Final Report

Maximising yield and reducing seasonal variation

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Maximising yield and reducing seasonal variation (AV16005)

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Public summary

The Australian avocado industry aims to increase production and profitability to build a sustainable and competitive supply of Australian avocados that will meet consumer requirements. Avocado is a semi-domesticated tree crop with a theoretical yield potential of 32 t/ha. Due to the irregular bearing behavior of avocado, which is primarily driven by a high rate of flower abscission due to poor fruit set and fruitlet abscission, average annual production levels across Australia are well below the theoretical yield potential. Due to a poor understanding of the physiological basis of flower and fruitlet abscission, practical management strategies for mitigating irregular bearing have yet to be developed. Therefore, to effectively mitigate irregular bearing, an in-depth physiological understanding of flower and fruitlet abscission set is required. New knowledge on flower and fruitlet abscission can be leveraged to develop innovative methods to reduce irregular bearing in order to maximize yield and reduce seasonal variation.

Avocado fruitlet abscission has been studied for over 40 years, yet little progress has been made in understanding the developmental and physiological processes that drive this production problem. Research from AV16005 uncovered key knowledge gaps on immature fruit abscission and identified management intervention points for future research. In this project, we show that management of summer fruitlet abscission has the potential to increase tree productivity by ≥24%. Fruitlet abscission, as well as flowering and fruit set, is impacted by the carbohydrate status of the tree and correlative dominance interactions between developing fruitlets and expanding vegetative shoots. Interestingly, we show that fruitlet growth arrest is a primary step in the abscission process. Therefore, managing summer fruitlet abscission will require methods to mitigate the growth potential of fruitlets. We addressed the physiological basis of fruitlet abscission and show that fruitlet growth arrest is associated with a significant decrease in carbohydrate supply and metabolism, as well as the initiation of a sugar signaling-starvation response. Further, fruit growth arrest is associated with an alteration in hormone profiles and metabolites in the maternal tissues of the fruit. To conceptualize the summer fruit drop, an integrative model was developed, which predicts that immature fruitlet abscission is caused by a signal(s) that act to promote a quasi-maturation pathway in the maternal tissues to arrest fruit growth and induce a conserved seed dormancy signaling pathway in the seed. Further, we propose that the activation of this quasi-maturation pathway allows fruits to acquire the competence to abscise. Interestingly, preliminary studies suggest that the physiological basis of the initial fruitlet drop event is not conserved with the factors that drive summer fruitlet drop. Thus, we propose that summer fruitlet abscission involves alternations in sugar and hormone signaling that impacts carbohydrate supply and metabolism necessary for fruit growth and development. Future research aimed at determining the hierarchy of hormone signaling, will provide the basis to develop a plant growth regulator application(s) aimed at limiting summer fruitlet abscission for managing irregular bearing in avocado.

Technical summary

Avocado is a semi-domesticated fruit tree crop with a theoretical yield potential of 32 t/ha. Due to the irregular bearing nature of avocado, which is primarily driven by a high rate of flower abscission caused by poor fruit set due and fruitlet abscission, average annual production levels across Australia are well below the theoretical yield potential. Methods to sufficiently reduce flower and fruitlet abscission are currently lacking due to a limited understanding of the physiological and developmental basis of fruit set and abscission. Therefore, to effectively manage irregular bearing, an in-depth understanding of flower and fruitlet abscission is required to develop practical applications to maximize yield and reduce seasonal variation.

Research in AV16005 is focused on understanding the physiological drivers of fruitlet abscission, which negatively impacts production in coastal regions of southwestern, WA, central QLD and Tristate area along the Murray River. While avocado fruitlet abscission has been studied for over 40 years, little progress has been made in understanding this experimentally challenging problem. Here, we uncovered key knowledge gaps on fruitlet abscission and identified management intervention points for future research. Here, we show that management of summer fruitlet abscission has the potential to increase tree productivity by 24% or more, which is extremely relevant, as fruits have acquired up to 40% of dry matter. Experimental trials support the hypothesis that carbohydrate availability and correlative dominance interactions are major factors that influence fruitlet abscission. As carbohydrates and correlative dominance interactions influence growth, we showed that fruitlet growth arrest is a primary step in the abscission process. Therefore, management intervention must target fruitlet growth arrest in order to limit immature fruit abscission. We investigated possible management strategies that could be used to limit fruitlet growth arrest and abscission. Results indicate that a decrease in carbohydrate supply and metabolism in the seed coat is associated with fruit growth arrest. Further, a sugar signaling – starvation response appears to be a casual determinant associated with fruit growth arrest. In addition, the suppression of auxin and increase in ethylene, jasmonic acid and abscisic acid in the maternal tissues likely functions to suppress growth. Based on studies in apple, it was proposed that immature fruitlet abscission was caused by nutritive deficiency signaling event that promotes developmental arrest, embryo abortion and abscission. Based on our studies, we propose that a quasi-maturation-like pathway is induced in the maternal tissues which functions in part to suppress growth but also allow the arresting fruitlets to acquire abscission competence. The activation of the quasi-maturation pathway in the maternal tissues also appears to activate a conserve dormancy signaling pathway to promotes embryo arrest. Thus, taken together, managing the activation of the quasi-maturation pathway will be critical for mitigating summer fruitlet abscission in avocado and possibly other tree crops. Future research aimed at determining the hierarchy of hormone signaling, will provide the basis to develop a plant growth regulator application(s) aimed at suppressing the quasi-maturation pathway(s) induced in the maternal tissues in order to limit summer fruit abscission for managing irregular bearing in avocado, as well as other tree crops.

Keywords

Horticulture, Avocado, Irregular Bearing, Fruit Abscission, Fruit Development, Seed Development

Introduction

The Australian Avocado Industry is aiming to improve production and profitability in order to build a sustainable and competitive supply of avocados that meet consumer requirements. However, annual avocado yields are well below the theoretical production potential due to irregular bearing, which is a major challenge for maximizing yields, stabilizing supply and maintaining market development efforts.

Avocado displays an irregular bearing habit that is heavily influenced by a high rate of immature fruit abscission (Slabbert, 1981; Davenport and Manners, 1982; Garner and Lovatt, 2008, 2016). Seasonal trends in fruit abscission appear to vary in which one to three waves of abscission occur over the course of the growing season (Adato and Gazit, 1977; Slabbert, 1981; Davenport and Manners, 1982; Perez et al., 1988; Garner and Lovatt, 2008, 2016). In California, the primary fruit drop event peaks approximately four weeks after flowering and the rate of abscission gradually declines throughout the growing season (Garner and Lovatt, 2008). As the early fruit abscission coincides with the outgrowth of the spring flush, it has been postulated that expansion of this vegetative flush diverts carbohydrates away from the immature fruits, causing them to drop (Salazar-García et al., 2013). It has also been proposed that the initiation of the summer vegetative flush may also be contributing factor that mediates fruit drop during the summer months (Whiley and Wolstenholme, 1990). Therefore, dominance interactions between expanding vegetative shoots and developing fruits appears to be a major factor that influences avocado fruit abscission. In addition, avocado fruit drop is likely influenced by dominance interaction among fruitlets, in which fruits with a high growth potential cause the abscission of fruits with a low growth potential, as this interaction is known to induce fruit abscission in other tree crops (Bangerth, 1989). While climate factors, such as high and low temperatures combined with low humidity, increase the rate of fruit abscission, results from Garner and Lovatt, 2008 indicate that fruit abscission is primarily mediated by physiological process(es).

Avocado fruit abscission has been studied for over 40 years, yet little progress has been made to identify and characterize the physiological drivers of fruit abscission (Adato and Gazit, 1977; Davenport, 1986; Garner and Lovatt, 2008, 2016). While these studies collectively identified differences in size, integrity and hormones produced between persisting and abscised fruits, a developmental and physiological understanding of fruit drop is lacking. In order to effectively manage fruit abscission, the physiological drivers underpinning the initial step(s) in the fruit abscission process must be addressed before this critical knowledge platform can be leveraged to develop new innovative management tools to mitigate irregular bearing.

In the AV16005 project, a desktop review was developed as a means to develop hypotheses and focus research aims in order to deliver a developmental and physiological understanding of fruit abscission. The second objective addressed the hypothesis that trees adjust crop load in response to carbohydrate status. The developmental process of fruit abscission was addressed in the third objective in order to better understand how fruit abscission can be managed. The last object addressed the physiological drivers under pinning the initial developmental events of fruit abscission in order to build an integrated model of fruit abscission. Based on the outputs and outcomes of this project, recommendations are discussed for future R&D investment.

Methodology

Target Audience

The target audience for this project includes growers and farm managers, as well as consultants.

Key Research Activities

Project activity was subdivided into seven activities addressing fruit abscission and one activity addressing relationship between tree carbohydrate status with flowering and fruit set.

Activity 1: Desktop review on reproductive development process in horticulture tree crops

The first activity involved the development of a desktop review on concepts and knowledge of reproductive development in horticultural cropping systems, including avocado. In this report, processes associated with fruit set, including floral bud initiation, pollination and fertilization, as well as fruit development and abscission were reviewed. To develop a meaningful review, current knowledge derived from model plant systems was integrated with information derived from horticulture tree crops to give the reader a picture of the basic understanding of these reproductive developmental processes. Following each of these topics, the current understanding of avocado reproductive process was presented followed by knowledge gaps. External reviewers were identified and they validated the review. Comments from each of the reviewers, as well as the Project Reference Group Committee, was incorporated into the review to develop a final desktop review.

Activity 2: Characterization of the seasonal patterns of fruit abscission

The first step in the project was to characterize the seasonal patterns of immature fruit abscission at orchard sites in which this irregular bearing component impacts yield. This activity is critical for identifying a period in the growing season for management intervention. Trials were initiated in a coastal region of Western Australia and the Riverland in South Australia. After selecting trees with uniform level of flowering, 100 and 240 fruits in total were tagged in three and five trees, respectively. As seed development is key for immature fruit development and a majority of immature fruits that abscise in the first week or two of the initial fruit drop event are unfertilized (Garner and Lovatt, 2016), trials were initiated when the average diameter of fruits was approximately $\geq$12 mm to ensure all fruits are fertilized. At regular time intervals over the course of the growing season, the number of tagged fruits that abscised was scored. In addition, the diameter of the tagged fruits was measured at regular time intervals to calculate growth rates over the course of the trials.

Activity 3: Relationship between tree carbohydrate status and fruit abscission

It has been hypothesized that trees adjust their crop load based on carbohydrate availability (Goldschmidt, 1999; Sawicki et al., 2015). Given the hypothesis that effective tree carbohydrate management is a pathway for increasing yields (Whiley and Wolstenholme, 1990), the project addressed the relationship between tree carbohydrate status and fruit abscission. Given that tools to estimate carbohydrate status throughout the tree under orchard management conditions are lacking, two trial systems predicted to negatively impact tree carbohydrate status were evaluated for their impact on fruit abscission. The two inducible fruit abscission systems identified for this activity were defoliation and shading. The overall goal of these trials was to determine whether the decline in tree carbohydrate status was associated with the onset of fruit abscission. Further, a treatment(s) causing a significant wave fruit abscission event would be leveraged to characterize the early and late developmental and physiological processes of immature fruit abscission. For this activity trees, were defoliated by removing all new flushes of vegetative growth. To negatively impact light levels in order to reduce CO_2 assimilation, trees were shaded using a cloth that blocked 77% of the sunlight. Light intensity in untreated and shaded trees was determined on a clear day at noon 21-days after the trial was initiated using the Quantum Flux Meter (MQ-500 Apogee Instruments, USA). Defoliated and shading trials were initiated in a coastal region of Western Australia and the Riverland in South Australia. Approximately, 4-6 trees were treated in each of these trials. In each tree, 40-50 fruits were tagged, and at regular time intervals, fruit abscission was scored and the diameter of fruits were measured for calculating the impact of these treatments on fruit growth and abscission. Stem carbohydrate levels were determined by harvesting 30 mm segments, 12 mm in width, from the summer/fall flush initiated in the previous season. Stem tissues were frozen on dry ice before transporting samples to Adelaide for carbohydrate analysis (see below).

Activity 4: Characterization of the fruit abscission developmental process

Studies in apple and lichee show that fruits undergo growth cessation prior to abscission (Greene et al., 2013; Zhao and Li, 2020). In avocado, a trial was performed in which the average diameter of persisting and abscised fruits was determined at four time points in the growing season (Garner and Lovatt, 2016). Results showed that the average size of abscised fruits were statistically smaller than the average size of persisting fruits at the peak of the initial fruit drop event in Hass. Further, in Fuerte, experimental evidence suggests that smaller sized fruits have a higher tendency to abscise compared to larger sized fruits, after the first wave of fruit drop (Perez et al., 1988). Therefore, we hypothesized that avocado fruits undergo growth cessation prior to abscission. This hypothesis was tested in a number of defoliation and natural abscission trials, as well as one shading trial, by tagging fruits (20-40 fruits/tree) and measuring the diameter of fruits at regular time intervals using hand-held digital calipers or digital fruit dendrometers. Subsequently, the fruit diameter measurements were used to calculate daily growth rates in order to determine if fruit growth arrest is associated with the abscission process.

Activity 5: Identification of physiological drivers of summer fruit growth arrest and abscission

To identify the physiological drivers of fruit growth arrest and abscission, we tested a number of hypotheses to explain summer fruit abscission, some of which, could have immediate outcomes for management intervention.

Is a change in fruit water potential associated with summer fruit growth arrest and abscission?

Irrigation strategies have been proposed as a means to increase the reproductive potential of a tree, as water stress conditions reduce yield (Silber et al., 2012). Therefore, to address if fruit growth arrest and abscission results from a decrease in water availability, the water potential between fruits with a normal growth rate and fruits undergoing arrest was measured. In this procedure, a normal growing or arresting fruit with a peduncle, as well as a leaf with a petiole, was removed from a tree, individually wrapped in a polyethylene bag and midday water potential ($\Psi_{fruit/leaf}$) was measured using a Schölander pressure chamber (Soilmoisture Corp, USA). Nine to ten biological replicates were used to calculate the average water potential for normal growing and arresting fruits, as well as mature leaves. Analysis of variance and Tukey's honest significant difference analysis were performed to determine if the difference in the means were statistically significant.

Is a deficiency in N or essential mineral elements associated with summer fruit growth arrest and abscission?

Mineral element(s) deficiency can negatively impact reproductive processes in avocado (Boldingh et al., 2016). If a deficiency in a mineral element is associated with summer fruit growth arrest and abscission, a plant nutrition strategy could be developed to mitigate irregular bearing. To address this hypothesis, mineral element profiles were compared between normal growing and arresting fruits. In this procedure, the seed coat, embryo and pericarp were dissected from normal growing and arresting fruits, as well as the pedicel, and immediately frozen on dry ice before storing tissues at -20^{0}C. After shipping samples to Adelaide on dry-ice, the fruit and pedicel samples were freeze-dried and ground to fine powder using a CryoMill-cryogenic grinder (Retsch, Germany). Total N content was analyzed by combustion of a 10 mg sample in a Europa 20-20 Stable Isotope Ratio Mass-Spectrometer (IRMS; Sercon Ltd, UK). Mineral elements were determined by emission spectroscopy using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Varian Vista Pro, UK) using 250 mg of freeze-dried material from each sample. Eight biological replicates were used for each sample to calculate the average % dry mass (DM) or mg/g DM. Two-tailed Student's t-test was performed to determine if the difference in the means were statistically significant.

Is a change in carbohydrate status in fruit tissues associated with summer fruit growth arrest and abscission?

Experimental studies indicate that fruit abscission is mediated by reduced carbohydrate availability in citrus (Mehouachi et al., 1995; Gómez-Cadenas et al., 2000) and apple (Botton et al., 2011). Given that carbohydrates are key substances require for growth, we hypothesized that fruit growth arrest could also be mediated by a decrease in the carbohydrate status of the seed and possibly the seed coat. To validate this hypothesis, the levels of sugar metabolites, sucrose, glucose, fructose, perseitol and mannoheptulose, as well as starch, were compared between arresting and normal growing fruits harvested from untreated and defoliated trees. Normal growing and arresting fruits harvested from untreated control trees were used to assess natural fruit abscission. Seven days after defoliation, fruits with a relatively normal growth rate and arresting fruits were harvested and both sets of samples were used to evaluate the early and late stages of fruit growth cessation,

respectively. After fruits were harvested, the embryo, seed coat and pericarp were dissected and immediately frozen on dry ice. Pedicel samples were also collected and frozen on dry ice. All fruit and pedicel samples were stored at -20^{0}C before shipment to Adelaide for sugar, starch and transcriptome analyses.

Quantification of sugars and starch from fruit, pedicel and stem tissues

Extraction and quantification of sugars and starch from the seed coat, pericarp, embryo and pedicel, as well as stems, was performed as previously described (Liu et al., 1999; Goetz et al., 2021). Briefly, fruit, pedicel and stem tissues were ground to a fine powder in liquid nitrogen using the CryoMill-cryogenic grinder (Retsch, Germany). 100-200 mg from each sample was freeze-dried for 20 - 24 h and the dry mass (DM) was determined. Soluble sugars were extracted three times with 80% ethanol at 80^{0}C for 30 min. Sorbitol was added to the extraction buffer (80% EtOH) at 100 ug/ml and used as an internal standard for High-Performance Liquid Chromatography (HPLC) analysis. The three soluble extracts were pooled and 1.0 ml of the combined extract was dried at 55^{0}C for 2 h using a Gene miVac Quattro (SP Industries, USA), while the insoluble pellet was used to measure starch (see below). The dried extract was redissolved in 50 µl of sterile water and 20 µl was analyzed on an HPLC system (Agilent 1200, USA) using the Sugar-Pak cation-exchange column to separate sucrose, perseitol, mannoheptulose, glucose and fructose (Waters Corp, AUS). Sugars were identified and quantified using the Agilent Technologies 1200 G1362A infinity refractive index detector (Agilent, USA) by comparing peak retention times with the sugar standards. Starch content was determined by using the Megazyme Total Starch Assay Kit (Megazyme, Ireland) as described by the manufacturer. At least five biological replicates per tissue type were used to calculate the average starch content (g/100g of DM) for normal and arresting fruit samples, as well as stem samples harvested from control, defoliated and shaded trees. Two-tailed Student's t-test or analysis of variance and Tukey's honest significant difference analysis were performed to determine if the difference in the means were statistically significant.

Activity 6: Hormone profiling from avocado fruit tissues

Hormones were quantified from fruit tissues ground in liquid nitrogen and extracted with ice-cold 50% (v/v) aqueous acetonitrile containing the appropriate hormone standards. After extraction, the samples were cleared and the supernatant was loaded onto a pre-treated HLB-SPE cartridge (30 mg, Waters, Wexford, Ireland) to remove contaminants. After drying, the samples were resuspended in 30% (*v*/*v*) acetonitrile and analysed by liquid chromatograpehy with tandem mass spectroscopy (LC-MS/MS) as previously described (Böttcher et al., 2010; Clayton-Cuch et al., 2021).

Activity 7: Transcriptome analysis

To identify candidate hormones and developmental pathways implicated in fruit growth arrest, a transcriptome analysis was performed, in which the global gene expression profiles between arresting and normal growing fruits were compared. Five biological replicates were used for the transcriptome analysis derived from normal growing/control and arresting fruit samples. Natural fruit abscission was examined by harvesting normal growing and arresting fruits from untreated control trees. Normal growing and arresting fruits were harvested from defoliated trees seven-days after treatment and compared to normal growing fruits from control trees. Normal growing and arresting fruits harvested from defoliated trees were used to assess gene expression profiles associated with early and late stages of fruit growth cessation, respectively. In this experiment, the seed coat, pericarp, embryo and pedicel, derived from normal growing/control and arresting fruit was ground to a fine powder in liquid nitrogen using the CryoMill-cryogenic grinder (Retsch, Germany). Total RNA was extracted from 100 mg of fruit and pedicel tissues using the Spectrum Plant Total RNA kit (SigmaAldrich/Merck). RNA samples were subsequently treated with DNAse I and purified using the TURBO DNA-free kit (ThermoFisher). Library preparation and RNA Sequencing were performed by the Australian Genome Research Facility Ltd (AGRF) in Melbourne according to their standard protocol. Raw sequencing data was processed using a standard bioinformatic procedure at the Stanford University Center of Genomics and California Polytechnic State University in collaboration with Drs Ramesh Nair and Jean Davidson, respectively. The processed RNA sequenced reads were mapped to the avocado reference genome to identify genes and their respective sequences that were subsequently annotated against the TAIR10 Arabidopsis database using the Basic Local Alignment Search Tool X (BLASTX).

RNA-Seq analysis was performed using the Bioconductor R (v. 4.0.2) package DESeq2 v.1.28.1 (Love et al., 2014). The DESeq2 package, based on the negative binomial distribution, was used to normalize the RNA-seq data and to identify

differentially expressed genes between normal growing/control and arresting fruits that abscised naturally or in response to defoliation with an adjusted *p*-value of 0.05 (Likelihood Ratio Test, sample size n = 5) and a > 2-fold-change in expression. The lists of differentially expressed genes from arresting fruit tissues were queried for genes involved in hormone biosynthesis, transport, deactivation and signaling. In addition, the lists of differentially expressed genes were also queried for developmental regulators known to regulate growth and developmental transitions through hormone and/or sugar signaling.

Activity 8: Is a change in fruit carbohydrate status associated with the initial fruit drop event?

