and GDH, proper chill hour accumulation is critical for fruit set, growth and development. Chill hour accumulation is the number of hours below 7°C. Each cultivar requires a certain number of chill hours to break bud dormancy (Lockwood & Coston. 2005). Low chill accumulation can result in poor fruit set and delayed bloom (Lockwood & Coston, 2005; Bassi & Monet, 2008; Luedling et al., 2013; Li et al., 2016). This study aims to create a prediction model using early spring temperatures, among other variables, to aid Georgia peach grower's in planning their management strategies for the season. For example, thinning is a common cultural practice used to improve fruit sizes (DeJong et al., 1991; Day & DeJong, 1998; Njoroge & Reighard, 2008).

Materials and Methods

Data Acquisition

Pack-Out and spray records to build the data set were provided by Pearson Farms (located in Fort Valley, Ga). From these records, data on 29 peach cultivars grown at several orchards in the surrounding area over the years 2013-2017 were obtained. Trees ranged from 2-14 years old. For each cultivar and year, a variety of parameters were collected or calculated. Bloom dates were found in Pearson Farms' spray records based on the application of Captan, Bravo Weather Stik, or Chlorothalonil. These pesticides are applied at full bloom (Bloom Management Guide, 2019). Bloom and harvest dates were used to calculate the fruit development period (FDP). The breakdown of size grades in the data set allowed for calculations of average fruit size (AFS) for each cultivar at each grove. The total amount of fruit harvested (picked yield) and the amount that was marketed (packed yield) were used to calculate the percent of the yield that was marketable and how much was culled. This information, along with the number of trees per orchard (not a measure of planting density), was used to calculate the pounds/tree or bushel/tree that were harvested. Their data also included information on the age of the trees (leaf year, LF).

The total chill hours (CH) received each year was determined using the Chill Hour Calculator on the UGA weather network **(**http://www.georgiaweather.net**)** using data from the Fort Valley weather station. A base temperature of 7°C was used. Each cultivar requires a certain number of chill hours; varietal requirements were found on the Peaches page of the UGA CAES website (https://peaches.caes.uga.edu) and in the Dave Wilson Nursery Catalog (https://www.davewilson.com/). From this information, the

difference in chill hours received and the chill hours required by each cultivar was calculated (Chill Hour Difference, CHD). This difference was also looked at as whether the chill hours were met or not (Chill Hour Met, CHM): 0 indicated chill hours were not met and 1 indicated sufficient CH accumulation. Because CHD and CHM take into account the varietal chill hour requirement, they provide a varietal effect that CH alone does not.

Historical weather data was obtained from the National Centers for Environmental Information (NCEI) for the nearest weather station (Warner Robins Air Force Base) with data covering the desired years. This station is ~23 miles from Pearson Farm. Hourly average temperatures from this data set were used to calculate growing degree hours (GDH) for the first 20, 30, 40, 50, and 60 days after bloom for each cultivar for each year. Similarly, daily average temperatures were used to calculate the growing degree days (GDD) for 20, 30, 40, 50, and 60 days after bloom. A base temperature of 7 °C was used for all calculations (Reighard & Rauh, 2015). Total GDD for the fruit development period was also calculated.

Model Development

All statistical analyses and model development were performed in JMP (Version 14.1.0. SAS Institute Inc., Cary, NC, 1989-2019). The dependent variables of interest were the FDP and AFS. Transformation of the dependent variables was attempted in several ways (log, ln, square root) to determine the best fit possible. The independent variables of interest were CH, CHM, CHD, GDH30, GDD30, and LY.

Initially, all possible independent variables were tested individually against the dependent variables (both transformed and untransformed) using simple regressions to

determine which ones were significantly correlated to the dependent variables ($p \leq 0.05$). This was largely done to narrow down the list of possible independent variables. For example, GDH for 20, 30, 40, 50, and 60 days after bloom were all tested, and 30 days showed the most significant correlation, so 30 GDH was used for future tests. If a variable was determined significant for at least one of the two interested dependent variables, then it was included in the list of possible variables for all dependent variables.

Next, a stepwise regression was used to determine which combination of factors were the best predictors for FDP and AFS respectively. The combination of variables that produced the lowest corrected Akaike Information Criteria (AICc) value was chosen. AICc is an estimator that reflects the relative quality of a model. These values can only be used in ranking the quality of the model (Burnham & Anderson, 2004; Snipes & Taylor, 2014). However, factors that measure the same parameter cannot both be included together in the model. For example, because GDH30 and GDD30 are both measures of early spring temperatures, both cannot be implemented in a model, therefore the one that least affected the AICc value remained. Subsequently, the chosen variables were used to create a generalized regression model. Models were selected based on their AICc values, adjusted R^2-values, p-values, and distribution of residuals. A Shapiro Wilk test was used to determine the normality of the distribution of residuals.

Results

The data used was not normal (data not shown). Several different transformations were tested to determine which would best normalize the data. This was determined through comparing R^2-values, distribution of residuals, and the normality of the residuals. The natural log transformations of FDP(GDD) and AFS were found to best normalize the

data. Only data and models pertaining to these transformed parameters are presented below. Six of the independent variables (CH, CHM, CHD, GDH30, GDD30, LY) were found to significantly associated with one or both of the desired dependent variables (AFS and FDP(GDD)), so all six were included when performing the stepwise regression for both dependent variables.

Fruit Development Period

The independent variables, LY, CH, CHD, and CHM were found to be associated with FDP(GDD), when each of these relationships were tested separately. The low adjusted R^2-values (Table 3.1) and the highly non-normal distribution of residuals (Fig 3.2) for each of these suggest they are not strong predictors for FDP(GDD) on their own. The variables GDH30 and GDD30 alone did not show a significant association with ln[FDP(GDD)] (Table 3.1).

These six parameters were then used to perform a stepwise regression to determine which combination would serve as the best predictor. The combination of CHD, CH, LY, and GDH30 were found to be the best predictors of ln[FDP(GDD)] because they produced the lowest AICc values without redundant parameters. These four parameters were used to create a generalized regression model. The estimates and p-values associated with them are presented in Table 3.2. The following equation was generated from the generalized regression model analysis:

$$\ln\big(FDP(GDD)\big) = 5.4038796 - 0.001708(CHD) + 0.0019012(CH) - 0.021259(LY) + 4.2832\mathrm{e}^{-5}(GDH30)$$

This model produced the most normally distributed residuals of those tested (Fig 3.4). The Shapiro-Wilk test indicated a p-value slightly below 0.05 (0.046) suggesting the

residuals are not normally distributed. However, visual analysis of the histogram and the normal quantile plot suggested that the residuals are near a normal distribution. An adjusted R^2-value of 0.3198 is associated with this model. This R^2-value is notably higher than those associated with the individual parameters (Table 3.1).

Average Fruit Size

When tested against AFS on their own, GDH30, GDD30, and CH were found to be significantly associated with the independent variable (Table 3.1). Again, while they showed significant correlations, the non-normal distribution of their residuals (Fig 3.3) and the low R^2-values associated with these simple regressions suggest they are not good predictors of average fruit size on their own (Fig 3.1).

These same six parameters as used with FDP(GDD) were used to perform a stepwise regression to determine the best combination to serve as predictors for AFS. The combination of CHD, CH, and GDH30 were found to best explain the variance in AFS data. This was determined by comparing AICc values, p-values, and R^2-values. These three independent variables were then used to create a generalized regression model. The following equation was generated using the estimates from the generalized regression model analysis:

$$\ln(AFS) = 0.8370062 - 0.000241(CHD) + 0.0002598(CH) - 1.366e^{-5}(GDH30)$$

This model produced residuals with the highest degree of normality (Fig 3.5). The distribution of residuals failed to reject the null hypothesis in the Shapiro-Wilk test suggesting the residuals are normally distributed (Fig 3.5). The residuals were also more normally distributed with the generalized regression model than those of the simple regressions with the induvial parameters (Fig 3.3). The R^2-value associated with this

model is 0.3129. This R^2-value is higher than those associated with the simple regressions of the individual parameters (Table 3.1). The higher R^2-value and the higher degree of normality seen with the generalized regression suggest it serves as a better predictor of AFS than each independent variable alone.

In the normal quantile plots for both generalized regression models, the tails where the data points diverged from normal the most were generally associated with early-season cultivars. This suggests a cultivar parameter or creating separate models for each ripening group may resolve some of the unexplained variation. Unfortunately, there is not enough data to confidently support such analyses at this time.

To serves as a case study, 'Carored', an early-season cultivar, and 'Augustprince', a late-season cultivar, were isolated and used to perform the same series of analyses described above. The model for FDP for 'Carored' relied on CH and CHM and had an R^2-value of 0.2916 while that for 'Augustprince' relied on CHD and GDD30 and had an associated R^2value of 0.8818. In terms of AFS, the model for 'Carored' relied on CHM, LY, and GDH30 with an R^2-value of 0.1292. The AFS model for 'Augustprince' relied on CHD, LY, and GDD30. The R^2-value associated with this model is 0.4386. The R^2-values associated with the 'Augustprince' are higher and those with 'Carored' are lower than those of the generalized regression models created including all cultivars. This, along with the reliance on different parameters, suggests that some of the unexplained variation seen in the overarching models could be explained by cultivar differences.

Discussion

Early spring temperatures significantly affected the length of the fruit development period and the average fruit size at harvest, consistent with other reports

regarding the importance of this parameter in peach fruit growth (Mimoun & DeJong, 1999; Lopez & DeJong, 2007; Lopez et al., 2007; Day et al., 2008; Reighard & Rauh, 2015). Higher GDH30 correlated with a decrease in AFS and in FDP. As discussed earlier, warmer spring temperatures have been shown to increase developmental and growth rates. However, early competition among fruit often prevents the full growth potential from being established. The increased developmental rate also shortens the window for growth further limiting fruit growth (DeJong & Grossman, 1995; Grossman & DeJong & Grossman, 1995; Lopez & DeJong, 2007). The increased developmental rate seen with higher spring temperatures during the first 30 days after bloom result in the shortened fruit development period observed in this analysis. The shortened growth window along with the resource-limited growth potential accounts for the smaller fruit observed with high early spring temperatures.

Adequate chill hour accumulation is critical for the success of the crop. Peaches undergo two dormancy periods. The first dormancy (endodormancy) is broken by the accumulation of chill hours. The second dormancy (ecodormancy) immediately follows the first and is broken by heat requirement (Lang, 1987; Lockwood & Coston, 2005; Luedling et al., 2013; Li et al., 2016). Cultivars differ in their chill hour requirements with low-chill requiring cultivars typically blooming earlier than high-chill requiring cultivars (Citadin et al., 2001; Okie & Blackburn, 2011; Li et al., 2016). High levels of chill accumulation have also been shown to reduce the heat requirement which results in earlier bloom times. Similarly, low levels of chill accumulation increase the heat requirement and results in later bloom times (Li et al, 2016). Failure to accumulate these chill hours can have adverse effects on the crop: delayed foliation and blooming, reduced

fruit set, buttoning (fruit sets but fails to size), and overall reduced fruit quality (Byrne & Bacon, 1992; Okie & Blackburn, 2011; Li et al., 2016). The effects CH accumulation can have on bloom times, fruit set, and fruit development can be observed through differences in the length of the fruit development period and average fruit sizes.

The age of the tree also impacts the productivity of the tree. Peach trees typically do not produce a large crop load until year 3 or 4. Productivity then increases over the subsequent years, usually peaking around year 6-8. Subsequently, production levels off before declining in later years (Vinyes et al., 2015). Photosynthetic capacity is greatly affected by the age of the tree and likely influences the productivity of the tree (DeJong & Moing, 2008). Older trees typically set less fruit (Robbie & Atkinson, 1994; Tredar et al., 2010; Volz et al., 2015).

While individually these factors are all correlated with the FDP and AFS, looking at them together served as an even better predictor. Understanding how these variables are working together to influence fruit sizes and the fruit development period can aid grower's in planning for the upcoming season. Thinning is a common cultural practice used to reduce competition during early fruit growth. The success of thinning is largely dependent on the timing. For example, in years where CH are adequate, the heat requirement will be reduced. In such years, bloom will likely occur earlier; the earlier bloom time will in turn result in relatively lower GDH30 owing to its occurrence earlier in the spring. In such years, longer fruit development periods and larger fruit can be expected. In such years, thinning can proceed as normal: ~30 DAFB and after the threat of spring freeze. Conversely, in low-chill years, bloom will likely be delayed. In such years, higher GDH30, poor fruit set, shorter fruit development periods, and smaller fruit

can be expected. The reduced fruit set in these years may eliminate the need for thinning. If there is still heavy fruit set after a low-chill winter, thinning earlier than ~30DAFB could help negate the effects of competition maximizing the fruit growth potential (Lopez & DeJong, 2007).

These models are limited in several ways. The amount of data was limited as only five years of data was available. This limited how detailed/complex analyses could be. For example, there was not enough power to support looking at interactions between terms or higher power regressions (i.e. quadratic).

The limited data also prevented a cultivar parameter from being utilized. As shown, the FDP and AFS of different cultivars are associated with different factors and are affected by these factors to varying extents. For example, 88% of the variation in FDP in ‘Augustprince’ could be accounted for by CHD and GDD30. In ‘Carored’, only 29% of the variation in FDP could be accounted for by CH and CHM. The high amount of unexplained variation in the early cultivar and the fact the tails are usually early cultivars suggest that these cultivars are highly impacted by factors other than those studied. Early-season cultivars typically suffer more damage in the occurrence of a late-spring freeze as they are blooming at this time (Murray, 2011). A cultivar parameter will be needed in the future.

Conclusion

The variables, CH, CHD, LY, and GDH30 could be used to predict the length of the fruit development period and the average fruit size at harvest. Such information will be useful to growers when planning management practices for the season. However, only five years of data was available and used to develop these models. There were not

sufficient degrees of freedom to explore more complex interactions between different variables or to evaluate cultivar-specific effects. The data set will continue to be updated each year to further improve the predictive power of the models. Additional data will also be added (rainfall, radiation, etc.) to look at the impact of other environmental factors on fruit growth and development.

Acknowledgements

Thank you to Pearson Farms for allowing us to use their data. Thank you also to Dr. Uttam Bhattarai for his help with the statistical analyses.

Tables:

Table 3.1. Regressions between Independent Variables and Dependent Variables of Interest

Independent Variables[2]	Dependent Variables ln[FDP(GDD)]			ln(AFS)		
	Estimate	Adjusted R^2	p-value	Estimate	Adjusted R^2	p-value
GDH30	0.0000289	0.003991	0.1502	**-0.000013**	**0.068475**	**<0.0001**[1]
GDD30	0.0006219	0.00304	0.1780	**-0.000281**	**0.06056**	**<0.0001**
LY	**-0.02062**	**0.025129**	**0.0051**	-0.001567	0.004366	0.1375
CH	**0.0002272**	**0.027787**	**0.0034**	**$4.5827e^{-5}$**	**0.055779**	**<0.0001**
CHD	**$2.656e^{-5}$**	**-0.0033**	**<0.0001**	$1.2815e^{-5}$	0.001406	0.2402
CHM	*	*	**0.0203**	**	**	0.2462

[1]Bolded Values indicate significant probabilities according to ANOVA/linear regression. $p \leq 0.05$

[2]All interactions were tested separately.

*Oneway ANOVA performed—mean of unmet chill (7.08345) was higher than mean of met chill (6.99016)

**Oneway ANOVA performed – no significant difference in means of met and unmet chill

Table 3.2. Parameter Estimates for ln[FDP(GDD)]

	Independent Variables				
	Intercept	CH Difference	Chill Hours	Leaf Year	GDH30
Estimate[1]	5.4038796	-0.001708	0.0019012	-0.021259	$4.2832e^{-5}$
p-value[2]	<0.0001	<0.0001	<0.0001	0.0010	0.0209

[1]Estimates are for the generalized regression model created using the parameters deemed significant by the step-wise regression

[2]$p \leq 0.05$. All values were determined significant.

Table 3.3. Parameter Estimates for ln[AFS]

	Independent Variables			
	Intercept	CH Difference	Chill Hours	GDH30
Estimate	0.8370062	-0.000241	0.0002598	$-1.366e^{-5}$
p-value[2]	<0.0001	<0.0001	<0.0001	<0.0001

[1]Parameter Estimates are for the generalized regression model created using the parameters deemed significant by the stepwise regression

[2]$p \leq 0.05$. All parameters were determined significant

Figures:

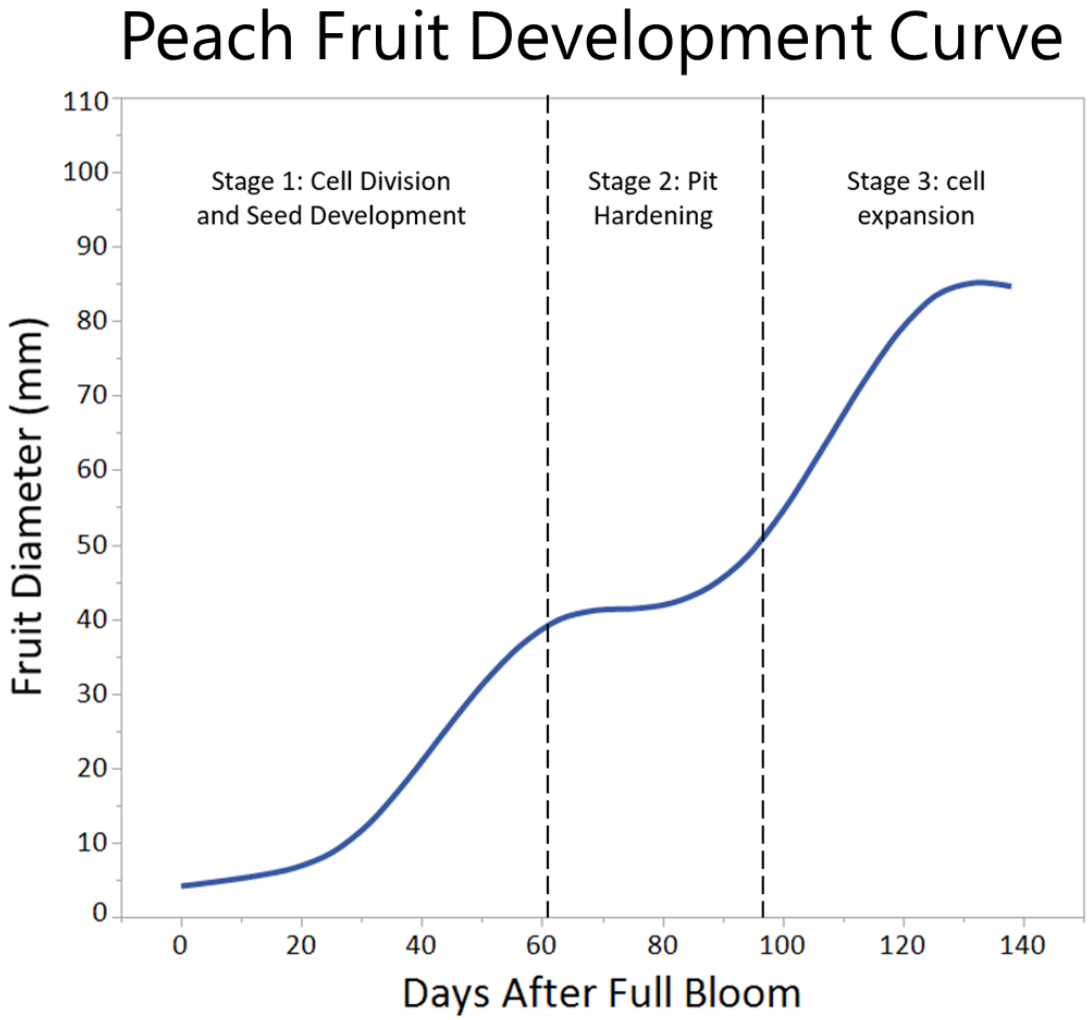


Figure 3.1) Peach fruit development curve. *In 2018, fruit diameters of 10 tagged fruit were taken regularly from bloom to harvest.*

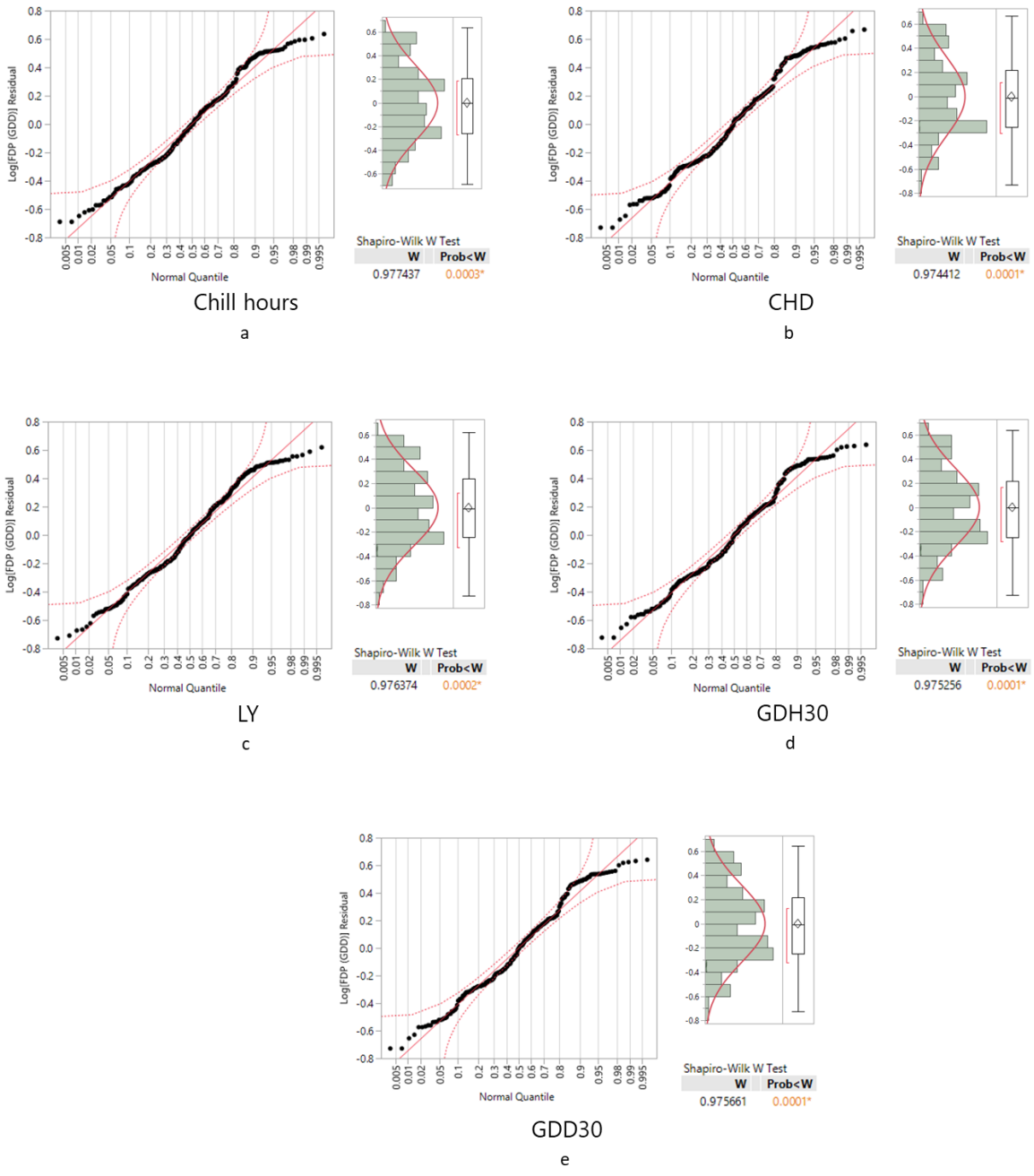


Figure 3. 2) Residual QQ plots, distributions, and Shapiro-Wilk Tests for ln[FDP(GDD)]. *a) Chill hours vs. ln[FDP(GDD)]. b) CHD vs. ln[FDP(GDD)]. c) LY vs. ln[FDP(GDD)]. d) GDH30 vs. ln[FDP(GDD)]. e) GDD30 vs. ln[FDP(GDD)]. The QQ plots and residual distribution curves serve as visual aids to determine how well the model fits the data. For shapiro-wilk tests $p \leq 0.05$. Small p-values reject the null hypothesis that data is normally distributed; small p-values indicate data is not normal.*