This activity addressed whether the initial fruit drop event was associated with changes in carbohydrate availability and metabolism during fruit growth arrest, as shown for summer fruit abscission. In this trial, normal growing and arresting fruits were harvested during the initial fruit drop event when the average diameter of fruits was ~12 mm. Fruits were harvested at this diameter to ensure that they were fertilized. Fruits were dissected into seed coat, pericarp and embryo and frozen immediately on dry ice. Pedicel samples from harvested fruits were also isolated and frozen on dry ice. Samples were stored at -20^{0}C, before being shipped to Adelaide. A preliminary transcriptome analysis was performed with all fruit samples. As all of the embryo sample was used for the transcriptome analysis, carbohydrate analysis was performed only with seed coat, pericarp and pedicel samples. Transcriptome, carbohydrate and statistical analyses were performed with five biological replicates as described above.

Activity 9: Understanding the relationship between flowering intensity, fruit set, fruit abscission and tree carbohydrate status

Irregular bearing caused by fruit abscission and/or poor fruit set is a major challenge for the Australian Avocado Industry. Research from AV16005 provides strong evidence that tree carbohydrate status, as well as dominance interactions, influences fruit abscission. As fluctuations in stem starch levels correlates with reproductive and vegetative growth throughout the season, we investigated whether changes in tree carbohydrate status influences flowering and fruit set. To score flowering, fruit set and fruit abscission, 10 shoots per tree were tagged. Flowering was evaluated by scoring the number of floral buds per shoot at peak flowering. In addition, the level of flowering was estimated using visual scale from 1 to 5, in which 1 and 5 represented low and high flowering, respectively. To estimate fruit set, the number of fruitlets per shoot at a late stage of flowering was scored. Fruit abscission was evaluated shortly after the initial fruit drop event by scoring the number of fruits per shoot. Trees defoliated early (DEF1) and mid-season (DEF2), as well as trees subjected to severe drought stress (DR) in mid-season, by turning off the irrigation for four weeks, were used in this trial. While ≥97% fruits abscised in defoliated trees, ~40% of fruits dropped in response to drought stress. Untreated trees, which carried a crop, were used as a control. We predicted that all of these treatments would impact winter starch accumulation creating sets of trees with different amounts of stored carbohydrates, which would allow us to assess how differences in tree carbohydrate status effects flowering, fruit set and early fruit retention. At bud burst, peak flowering and shortly after the initial fruit drop event, stems derived from the previous seasons flush were isolated from each of the treated and control trees. Harvested stems were approximately 30 mm in length and 12 mm in diameter. Stems were frozen on dry ice and stored at -20^{0}C before being shipped to Adelaide on dry ice. Carbohydrate analysis was performed as described above. Analysis of variance and Tukey's honest significant difference analysis were performed to determine if the difference in the means were statistically significant.

Extension Activities

To disseminate research activities and engage with the Australian Avocado Industry, the AV16005 project was linked to the avocado extension and communication projects, AS17005 and AV18003, respectively. Extension activities were performed through invited annual presentations at Regional Avocado forums in Western Australia, South Australia, Victoria and Queensland, not only to communicate findings from AV16005 to industry but presentations were also given on limiting factors of fruit set, as well as plant growth regulators (see appendix E). In addition, two industry articles were published in Talking Avocados (AV18003; see appendix C).

Results and discussion

Results

Desktop review on avocado reproductive development

A desktop study reviewing key reproductive developmental processes including floral bud initiation, pollination and fertilization, fruit set, fruit development and abscission. This comprehensive review integrated basic reproductive derived from model plants with a current understanding of reproduction in horticultural tree crops including avocado. In this review, key knowledge gaps were identified for the underlying developmental and physiological processes involved in floral bud initiation, fruit set, development and abscission. Moreover, knowledge gaps identified for fruit abscission laid the groundwork for addressing this irregular bearing driver in avocado. The stage 1 review was 'validated' by Drs Inaki Hormaza (IHSM La Mayora – CSIC – University of Malaga, Spain) and Alon Samach (Institute for Plant Sciences and Genetics in Agriculture, Rehovot, Israel). Comments from Drs Hormaza and Samach were incorporated into the final version, which also includes input from Byron de Kock (Hort Innovation), Simon Newett (QDAF, AV17005) and Neil Delroy (Jasper Farms). Please refer to Appendix A for the review and letters of support from Drs Hormaza and Samach.

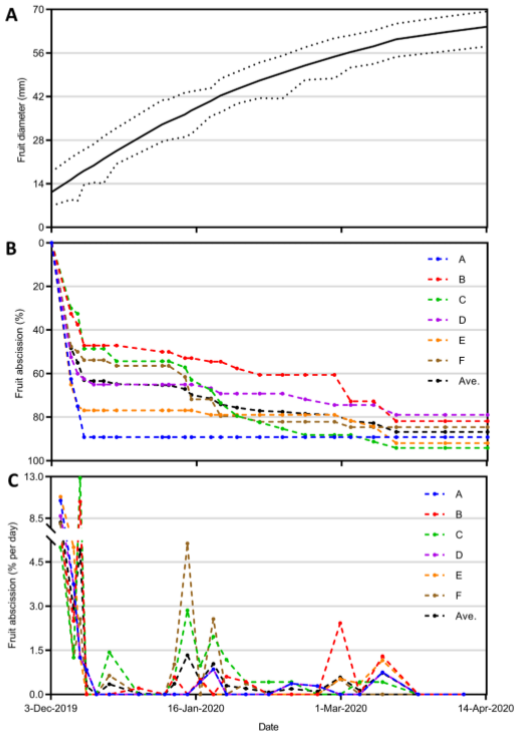

Figure 1. Seasonal patterns of natural fruit abscission in WA

(A) The diameter of fruits measured over the course of the natural abscission trial I using hand-held digital calipers. Values are the mean (line) and range (dots) of 240 fruits distributed on six different trees. (B) Percent of natural fruit abscission. This graph includes abscission in each of the six trees used in this evaluation, as well as the average percentage of fruit abscission. (C) Natural fruit abscission displayed as percent of abscission per day. Abscission in each of the six trees is displayed, as well as the average percent of fruit abscission per day.

Seasonal patterns of fruit abscission

Fruit abscission is a major driver of irregular bearing, and limits production major growing regions including coastal regions of WA, central QLD and in hot summer climates in the Tristate region. To identify a fruit abscission management intervention point(s), the seasonal pattern of fruit drop was evaluated at a coastal site in WA, as well as an orchard in the Riverland, SA. At the field site in WA, results showed that a significant wave of fruit abscission occurred at the start of the trial when the average diameter of the fruits was 12 mm (Figure 1A). During this initial wave of fruit abscission, trees dropped on average 63% of the crop that set (Figure 1B), with an average abscission rate of 4-8 fruits per day (Figure 1C). After this initial fruit abscission event, an additional 24% of fruits dropped from mid-December to the beginning of April

(Figure 1B). Based on the pattern of summer fruit drop in individual trees, a small peak of fruit drop occurred in mid-January in two trees, while another peak of fruit drop occurred at the beginning of March in two other trees (Figure 1C).

At the field site in SA, summer fruit abscission was monitored when the average diameter of fruits at the beginning and end of the trial was approximately 33 and 62 mm, respectively (Figure 2A). It should be noted that this trial was initiated after the initial fruit drop event. Results from this study showed that the average rate of fruit drop at the end of the trial was 46% (Figure 2B). While the percent of fruit drop per day was variable across the trees, a small peak of immature fruit abscission occurred in mid-January (1-1.4% of fruits per day; Figure 2C), which is similar in timing as the first summer fruit drop in WA the previous year.

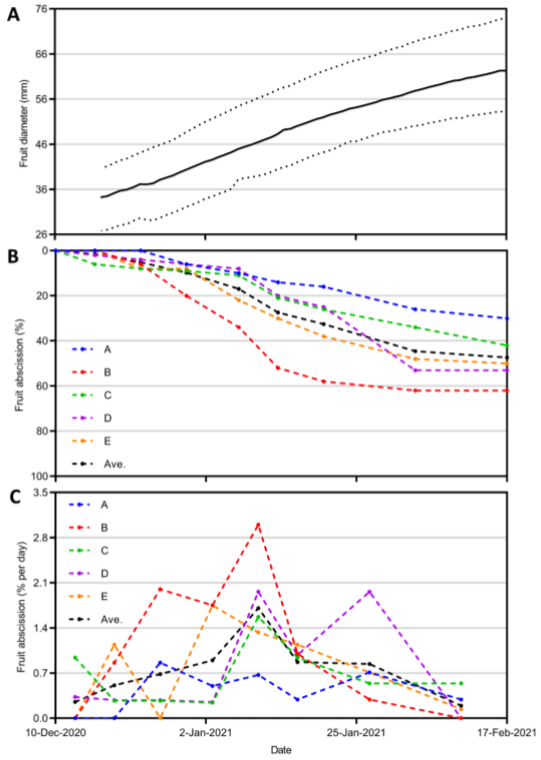

Figure 2. Seasonal patterns of natural fruit abscission in SA
(A) The diameter of fruits measured over the course of the natural abscission trial II using digital fruit dendrometers. Values are the mean (line) and range (dots) of 36 fruits distributed on five different trees. (B) Percent of natural fruit abscission. This graph includes abscission in each of the five trees used in this trial, as well as the average percentage of fruit abscission. (C) Natural fruit abscission displayed as percent of abscission per day. Abscission in each of the five trees is displayed, as well as the average percent of fruit abscission per day.

Association between tree carbohydrate status and fruit abscission

The association between tree carbohydrate status and fruit abscission was investigated in defoliation and shading trials. These trials were utilized to determine if a reduction in tree carbohydrate status was associated with the onset of fruit abscission.

Defoliation of trees and shoots is an effective method to induce fruit abscission in apple, citrus and lichee (Llewelyn, 1968; Mehouachi et al., 1995; Zhao and Li, 2020). To evaluate the effectiveness of defoliation in avocado, newly initiated vegetative shoots were removed (Figure 3A and B). Fruit drop was monitored by tagging fruits in control and defoliated trees and scoring whether each fruit persisted or abscised at regular time intervals over six-weeks. Results showed that fruit abscission was induced in defoliated trees approximately 10-days after the vegetative shoots were removed (Figure 3C). A sharp increase in fruit abscission occurred over the next 15-days in which trees dropped on an average 80% of the fruits. Eighteen days later at the end of the trial, trees drop approximately 97% of their fruit. In contrast, the average rate of fruit drop at the end of the trial in control trees was 8%.

Results showed that a marked reduction in stem starch levels occurred in defoliated trees compared to control trees eight

days after the trial was initiated (Figure 3D). Subsequently, starch levels in the stems of defoliation trees remained low at day 15 compared to levels in control stem segments. Sucrose levels were significantly reduced in stem segments from defoliated trees compared to control at day 2, 8 and 15 (Figure 3E). Perseitol and mannoheptulose are two major sugars produced in avocado that are predicted to have a storage function (Liu et al., 2002; Tesfay et al., 2012). Results from the carbohydrate analysis showed that perseitol was significantly reduced at day 8 and 15 in defoliated trees compared to control (Figure 3F). Compared to perseitol, the levels of mannoheptulose were highly variable at day 2 and 15 in the stems of defoliated trees. As a result, no significant differences between defoliated and control could be detected at these time points (Figure 3G). However, a small but significant difference in mannoheptulose was identified in defoliated trees at day 8. The levels of glucose and fructose were highly reduced in the stems of defoliated trees at day 8 compared to control (Figure 3H and I). Taken together, defoliation-induced fruit abscission correlated with a marked decline in starch, sucrose and perseitol levels, which remained low throughout the trial.

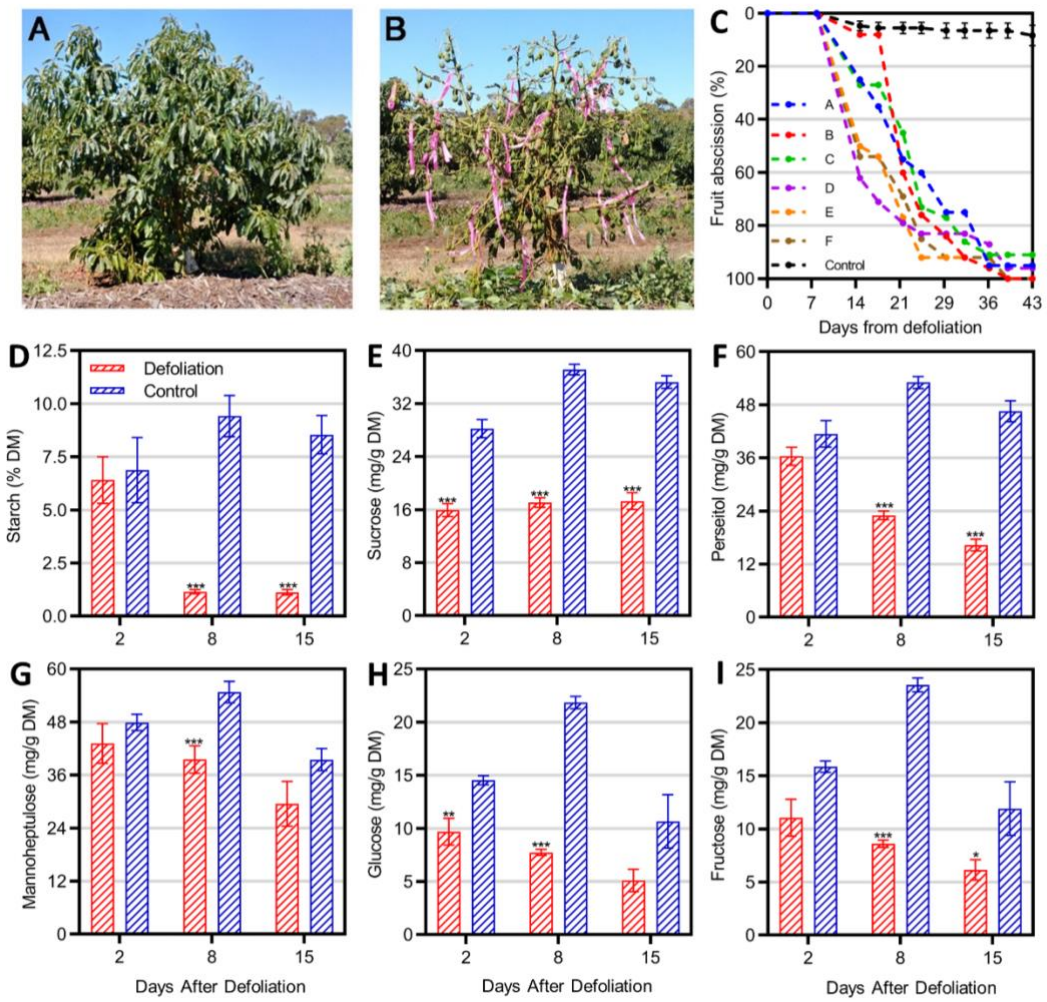

Figure 3. The effect of defoliation on fruit abscission and tree carbohydrate status
Image of tree (A) before and (B) after defoliation. (C) The average percent of fruit abscission in the six control and each of the six defoliated trees. The rate of fruit abscission was determined by evaluated by tagging 18 or 26 fruits per tree and scroring fruit drop in control and defoliated tree, respectively. The levels of (D) starch, (E) sucrose, (F) perseitol, (G) mannoheptulose, (H) glucose and (I) fructose 2, 8 and 15 days after the trial was initiated. Sugar metabolites were measured in mg/g of dry mass (DM), while starch is displayed as % DM. (D-I) Numbers are mean values are derived from 6 (for day 2) or 12 (for days 8 and 12) biological replicates, ± standard error of the mean (bars). Asterisks indicate a significant statistical difference between treatments on the specified day (*p=≤0.05, **p=≤0.01, ***p=≤0.001).

Shading trees and shoots, which reduces light required for photosynthesis/CO_2 assimilation, induces fruit abscission in tree crops (Mehouachi et al., 1995; Zhao and Li, 2020). Fruit abscission was evaluated by shading trees with a shade cloth (Figure 4A and B), which blocked light levels by 77% (Figure 4C). Results showed that over the 100-day period of the trial, control trees dropped on average 7% of the crop (Figure 5A). In contrast, a significant increase in fruit abscission occurred in response to shading. However, compared to defoliation, the abscission response across the four shaded trees was highly variable and was initiated between 25-42 days after the trial was commenced (Figure 5A). At the end of the trial, shaded trees 'A' and 'C' retained approximately 75% and 60% of the fruits, respectively (Figure 5A). In these trees, fruit abscission occurred in small increments over the course of the trial. In contrast, shaded trees 'B' and 'D' showed a significant increase in fruit abscission, in which a wave of fruit drop occurred between 25 and 75 days after the trial was initiated (Figure 5A). As a result, shaded trees 'B' and 'D' dropped 74 and 69% of their fruit, respectively, by the end of the trial. The diameter of the tagged fruits was measured at regular time intervals over the 100-day trial. Interestingly, the average diameter of the retained fruits was similar in shading and control trees (Figure 5B). This result suggests that the abscission of fruits allows the retained fruits to maintain a constant growth rate. Therefore, we propose that a threshold of fruit growth is required for fruits to maintain development and persist on the tree.

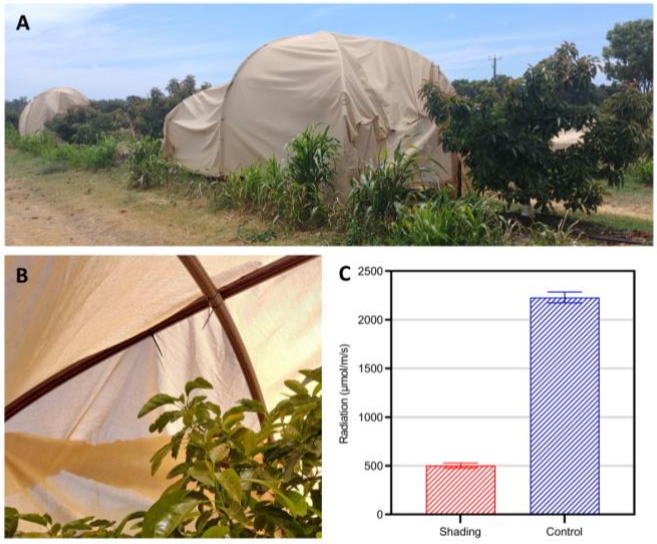

Figure 4. Shading reduced light levels
To reduce tree carbohydrate status by limiting CO_2 assimilation, (A and B) trees were enclosed in a structure covered in shade cloth to reduce light energy required for photosynthesis. (C) Twenty-one days after the trial was initiated, light intensity was measured on a clear day using a Quantum Flux Meter (MQ-500, Apogee Instruments, USA). Numbers are mean values derived from 9-10 independent measurements, ± standard error of the mean (bars).

The carbohydrate status of the shaded and control trees was evaluated by measuring stem starch reserves, as well as perseitol and mannoheptulose, which are all predicted to have a storage function in avocado (Liu et al., 2002; Tesfay et al., 2012). In addition, the translocated sugar, sucrose, was also measured, as well as glucose and fructose. At day 14, the levels of starch were highly variable in control and shaded trees, as a result no significant differences in this storage carbohydrate were detected (Figure 5C). However, at day 36 and 65, a significant reduction in starch occurred in shaded trees compared to control. A significant reduction in sucrose, perseitol and mannoheptulose occurred 14 days after the trial was initiated (Figure 5D, E and F). However, the differences at day 36 were less significant, while no differences were detected at day 65. Glucose and fructose levels were low in shaded trees throughout the trial with the most significant difference occurred at day 36 (Figure 5H and I). Taken together, a decrease in stem starch levels is associated with an increase in fruit abscission when trees are shaded. Further, shading is less effective at inducing fruit abscission than defoliation.