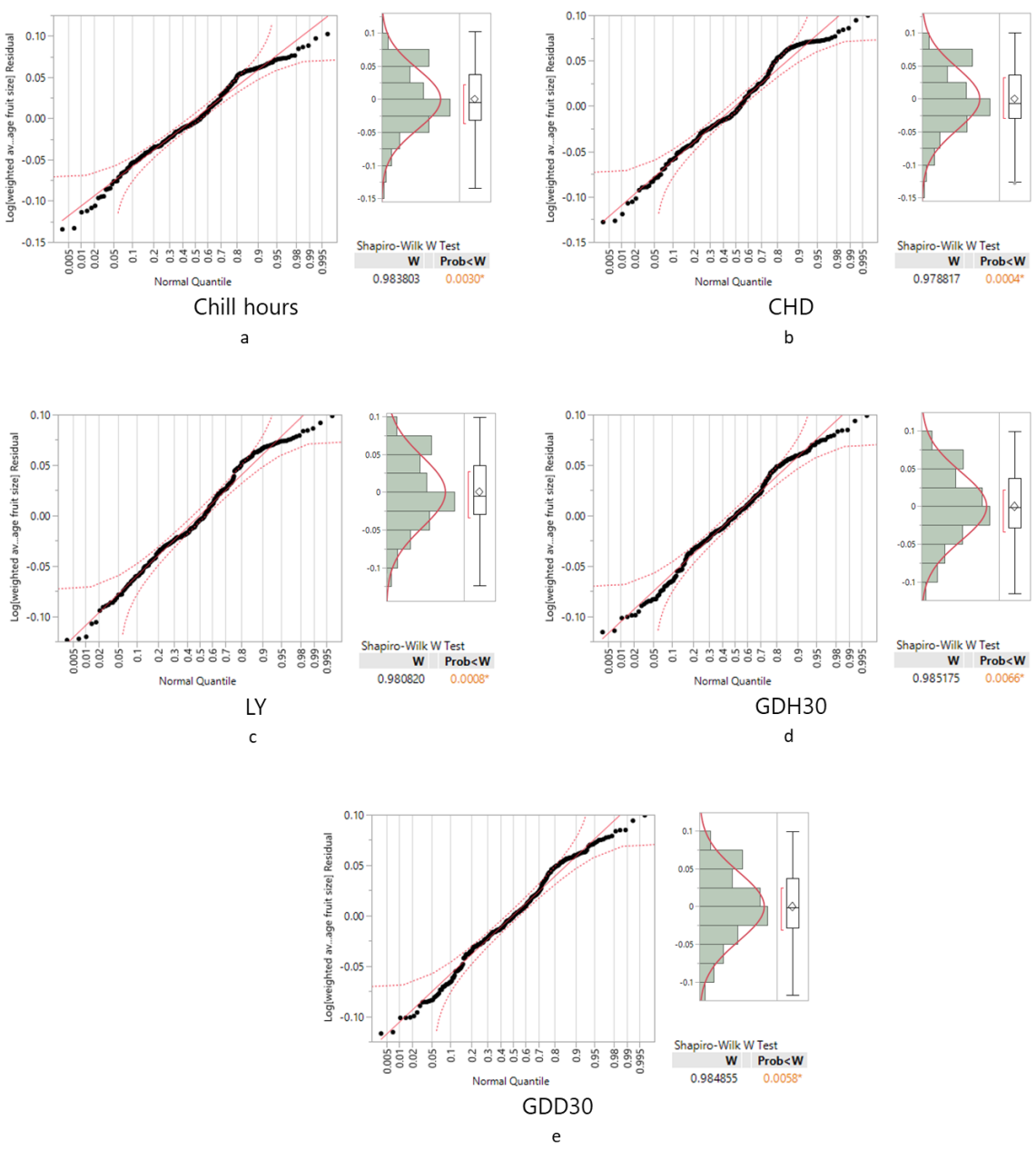
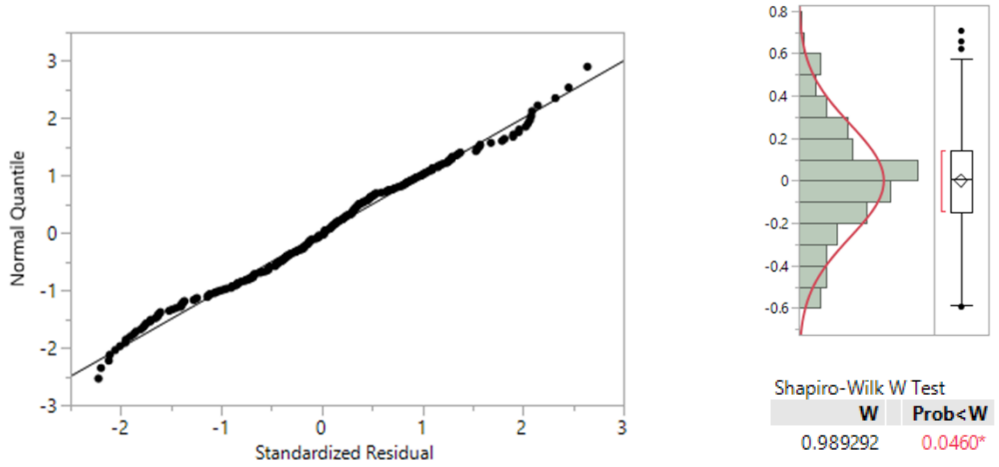
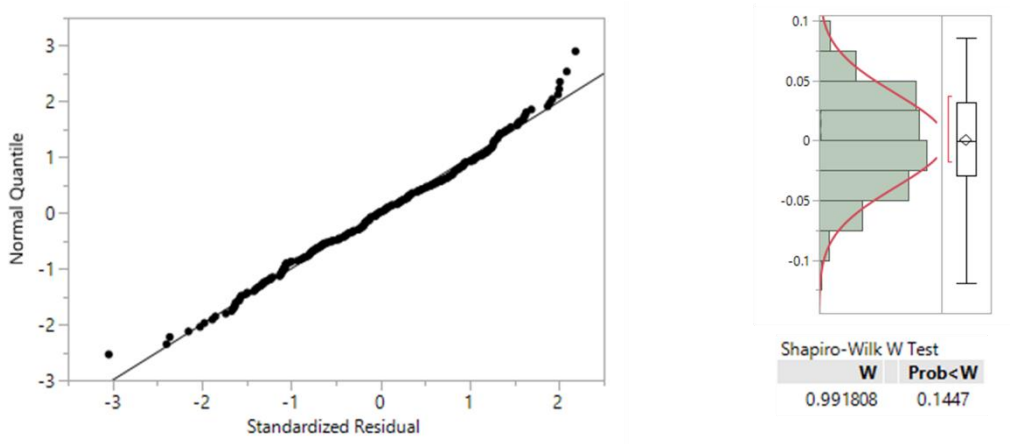


Figure 3. 3) Residual QQ plots, distributions, and Shapiro-Wilk Tests for ln(AFS). *a) Chill hours vs. ln[AFS). b) CHD vs. ln(AFS). c) LY vs. ln(AFS). d) GDH30 vs. ln(AFS). e) GDD30 vs. ln(AFS). The QQ plots and residual distribution curves serve as visual aids to determine how well the model fits the data. For shapiro-wilk tests $p \leq 0.05$. Small p-values reject the null hypothesis that data is normally distributed; small p-values indicate data is not normal.*



a b

Figure 3. 4) Residual plots for ln(FDP(GDD)) generalized regression model. *a) Normal Quantile Plot of the residuals for ln(FDP(GDD) generalized regression model. b) Distribution curve of residuals with Shapiro-Wilk Test for normality. The Normal Quantile Plot and residual distribution curves serve as visual aids to determine how well the model fits the data. For shapiro-wilk tests $p \leq 0.05$. Small p-values reject the null hypothesis that data is normally distributed; small p-values indicate data is not normal.*



a b

Figure 3. 5) Residual plots for ln(AFS) generalized regression model. *a) Normal Quantile plot of the residuals for ln(AFS) generalized regression model. b) Distribution curve of residuals with Shapiro-Wilk Test for normality. The Normal Quantile Plot and residual distribution curves serve as visual aids to determine how well the model fits the data. For shapiro-wilk tests $p \leq 0.05$. Small p-values reject the null hypothesis that data is normally distributed; small p-values indicate data is not normal.*

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CHAPTER 4

QUANTIFYING CYTOKININ EXPRESSION DURING STAGE I OF PEACH GROWTH: THE EFFECTS OF FRUIT LOAD REDUCTION

Introduction

Early peach (*Prunus persica* L.) growth is characterized by rapid growth due to cell division. A lag phase follows during which the pit lignifies. A second period of rapid growth is brought on by cell expansion after this lag phase (Conners, 1919; Day & DeJong, 1998; Lockwood & Coston, 2005). Stage I growth is critical as the number of cell divisions that occur largely define the size potential of the fruit; fruit size is a measure of the number of cells in a fruit and the size of those cells (Grossman & DeJong, 1995; Wu et al., 2005). Factors influencing the rate of cell division can ultimately affect final size of the fruit.

Thinning is a common cultural practice used to remove some portion of the fruit load during early growth to reduce competition among fruit for limited resources. This removal of competition allows more efficient use of the available resources and allows for more growth during early development. Reports show that thinning affects the rate of cell division during early fruit growth (Denne, 1960; Dash et al., 2013; Samuolienė et al., 2016). Cytokinin application early on also increases cell division during early fruit growth (Wismer et al., 1995; Lewis et al., 1996; Xu et al., 2016).

Cytokinins are a large, well-known class of phytohormones that play a major role in cell division. Most cytokinins exist in their free base form alongside their

corresponding nucleotides and nucleosides (Arnau et al., 1999; Haberer & Kieber, 2002; Kakimoto, 2003). These cytokinins share very similar structures with their activity dependent on the structure and conformation of their side chain (Mok & Mok, 2001; Haberer & Kieber, 2002; Schäfer et al., 2015).