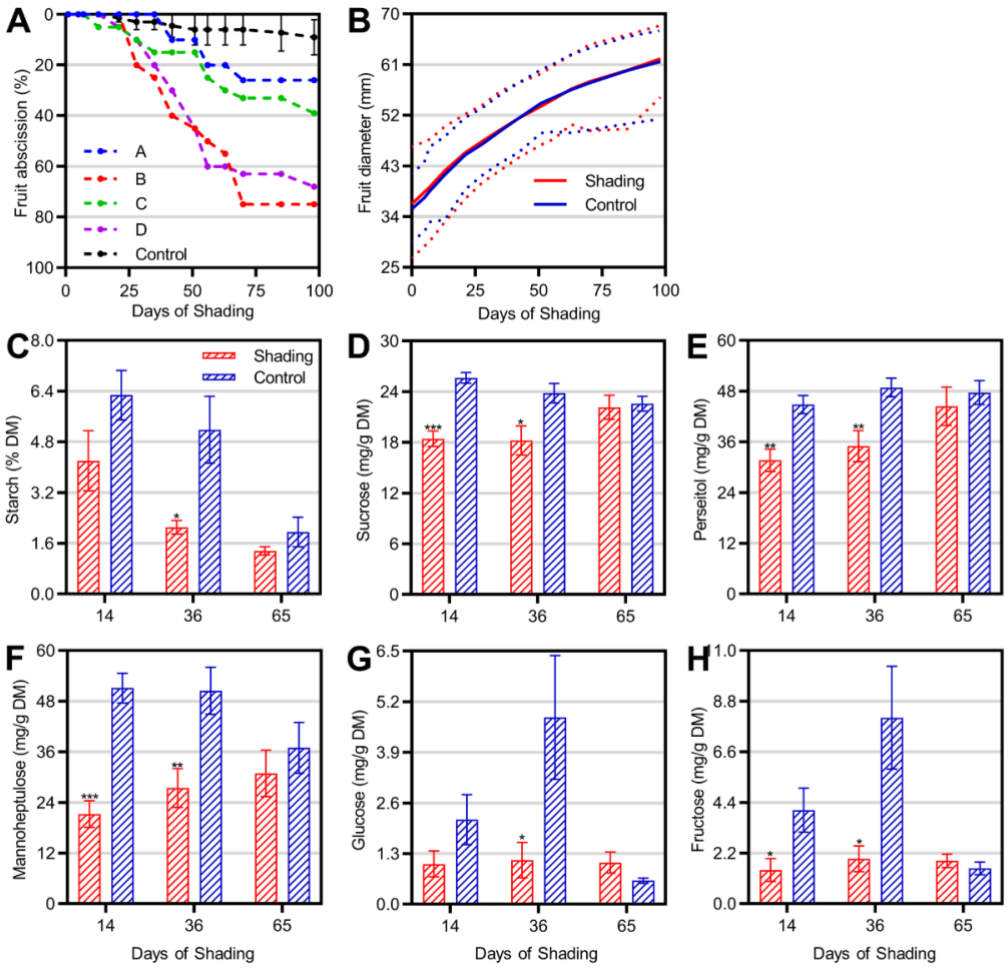

Figure 5. The effect of shading on fruit abscission and tree carbohydrate status
(A) The average percent of fruit abscission in four control and shaded trees over a 100-day period. Twenty fruits per tree were tagged and used to evaluate fruit abscission. (B) The rate of fruit growth displayed for control and shaded trees derived from the average diameter of fruits over the course of the trial. Values are the mean (line) and range (dots) for the growth rate for 80 fruits in control and shaded trees. The levels of (C) starch, (D) sucrose, (E) perseitol, (F) mannoheptulose, (G) glucose and (H) fructose were quantified in stems 14, 36 and 65 days after the trial was initiated. Soluble stem carbohydrates were measured in mg/g of dry mass (DM), while starch is displayed as % DM. (C-H) Mean values were derived from eight biological replicates (shoots), ± standard error of the mean (bars). Asterisks indicate a significant statistical difference between treatments on the specified day (*p=≤0.05, **p=≤0.01, ***p=≤0.001).

Dominance interaction exhibited by vegetative shoots impacts carbohydrate availability for fruit retention

Growth of the spring and summer flush is associated with the early and summer fruit abscission events (Whiley and Wolstenholme, 1990; Salazar-Garcia et al., 1998). Therefore, dominance exerted by the outgrowth of vegetative flushes, is thought to divert carbohydrates away from fruits, which results in fruit abscission. Trials were established in 2018 and 2019 to test this dominance hypothesis, in which the spring flush was removed or tipped at an early stage of expansion (<15 mm in length) and fruit retention was compared to control shoots. Results showed that removal of the spring flush prior to the first abscission event resulted in an increase in fruit retention throughout each of the trials (Figure 6A and B). It should be pointed out that fruit retention was higher in tipped shoots at an early point in the first abscission event indicating that tipping shoots also increased fruit set. Further, control and tipped shoots underwent fruit abscission with the tipped shoots retaining significantly more fruit than control shoots after the fruit drop event was completed. As a decrease in stem starch reserves correlates with the expansion of vegetative and inflorescence shoots, as well as flower development, carbohydrate levels were compared between control and tipped shoots.

Sugars are delivered to growing shoots and developing organs, such as fruits, in the form of sucrose (Ruan, 2012; Bihmidine et al., 2013), as well as mannoheptulose and perseitol in avocado (Liu et al., 2002; Tesfay et al., 2012). We examined the

levels of these three translocated sugars in stems to determine if tipping shoots altered carbohydrate availability to fruits. Results showed that the levels of sucrose and mannoheptulose were similar between control and tipped shoots in 2018 (Figure 7A and C). However, in 2019, sucrose levels significantly lower in tipped shoots after the first fruit abscission event was completed (Figure 7A). In 2018, the level of perseitol was significantly lower in tipped shoots after the first fruit abscission event ceased, while the level of this C7-sugar was unchanged in 2019 (Figure E and F). Given that there was not a conserved response to tipping for each of the translocated sugars in 2018 and 2019, it doesn't appear that tipping shoots reduces or alters carbohydrate availability.

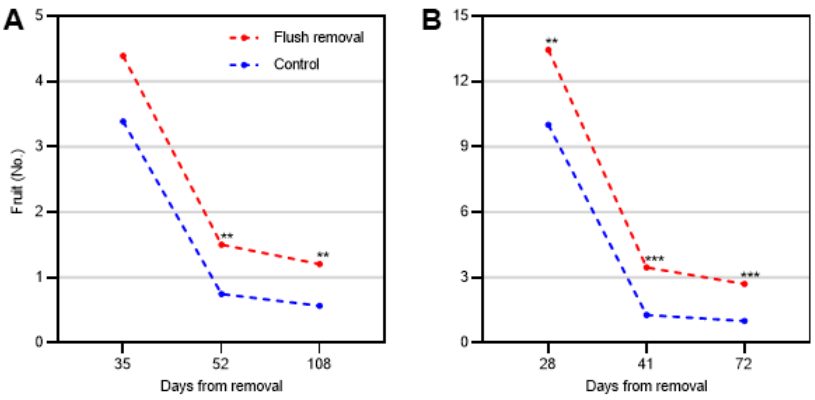

Figure 6. Tipped shoots display an increase in fruit retention.
The average number of fruits per shoot was evaluated at three time points in indeterminate control (blue) and tipped shoots (red) in (A) 2018 and (B) 2019. Values are means of derived from 54-59 biological replicates (shoots) distributed over 7-8 trees. Asterisks indicate a significant statistical difference between treatments for each day (**p*=≤0.05, ***p*=≤0.01, ****p*=≤0.001).

Hexoses, including glucose and fructose, are key sugar metabolites used for respiration (energy) and the synthesis of primary and secondary metabolites (Buchanan et al., 2015). In the tipping trial performed in 2018, the levels of glucose and fructose in stems tended to be lower after the initial fruit abscission event in 2018 (Figure 7G and I) but not in 2019 (Figure H and J). The lack of a conserved response to the levels of glucose and fructose in stems during the 2018 and 2019 trials indicates that tipping doesn't appear to alter the hexose pool in shoots.

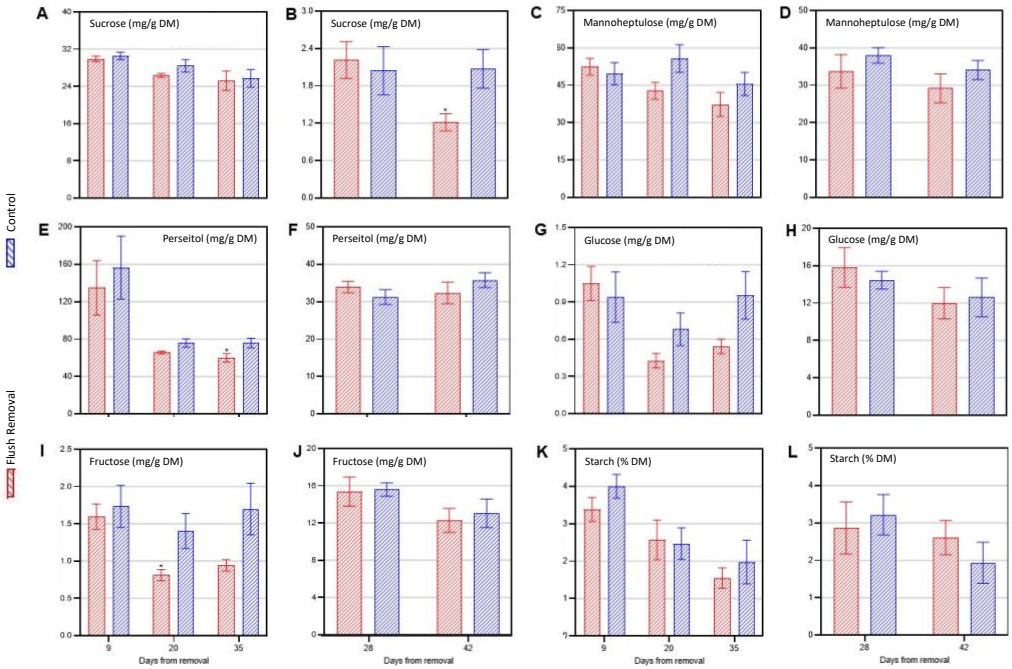

Figure 7. Stem carbohydrate profiles in tipped and control shoots
The levels of stem (A-B) sucrose, (C-D) mannoheptulose, (E-F) perseitol, (G-H) glucose and (I-J) fructose in mg/g dry mass (DM). (K-L) Starch was measured in % dry mass (DM) in stem tissue. Sugar and starch measurements in control (blue) and tipped (red) shoots at three and two-time intervals from trials performed in 2018 and 2019, respectively. Numbers are mean values derived from 7-8 biological replicates (shoots) sampled from 7-8 different trees, ± standard error of the mean (bars). Asterisks indicate a significant statistical difference between treatments on the specified day (*p=≤0.05, **p=≤0.01, ***p=≤0.001).

Stem starch levels fluctuate with the temporal patterns of vegetative flushes and reproductive cycles in avocado (De Jong, 2018). Further, stem starch levels may be used as an indicator to assess carbon reserves. Results showed that tipped shoots did not display a conserved alteration in stem starch levels in 2018 and 2019 (Figure 7K and L). Given that tipped shoots set and retained more fruit than control shoots, we reasoned that removing the young spring flush resulted increased carbohydrate availability likely derived from starch, as well as other sugars, which was used to support reproductive growth.

Fruit growth arrest is an initial step in the developmental process of fruit abscission

In apple and lichee, growth arrest is an initial step in the fruit abscission process (Greene et al., 2013; Zhao and Li, 2020). Interestingly, at the peak of the summer fruit abscission event in California, abscised fruits were statistically smaller than retained fruits in Hass (Garner and Lovatt, 2016). Further, smaller sized fruits have a higher tendency to abscise compared to larger sized fruits, after the first wave of fruit drop in Fuerte (Perez et al., 1988). Based on these studies in apple, lichee and avocado, we investigated the possibility that fruits under growth arrest prior to abscission.

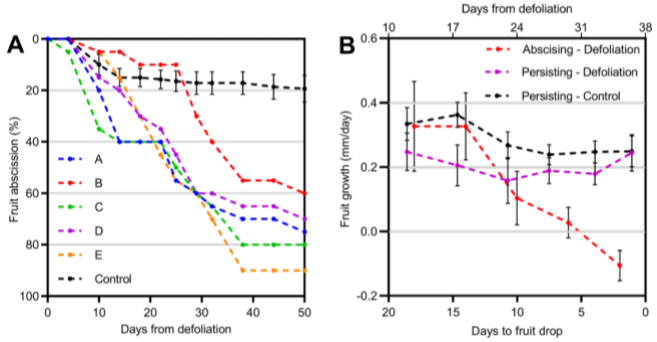

Figure 8. Defoliation effect on fruit abscission and growth rate.
In this defoliation trial, (A) the average percent of fruit abscission in five control and defoliated trees, ± standard error of the mean (bars), was evaluated at 12 time-intervals over a 50-day period following defoliation. The rate of fruit growth was based on fruit diameter measurements derived from 20 fruits per tree. (B) The average daily growth rate of fruit in defoliated trees fated to abscise is displayed in the days before the physical drop (below X-axis). The average daily growth rate of persisting fruits in defoliated and control trees is also shown (above X-axis). Numbers are mean values derived from 23-45 biological replicates (fruit), ± standard error of the mean (bars).

This hypothesis was first tested in a defoliation trial established in early March, 2019 in WA. In contrast to defoliation performed in December, which caused an average 97% of the crop to abscise (Figure 3C), only an average 70% of the fruits abscised in response to defoliation in March (Figure 8A). Using fruit diameter measurements collected during the trial, the average daily fruit growth rates in control and defoliated trees were estimated starting on day 14 of the trial (Figure 8B). In control trees, the average growth rate of immature fruits was ~0.35 mm/day during the third week of the trial (days 14-21; Figure 8A). By the fourth week of the trial, the growth rate of immature fruits declined to an average rate of ~0.24 mm/day, which was maintained until the trial was completed. In defoliated trees, retained fruits displayed growth rates slight lower than control trees (Figure 8B). Moreover, collation of fruit growth rates from abscised fruits in defoliated trees suggested that fruits undergo growth cessation prior to abscission (Figure 8B). In fact, many of the fruits shrink before dropping off the tree. Results from this preliminary study supported the hypothesis that fruits undergo growth cessation prior to abscission.

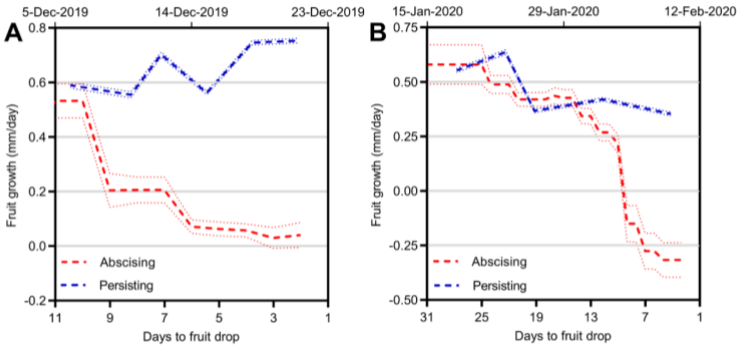

Figure 9. Fruit growth arrest is the initial developmental stage of early and summer fruit abscission
Growth rates of persisting and abscising fruits during the (A) initial and (B) summer fruit drop events. The average daily growth rates of persisting fruits are displayed in blue over the specified time intervals (top x-axis). Daily growth rates of fruits undergoing abscission are displayed in red over the days to fruit drop (bottom x-axis). Values are the mean (dashed line) and range (dots) derived from 224 or 129 persisting and 31 or 37 abscising fruits in (A) and (B), respectively.

To further test if fruit growth arrest is an initial step in the fruit abscission process, a trial was established in which 240 fruits were tagged in six trees when the average diameter of the immature fruits was 12.2 mm. This time point was selected, as fruits with a diameter >10 mm are fertilized (Sedgley, 1980; Garner and Lovatt, 2016) . After measuring the diameter of tagged fruits every three to four days, the average growth rate was determined during early and later stages of growth arrest (Figure 9A and B). The average growth rate of persisting fruits increased over the three-week period during an early stage of fruit development, which overlapped with the initial fruit drop event (Figure 9A). In contrast, a slight decrease in the average growth rate of persisting fruits occurred during mid-summer (Figure 9B). As fruits abscise at various time points in these two trials, growth rates of abscising fruits were combined to calculate the average growth rates prior to abscission. Results showed that immature fruits undergo growth arrest prior to abscission during the initial fruit drop event, as well as in the summer (Figure 9A and B). Taken together, our results show that the cessation of fruit growth is a universal phenotype that is initiated prior to abscission during the early and summer fruit drop events.

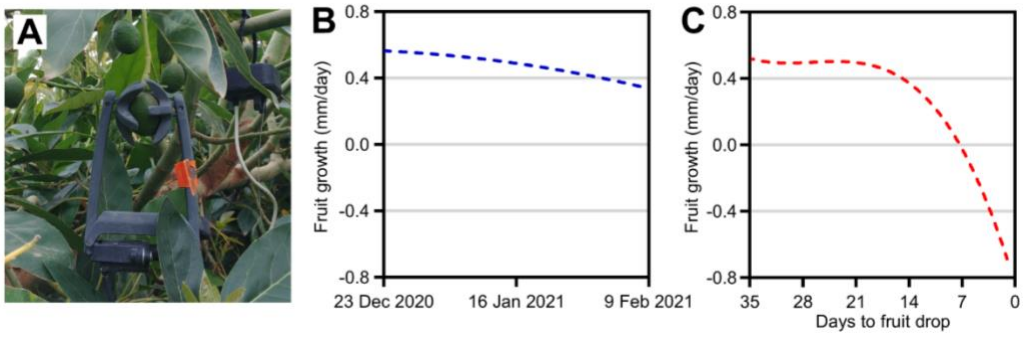

Figure 10. High-resolution phenotyping of fruit growth arrest
(A) Fruit growth was measured using digital fruit dendrometers, which record the diameter of fruits on an hourly basis. (B) Average daily growth rates were derived from (B) persisting fruits over the course of the trial. (C) A switch from high to low growth rate occurred in fruits prior to fruit drop. Average daily fruit growth rates displayed in days to fruit drop. Values are the means derived from (B) 34 persisting and (C) 22 arresting/abscising fruits and each fruit was treated as replicate.

Based on studies from above, fruit growth arrest precedes fruit abscission. This was achieved by measuring the diameter of fruits every 3-4 days using hand-held digital calipers. To further characterize the growth cessation phenotype, a digital fruit dendrometer trial was established to monitor fruit growth rates on a daily basis (Figure 10A). Using fruit dendrometers, the average daily diameter of growing fruits was used to calculate daily growth rates during avocado fruit development

(Figure 10B). Results showed that fruit growth arrest was initiated approximately 15-days before abscission (Figure 10C). Initially, the decrease in growth rate is gradual. However, approximately 10-days before abscission, the rate of fruit growth arrest increased. Approximately 5-days before abscission, fruit growth ceased and the arrested fruits shrunk before they abscised (Figure 10C). These results suggest that digital based dendrometers can be used to detect abscising fruits at an early point in the growth cessation process.

Physiological drivers of summer fruit growth arrest and abscission

The AV16005 project developed four hypotheses aimed at predicting the physiological drivers of summer fruit growth arrest and abscission. The first two of the hypotheses tested had the potential to have an immediate impact for the development of a practical application, while the other two hypotheses required additional basic research due to the lack of understanding of this irregular bearing process. In this section, results addressing each of the hypotheses are discussed.

Is a change in water potential involved in fruit growth arrest and abscission?

Irrigation strategies were proposed as a means to increase the reproductive potential of a tree (Silber et al., 2012). Therefore, we addressed whether a change in fruit water potential was associated with fruit growth arrest and abscission. In this trial, the water potential of arresting and control fruits with a normal growth rate at mid-day was measured with a pressure chamber device. Results showed that there was no significant difference in the water potential between arresting and control fruits with a normal growth rate (Figure 11). Taken together, our results suggest that summer fruit abscission is not mediated by a change in fruit water potential.

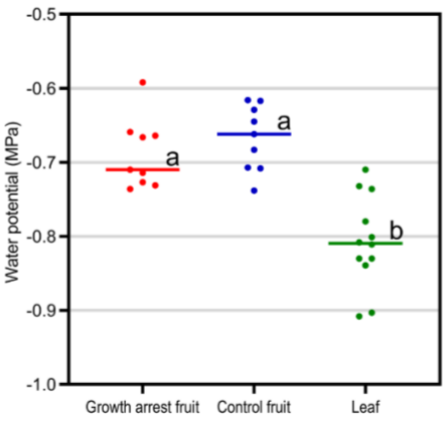

Figure 11. Water potential in arresting and developing fruits

Average mid-day water potential for arresting (red) and developing/persisting (blue) fruits, as well as leaves (green). Arresting and normal growing fruits had an average daily growth rate of 0.05 mm/day and 0.37 mm/day, respectively. Fruits were collected on 11 March 2020, and the water potential was measured using a Schölander pressure chamber at mid-day. Horizontal lines show the median and the dots represent individual measurements from each of the 8 biological replicates (fruits/leaves). Lines with the same letter are not significantly different as determined by Analysis of Variance with post-hoc Tukey's Honestly Significant Difference test, $p \leq 0.05$.

The impact of water stress on fruit abscission during the summer growing period

To further investigate the possible interplay between water relations and fruit abscission, we examined the impact to water stress on fruitlet abscission during the summer growing period. In this trial, the irrigation was shut off for 29-days. Over the course of the trial, the weeds adjacent wilted and died from the lack of water (Figure 12A), indicating that water availability was significantly reduced. In well irrigated control trees, the shoots and leaves were undergoing expansion and the mature leaves appeared healthy at day 29 (Figure 12B). In contrast, at the end of the water stress treatment, shoot and leaf expansion was highly reduced and mature leaves displayed a substantial amount of tip burn (Figure 12C). At this time point, differences in fruitlet retention were apparent (Figure 12D). In addition to the increase in fruitlet drop, leaf abscission occurred in the water stress trees (data not shown). As the trees appeared to be on the verge of collapse, the irrigation was

turned on at day 29 (Figure 12D, green arrow). After the irrigation was turned on, the decrease in the rate of fruit retention was reduced and ceases at day 32 and onwards (Figure 12D).

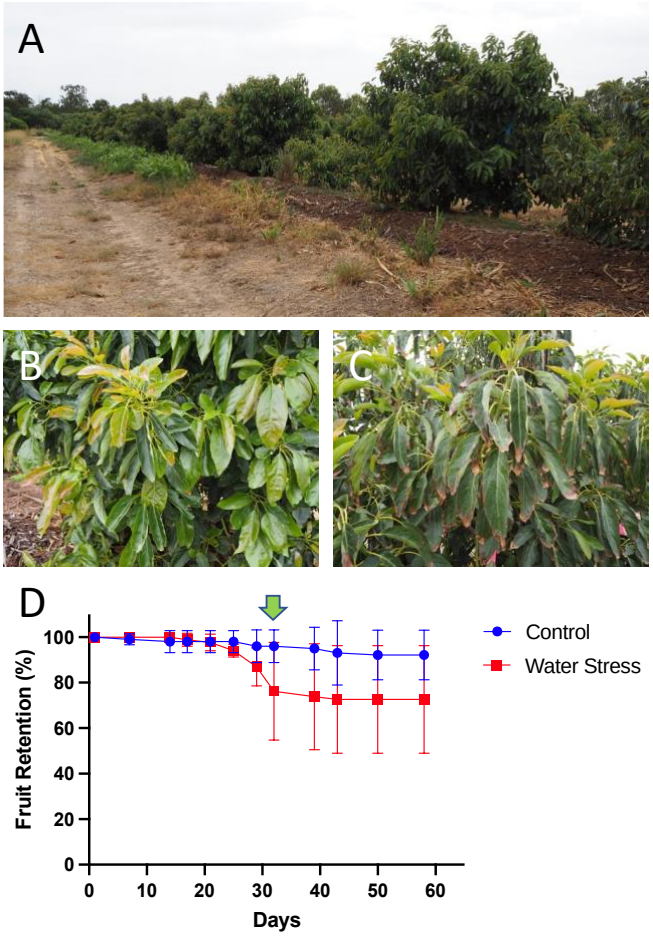

Figure 12. Severe water stress can induce fruit abscission but is reversed in a fairly rapid manner
(A) Image of water stressed trees (note, vegetative on the backs marks the region of the row where the irrigation was turned off. (B) Image of leaves and shoots in well irrigated control trees. (C) Image of leaves and shoots in water stressed tress 29-days after the irrigation was turned off. (D) Fruit retention (%) was determined for control (blue line/circles) and water stress (red line/circles) trees. The irrigation was turned off for 29-days. The green arrow shows the time in which the irrigation as turned on.

Is irrigation a viable method to mitigate summer fruit abscission in well-managed orchards

Results from the water potential and water stress trials, indicate that the summer fruit drop event may not be caused by a lack of water availability in well managed orchards. This is particularly evident in the water stress trial in which fruitlet abscission only significantly deviated from the control trees 29-days after the irrigation was turned off. At this time, the leaves displayed a substantial amount of tip burn and trees were also shedding leaves. Therefore, leaf and fruit abscission induced at this time is likely due to severe water stress, a condition that would not be permitted in well managed orchards. Interestingly, when the irrigation was turned on, the rate of abscission slowed considerably and ceased within 5-days. While these trials should be repeated in another season(s), these results indicate that modifying irrigation strategies in a well-managed orchard may not provide a viable pathway to mitigate summer fruit abscission.

Is a deficiency in a mineral element(s) associated with fruit growth arrest

Mineral elements acquired from the soil are essential for plant growth and development (Hansch and Mendel, 2009; White and Brown, 2010). Further, the impact of mineral element deficiency in crops not only effects plant growth and development but also yield. A previous study examined whether the nutrient status of the tree influences avocado fruit

abscission (Garner and Lovatt, 2008). Tree nutrient status was determined by measuring nitrogen and other essential mineral elements in leaves. Results from this study indicate fruit abscission did not associate with changes in tree nutrient status. To further determine whether nutrients may be involved in fruit abscission, the levels of key mineral elements were measured in arresting and control fruits with a normal growth rate. Results showed that nitrogen levels were higher in the embryo, seed coat and pericarp of fruits undergoing arrest compared to actively growing fruits (Figure 13A). A similar trend was also observed for the levels of phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, zinc, and sodium which were generally higher in fruit undergoing growth arrests relatively to control fruit (Figure 13B-L). Thus, results from our study, as well as Garner and Lovatt, 2008, indicates that mineral-deficiency is not a major driver of fruit abscission in well-managed orchards. Therefore, a modified plant nutrition strategy doesn't appear to be a feasible solution to mitigate summer fruit abscission in well managed orchards.

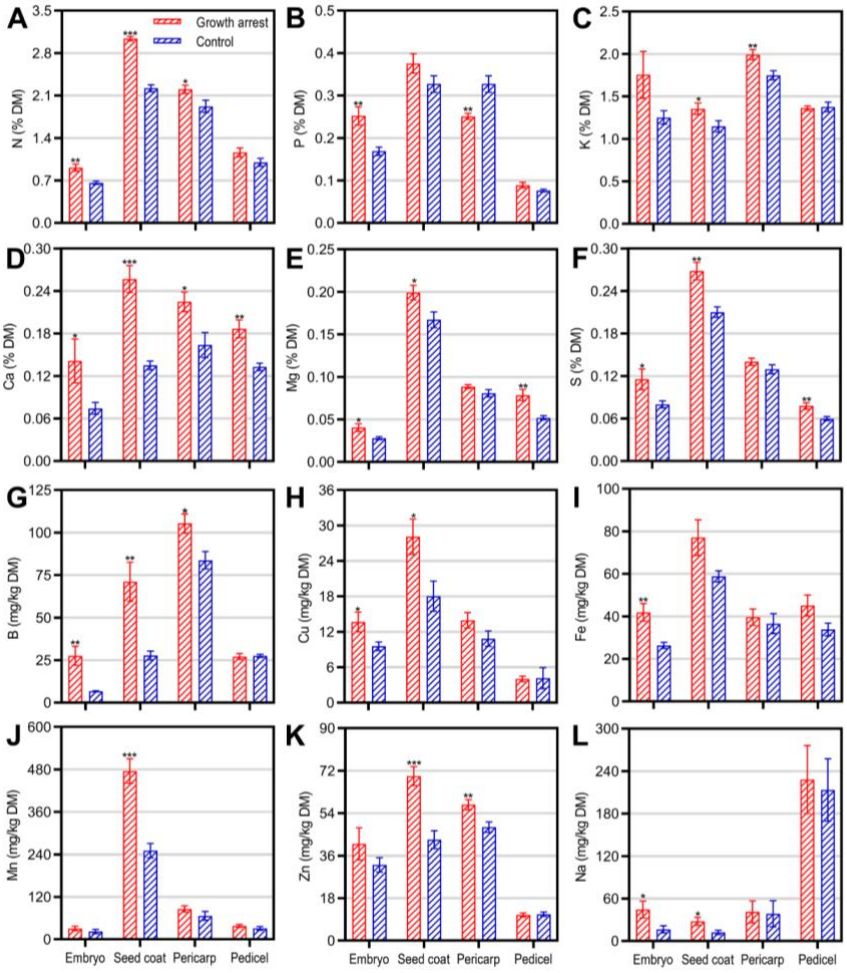

Figure 13. Mineral nutrient profiles in high and low growing fruits
The levels of (A) nitrogen (N), (B) phosphorus (P), (C) potassium (K), (D) calcium (Ca), (E) magnesium (Mg), (F) sulfur (S), (G) boron (B), (H) copper Cu), (I) iron (Fe), (J) manganese (Mn), (K) zinc (Zn) and (L) sodium (Na) were measured in normal growing/control (blue) and arresting (red) fruits in either % dry mass (DM) or mg/kg DM. Normal growing/control and arresting fruits had an average growth rate of 0.79 mm/day and 0.05 mm/day, respectively. collected on 16 January 2020. Mineral elements were measured in the embryo, seed coat, pericarp, and pedicel. Numbers are mean values derived from 8 biological replicates (fruits), ± standard error of the mean (bars). Asterisks indicate a significant statistical difference between normal growing/control and arresting fruit tissues (*p=≤0.05, **p=≤0.01, ***p=≤0.001).

Is a change in carbohydrate status associated with growth arrest and abscission?

Experimental studies indicate that fruit abscission is mediated by reduced carbohydrate availability, signaling and availability in citrus (Mehouachi et al., 1995; Gómez-Cadenas et al., 2000) and apple (Botton et al., 2011). Given that

carbohydrates are key substances require for growth, we hypothesized that the switch from a high to low growth rate that occurs early in the immature fruit abscission process would be mediated by a decrease in the carbohydrate status of the seed and possibly seed coat.

Fruit carbohydrate status is altered in response to defoliation

As a first step toward testing the hypothesis that the carbohydrate status of the immature fruit influences growth arrest and abscission, fruits from a defoliated trees were isolated and compared to fruits from control trees. It should be noted that this trial was performed after the initial fruit drop event, when the abscission rate in control trees was low. As defoliation induced a ~97% fruit drop event (Figure 3C), we reasoned that significant change in sugar metabolites would occur in fruits isolated from defoliated trees prior to the time in which the fruit drop event was initiated, 10-days after the trial was initiated (Figure 3C). Results showed defoliation caused a significant reduction of sucrose in the embryo and seed coat 2 and 8 days after the trial was initiated (Figure 14A). The reduction of sucrose was most evident in the seed coat at both time points in defoliated trees. In the pericarp, a significant reduction in sucrose only occurred 2 days after defoliation. However, the levels of this disaccharide increased in defoliated pericarp samples similar to the levels in the pericarp of control trees (Figure 14A).

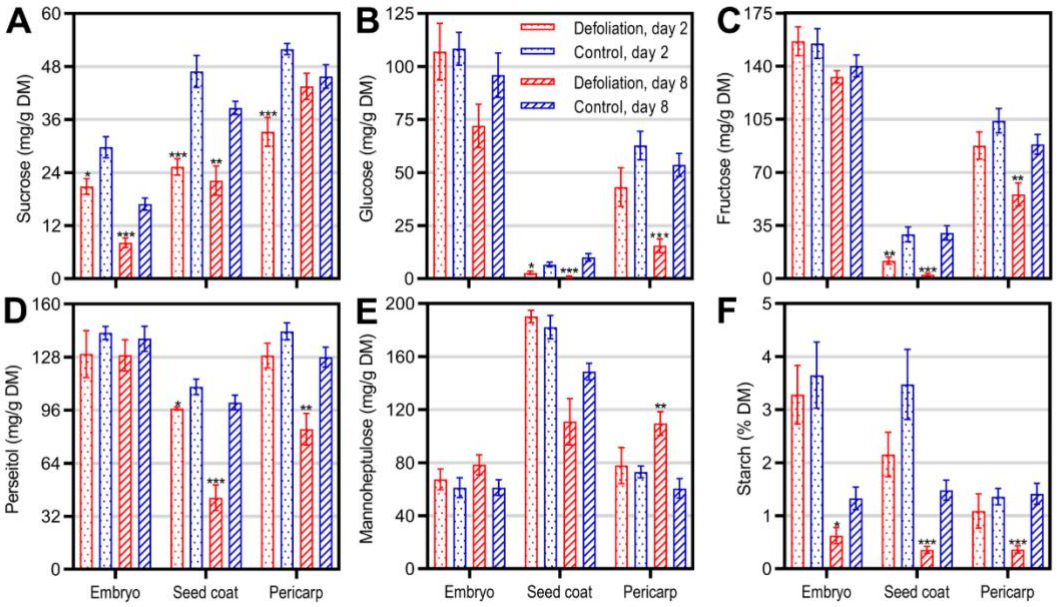

Figure 14. Carbohydrate metabolites measured in fruits from defoliated trees

The levels of (A) sucrose, (B) glucose, (C) fructose, (D) perseitol, (E) mannoheptulose and (F) starch were measured in fruits sampled two and eight days after defoliation in control and treated trees. Carbohydrates were measured in mg/g of dry mass (DM), except starch which is presented in % dry mass. (*p=≤0.05, **p=≤0.01, ***p=≤0.001).

In sink tissues, sucrose is metabolized to glucose and fructose to maintain the flow of photosynthates and nutrients to growing tissues (Bihmidine et al., 2013; Osorio et al., 2014). To examine whether sucrose uptake and metabolism was affected by defoliation, sucrose, glucose and fructose were measured in fruit tissues from control and defoliated trees. Two and eight days after defoliation, the seed coat of fruits displayed a significant reduction in the levels of sucrose, glucose and fructose (Figure 14A-C). While the levels of sucrose decreased two and eight days after defoliation in the embryo (Figure 14A), there was no significant difference in the levels of glucose and fructose in this tissue (Figure 14B and C). In the pericarp, a decline in sucrose, glucose and fructose was not significant until eight days after defoliation (Figure 14B and C). Taken together, these results suggest that under carbon limiting conditions, the supply of sucrose to the seed coat is reduced. In addition, sucrose metabolism may also be reduced and targeted when trees experience a severe stress event. Furthermore, sucrose metabolism may be targeted early in the growth arrest process, as the levels of glucose and fructose are significantly low in the seed coat two-days after defoliation. The significant decline in glucose and fructose in the pericarp

eight days after defoliation shows that the sucrose metabolism response to defoliation is delayed compared to the seed coat. Finally, sucrose metabolism in the embryo appears to be functional, as there were no significant changes in the two hexoses levels eight days after defoliation.

In addition to sucrose, glucose and fructose, avocado fruits accumulate perseitol and mannoheptulose, which have been predicted to play a role in suppressing ripening when fruits reach maturity (Liu et al., 2002; Tesfay et al., 2012). However, how these sugars respond to fruit abscission has yet to be investigated. Results showed that the levels of perseitol was significantly reduced in the seed coat two and eight days after defoliation (Figure 14D). In response to defoliation, the level of perseitol was unchanged in the embryo at day two and eight of the trial, while a decrease in this seven-carbon sugar occurred in the pericarp eight days after defoliation (Figure 14D). Eight days after defoliation, mannoheptulose levels increased in the pericarp, but no significant changes in this seven-carbon sugar was detected in the embryo and seed coat in response to defoliation (Figure 14E). Taken together, defoliation induces changes in the metabolism of perseitol in the seed coat and pericarp. Furthermore, it is tempting to speculate that increase in mannoheptulose in the pericarp in response to defoliation could be mediated by the conversion of perseitol to mannoheptulose, which is known to occur during at late stages of fruit development when the seed coat dries up (Tesfay et al., 2012).

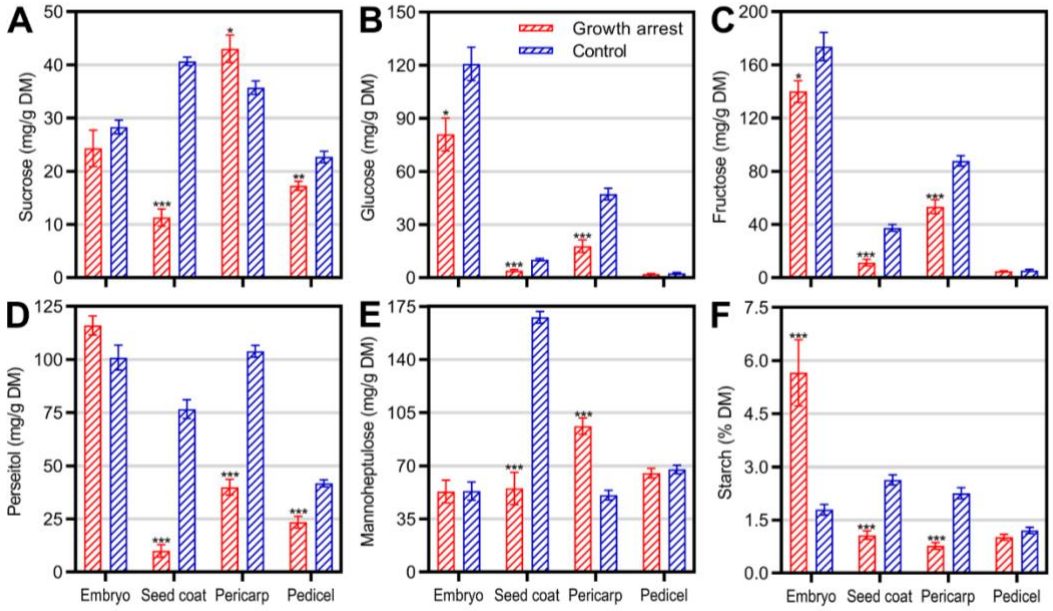

Figure 15. Carbohydrate metabolites in arresting and control fruits
The levels of (A) sucrose, (B) glucose, (C) fructose, (D) perseitol, (E) mannoheptulose and (F) starch were measured in control fruits with a normal growth rate and arresting fruits during the summer after the initial fruit drop event. Sugar metabolites were measured in mg/g of dry mass (DM), except starch which is presented in % DM. Numbers are mean values derived from six biological replicates (fruit), ± standard error of the mean (bars). Asterisks indicate a significant statistical difference between treatments on the specified day (*p=≤0.05, **p=≤0.01, ***p=≤0.001).

Experimental studies indicate that starch reserves are used as a buffer to supplement growth when carbohydrate availability is reduced (Smith and Stitt, 2007). In all fruit tissues, a significant decline in starch occurred in the embryo, seed coat and pericarp eight days after defoliation (Figure 14F). Together, these results indicate that defoliation results in an increase in starch catabolism across the three different fruit tissues.

Natural fruit growth cessation is associated with decline in carbohydrate status and metabolism

To better address whether changes in carbohydrate status and metabolism is associated with fruit growth arrest, two sets of fruits were harvested. Fruits with a normal growth rate were harvested, as these fruits displayed a high propensity for retention. In addition, fruits undergoing growth arrest were also harvested, as these fruits have a high tendency to abscise. After harvesting, fruits were dissected into embryo, seed coat, pericarp and pedicel. Subsequently, soluble sugars, including

sucrose, glucose, fructose, perseitol and mannoheptulose, as well as starch were measured in each of the fruit tissues, as well as the pedicel. Results showed that in contrast to the embryo and pericarp, sucrose was highly reduced in the seed coat in arresting fruits (Figure 15A). In addition, sucrose levels were reduced in the pedicel of fruits with a low growth rate indicating that sucrose uptake may be reduced when actively growing fruits undergo growth cessation (Figure 15A). The levels of glucose and fructose were significantly reduced in the embryo, seed coat and pericarp (Figure 15B and C), which suggests that sucrose metabolism is reduced when fruits undergo growth arrest. In arresting fruits, a significant reduction in perseitol and mannoheptulose occurred in the seed coat, while no changes were observed in the embryo (Figure 15D and E). Interestingly, the pericarp of arresting fruits displayed a significant decrease and increase in the levels of perseitol and mannoheptulose, respectively (Figure 15D and E). The decline in perseitol and increase in mannoheptulose suggests that an aldolase, which converts perseitol to mannoheptulose, may be activated in the pericarp during growth cessation. In the pedicel of arresting fruits, only perseitol was reduced; whereas, mannoheptulose was unaffected by the change in fruit growth (Figure 15D and E). Interesting, a hyper-accumulation of starch was detected the embryo of fruits undergoing growth arrest (Figure 15F). In contrast, starch was significantly decreased in the seed coat and pedicel of arresting fruits (Figure 15F). As an increase in starch is associated with later stages of seed maturation, the hyper-accumulation of starch in the embryo suggests that a decrease in fruit growth rate and carbon status of the seed coat induces a late-stage maturation process in the embryo.

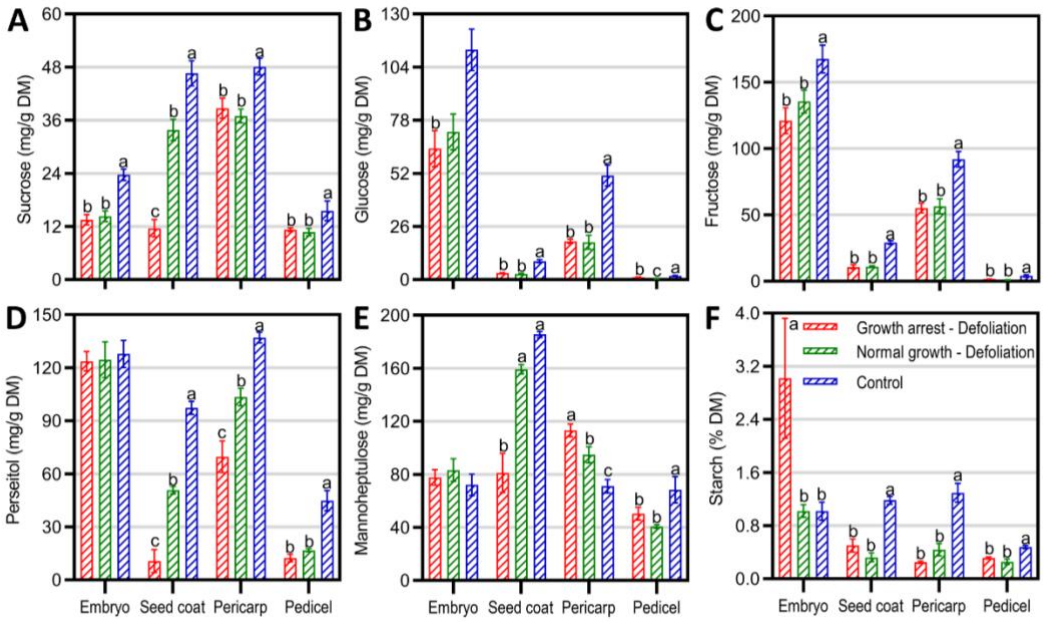

Figure 16. Carbohydrate profiles in normal growing and arresting fruits induced to abscise via defoliation
The levels of (A) sucrose, (B) glucose, (C) fructose, (D) perseitol, (E) mannoheptulose and (F) starch were measured in normal growing fruits with average daily growth rates of 0.65 mm/day and 0.59 mm/day derived from control and defoliated trees respectively. In addition, sugar metabolites and starch profiles were also shown for arresting fruits with an average daily growth rate of 0.08 mm/day derived from defoliated trees. All fruits were sampled from control and defoliated trees seven days after the trial was initiated. Sugar metabolites were measured in mg/g of dry mass (DM) and starch is presented in % DM. Numbers are mean values derived from 6-8 biological replicates (fruit), ± standard error of the mean (bars). Lines with the same letter are not significantly different as determined by Analysis of Variance with post-hoc Tukey's Honestly Significant Difference test, $p \leq 0.05$.