Isopentenyltransferase (IPT) is largely responsible for catalyzing the first step of cytokinin biosynthesis through the prenylation of adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP), and adenosine 5'-triphosphate (ATP) to produce isopentenyladenosine-5′-monophosphate (iPMP), isopentenyladenosine-5′-diphosphate (iPDP), and isopentenyladenosine-5′-triphosphate (iPTP), respectively. In this reaction, ADP and ATP are the main prenyl acceptors (AMP to a lesser extent) and dimethylallyl diphosphate (DMAPP) acts as the acceptor (Kakimoto, 2003; Sugawara et al., 2008; Kamada-Nobusada & Sakakibara, 2009). IPT is responsible for the majority of isopentenyladenine (iP) and trans-Zeatin (tZ) production (Matsumoto-Kitano et al., 2008; Immanen et al., 2013) Overexpression of *IPT* through genetic modification increases cytokinin levels in fruit during early development (Mao et al., 2002; Luo et al, 2005; Xu et al., 2016).

The enzyme LONELY GUY (LOG) dephosphorylates iPMP, trans-Zeatin riboside monophosphate (tZMP), dihydrozeatin riboside monophosphate (DZMP), and cis-Zeatin riboside monophosphate (cZMP) converting these cytokinins to their active base forms: iP, tZ, dihydrozeatin (DZ), cis-Zeatin (cZ), respectively (Kamada-Nobusada & Sakakibara, 2009; Tokunaga et al., 2011). Overexpression of LOG has been shown to increase cell division during early growth in *Arabidopsis*, particularly in the embryo (Kuroha et al., 2009).

Cytokinin Oxidase (CKX) is a critical enzyme for cytokinin degradation. CKX cleaves the side chain of cytokinin bases releasing adenine and 3-methyl-2-butenal (Schmülling et al., 2003; Avalbaev et al., 2012). CKX is typically highly expressed in regions undergoing active cell division (Avalbaev et al., 2012). Certain CKX concentrations peak during early fruit development in kiwi (Pilkington et al., 2013) and tomato (Matsuo et al., 2012).

Due to their known involvement in cell division, cytokinins are expected to be in great quantities during stages of rapid cell division (stage I in peach) during fruit growth. Few studies have described the abundance of cytokinins during early stages of fruit growth and development (Lewis et al., 1996; Arnau et al., 1999). In peach, four cytokinins have been shown to peak during stage I: dihydrozeatin riboside (DHZR), iPMP, dihydrozeatin riboside-5'-monophosphate (DHZMP), and zeatin riboside (ZR).

This study aims to further explore the role of cytokinins in early peach fruit growth and development and determine if expression of their metabolism-related genes is altered by thinning treatments.

Materials and Methods

Plant Material

Twelve 10-year-old 'Cresthaven' trees at Lane's Southern Orchard in Fort Valley, Ga, were used for this study in 2019. The care and maintenance of the trees was performed according to standard commercial cultivation practices for Georgia by the orchard crew.

Thinning Treatments

The twelve trees were split into blocks of three trees. Within each block, each tree was randomly assigned to one of three treatments: thinning at 22 days after full bloom (DAFB), thinning at 29 DAFB, and no thinning control. All thinning was performed manually to 15 cm spacing between fruit on a shoot.

Sample Collection

At bloom, ten blooms were randomly tagged on each tree. Samples were collected at 0, 6, 12, 19, 26, 52, 95, 125, 135 DAFB with the last two dates being harvest dates. At each sample date, two diameters were taken from these fruit. If a fruit was missing at the sample date, the next nearest fruit was tagged in its place. At each sample date, 3-10 blooms/fruit (based on the developmental stage) were collected and transported back to Athens to take individual fruit weights. At each sample date, 3-10 fruit were also collected and immediately frozen in liquid nitrogen for transport back to Athens. This set of fruit was kept at -80°C until testing.

Gene Selection and Primer Design

IPT, *LOG,* and *CKX* sequences in peach were identified by searching the Genome Database for Rosaceae (GDR: https://www.rosaceae.org/) using corresponding amino acid sequences from *Arabidopsis* (TAIR: arabidopsis.org). Selected genes were numbered based on their order in the genome. The sequence information provided through GDR was used to design primers for segments of the coding region to be used for PCR amplification. Information regarding gene identity, accession numbers, and primer sequences are presented in Table 4.1.

RNA Extraction and Quantification

RNA extraction was performed following the procedure described by Vashisth et al., (2011). Extractions were performed on the tissue samples collected at 0, 6, 12, 19, 26, and 52 DAFB. Quality and quantity of RNA was determined using a NanoDrop 8000 spectrophotometer and gel electrophoresis. cDNA was prepared using reverse transcription. The designed primers and cDNA were used for qRT-PCR to determine relative gene expression levels. B-Actin, Ky-Actin, and RNA Polymerase II were used as reference genes and used for normalizing.

Statistical Analysis

All statistical analyses were performed in JMP (Version 14.1.0. SAS Institute Inc., Cary, NC, 1989-2019). Collection dates were analyzed separately when determining treatment effects. ANOVA followed by Tukey's HSD were used to determine significant differences between treatments and between collection dates. A 2-fold or more difference in relative expression levels was considered to be biologically significant.

Results

Fruit size

Across treatments, fruit sizes did not differ during the first 12 DAFB. At 19 DAFB, differences in treatments began to arise. However, as these differences appeared before either of the thinning treatments are applied, these differences in size were likely not related to the thinning treatments. At 26 DAFB, the 22 DAFB thinned trees displayed significantly larger fruit diameter than the later thinning and control treatments. However, the two thinned treatments had similar fruit weights and both were significantly greater than that of the control. However, for the later thinning treatment which had not yet been

performed, the difference observed from the control is not due to thinning. From 52 DAFB onwards, the fruit on thinning treated trees showed a range of fruit from significantly larger to comparable fruit diameters relative to that of the control trees. Between the two thinning treatments, the 29 DAFB thinning treatment showed significantly larger to comparable fruit diameter to the 22 DAFB thinning treatment. At 52 and 95 DAFB, fruit weights did not differ significantly between the three treatments. However, at the first harvest date, the two thinning treatments showed significantly higher fruit weights than the control. Similarly, at the second harvest date, the thinned trees displayed significantly heavier fruit than that in the control. Fruit on the 29 DAFB thinned trees were also significantly heavier than those on the 22 DAFB thinned ones at this harvest (Fig 4.1a, 4.2a).

Over the course of the season, the changes in fruit weight and size over time were consistent over time. Because the same trends over time were seen across treatments, only the control is presented at this time (Fig 4.1b, 4.2b). Fruit diameters significantly increased between 6 and 12 DAFB, 19 and 26 DAFB, 26 and 52 DAFB, and 95 DAFB and harvest. There is an apparent decrease in fruit diameters between 12 and 19 DAFB, but it was deemed not significant. At 12 DAFB, all random fruit were removed and taken back to the lab for diameter measurements instead of measuring the tagged fruits. At the subsequent collection date, measurements were resumed with the tagged fruits. It is possible the decrease in fruit diameter observed is due to natural variations in fruit size (Fig 4.1b). While fruit weights increased across the season, they did not significantly differ until 52 DAFB. Fruit weight then again increased at 95 DAFB and at harvest (Fig 4.2b).

ISOPENTENYLTRANSFERASE

All the treatments followed the same trend over time in *IPT* expression. For the sake of simplicity, only the control is shown (4.3). *IPT2* and *IPT5* expression levels did not differ significantly over the course of early fruit growth (Fig 4.3b,d). *IPT1* expression increased throughout early fruit growth with a ~55.7-fold increase from bloom to 52 DAFB (Fig 4.3a). *IPT6* expression significantly increased from bloom to 12 DAFB (~20.7-fold) and then leveled off (Fig 4.3e). *IPT3* expression was relatively constant from bloom to 26 DAFB before falling significantly lower at 52 DAFB. *IPT3* expression was highest numerically at 12 DAFB and was ~3.8-fold higher than expression at 52 DAFB (Fig 4.3c). Significant differences observed over time were also biologically significant.

IPT expression did not differ across treatments across time (Fig 4.4) except at 26 DAFB where *IPT3, IPT5,* and *IPT6* expression was significantly higher in the control fruit compared to the 22 DAFB thinned fruit (2.1-, 1.4-, 2.0-fold higher, respectively) (Fig 4.4c,d,e).

LONELY GUY

Because the same general trends in *LOG* expression were observed across treatments over time and because of the high degree of variability within treatments at each time point, only the data for the control is present in Fig 4.5. *LOG1* and *LOG2* expression did not significantly differ across early fruit growth (Fig 4.5a,b). *LOG3, LOG4, LOG6, LOG7,* and *LOG8* expression tended to be significantly higher during the first 12 DAFB and then declined in the following weeks (Fig 4.5c-g). *LOG3* and *LOG4* expression peaked at 12 DAFB and then declined through the remainder of early fruit growth (Fig 4.5c,d). *LOG3* expression was ~7.9-fold higher at 12 DAFB than at 52

DAFB and *LOG4* was ~5.1-fold higher at 12 DAFB than 19 DAFB. *LOG6* expression was highest at 6 DAFB and lowest at 52 DAFB (~148.8-fold difference) (Fig 4.5e). *LOG7* and *LOG8* expression were ~97.1-fold and ~14.1-fold, respectively, at their highest (6 DAFB) than at their lowest (52 DAFB) (Fig 4.5f,g). Hence, with the exception of *LOG1*, LOG expression appears to peak during the very early stages of fruit growth and peaks at 6 and/or 12 DAFB.

With few exceptions, *LOG* expression did not differ between treatments at the different time points (Fig 4.6). *LOG3, LOG4,* and *LOG8* did show a significant difference between treatments at 26 DAFB. The control fruit displayed higher *LOG3* and *LOG4* transcript abundance than the 29 DAFB thinning treatments at 26 DAFB (1.8- and 1.7-fold higher, respectively) (Fig 4.6c,d). *LOG8* expression was significantly higher in the control than the 22 DAFB and 29 DAFB thinning treatment at 26 DAFB (2.4- and 2.1-fold higher, respectively) (Fig 4.6g). At 52 DAFB, *LOG6* expression was significantly higher in the 22 DAFB treatment than the control and the 29 DAFB treatment trees (3.8- and 6.2-fold higher, respectively) (Fig 4.6e).

CYTOKININ OXIDASE

Because treatments showed similar trends in *CKX* expression over time, only the control is presented for the sake of simplicity (Fig 4.7). *CKX2* and *CKX3* expression did not significantly differ over the course of early fruit growth (Fig 4.7b,c). *CKX1* expression significantly increased throughout the course of early fruit growth (~7.2-fold increase from bloom to 52 DAFB); expression was highest at 52 DAFB and lowest from 0 to 12 DAFB (Fig 4.7a). *CKX4* expression peaked at bloom and then decreased rapidly with the lowest expression levels at 19 DAFB (~156.8-fold) (Fig 4.7d) *CKX5* expression

also peaked early (0 and 6 DAFB) before declining (~12.7-fold difference between 6 DAFB to 52 DAFB) (Fig 4.7e).

Treatments only significantly differed in *CKX3* expression at 19 DAFB with the 22 DAFB thinned treated fruit showing 2.6-fold higher expression levels than the 29 DAFB thinned treated fruit (Fig 4.8c), but this was likely not related to the thinning treatment. While there appear to be other observable differences between treatments, they are not significant. There are several instances where there is a high degree of variation within a treatment at a given time point (Fig 4.8).