Defoliated induced fruit growth arrest is associated with decline in carbohydrate status and metabolism

Results from above indicate that carbohydrate status and metabolism is altered during growth arrest. In this experiment, we wanted to determine if changes in carbohydrate status and metabolism occurred early in the fruit growth arrest process. While defoliation induces a massive wave of fruit drop (Figure 3C and Figure 6B), abscission is not synchronized as the process occurs over 25 to 30-day period. Therefore, defoliated induced fruit abscission provides an opportunity to capture

and metabolically analyze fruits undergoing growth arrest early and later in this process. To achieve this objective, fruits with a normal growth rate and fruits undergoing growth arrest were harvested from defoliated trees, seven days after treatment. We reasoned that fruit harvested with a normal growth rate from defoliated trees would correspond to an early time in the growth arrest process. Subsequently, fruits harvested with a low growth rate would represent a later time point in the growth arrest process. In this trial, fruits with a normal growth rate were harvested from untreated control trees, seven days after treatment for comparison to the two defoliated fruit samples. Harvested fruits were dissected into embryo, seed coat, pericarp and pedicel and soluble sugars, including sucrose, glucose, fructose, perseitol and mannoheptulose, as well as starch were measured. Results showed sucrose levels were reduced in all fruit tissues derived from early and late arresting fruits compared to normal growing from control trees (Figure 16A). Further, a dramatic reduction in seed coat sucrose levels occurred from early to late growth arrest in defoliated trees (Figure 16A). Glucose and fructose levels were significantly reduced in all the fruit tissues; however, this decrease was more pronounced in the seed coat (Figure 16B and C). Therefore, a decrease in sucrose metabolism appears to occur early in the growth arrest process. The levels of perseitol were significantly reduced in the seed coat, pericarp and pedicel of fruits from defoliated trees with significant decline in this C7-sugar occurring from early to late fruit growth arrest (Figure 16D). Seed coat and pedicel mannoheptulose levels were also reduced during defoliated mediated growth arrest (Figure 16E). Interestingly, the levels of this C7-sugar displayed a significant decline when fruits transition from early to late stages of fruit growth arrest. In contrast to the seed coat and pedicel, the levels of mannoheptulose significantly increase in the pericarp during the growth arrest process, possibly due to the interconversion of perseitol to mannoheptulose (Figure 16E). The levels of these C7-sugars in the embryos of defoliated fruits were similar to the embryo in control fruits (Figure 16D and E). While starch levels decreased in the seed coat, pericarp and pedicel from fruits in defoliated trees, the levels of this carbohydrate reserve increased in the embryo relative to the control (Figure 16F). The accumulation of starch at the late stage of growth arrest indicates carbohydrate metabolism in the embryo is distinct from that of the seed coat and pericarp.

Alteration in the expression of genes involved in carbohydrate metabolism that are associated with fruit growth arrest

Sugars are key metabolites required for plant growth and development that are used to generate cellular energy, reducing power and serve as building blocks for amino acids, nucleic acids, lipids and secondary metabolites (Buchanan et al., 2015). Moreover, sugars, such as glucose, sucrose and trehalose-6-phosphate also function as signals used to regulate plant growth and development in response to carbohydrate availability (Wang and Ruan, 2013; Ljung et al., 2015). Results from above showed that fruits undergoing growth arrest displayed alterations in the levels of sugar metabolites, particularly in the seed coat. Transcriptome analysis was used to better link changes in the level of sugar metabolites with alterations in the expression of genes involved in carbohydrate transport, metabolism and sugar signaling.

Results from the transcriptome analysis showed that genes involved in sucrose transport and metabolism were differentially expressed during fruit growth arrest (Figure 17A and B). It is highly likely that the differences in gene expression levels is due to a feedback mechanism(s), which functions to maintain sucrose homeostasis during fruit development. For example, a gene that encodes an invertase (INV), sucrose synthase (SUS) and/or sucrose transport proteins may be up-regulated when the levels of sucrose drop below a threshold. Alternatively, hormones that inhibit growth may act to down-regulate an *INV*, *SUS* and/or sucrose transport gene(s) in response to a reduction in sucrose availability. Lastly, INVs, SUSs and/or sucrose transport proteins may also be regulated post-transcriptionally; therefore, changes in the expression of these genes may not reflect the function of these proteins.

Starch is a key non-structural carbohydrate that buffers plant tissues when sugar availability from photosynthetic tissues is limiting (Smith and Stitt, 2007). Results from the starch analyses showed that this non-structural carbohydrate was significantly reduced in the maternal tissues, seed coat and pericarp (Figures 13-15). Consistent with this result a number genes that encode amylases, which breakdown starch, were up-regulated in the seed coat and pericarp, during growth arrest (Figure 17C). Moreover, many of these amylase genes were up-regulated early in the growth arrest process. In contrast, a number of amylases genes were also down-regulated in the seed coat and pericarp (Figure 17C). However, the down-regulation of these amylase genes occurred late in the growth arrest process and may be targeted as part of a feed-back mechanism. Interestingly, the embryo displayed a similar pattern of amylase gene expression as the seed coat and pericarp during growth arrest (Figure 17C). Given that the embryo accumulates starch during growth cessation, the up-regulation of amylase genes may act to maintain sugar homeostasis, when carbohydrate availability is limiting.

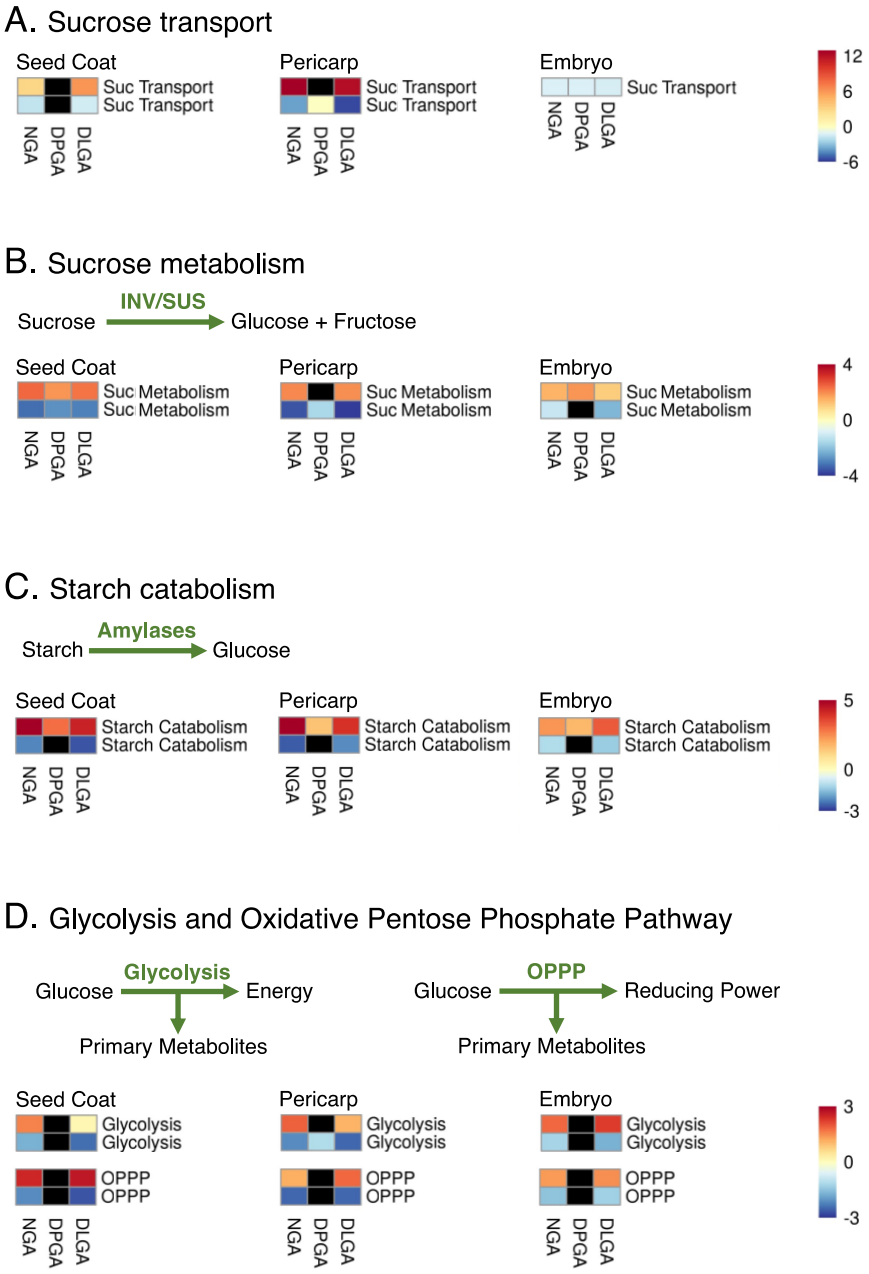

Figure 17. Fruit growth arrest is associated with changes in the expression of genes involved in carbohydrate metabolism and transport

Heat map used to display the collective expression of genes associated with the (A) transport and (B) metabolism of sucrose, (C) starch catabolism and (D) glycolysis and the oxidative pentose phosphate pathway (OPPP) in the seed coat, pericarp and embryo of fruits undergoing natural growth arrest (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heat map. Black boxes indicate that a gene is not differentially expressed. SUC, sucrose; INV, invertase; SUS, sucrose synthase; OPPP, oxidative pentose phosphate pathway.

Glycolysis and the oxidative pentose phosphate pathway (OPPP) are key carbohydrate metabolic pathways used to generate cellular energy and reducing power, respectively, as well as produce primary metabolites (Kruger and von Schaewen, 2003; Lunn, 2007; Obata, 2019). Results from the transcriptome data showed that genes involved in glycolysis and OPPP pathways were differentially expressed (Figure 17D). In all but the pericarp tissue, the differential expression of

the genes involved in glycolysis occurred late in the growth arrest process. Similarly, the change in expression of OPPP genes appears to be a late growth arrest response.

Taken together, changes in the expression of genes that breakdown sucrose and starch, INVs/SUSs and amylases, respectively, to ultimately generate glucose, which feeds into glycolysis and OPPP to maintain cellular function, appear to be central targets that are impacted early in the growth arrest process. However, as the growth arrest proceeds, sucrose transport, glycolysis and OPPP are impacted, which likely effects metabolic function of cells and fate of these different fruit tissues.

Changes in the expression of key sugar signaling genes is associated with early and late stages of fruit growth arrest

In the seed coat, embryo and pericarp, results showed that genes involved in trehalose-6-phosphate homeostasis and sugar signaling were differentially expressed during growth arrest (Figures 18). As the production of trehalose-6-phosphate depends upon the activity of trehalose-6-phosphate synthase (TPS) and trehalose 6-phosphate phosphatase (TPP) (Lunn et al., 2014; Figueroa and Lunn, 2016), transcriptome analysis showed that a number of *TPS-like* and *TPP-like* genes were differentially expressed across the fruit tissues (Figure 18A). Moreover, a small subset of sugar signaling genes involved in regulating growth in response to carbohydrate and nutrient availability were also differentially expressed (Figure 18B). In the maternal tissues, seed coat and pericarp, changes in the expression of genes that control trehalose-6-phosphate levels and sugar signaling occurred early in the growth arrest process (Figure 18A and B). Therefore, based on the sugar metabolite and transcriptome analyses, we propose that a sugar starvation response is activated early and functions to mediate fruit growth arrest by inducing metabolic changes in carbohydrate metabolism.

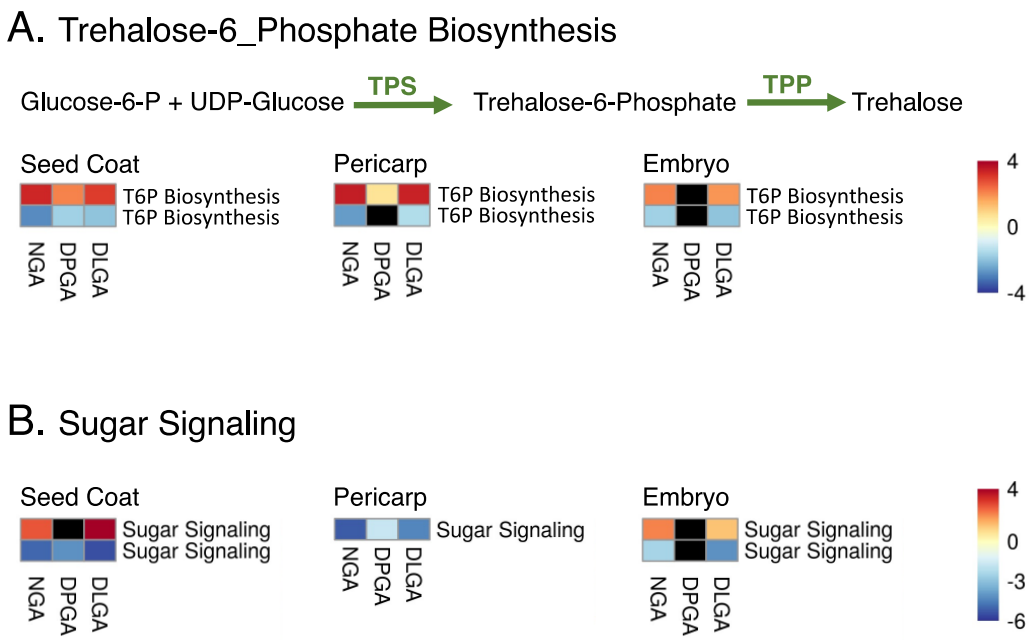

Figure 18. Fruit growth arrest is associated with changes in the expression of genes associated with trehalose-6-phosphate biosynthesis and sugar signaling

Heat map used to display the collective expression of genes associated with (A) trehalose-6-phosphate biosynthesis and (B) sugar signaling in the seed coat, pericarp and embryo of fruits undergoing natural growth arrest (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heat map. Black boxes indicate that a gene is not differentially expressed. T6P, trehalose-6-phosphate.

Down-regulation of genes that control cell division is associated with summer the fruit growth arrest process

In contrast to other horticultural crops, cell division is the primary driver of avocado fruit growth throughout development (Schroeder, 1953; Taylor and Cowan, 2001). Therefore, we speculated that fruit growth arrest would involve and down-

regulation of cell cycle genes that promote cell division in plants (Inze and De Veylder, 2006). To test this hypothesis, the transcriptome data was interrogated for changes in the expression of cell cycle genes, including cyclins and cyclin dependent kinases (CDKs), as well as genes that control DNA-synthesis. Results showed that the collective expression of genes encoding cyclins, CDKs and DNA-synthesis regulators, were down-regulated during fruit growth arrest (Figure 19A-C). Moreover, the down-regulation of cyclins, CDKs and DNA-synthesis regulatory genes occurred early in the growth arrest process (Figure 19A-C). Taken together, fruit growth arrest is tightly associated with the suppression of cell cycle and DNA-synthesis regulatory genes. Given that the expression of cell cycle regulators is under the control of sugar availability, (Inze and De Veylder, 2006), suggests that the decline in carbohydrate availability and metabolism is a key factor in the suppression of genes that regulate cell division during growth arrest. As hormones also play a critical role in regulating cell division in plants (Inze and De Veylder, 2006), it is highly likely that fruit growth arrest is also under hormonal control.

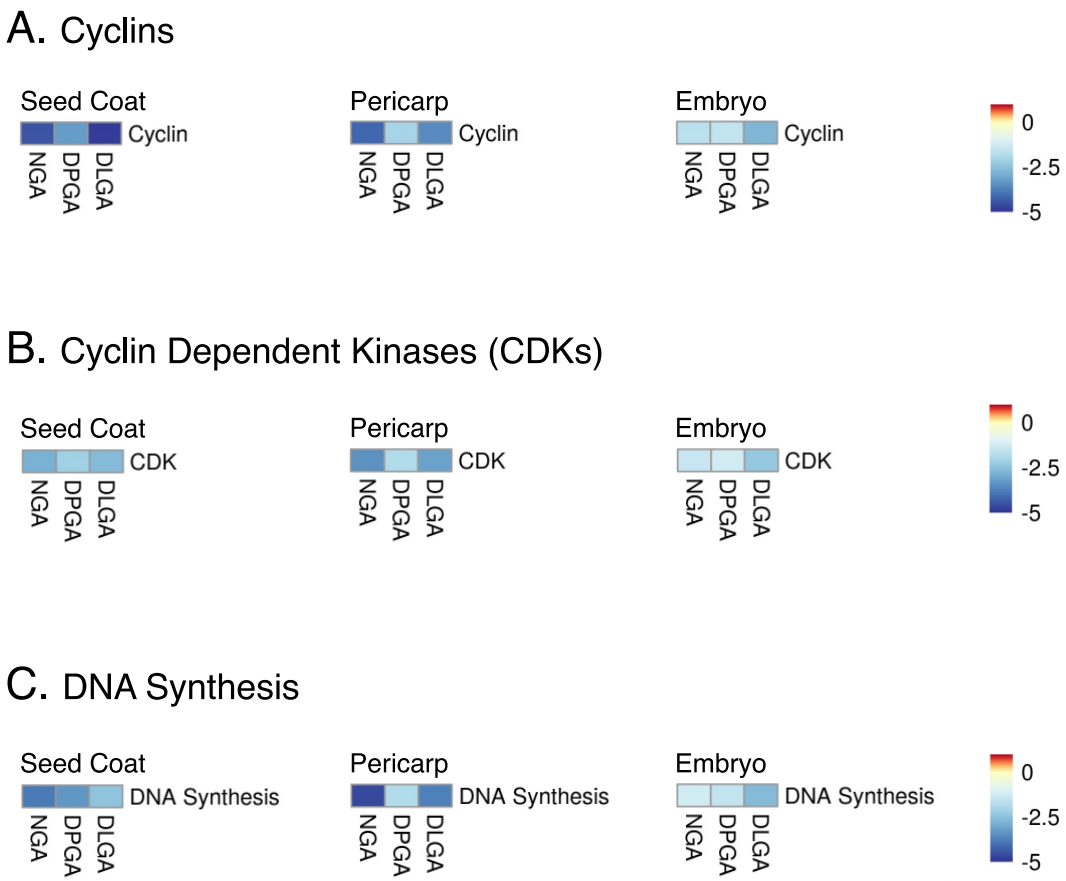

Figure 19. Fruit growth arrest is associated with a down-regulation in genes that regulate cell division
Heat map used to display the collective expression of genes associated with the regulating cell division including (A) cyclins and (B) cyclin dependent kinases (CDK), as well as (C) DNA-synthesis in the seed coat, pericarp and embryo of fruits undergoing natural growth arrest (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heat map. Black boxes indicate that a gene is not differentially expressed. Note: the collective expression of CDK genes also includes other key kinases that control cell cycle checkpoints.

Hormonal control of fruit development

Fruits undergo sequential phases of development starting with the growth phase, which is initiated upon fruit set (Kumar et al., 2014; Fenn and Giovannoni, 2021). The growing phase of fruit development is often mediated by a period of cell division followed by a duration of cell expansion. During the later period of cell expansion mediated growth, fruits undergo maturation, which is necessary for ripening (McAtee et al., 2013; Kumar et al., 2014). Auxin is a key hormone that controls fruit set, cell division and cell expansion during the growth phase of fruit development ((Kumar et al., 2014; Fenn and Giovannoni, 2021). Auxin biosynthesis and signaling, which is mediated by YUCCAs (YUCs) and the AUX/IAAs and ARFs

transcription factors (Zhao, 2018; Cance et al., 2022), respectively, as well as conjugation, regulates the activity of this hormone (Zhang and Peer, 2017). GH3 enzymes modulates auxin action via conjugation with amino acids, which functions to inactivate auxin (Zhang and Peer, 2017). Moreover, polar auxin transport system mediated by auxin transport proteins controls cellular influx, efflux and intracellular transport required for tissue patterning and cell division during fruit development (Pattison et al., 2014; Semeradova et al., 2020).

In model plants, ARF and AUX/IAA signaling proteins act to promote fruit growth in part through the activation of genes that mediate cell division. In addition, as proliferating cells switch to cell expansion, ARF and AUX/IAA signaling proteins act to up-regulate gibberellin (GA) biosynthesis genes, which encode GA-20 oxidases and GA-3 oxidases (Fenn and Giovannoni, 2021). Once GA biosynthesis is induced, this hormone functions together with auxin to promote cell expansion. The decline in auxin during the later stage of cell expansion is critical for maturation (McAtee et al., 2013; Kumar et al., 2014). Cytokinin and brassinosteroid are also implicated in regulating fruit growth but their precise role is not well defined (Fenn and Giovannoni, 2021).

While a hormonal framework for avocado fruit development is incomplete, experimental studies suggest that the seed coat, endosperm and embryo contain high levels of active auxin, gibberellin and cytokinin(s) (Blumenfeld and Gazit, 1970, 1972; Gazit and Blumenfeld, 1972). In contrast, auxin and gibberellin activities were not detected in the mesocarp (Blumenfeld and Gazit, 1972; Gazit and Blumenfeld, 1972). Further, the mesocarp appears to contains measurable levels of an inactive form of cytokinin (Gazit and Blumenfeld, 1970). These preliminary studies provide evidence that the avocado seed produces key growth promoting phytohormones used to regulate fruit development, which is consistent with the seed control hypothesis (Crane, 1964; Gillaspy et al., 1993; McAtee et al., 2013). Further, these studies support a hypothesis that the seed coat is the major hormone production site in the seed, which facilitates the development of the seed and the pericarp.

It is highly likely that summer fruit growth arrest and abscission will be managed via a plant growth regulator (PGR) application(s). Therefore, it is essential to understand the hormonal framework of fruit growth arrest. To achieve this goal, the transcriptome of arresting fruits was interrogated for changes in the expression of genes involved in auxin, gibberellin, cytokinin and brassinosteroid function to assess how growth cessation effects these hormone activities. The ability to supplement a growth promoting hormone activity(ies) required to overcome growth arrest will be key in the development of a PGR based application(s).

Candidate growth promoting phytohormones targeted by fruit growth arrest

Auxin

Fruits undergoing growth arrest displayed marked changes in the expression of genes implicated in auxin biosynthesis, metabolism/catabolism and signaling, as well as transport (Figure 20 and 21). In maternal tissues, the seed coat and pericarp, *YUC-like* auxin biosynthesis genes were down-regulated during fruit growth arrest (Figure 20A). The repression of *YUC-like* genes occurred during early and later stages of seed coat arrest. In contrast, down-regulation of *YUC-like* genes occurred in the pericarp only during the later stages of growth arrest. In the seed coat, pericarp and embryo, a number of *GH3-like* genes were upregulated during fruit growth arrest (Figure 20B). In all three tissues, these *GH3-like* genes were up-regulated early in the growth arrest process. In the maternal tissues, seed coat and pericarp, a number of *GH3-like* genes were down-regulated during fruit growth arrest (Figure 20B). Interrogation of the transcriptome data showed that a large number of *AUX/IAA-like* and *ARF-like* genes were differentially expressed during fruit growth arrest (Figure 20C). In many cases, this expression occurred early in the cessation of growth process (Figure 20C). Taken together, the mechanisms that control auxin levels and signaling appears to be altered during early and later stages of fruit growth arrest.

To further examine the impact of fruit growth arrest on auxin, the levels of the primary auxin, indole-3-acetic acid (IAA), was measured in arresting and normal growing fruitlets. Results showed that IAA levels were the highest in the seed coat compared with the embryo and pericarp in normal growing fruits (Figure 20D). Further, at a late stage of growth arrest, the level of IAA was significantly reduced in the seed coat, as well as the pericarp. The apparent decrease in IAA levels in the embryo was not significant. Taken together, the reduction in *YUC-like* gene expression and IAA levels in the seed coat and pericarp suggests that the growth arrest signal(s) reduce free IAA levels via negatively regulating auxin biosynthesis. Given that *GH3.1-like* were induced during growth arrest, we also measured IAA-Asparagine (IAA-Asp) levels in the seed coat,

pericarp and embryo of arresting and normal growing fruitlets. Results showed that IAA-Asp levels significantly increased in the seed coat (Figure 20D). Although the levels of IAA-Asp increased in the pericarp, the average value was not significant. Thus, our results suggest that free IAA levels are also reduced in the seed coat via conjugation with Asp, which is likely mediated by *GH3-like* at a late stage of growth arrest.

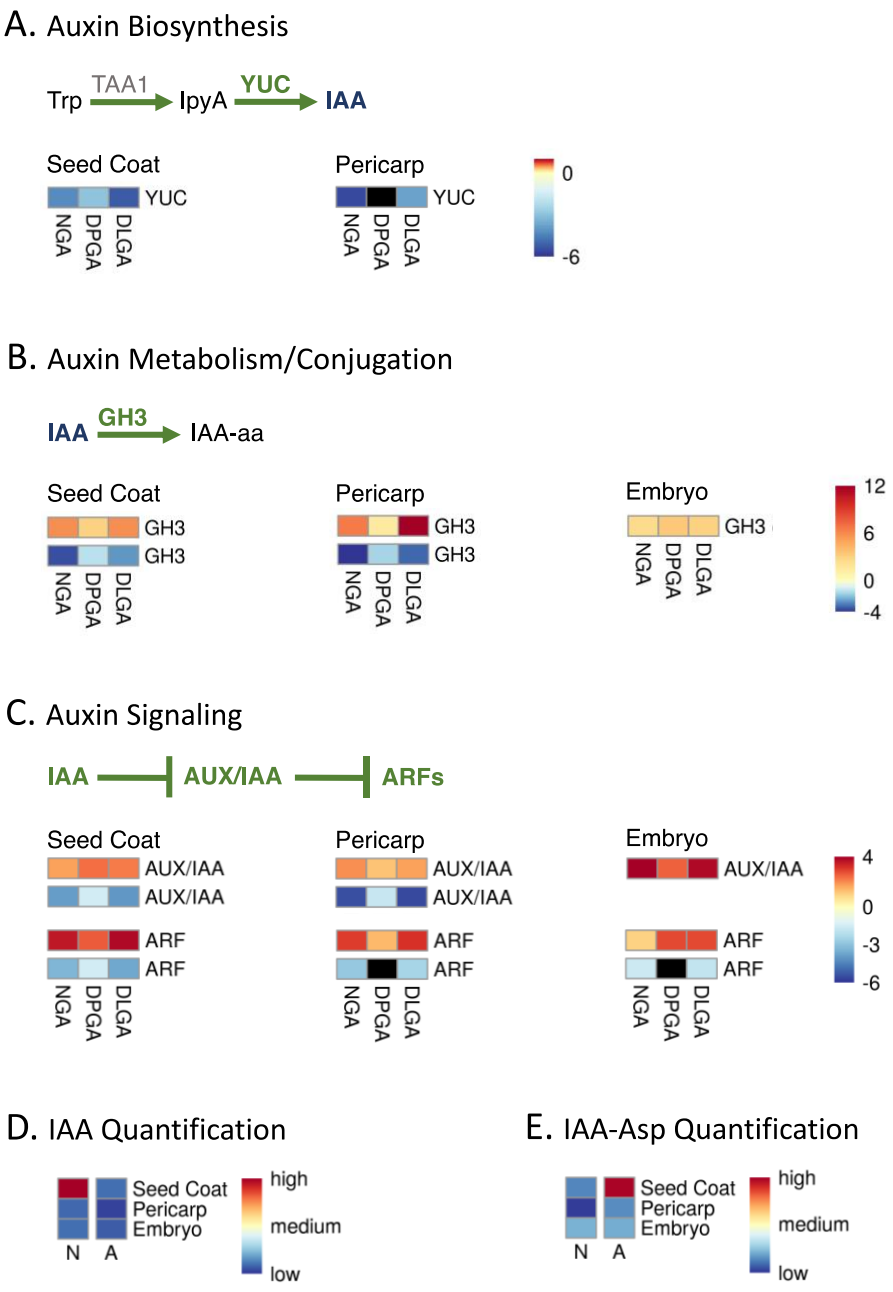

Figure 20. Collective changes in the expression profiles involved in auxin activity and regulation of free IAA levels during fruit growth arrest

Heatmap used to display the collective expression of genes involved in (A) auxin biosynthesis, (B) metabolism/conjugation and (C) signaling in the seed coat, pericarp and embryo fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed. (D). Heatmap used to display changes in free IAA levels in the seed coat, pericarp and embryo. (E) Heatmap used to display changes in IAA-Asp levels in the seed coat, pericarp and embryo of (N) normal growing and (A) arresting fruit samples.

The transport of auxin between the filia (embryo and endosperm) and maternal tissues of the seed, as well as from the seed to the fleshy part of the fruit is critical for coordinating growth and development of the fruit and seed (Figueiredo and Kohler, 2018; Robert, 2019; Guo et al., 2022). To determine if fruit growth arrest is associated with an alteration in auxin transport, we queried the transcriptome data for the differential expression of genes that encode auxin transport proteins. In the seed coat and pericarp, a number of genes that encode auxin influx proteins were differentially expressed during growth arrest suggesting that the auxin influx was altered in maternal tissues (Figure 21A). The expression of genes implicated in the efflux or export of auxin out of cells was altered in the seed coat, pericarp and embryo during fruit growth arrest (Figure 21B). The transport of auxin across the tonoplast membrane, which surrounds the vacuole, likely functions to regulate cellular levels auxin (Ranocha et al., 2013). The overall decrease in expression of genes that encode vacuolar auxin transport proteins suggests that the internal levels of auxin may be altered during fruit growth arrest (Figure 21C). Lastly, auxin efflux proteins are regulated by a set of proteins that modify the activities of these auxin transport proteins (Armengot et al., 2016). Results showed that genes encoding these regulatory proteins are differentially expressed during fruit growth arrest (Figure 21D). Taken together, results suggest that auxin transport and cellular homeostasis of this hormone are altered during growth arrest, which may be a critical factor in the cessation of cell division and patterning required for fruit development.

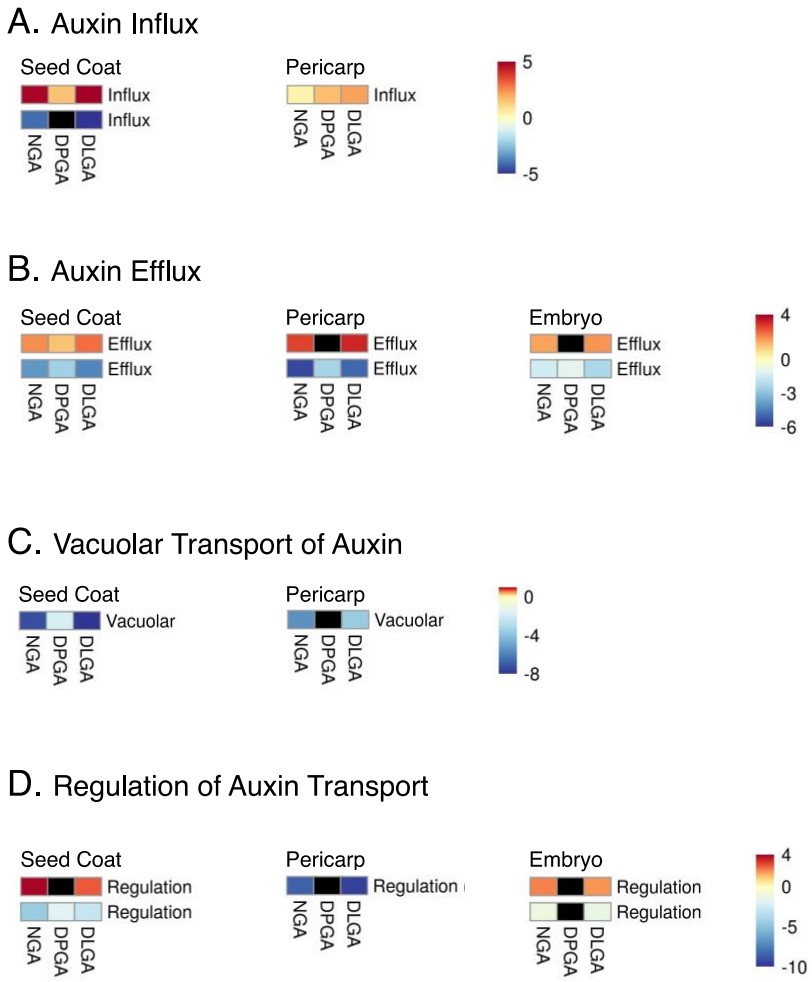

Figure 21. Collective changes in the expression profiles for genes involved in auxin transport
Heatmap used to display the collective expression of genes involved in the transport of auxin (A) into (influx) and (B) out (efflux) of cells, as well as across the (C) tonoplast membrane of vacuoles. (D) The collective expression of genes that regulate auxin transport proteins are displayed. The gene expression data was derived from the seed coat, pericarp and embryo fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed.

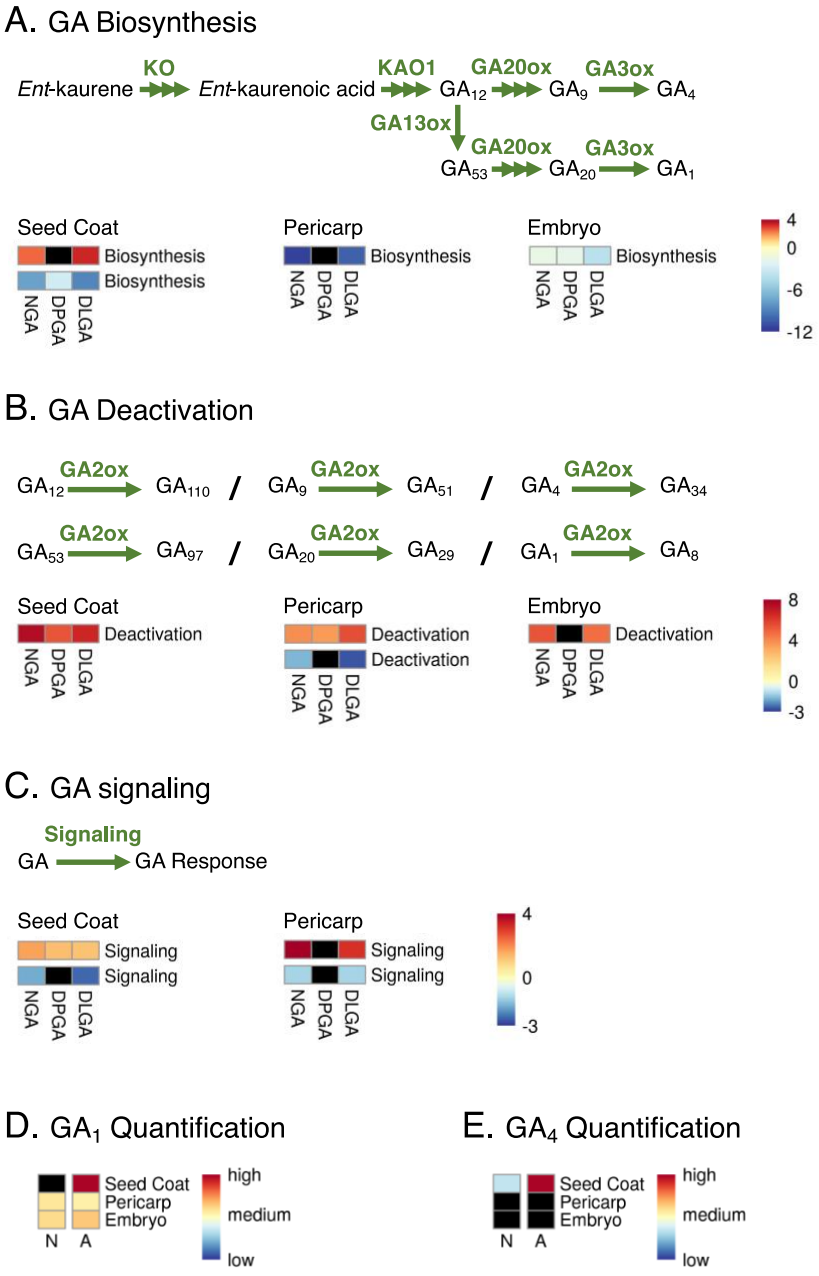

Figure 22. Collective changes in the expression of genes involved in GA activity and GA_1/GA_4 levels during fruit growth arrest
Heatmap used to display the collective expression of genes involved in (A) GA biosynthesis, (B) deactivation and (C) signaling in the seed coat, pericarp and embryo of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The log_2 fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed. Heatmap used to display changes in (D) GA_1 and (E) GA_4 levels in the seed coat, pericarp and embryo of (N) normal growing and (A) arresting fruit samples.

Gibberellin

While experimental studies indicate that the bioactive GAs, GA_1 and GA_4, promotes the cell expansion with auxin during the growth phase of tomato fruit development (Kumar et al., 2014; Fenn and Giovannoni, 2021), it is unclear if GA is involved in avocado fruit development, which is primarily mediated by cell division (Schroeder, 1953; Cowan et al., 2001; Dahan et al., 2010). In the GA biosynthesis pathway, GA_1 and GA_4 are derived from ent-kaurene (Figure 22A). In the seed coat GA biosynthesis genes, including *GA20OX*-like *GA3OX*-like, were differentially expressed in arresting fruits (Figure 22A). However, in the pericarp and embryo of arresting fruits, there was an overall down-regulation of GA biosynthesis genes. Gibberellin levels are also controlled in part by GA2 oxidases, which function to deactivate the bioactive forms of this

hormone (Hedden, 2020) (Figure 22B). Results showed that a number of GA deactivation genes, including *GA2OX-like* were upregulated in seed coat, pericarp and embryo during fruit growth arrest (Figure 22B). In the pericarp, a number of GA deactivation genes were also down-regulated during growth arrest (Figure 22B). Finally, an alternation in the expression of GA signaling genes was evident in the maternal tissues, seed coat and pericarp, during fruit growth arrest (Figure 22C). Taken together, the differential expression of genes that encode GA biosynthesis, deactivation and signaling proteins were indicates that GA levels may be altered during fruit growth arrest.

To better understand if GA is playing a role in growth arrest, we quantified the levels of GA_1 and GA_4 in normal growing and arresting fruits. In normal growing fruits, GA_1 is detected in embryo and pericarp; however, the levels of this active GA is not detectable in the seed coat (Figure 22D). During a late stage of fruit growth arrest, the levels of GA_1 did not change in the embryo and pericarp. In contrast, the levels of GA_1 significantly increased in the seed coat at a late stage of growth arrest (Figure 22D). Results showed that GA_4 was only detected in the seed coat in persisting fruits with a normal growth rate (Figure 22E). While GA_4 appeared to also increase in the seed coat during growth arrest, this change is not significant (p=0.09; Figure 22E). The increase in the levels of GA_1 in the seed coat is interesting in that GA plays a significant role not only in mediating cell expansion but also maturation. Further, once plant cells exit cell proliferation and switch to cell expansion/maturation, cell division in inhibited. Therefore, it is highly probably that in order for avocado fruits to maintains a mode of growth primarily mediated by cell proliferation, a mechanism(s) must exist to suppress GA mediated cell expansion/maturation. Moreover, the increase in GA_1 suggests that fruit growth arrest may be mediated by the activation of a maturation-like pathway(s), which not only acts to suppress cell proliferation but promotes the competence to abscise.

Cytokinin

As avocado fruit development is primarily mediated by cell division, we queried the transcriptome data to determine if changes in the expression of genes that control cytokinin activity were altered during growth arrest. Analysis of the transcriptome data indicated that growth arrest involves changes in cytokinin biosynthesis, signaling and catabolism in the seed coat, embryo and pericarp (Figure 23). In the seed coat, a number of cytokinin biosynthesis genes were up-regulated during growth arrest (Figure 23A). The up-regulation of the cytokinin biosynthesis genes does not occur early in the growth arrest process, indicating this response may be activated as part of a feedback loop activated late in the growth arrest process. In the seed coat, pericarp and embryo and number of cytokinin deactivation genes were differentially expressed (Figure 23B). Of note, a cytokinin oxidase (*CKX-like*) was highly induced in all three tissues and the activation of this gene occurred early in seed coat arrest. Genes involved in cytokinin signaling were differentially regulated during growth arrest (Figure 23C). Taken together, the transcriptome study shows that changes in the expression of genes involved in cytokinin biosynthesis, deactivation and signaling occurs during growth arrest process.

To further evaluate a role for cytokinin in fruit growth arrest, we compared the levels of the active cytokinins, trans-Zeatin (tZ) and isopentyladenine (iP) in arresting and normal growing fruitlets. Results showed that tZ levels were detected in the seed coat and embryo derived from normal growing fruits (Figure 23D). In contrast, the level of this cytokinin was extremely low in the pericarp. In arresting fruits, there was no significant change in the levels of this hormone in the seed coat and embryo, as well as in the pericarp (Figure 23D). We also examined the levels of cis-Zeatin (cZ), and the level of this hormone was well below the detection threshold in the seed coat, pericarp and embryo derived from arresting and normal growing fruits (data not shown). The levels of iP were also quantified in the tissues of arresting and normal growing fruits. Results showed that the average level of iP was significantly low in the seed coat, pericarp and embryo of normal growing fruits (Figure 23E). Further, there was no significant change in the levels of iP in arresting and normal growing fruits. Therefore, fruit growth arrest doesn't appear to target free levels of active cytokinins in fruits fated to abscise. However, given that changes in cytokinin response are evident in the transcriptome of arresting fruits suggests that the fruit growth arrest signal(s) may target cytokinin response.