Discussion

The observed pattern in fruit growth was consistent with previous reports (Conners, 1919; Day & DeJong, 1998; Lockwood & Coston, 2005). Thinned trees showed larger fruit than the unthinned controls, consistent with studies indicating that thinning during early fruit growth results in larger fruit (Day & DeJong, 1998; Lockwood & Coston, 2005; Njoroge & Reighard, 2008; Malhotra & Deshmukh, 2017). However, some significant differences in fruit weights and diameters were observed before treatments were implemented. The differences observed between treatments in fruit weights and diameters were not the same. For example, at 26 DAFB, the 29DAFB treatment had fruit with significantly smaller diameters than the 22 DAFB treatment while the 26 DAFB treatment had fruit of a comparable weight to the 22 DAFB treatment. The observed differences also did not persist throughout the rest of the season. For these reasons, it is possible that the premature differences in fruit weights and diameters between treatments were due to variation across the trees selected for the study.

Expression patterns of *IPT* genes were not consistent with one another across early fruit growth and development (Fig 4.4). However, their general transcript abundance during this period suggests that there is some level of endogenous cytokinin biosynthesis within the young fruit, although this needs to be determined through cytokinin quantification. This is consistent with reports of iPMP production (by-product of the reaction catabolized by IPT) throughout early fruit growth (Arnau et al., 1999). Increased *IPT* expression through genetic modification was also shown to improve fruit set and increase overall cytokinin concentrations in tomato (Martineau et al., 1994; Srivastava & Handa, 2005). Further work will need to be done to determine how endogenous *IPT* expression affects cytokinin metabolism during early fruit growth.

The thinning treatments did not affect the levels of *IPT* expression throughout early fruit growth and development with a few exceptions. At 26 DAFB, however, the control treatment showed significantly higher levels of *IPT3*, *IPT5,* and *IPT6* expression than the 22 DAFB thinned treatment. This may potentially suggest an earlier decline in cytokinin synthesis in response to thinning. The significance of such a decrease remains unclear.

LOG expression was generally higher during the first 12 DAFB and declined throughout the rest of stage I (Fig 4.6c-g). The higher *LOG* expression during this time was determined to be both statistically and biologically significant. *LOG* expression during this time correlates with rapid cell division during early fruit growth and development. LOG is responsible for the conversion of inactive cytokinins to their active base forms. This is consistent with reports of these active cytokinins (iP and tZ) being highest at bloom and during the following few weeks in peaches (Arnau et al, 1999) and

in tomato (Matso et al., 2012). Endogenous *LOG* expression appears to be important for early cell division and fruit set during stage I peach growth.

LOG expression generally did not differ between the different thinning treatments across early fruit growth and development. The control had higher *LOG3* and *LOG4* expression levels than the 29 DAFB treatment and higher *LOG8* expression levels than both thinning treatments at 26 DAFB (Fig 4.5c,d,g). This was somewhat inverse to what was observed in fruit weight and diameter at this point (the control had the smallest diameters and weights) (Fig 4.1a, Fig 4.2a). As the differences in expression are resolved at the next time point and not present in all the *LOG* genes studied, the significance of this variation in terms of fruit growth remains unclear. Measurement of active cytokinin concentrations is needed to better understand the roles of these phytohormones in regulating early fruit growth.

There were no consistent trends in *CKX* expression throughout stage I peach growth (Fig 4.8). Expression appeared higher early on (0-12 DAFB), but this was neither a significant or consistent trend (Fig 4.8b-e); *CKX1* was highest at 52 DAFB and lowest early on, while *CKX5* displayed the opposite trend. The higher *CKX5* expression observed during early peach growth is consistent with other reports of elevated *CKX* expression during early fruit growth (Matsuo et al., 2012; Pilkington et al, 2013). *CKX* expression is typically found in conjunction with *IPT* expression in regions undergoing active cell division (Avalbaev et al., 2012); general *IPT* and *CKX* expression was seen during early fruit growth, supporting the idea that these two genes likely work to balance cytokinin levels during active cell division. However, higher *CKX1* abundance at later stages may suggest a need for cytokinin inactivation at that stage. Together, the patterns

of expression of various CKX genes suggest that cytokinin oxidation may be mediated by different genes at different times of fruit development. Further, their gene products may display differential oxidation capacity which may alter cytokinin metabolism differentially during the developmental changes. One way of determining their roles is to quantify the cytokinin metabolites (degradation products) during these stages, in frutre studies. The different thinning times did not consistently affect *CKX* expression.

Conclusion

Different thinning times did not appear to significantly or consistently alter the expression of key cytokinin genes throughout stage I growth. There were no consistent trends across the multiple gene family members in terms of *IPT* and *CKX* expression. Additional work will need to be done to determine if their expression impacts cytokinin metabolism within the developing fruit. Expression of multiple *LOG* family members was consistently highest during the first few weeks suggesting active cytokinin formation during this period. Hence, these *LOG* genes may play a role in regulating active cytokinin levels during early peach fruit growth and development.

Acknowledgements

Thank you to Lane's Southern Orchard for allowing the use of their field and for the care and maintenance of the trees.

Tables:

Table 4.1) Summary of Gene Identities, similarity to *Arabidopsis,* primer sequences

Isopentenyltransferase

Gene	Gene Identity	% ID to ATIPT 1	% positives to ATIPT1	Forward primer 5'-3'	Reverse Primer 5'-3'
IPT1	Prupe.1G150800.1	43.20%	60.20%	CGGTGGCTCGAATTCCTACA	GCCTTCCTGATTCCGTGAGT
IPT2	Prupe.1G151100.1	50.00%	66.04%	GCAAGTCTACAGAGGCCTGG	CCACCGTCCGATCGATGTAG
IPT3	Prupe.4G170400.1	38.39%	61.61%	TTGATAGTGGCCGTGTTCCC	CAGCTGCTTCCCAGTCTTCA
IPT5	Prupe.6G12800.1	44.88%	74.02%	GATGCTTCTCTCCCTGTGCT	ATCAGGCAGGTCTACTGACG
IPT6	Prupe.8G243600.1	43.05%	64.57%	TCCATTTTAGGCCGGGATCG	TGTCCACCCGTTTTGACACA

Cytokinin Oxidase

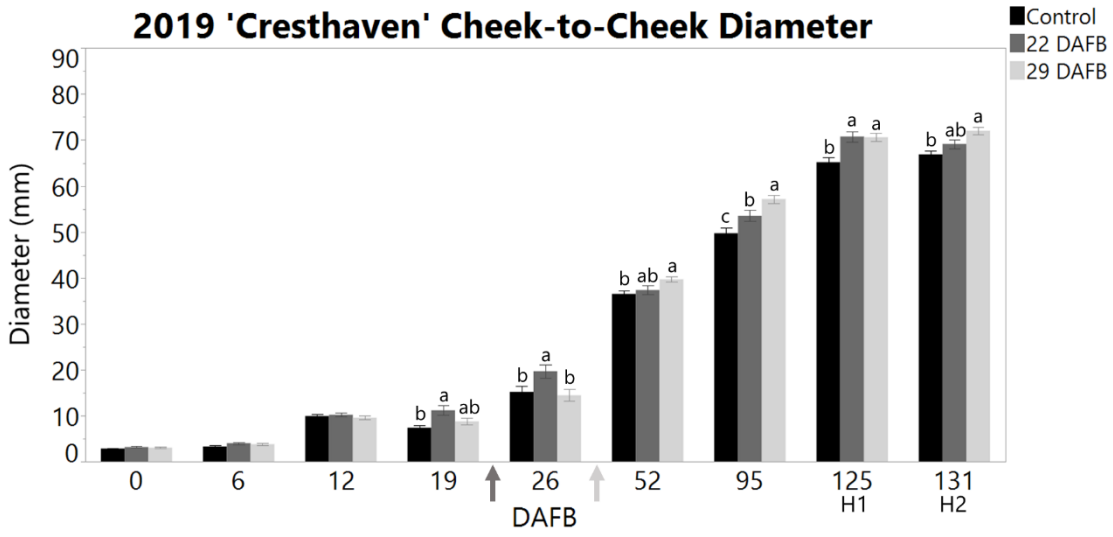
Gene	Gene Identity	% ID to ATCKX1	% positives to ATCKX 1	Forward primer 5'-3'	Reverse Primer 5'-3'
CKX 1	Prupe.1G373300.1	53.17%	71.23%	CTGTTCTTGGTGGGCTAGGG	AACTTGTGGGTGGAGGGTTG
CKX 2	Prupe.1G404300.1	43.33%	61.96%	GGACGAGGAGGACTCGTTTG	AGTAGAGCATTGATCCGGCG
CKX 3	Prupe.2G026700.1	66.10%	80.15%	CGGGCGAGAATATCGTTGGA	CCTGAACCGGGTCCTTTGAA
CKX 4	Prupe.7G052300.1	67.29%	79.63%	TTCGCCTATGTACGGCTTCC	TGTTGGTGTGGCAAGGAGTT
CKX 5	Prupe.7G208400.1	43.89%	63.53%	AACATTACCACCGCACCAGT	CAAGTGCCTCCCACTCATCA

Lonely Guy

Gene	Gene Identity	% ID to ATLOG1	% positives to ATLOG 1	Forward primer 5'-3'	Reverse Primer 5'-3'
LOG1	Prupe.1G367500.1	74.06%	87.26%	CTGGGAAGAACCCGAGCTAC	AGACAGCTTGGGAGACCTGA
LOG2	Prupe.1G409400.1	73.37%	85.43%	GACATGCATGAGCGTAAGGC	CCGAGCACATGGCTTGATGA
LOG3	Prupe.4G230500.1	29.31%	34.48%	GGGTCAGAGCTTCCAGTTCC	TGCTGGGCTTTGTCAGTAGG
LOG4	Prupe.6G005100.1	57.36%	79.70%	GGGACTCAACTGGTGGAGAG	TCAAGTGGAACAAGGGCTGT
LOG6	Prupe.6G236000.1	84.86%	91.74%	TAAACCGGTGGGATTGCTGA	GTGTAGCCAAGCTGCTCCAT
LOG7	Prupe.7G097700.1	71.21%	83.84%	CCTCTTGAATGTGGACGGCT	GCCAACACATCACGTGGTTC
LOG8	Prupe.8G174600.1	76.77%	88.38%	GGGCACAGCTTGGTATCCAT	GCTCAACCTCCCATCTTGCT

[1]Genes were numbered based on their position in the genome

Figures:



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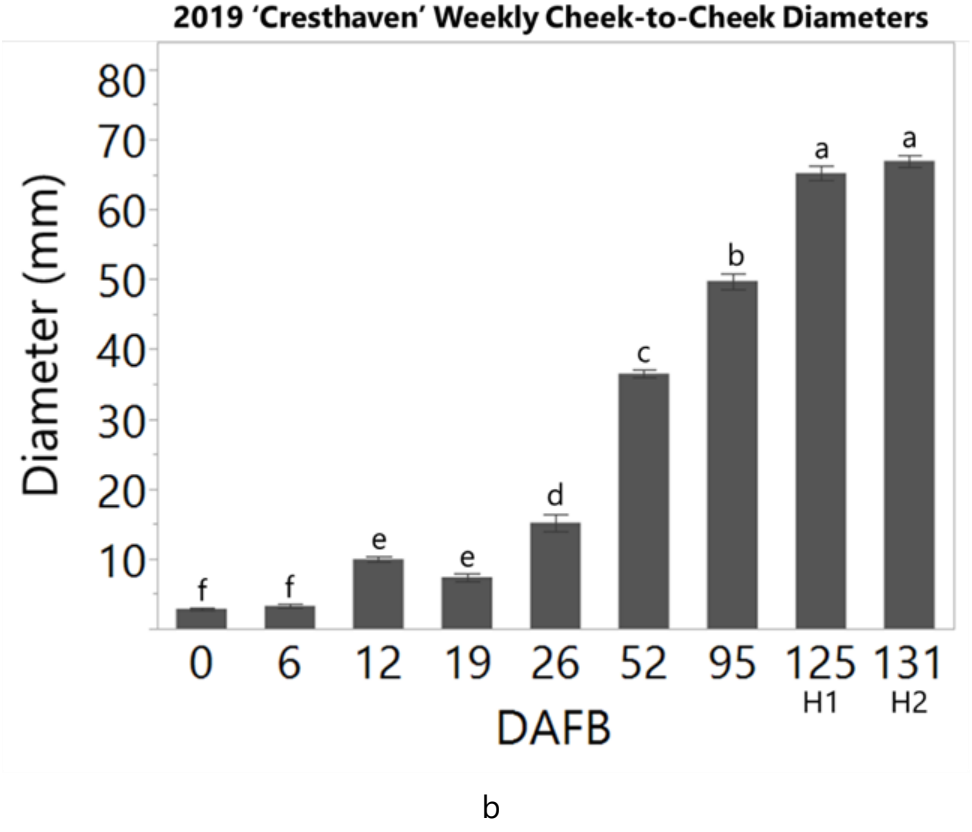
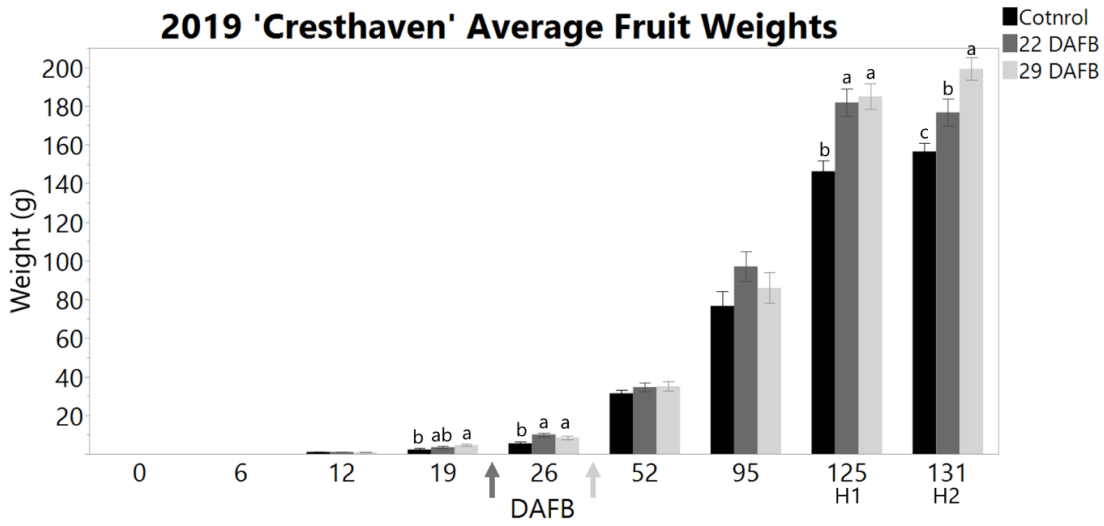


b

Figure 4.1) 2019 'Cresthaven' Cheek-to-Cheek Diameter Across Time and Treatments. a) *Differences thinning treatments. Each timepoint was analyzed separately. Arrows indicate where the two thinning treatments fell. The last two dates are harvest dates.* ***b)*** *Differences over time. Treatments showed the same trend over time so only the control is presented. The last two dates are harvest dates. Different letters indicate values that differ significantly. p≤0.05. Arrows indicate when thinning treatments were implemented. The last two time points are harvest dates.*



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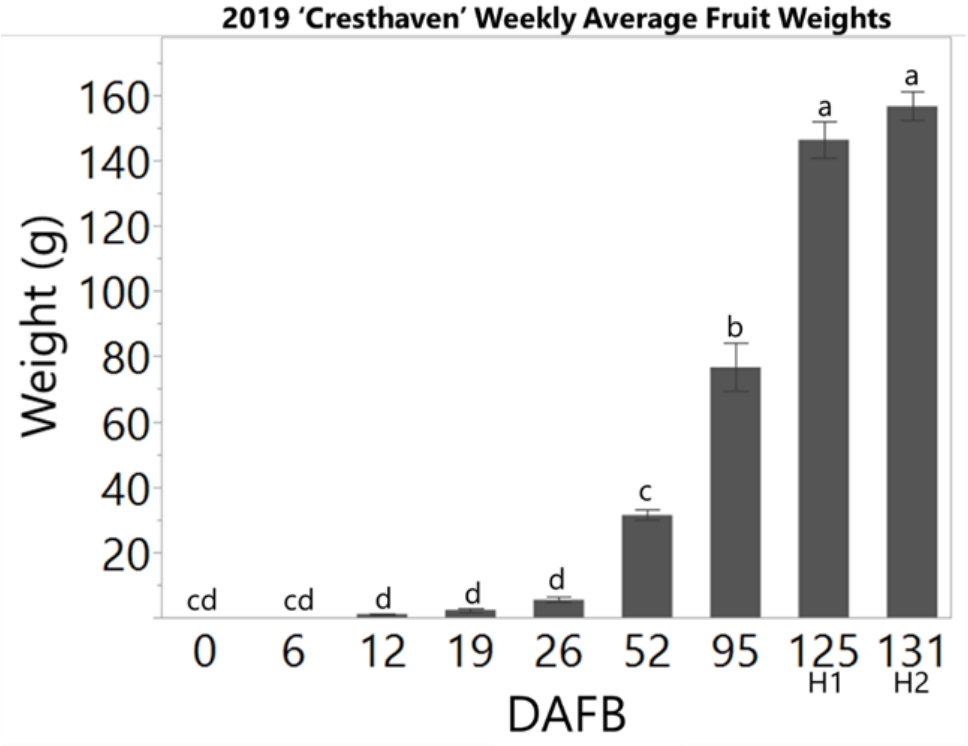

b

Figure 4.2) 2019 'Cresthaven' Fruit Weights Across Time and Treatments. a) *Differences thinning treatments. Each timepoint was analyzed separately. Arrows indicate where the two thinning treatments fell. The last two dates are harvest dates.* ***b)*** *Differences over time. Treatments showed the same pattern so only the control is presented. The last two dates are harvest dates. Different letters indicate values that differ significantly. $p \leq 0.05$. Arrows indicate when thinning treatments were implemented. The last two time points are harvest dates.*

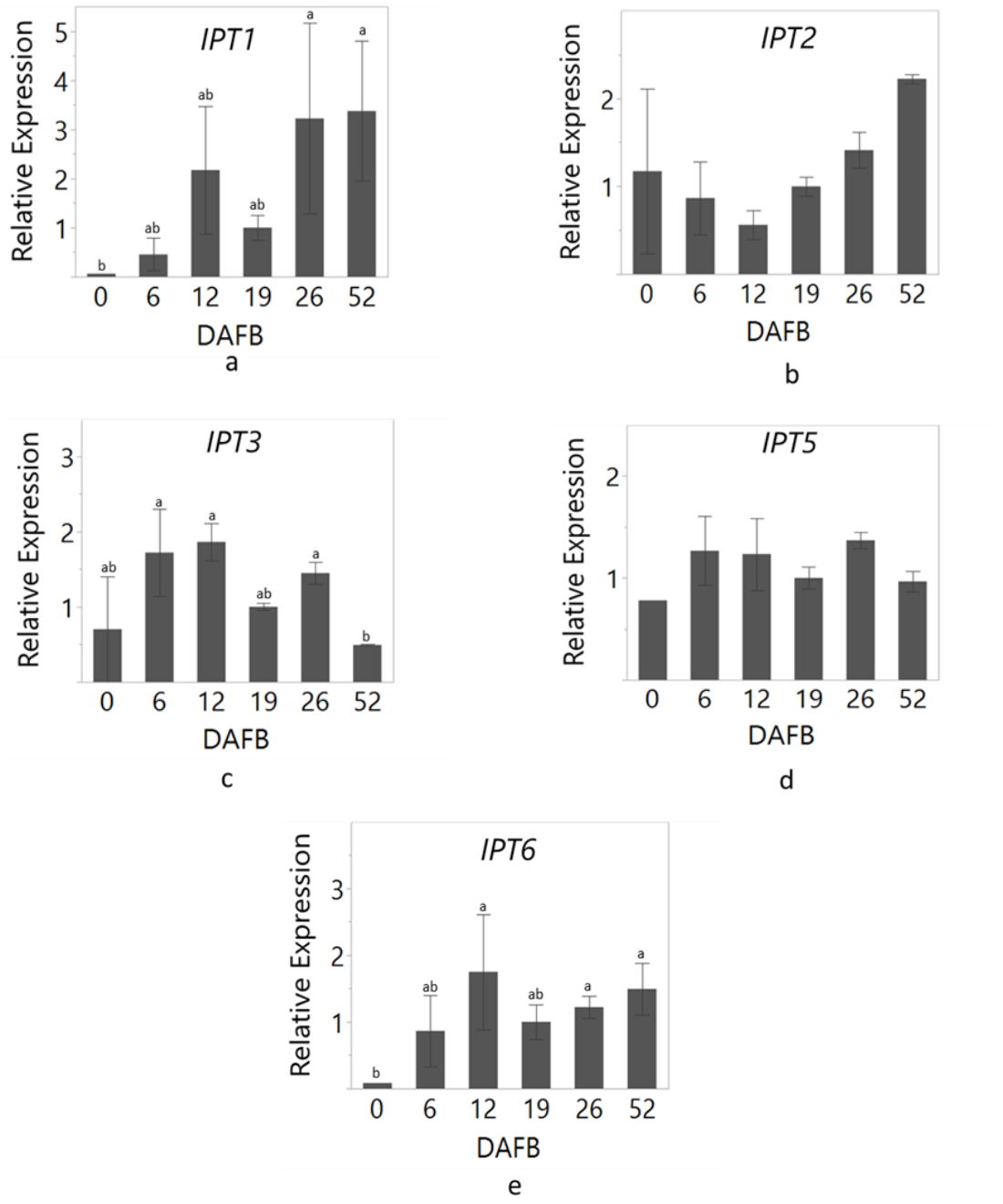

Figure 4.3) Relative expression levels of IPT throughout early fruit growth. a) *Expression of IPT1* ***b)*** *Expression of IPT2* ***c)*** *Expression of IPT3* ***d)*** Expression of IPT5 **e)** Expression of IPT6. *Only the control treatment was used for analysis. Different letters indicate significantly different values. p≤0.05*