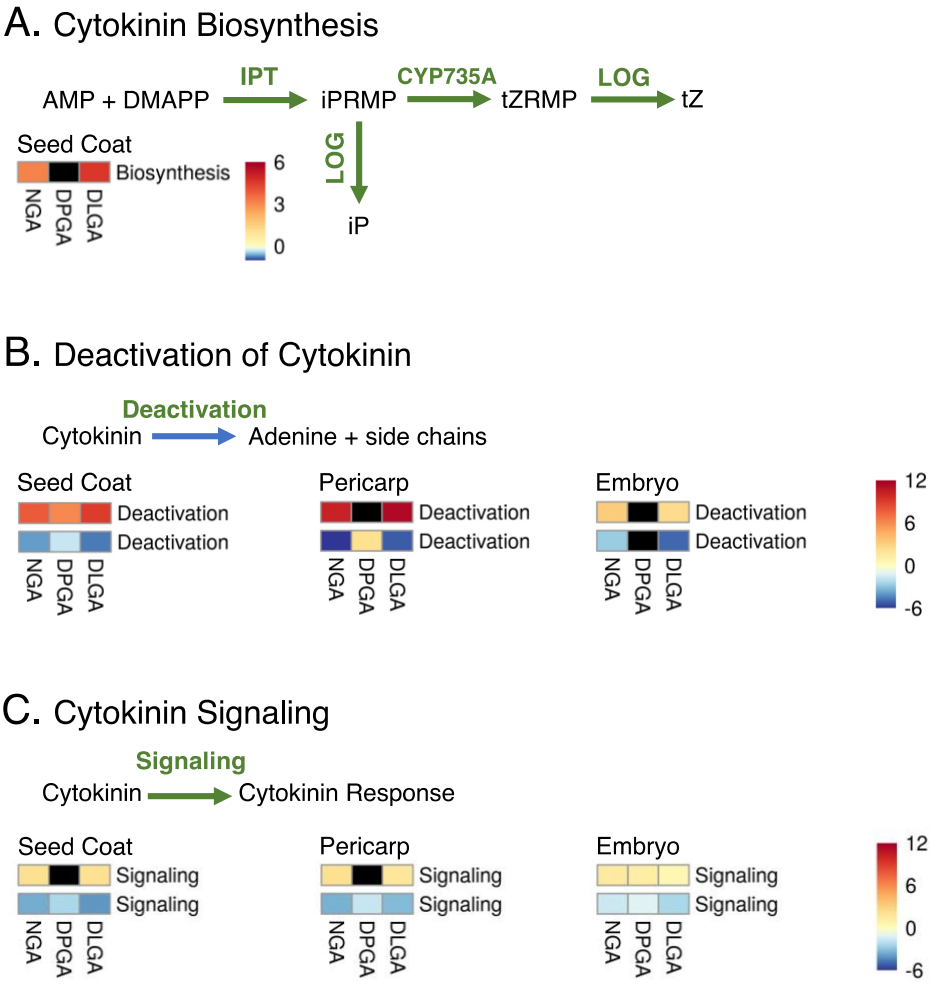

Figure 23. Collective changes in the expression of genes involved in cytokinin activity and tZ/iP levels during fruit growth arrest
Heatmap used to display the collective expression of genes involved in (A) cytokinin biosynthesis, (B) deactivation and (C) signaling in the seed coat, pericarp and embryo of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed.

Brassinosteroid

As brassinosteroid (BR) signaling has been implicated in fruit growth and development (Fenn and Giovannoni, 2021), the transcriptome was queried for the differential expression of genes involved in brassinosteroid (BR) biosynthesis, signaling and metabolism in the seed coat, embryo and pericarp of fruits undergoing growth arrest. In the BR biosynthesis pathway, the active forms, castasterone and brassinolide, are synthesized from campesterol in a multi-step process (Figure 24A). A number of genes that mediate BR biosynthesis were differentially expressed in the seed coat and pericarp during fruit growth arrest (Figure 24A). In the embryo of arresting fruits, BR biosynthesis genes were down-regulated during fruit growth arrest (Figure 24A). The overall reduction in expression of BR biosynthesis genes in arresting embryos suggests that the level of an active BR may be reduced during late growth arrest. BR deactivation plays a fundamental role in reducing the level of active BRs and the signaling processes associated with growth (Wei and Li, 2020). Results showed that BR deactivation genes were highly induced in the seed coat during fruit growth arrest (Figure 24B). In contrast, fruit growth cessation resulted in the down-regulation of BR deactivation genes in the pericarp and embryo (Figure 24B). Overall, BR signaling genes were induced in the seed coat, pericarp and embryo during fruit growth cessation (Figure 24C). Taken together, these results indicate that BR function is altered during fruit growth arrest and the levels of an active BR may be reduced in the embryos of arresting fruits.

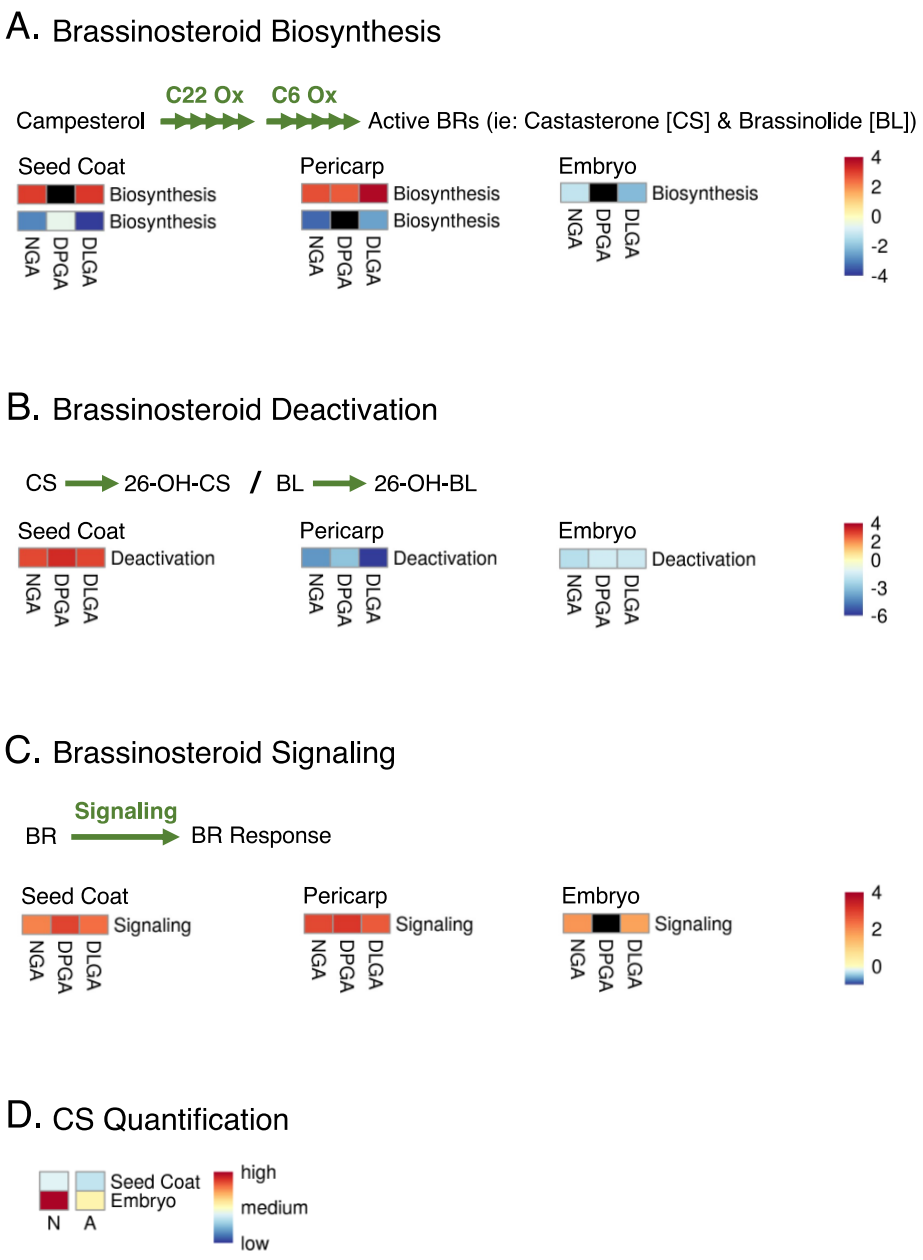

Figure 24. Collective changes in the expression of genes involved in BR activity and castasterone (CS) levels during fruit growth arrest
Heatmap used to display the collective expression of genes involved in (A) BR biosynthesis, (B) deactivation and (C) signaling in the seed coat, pericarp and embryo of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed. (D) Heatmap used to display changes in CS (castasterone) levels in the seed coat, pericarp and embryo of (N) normal growing and (A) arresting fruit samples.

To assess the possible involvement of BR activity in fruit growth arrest, we quantified castasterine (CS) and brassinolide (BL) in normal growing and arresting fruit tissues. Results from our study show that BL is not detected in normal growing and arresting fruits (data not shown) indicating that this active BR is not involved in avocado fruit growth and development, as well as abscission. However, CS accumulated in the embryo and seed coat of normal growing fruits (Figure 24D). In response to growth arrest, the level of CS significantly declined in the embryo but not in the seed coat (Figure 24D). Thus, the growth arrest signal(s) appears to target CS biosynthesis and/or catabolism during a late stage of growth cessation.

Candidate growth inhibiting phytohormones identified for fruit growth arrest

Apple has been used as a model system to study immature fruit abscission. It has been hypothesized that apple fruit abscission is mediated by dominance interactions among developing fruits within an inflorescence (Bangerth, 1989). In this system, development of the dominant apical fruitlet(s) causes the youngest basal fruitlets to abscise. Experimental studies suggest that fruitlet abscission is mediated by 'nutritional stress', in which the dominant apical fruitlet diverts carbohydrates away from the younger lateral fruitlets (Botton et al., 2011; Botton and Ruperti, 2019). In turn, nutritional stress promotes ethylene and abscisic acid (ABA) production in fruits fated to abscise (Dal Cin et al., 2005; Botton et al., 2011; Eccher et al., 2013; Eccher et al., 2015). Further, it was proposed that ethylene production in the cortex diffuses to the seed to promote embryo abortion, which impairs polar auxin transport in the pedicel (Eccher et al., 2015). As a result of reduced auxin levels in the pedicel, ethylene initiates cell separation processes to in the abscission zone to stimulate fruit drop (Botton et al., 2011; Eccher et al., 2015; Botton and Ruperti, 2019). Currently, the nature of the nutritional stress that promotes the accumulation of ethylene and ABA in fruits is not understood, as studies in apple indicate that abscising fruits have similar sugar profiles as persisting fruits during early stages of development (Ackerman and Samach, 2015). Moreover, the role of ABA in the abscission process has yet to be characterized (Botton and Ruperti, 2019).

Studies in avocado show that abscised and weakly attached immature fruits accumulate high levels of ethylene (Adato and Gazit, 1977), as well as ABA (Davenport and Manners, 1982; Garner and Lovatt, 2016). These studies indicate that ethylene is primarily produced in the seed and seed coat, while ABA is synthesized in the mesocarp. Therefore, it was hypothesized that accumulation of ethylene and ABA in fruits fated to abscise would prevent nutrient uptake causing the nutrient deficient fruit to abscise.

Ethylene

Analysis of the transcriptome data indicated that growth arrest involves changes in ethylene biosynthesis and signaling in the seed coat, embryo and pericarp (Figure 25). Results showed that ethylene biosynthesis genes were highly induced in the seed coat, pericarp and embryo (Figure 25A). The increase in expression of these ethylene biosynthesis genes occurred early in fruit growth arrest, indicating that this hormone may play a key role in this process. In contrast to the seed tissues, a number of ethylene biosynthesis genes were also down-regulated in pericarp (Figure 25A). The down-regulation of ethylene biosynthesis genes occurred late in the growth arrest process and may be regulated by a feedback mechanism. In the maternal tissues, seed coat and pericarp, a number of ethylene signaling genes were induced, as well as repressed (Figure 25B). However, in the embryo, ethylene signaling genes were only induced (Figure 25B). Thus, the gene expression results indicates that ethylene acts early in the growth arrest process throughout the fruit tissues. Given the seed coat exhibits the highest up-regulation of ethylene biosynthesis genes early the growth arrest process suggests that growth cessation is initiated in the seed coat. This hypothesis is in contrast from the mechanism proposed in apple (Botton et al., 2011), which speculates that ethylene is produced in the cortex to promote fruit abscission via embryo abortion.

To further address a role for ethylene in immature fruit abscission, we aimed to determine the tissue(es) where this hormone was produced during fruit growth arrest. Due to gaseous nature of ethylene and difficulties with estimating this hormone, we quantified the levels of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of this hormone. By quantifying the precursor of ethylene, we can determine the tissues where ethylene biosynthesis is activated. Results showed that ACC was detected in the seed coat, pericarp and embryo of normal growing fruits, with the highest levels occurring in the pericarp (Figure 25C). In response to growth arrest, ACC levels significantly increased in the seed coat, but not in the pericarp and embryo (Figure 25C). Thus, ethylene biosynthesis appears to be activated primarily in the seed coat during fruit growth arrest. However, we cannot rule out the possibility that ethylene is also produced in the pericarp and embryo if ACC is rapidly converted to ethylene in these tissues.

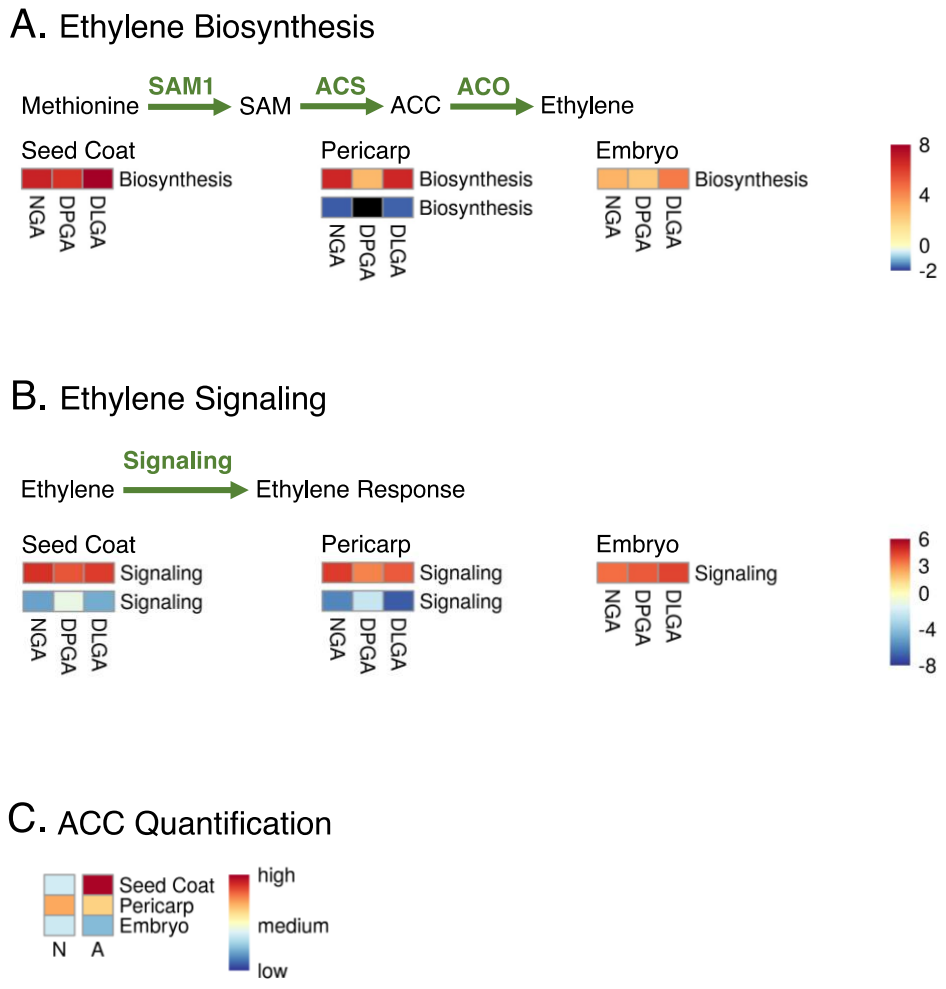

Figure 25. Collective changes in the expression of genes involved in ethylene activity and ACC levels during fruit growth arrest
Heatmap used to display the collective expression of genes involved in (A) ethylene biosynthesis and (B) signaling in the seed coat, pericarp and embryo of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed. (C) Heatmap used to display changes in ACC (1-aminocyclopropane-1-carboxylate) levels in the seed coat, pericarp and embryo of (N) normal growing and (A) arresting fruit samples.

Abscisic Acid (ABA)

As ABA is implicated in apple immature fruit abscission (Botton et al., 2011; Eccher et al., 2013), we queried the transcriptome data to determine if the expression of genes that control ABA activity were altered during growth arrest. In the seed coat, pericarp and embryo, ABA biosynthesis genes were up-regulated during fruit growth arrest (Figure 26A). This up-regulation occurred early in the growth arrest process. In the pericarp only, a number of ABA biosynthesis genes were down-regulated during fruit growth cessation. Results show that the down-regulation of these ABA biosynthesis genes occurred late in the growth arrest process in the pericarp (Figure 26A). ABA deactivation genes were up-regulated in the seed coat, pericarp and embryo, which may function to regulate the levels of this hormone during the growth arrest process (Figure 26B). Interestingly, the ethylene deactivation genes were up-regulated early in the growth arrest process in the seed coat and embryo but not in the pericarp. In addition, ABA signaling genes were differentially expressed in the seed coat and pericarp during fruit growth arrest (Figure 26C). Overall, in the embryo, ABA signaling genes were up-regulated during fruit growth cessation (Figure 26C). Taken together, the transcriptome studies suggests that ABA plays a role in fruit growth arrest, and likely acts early, as well as late in the process.

Figure 26. Collective changes in the expression of genes involved in ABA activity and ABA levels during fruit growth arrest
Heatmap used to display the collective expression of genes involved in (A) ABA biosynthesis, (B) deactivation and (C) signaling in the seed coat, pericarp and embryo of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed. (D) Heatmap used to display changes in ABA levels in the seed coat, pericarp and embryo of (N) normal growing and (A) arresting fruit samples.

To further address a role for ABA in immature fruit abscission, we quantified the levels of this hormone, as well as the catabolites, phaseic acid (PA) and dihydro phaseic acid (DPA), in normal growing and arresting fruits. Results showed that the levels of ABA were low in the seed coat, pericarp and embryo in normal growing fruits (Figure 26D). However, during growth arrest a significant increase in ABA occurred in the pericarp only (Figure 26D). In normal growing fruits, PA and DPA levels were highest in the embryo and pericarp, respectively (Figure 26E and F). During fruit growth arrest, a significant increase in PA and DPA occurred in the seed coat and embryo (Figure 26E and F). Therefore, results suggest that ABA may

play a role in mediating fruit growth arrest in the pericarp. However, in the seed coat and embryo, ABA levels are tightly regulated during fruit growth arrest.

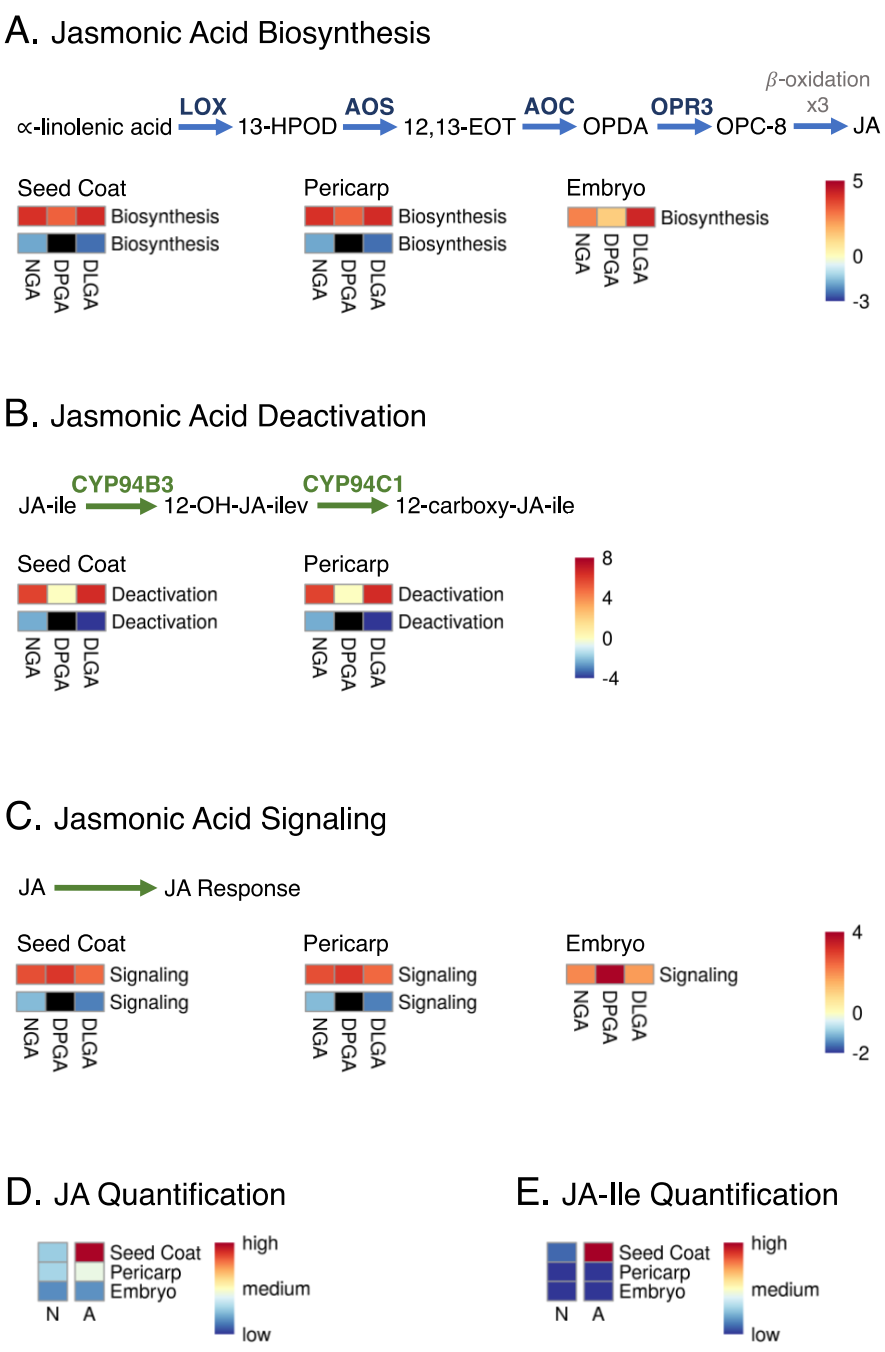

Figure 27. Collective changes in the expression of genes involved in jasmonic acid (JA) biosynthesis, deactivation and signaling during fruit growth arrest

Heatmap used to display the collective expression of genes involved in (A) JA biosynthesis, (B) deactivation and (C) signaling in the seed coat, pericarp and embryo of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DLGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed. Heatmap used to display changes in (D) JA and (E) JA-Ile levels in the seed coat, pericarp and embryo of (N) normal growing and (A) arresting fruit samples.