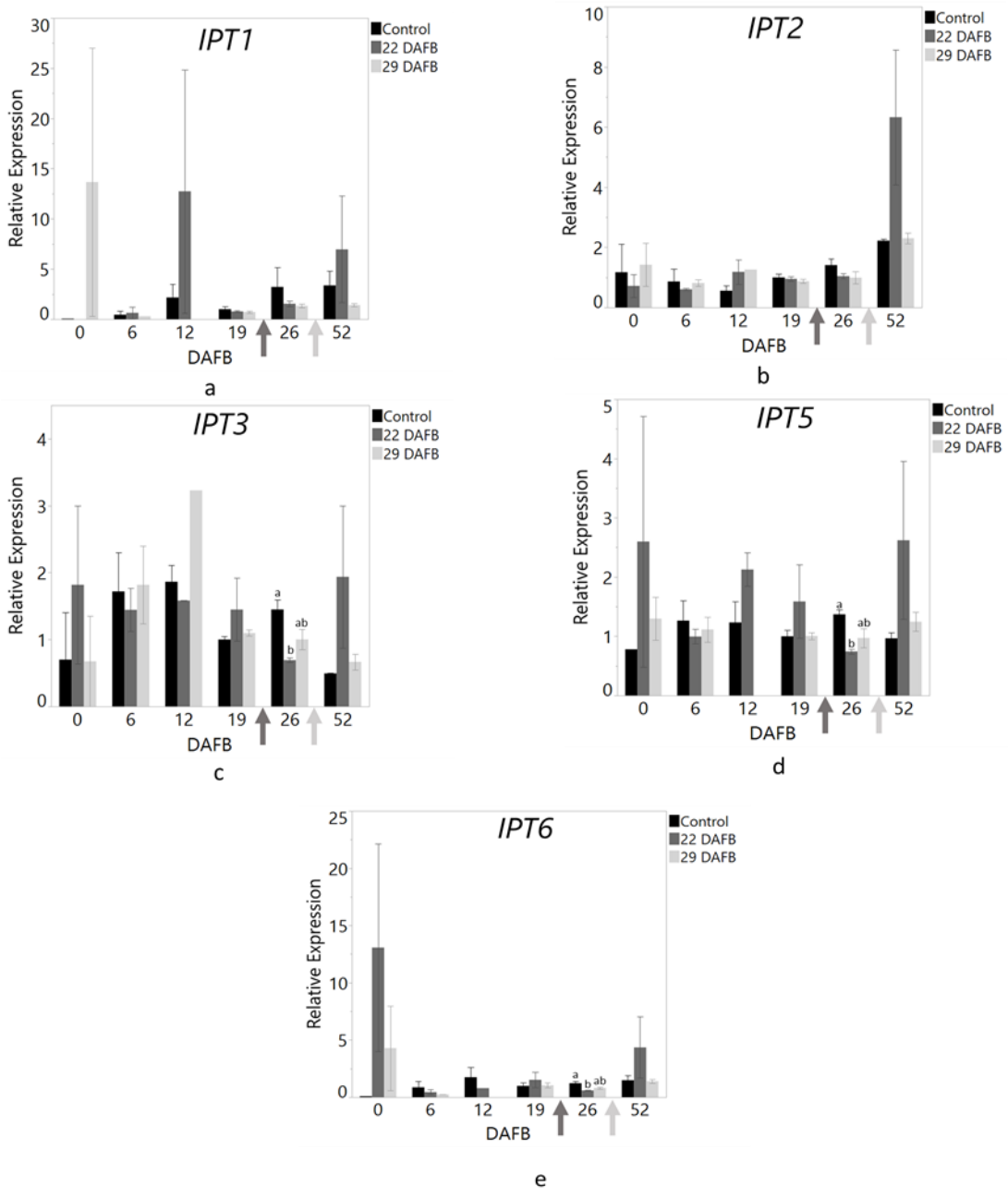

Figure 4.4) Relative expression of 5 IPT genes across thinning treatments at each time point. a) *Expression of IPT1* ***b)*** *Expression of IPT2* ***c)*** *Expression of IPT3* ***d)*** Expression of IPT5 **e)** Expression of IPT6. *Each time point was analyzed separatley. Different letters indicate significantly different values. p≤0.05. Arrows indicate when thinning treatments were implemented.*

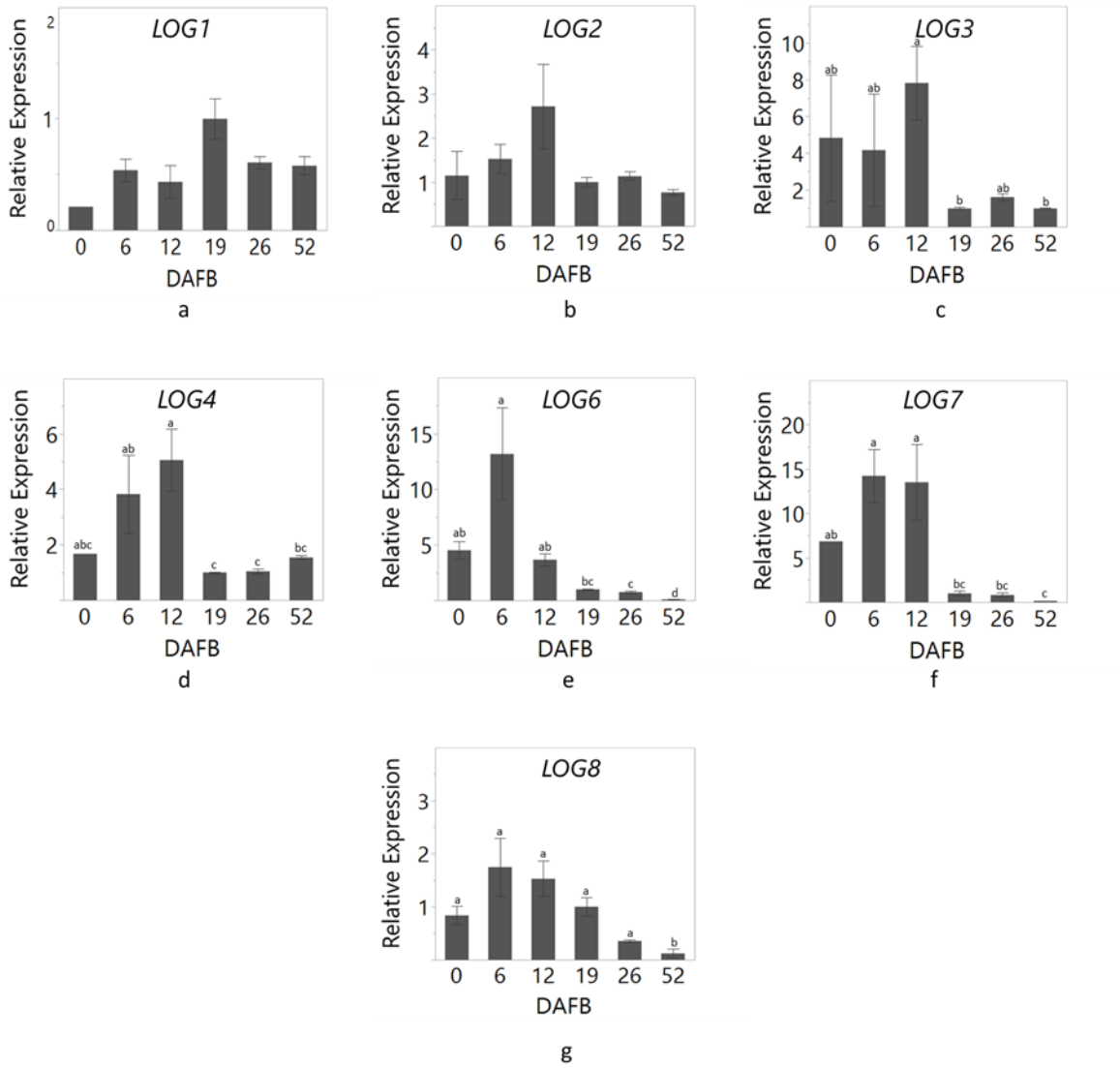

Figure 4.5) Relative expression levels of LOG throughout early fruit growth. a) *Expression of LOG1* ***b)*** *Expression of LOG2* ***c)*** *Expression of LOG3* ***d)*** Expression of LOG4 **e)** Expression of LOG6 **f)** Expression of LOG7 **g)** Expression of LOG8. *Only the control treatment was used for analysis. Different letters indicate significantly different values. p≤0.05*

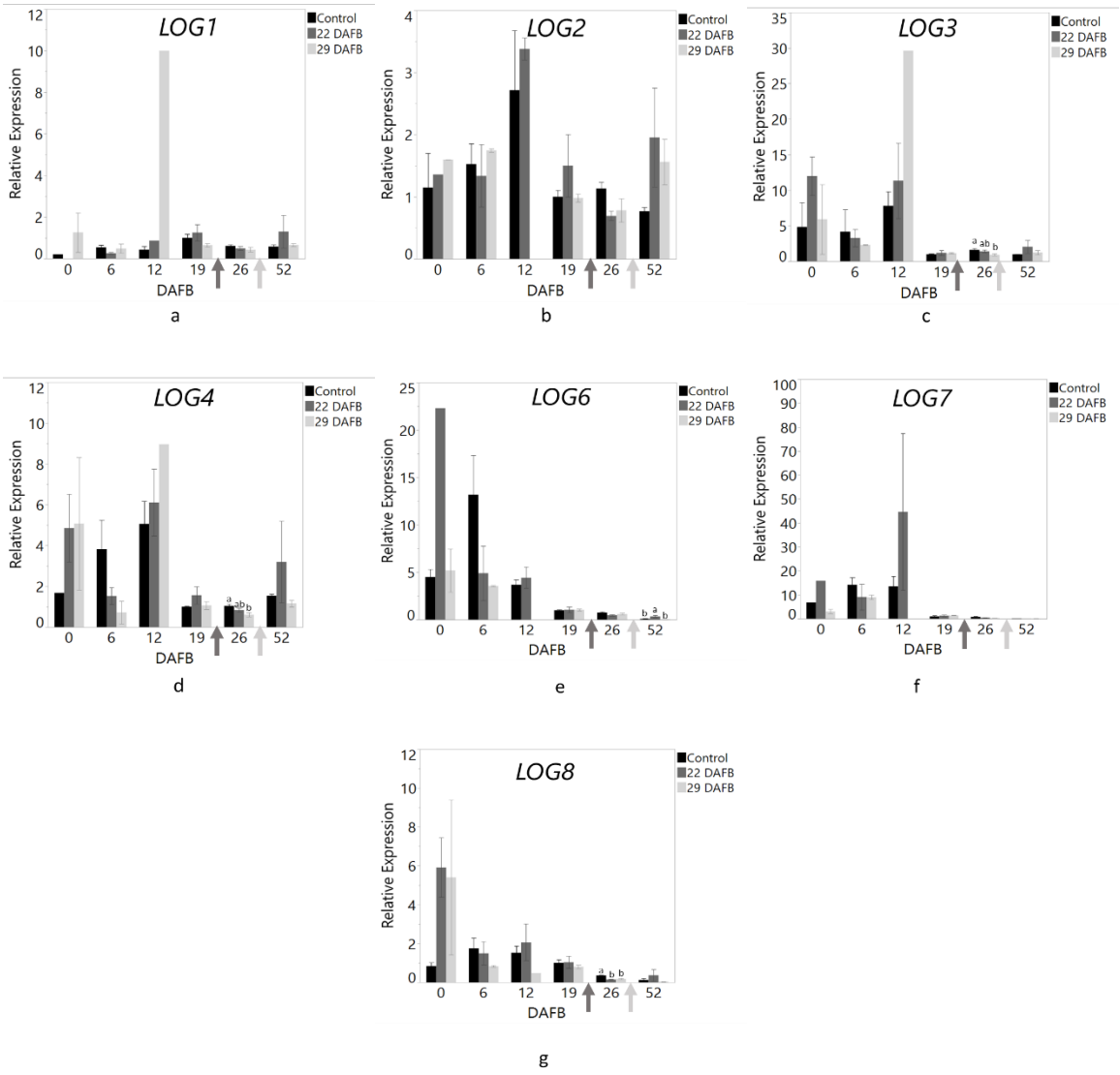

Figure 4.6) Relative Expression Levels of 7 LOG genes across thinning treatments at each time point. a) *Expression of LOG1* ***b)*** *Expression of LOG2* ***c)*** *Expression of LOG3* ***d)*** Expression of LOG4 **e)** Expression of LOG6 **f)** Expression of LOG7 **g)** Expression of LOG8. *Each time point was analyzed separatley. Different letters indicate significantly different values. p≤0.05.Arrows indicate when thinning treatments were implemented.*

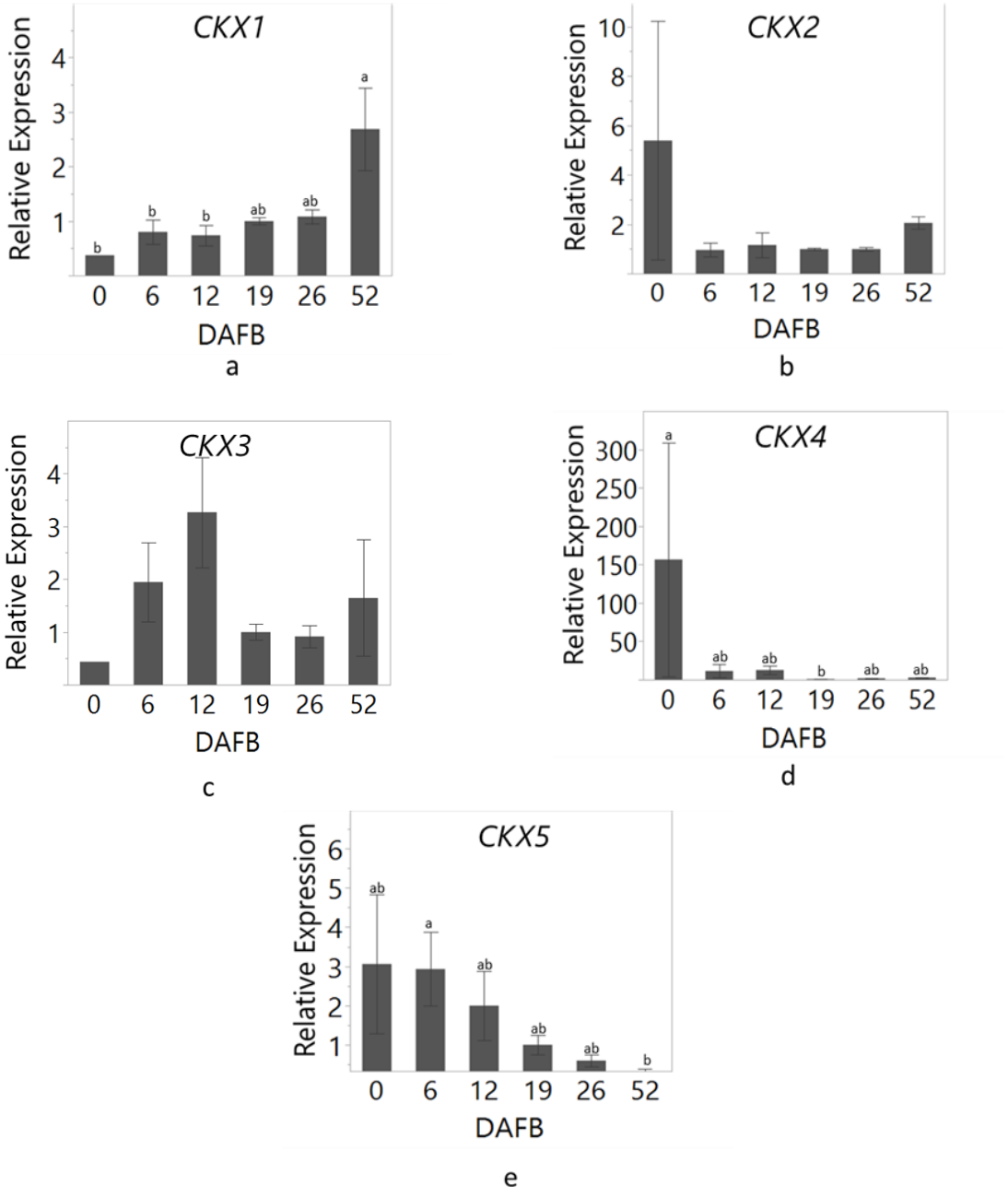

Figure 4.7) Relative expression levels of CKX throughout early fruit growth. a) *Expression of CKX1* ***b)*** *Expression of CKX2* ***c)*** *Expression of CKX3* ***d)*** Expression of CKX4 **e)** Expression of CKX5. *Only the control treatment was used for analysis. Different letters indicate significantly different values. $p \leq 0.05$.*

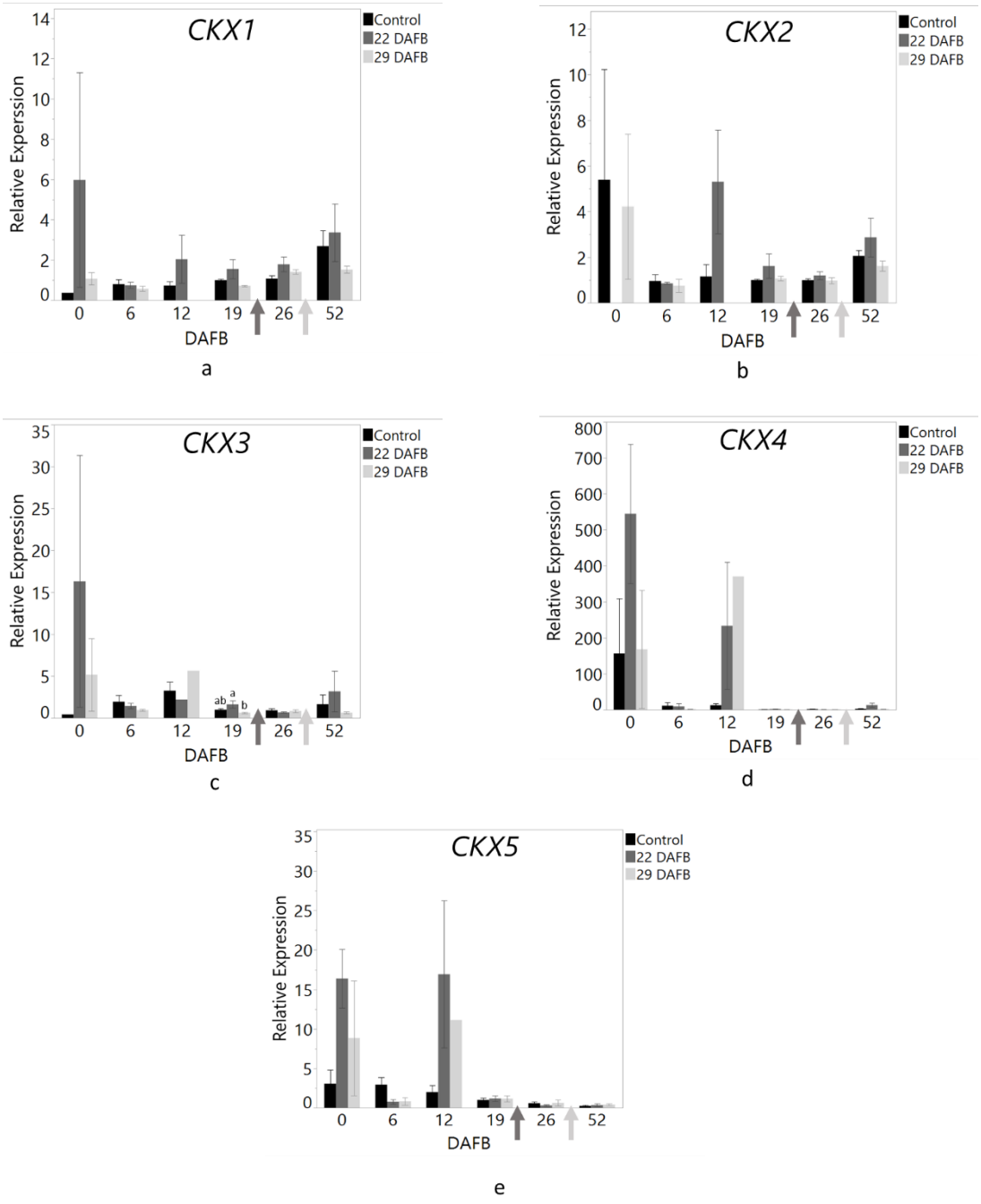

Figure 4.8) Relative Expression Levels of 5 CKX genes across thinning treatments at each time point. a) *Expression of CKX1* ***b)*** *Expression of CKX2* ***c)*** *Expression of CKX3* ***d)*** Expression of CKX4 **e)** Expression of CKX5. *Each time point was analyzed separatley. Different letters indicate significantly different values. p≤0.05.Arrows indicate when thinning treatments were implemented.*

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CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

The goal of this study was to better understand stage I growth in peaches and to determine the effects of thinning on various aspects of fruit growth, development, and quality. This was accomplished through three different projects. Firstly, by performing thinning trials were implemented at different times and intensities and the effect of thinning on crop yield and fruit quality were evaluated. Secondly, prediction models were created to determine the effect of chilling accumulation and early spring temperatures during the first 30 DAFB on fruit size and the fruit development period. Lastly, the expression of three key cytokinin biosynthesis genes were monitored throughout stage I growth.

With the thinning trials, it was apparent thinning is not a 'cure-all'. In years with inadequate chill accumulation and late freezes such as in 2017, thinning may not rescue the crop and may in fact not be necessary. In other years, thinning did have a significant effect on fruit sizes and yields; bloom thinning was associated with higher fruit weights and diameters, but reduced yields. No thinning resulted in smaller, but still commercially-sized, fruit and higher yields. The fruit thinning treatments resulted in fruit weights and crop yields midway between the bloom thinning treatments and control. For this reason, bloom thinning does not appear to be the best option for Middle Georgia. Waiting to thin until after the threat of freeze and until fruit can be selectively thinned (~28 DAFB) may be the better option. There was no superior spacing of fruit in relation to thinning

intensity. Future studies could look at thinning on a crop load basis/fruit per tree basis instead of a set spacing. The thinning schedule could also be done on a degree day or hour basis instead of a calendar one. Future studies may also need to evaluate combinations of limited bloom thinning with that of fruit thinning.

A prediction model was created for the length of the fruit development period using GDH30, the number of chill hours accumulated (CH), the difference in the number of chill hours required by a certain cultivar and the chill hours received (CHD), and the age of the tree (LY). Similarly, another model was created for the average fruit size at harvest using GDH30, CH, and CHD. These models serve to aid growers in making decisions regarding their management practices for the season. In the future, additional data will need to be collected to update and refine the models.

Expression of five *IPT* genes (cytokinin biosynthesis), 7 *LOG* genes (cytokinin activation), and 5 *CKX* genes (cytokinin degradation) were monitored throughout early peach growth and development. Thinning treatments were also implemented to determine if fruit load reduction affected cytokinin metabolism. Thinning treatments did not consistently alter expression of these genes across stage I growth. *IPT* and *CKX* showed inconsistent trends throughout early growth and development. *LOG* expression was generally higher during the first 12 DAFB. Their expression during this period suggests that cytokinin activation during stage I growth is regulated at the transcriptional level. Quantifying the amounts and concentrations of different cytokinins at each time point would offer additional insight into cytokinin metabolism during early fruit growth and development.