Jasmonic acid (JA)

While jasmonic acid (JA) and the active conjugate (JA-Ile) play a significant role in inhibiting growth and promoting seed

maturation (Li et al., 2004; Sohn et al., 2022), the role of this hormone in immature fruit abscission is poorly understood. To assess a function for JA activity during fruit growth arrest, we examined the expression of genes associated with the function of this hormone. To this end, genes that encode proteins involved in JA biosynthesis were differentially expressed in the seed coat and pericarp during fruit growth cessation (Figure 27A). Overall, JA biosynthesis genes were up-regulated during growth arrest in the embryo. Genes related to JA deactivation and signaling were differentially expressed in the maternal tissues, seed coat and pericarp, during growth cessation with a set of genes up-regulated and another set down-regulated (Figure 27B and C). While there was no change in the expression profiles of JA deactivation genes in the embryo, the transcriptome study suggests that JA signaling was induced throughout the growth arrest process in this seed tissue (Figure 27C). Taken together, results suggest that JA plays a role in mediating fruit growth arrest throughout the developmental process.

To further characterize a role for JA in fruit growth arrest, the levels of JA and JA-Ile were quantified in the seed coat, pericarp and embryo of normal growing and arresting fruits. While JA was detected in the tissues of normal growing fruits, the levels of this hormone significantly increased in the seed coat only (Figure 27D). Further, a significant increase in JA-Ile also occurred in the seed coat. Thus, our results suggest that JA and JA-Ile play a role in mediating growth arrest during immature fruit abscission Figure 27E). The differential expression of JA biosynthesis and signaling genes in the pericarp and embryo may be the result of mobile nature of this hormone and its ability to control its own production. However, we predict that a mechanism(s) may exist to prevent this hormone accumulating in the pericarp and embryo during growth arrest.

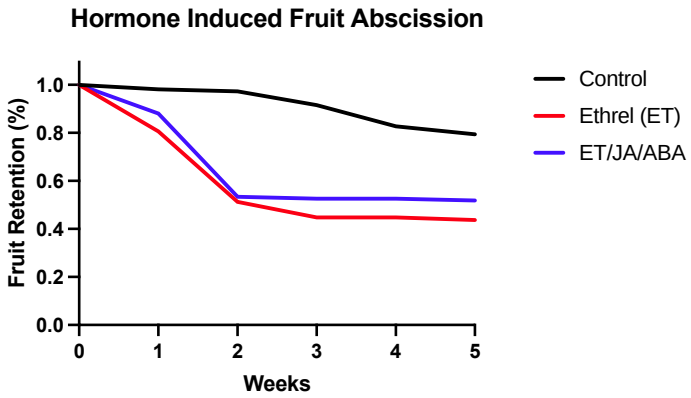

Figure 28: Validating a role for hormones implicated in immature fruit abscission
Fruit retention (%) was monitored on a weekly basis for five weeks. Ethrel was used as an ethylene (ET)-evolving plant growth regulator to assess the role of this hormone in immature fruit abscission (red line). A mixture of hormones implicated in immature fruit abscission was also applied, which included Ethrel, JA and ABA (blue line). Control trees were treated with the wetting agent used in our hormone applications (black line).

Validating the role of ethylene, JA and ABA in immature fruit abscission

A trial was established to determine if ethylene, as well as JA and ABA, mediate immature fruit abscission. To examine the impact of ethylene on immature fruit abscission, the ethylene-evolving plant growth regulator, Ethrel, was applied to a set of trees. In addition, we also applied a Ethrel, JA and ABA to another set of trees to compare with the Ethrel treatment alone and control trees. Fruit abscission was monitored by tagging a subset of fruits in each of the trees for each treatment as well as the control. Results showed the control trees experienced a fruit drop event 2 to 4 weeks after the trial was initiated (Figure 28). During this period, fruit retention was reduced by 20.6% in control trees. In the Ethrel treated trees, immature fruit abscission was induced from 0 to 3 weeks after this PGR was applied to the trees (Figure 28). At the end of the trial, the fruit retention in the Ethrel treated trees was significantly reduced by 56.3%. In trees treated with Ethrel, JA and ABA, immature fruit abscission was induced from 0 to 2 weeks after application (Figure 28). At the end of the trial, fruit retention was reduced by 48.2% in the Ethrel, JA and ABA treated trees, which was not significantly different from Ethrel treated trees. These results indicate that ethylene is an inducer of immature fruit abscission in avocado. The fact that fruit abscission was not enhanced Ethrel plus JA and ABA application indicates that (1) JA and ABA play a minor role in fruit

abscission, (2) ABA and/or JA may counter act the abscission potential of fruits and/or (3) a subset of fruits, ~48-50%, may be resistant to the actions of these hormones.

Developing a PGR application to limit summer fruit abscission

The seed coat is an essential organ that facilitates the uptake of nutrients from the mother plant and distributes this material to the embryo, endosperm and pericarp tissues (Radchuk and Borisjuk, 2014). In addition, the seed coat also produces hormones that are transported the other parts of the seed and fruit to regulate growth. Thus, the seed coat is essential for coordinating physiological and developmental processes that take place during fruit development (Radchuk and Borisjuk, 2014). Research from our project shows that the seed coat is the major site of auxin production. Moreover, a significant decline in carbohydrates and free auxin levels in the seed coat is associated with fruit growth arrest. Based on our studies, we propose that fruit growth arrest is mediated by a maturation-like pathway, which functions in part to repress growth and promote the competence to abscise. Taken together, we hypothesize that maintaining seed coat function is essential for limiting immature fruit abscission.

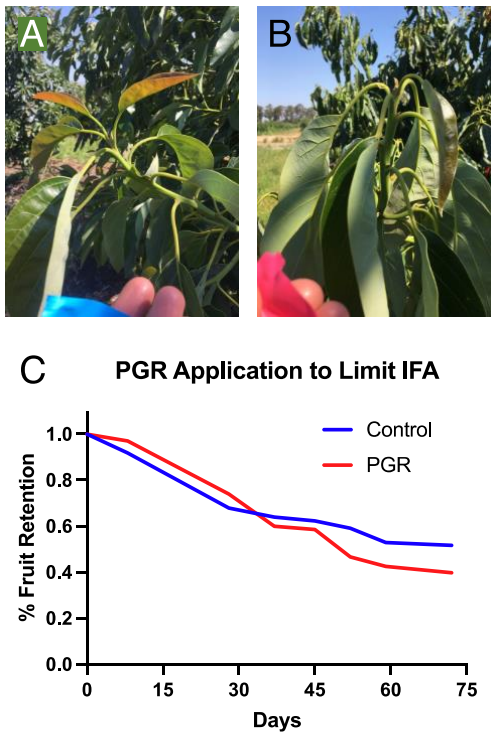

Figure 29: Validating a role for hormones in immature fruit abscission

(A) Image of a control vegetative shoot 3-days after the wetting agent was applied at the onset of the PGR trial. (B) Image of PGR treated shoot 3-days after the PGR application was applied. Fruit retention (%) was monitored on a regular basis over the course of the trial. A PGR application containing NAA (1-naphthaleneacetic acid), AVG (aminoethoxyvinylglycine) and/or DIECA (diethyldithiocarbamate) was applied at regular intervals (red line) (note: NAA is a stable synthetic auxin, AVG inhibits ethylene biosynthesis and DIECA is an inhibitor of JA biosynthesis). Control trees were treated with the wetting agent used in our plant growth regulator application (black line).

To maintain seed coat function, three hormones whose levels were significantly altered during fruit growth arrest in the seed coat were targeted. Given that auxin is not only critical for mediating cell division but also suppresses maturation (Kumar et al., 2014; Fenn and Giovannoni, 2021), we reason that maintaining auxin levels in the seed coat is critical for protecting fruits from a maturation-like signal(s) that inhibits growth. As we showed that ethylene promotes immature fruit abscission in avocado (Figure 28), this hormone was targeted to limit immature fruit abscission. Given that JA promotes seed maturation and senescence, as well as inhibits leaf and petal expansion (Li et al., 2004; Huang et al., 2017; Sohn et al., 2022), we reasoned that targeting this hormone may also be important for managing immature fruit abscission. In this trial, a PGR mixture AVG (aminoethoxyvinylglycine) and DEICA (diethyldithiocarbamate) at 150 mg/L and 22 mg/L, respectively, was applied every 7-10 days. At the onset of the trial and every three weeks thereafter, NAA (1-napthaleneacetic acid; 25

mg/mL) was included in the AVG/DEICA application. This trial was initiated when the average diameter of fruits was 37.1 mm. Results showed that 3-days after application of the NAA/AVG/DEICA mixture the vegetative shoots wilted compared to the control trees treated with the wetting agent (Figure 29A and B). As applications of NAA can stimulate ethylene production, which is dependent upon the sensitivity of a plant to this synthetic auxin (Iqbal et al., 2017; Clayton-Cuch et al., 2021), it is highly likely that treatment of avocado trees with 25 mg/L stimulated ethylene production, which resulted in the wilting of vegetative shoots. After this initial application, NAA was applied at 5 mg/L every third week. At this concentration, the NAA/AVG/DEICA had no effect on shoots (data not shown). Throughout and at the end of the trial, results showed that the percentage of fruit retention was similar in both control and PGR treated trees (Figure 29C). While this application did not yield a positive result, it is imperative in the next project to determine the upper concentration of NAA that can be applied without stimulating an ethylene-like response. It should also be pointed out the presumed release of ethylene in response to 25 mg/L application failed to induce a significant immature fruit abscission event. Therefore, the concentration of AVG may be effective at limiting the impact of ethylene induced by NAA.

Preliminary data on the temporal hormone profiles in the seed coat during growth arrest

In order to effectively develop a plant growth regulator application to mitigate summer fruit abscission, it is critical to determine the changes in hormone profiles that occur early in the fruit growth arrest process. As defoliation can be used to induce immature fruit abscission, this system can be utilized to capture fruits at early to late stages of growth arrest. Further, as the seed coat appears to the critical tissue that transmits information to the embryo and pericarp, we focused on examining hormones in this tissue. In this trial, a set of trees were selected for defoliation, as well as another set to collect fruits with a normal growth rate, termed “control fruits”. After tagging, two fruit diameter measurements were recorded to estimate normal growth rates in control and treated tree prior to defoliation. Five-days after defoliation, fruits with a normal growth rate were harvested from control and defoliated trees. In addition, fruits with an intermediate growth rate, as well as fruits that had nearly undergone growth cessation, were harvested from defoliated trees. Thus, in this trial, we were able to capture fruits at early, mid and late stage of growth arrest. After isolating the seed coat, IAA, IAA-Asp, ACC and JA were quantified. Unfortunately, many of the measurements were based on two biological replicates (n = 2). Therefore, the results presented in figure 30 are preliminary. The preliminary results suggest that free IAA and IAA-Asp levels decline and increase, respectively, early the growth arrest process in the seed coat (Figure 30 A and B). Therefore, the early reduction in free IAA suggests that this hormone is critical for fruit growth and must be targeted for management intervention to limit growth arrest. During fruit growth arrest, preliminary results indicate that ACC substantially increases late in this process (Figure 30C). In addition, preliminary results suggest that a peak of JA was induced at an intermediate stage of growth arrest (Figure 30D). Thus, the preliminary data presented indicates that ethylene and JA may not be acting early in the fruit growth arrest process.

Developmental mechanisms that control growth and maturation are associated with fruit growth arrest

Fruits undergo sequential phases of development to ensure that these reproductive structures and seeds reach their final size and mature before ripening and abscission is initiated (Kumar et al., 2014; Fenn and Giovannoni, 2021). The maturation transition occurs at the end of the growing phase and is necessary for the competence to ripen/senescence, which ends with abscission (McAtee et al., 2013; Kumar et al., 2014). A model that describes the hormonal control of immature fruit abscission was developed in apple and serves as a basis to understand fruitlet abscission in other crops. According to this model, ethylene produced in the cortex moves to the seed to induce embryo abortion (Eccher et al., 2015; Sawicki et al., 2015; Botton and Ruperti, 2019). As the fruits undergo developmental arrest in response to embryo abortion, transport of auxin through the pedicel diminishes allowing ethylene to activate fruit abscission. While this model describes the hormone signaling events linking ethylene with embryo abortion and abscission, it doesn’t provide any information regarding the developmental events that are associated with fruit growth arrest. In this section, we provide evidence of how fruits undergo fruit growth arrest.

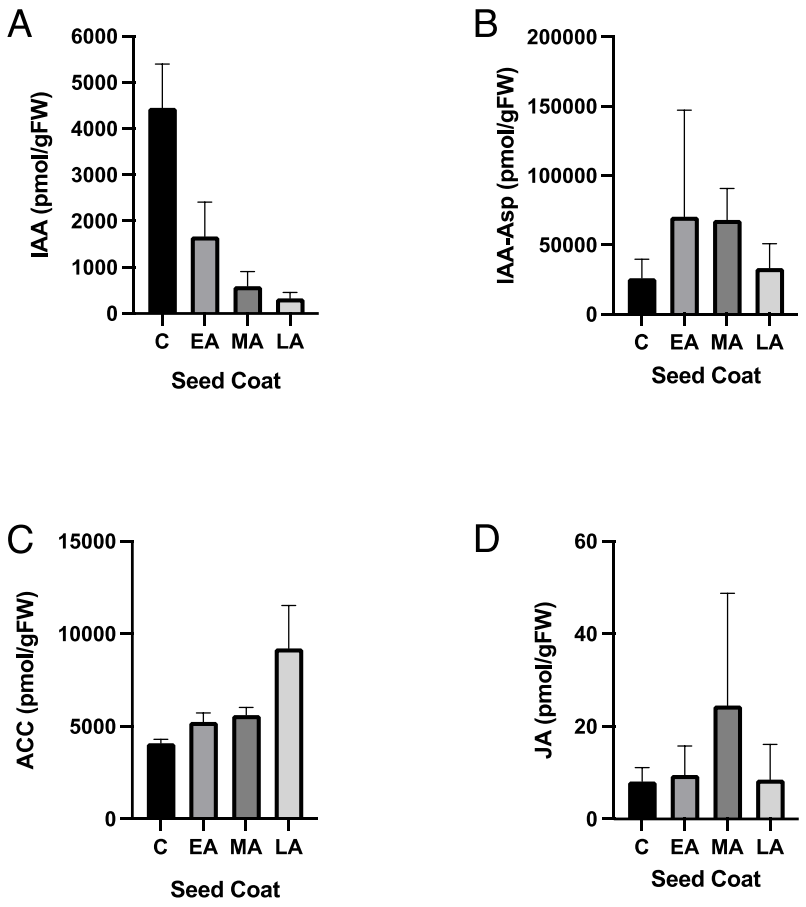

Figure 30: Preliminary temporal hormone profiling in the seed coat during fruit growth arrest
Defoliation was used to induce summer fruit abscission in order to determine a preliminary temporal pattern of the hormonal control of fruit growth arrest in the seed coat. The levels of (A) IAA, (B) IAA-Asp, (C) ACC and (D) JA was quantified in the seed coat of normal growing fruits from control trees (C) and compared to with the seed coat derived from fruits undergoing arrest at an early (EA), mid (MA) and late (LA) stage of growth arrest. As this work is preliminary, this trial will need to be repeated.

Alteration in organ polarity gene expression is associated with fruit growth arrest

After fertilization, the developmental mechanisms that mediate the expansion of the seed coat and pericarp is poorly understood. In leaves, expansion is mediated by the juxtaposition of the adaxial (top) and abaxial (bottom) domains (Figure 30), which allows these structures to grow in a lateral direction (Du et al., 2018). The interplay between the polarity regulators that specify the adaxial and abaxial domains creates a boundary or middle domain where auxin activity is confined (Heisler, 2021). In addition to leaves, genetic studies show that the adaxial/abaxial polarity regulators also mediate expansion of floral organs, including carpels, as well as integuments, which give rise to the seed coat (Du et al., 2018). Given the interplay between polarity regulators with auxin activity, we queried the gene expression data to determine if adaxial/abaxial polarity factors are differently expressed in the maternal tissues during growth arrest. Results showed that overall adaxial regulatory-like genes are down-regulated in the seed coat and pericarp during growth arrest (Figure 31). In the seed coat and pericarp, abaxial regulatory-like genes are differentially expressed during growth arrest (Figure 31). Taken together, the gene expression data suggests that the fruit growth arrest signal(s) target adaxial/abaxial polarity regulatory-like genes, which may act to suppress the expansion of the seed coat and pericarp.

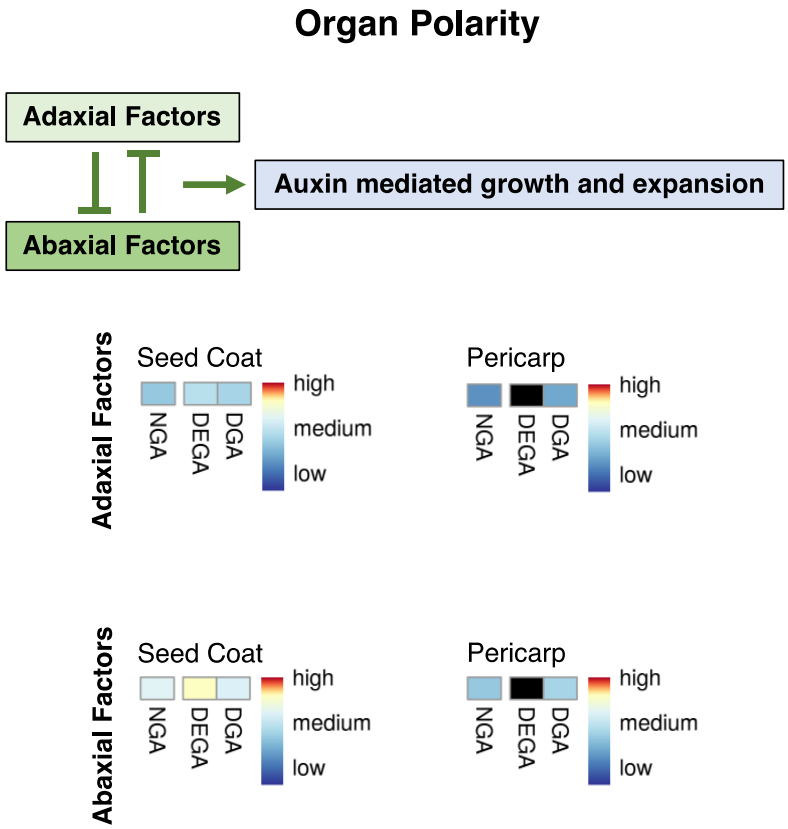

Figure 31. The expression of organ polarity genes were altered during fruit growth arrest
Auxin mediated growth and expansion is mediated by the interplay between factors that specify adaxial and adaxial domains of leaves, floral organs and integuments.Heatmap used to display collective changes in the expression of genes that control adaxial and abaxial identity in the seed coat and pericarp of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DEGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed.

Fruit growth arrest is associated with an increase in the expression of genes that negatively regulate meristem activity

In leaves, a high rate of cell division occurs at the margins where auxin production is confined, as well as the inner tissues (Heisler, 2021; Tsukaya, 2021). Together these regions of high cell division are term as the "leaf meristem". Given that regulators of the leaf meristem also impacts the growth of floral organs, integuments and/or seeds, suggest that leaf-like meristems also control the growth of structures related to leaves including the seed coat and pericarp tissues (Figure 32) (Tsukaya, 2021). As avocado fruit growth is primarily mediated by cell division (Schroeder, 1953; Cowan et al., 2001; Dahan et al., 2010), we examined the possibility that conserved meristem regulators mediate growth arrest during immature fruit abscission. Results showed that negatively and positively regulators of meristem activity and cell proliferation were up- and down-regulating, respectively, during fruit growth arrest (Figure 32). Further, a subset of negative regulatory-like genes were up-regulated early in the seed coat suggesting that the growth arrest signal(s) may target these genes to repress cell proliferation (Figure 32). Given that these negative regulators promote maturation (Du et al., 2018), suggests that growth arrest may be caused by the activation of a quasi-maturation process.

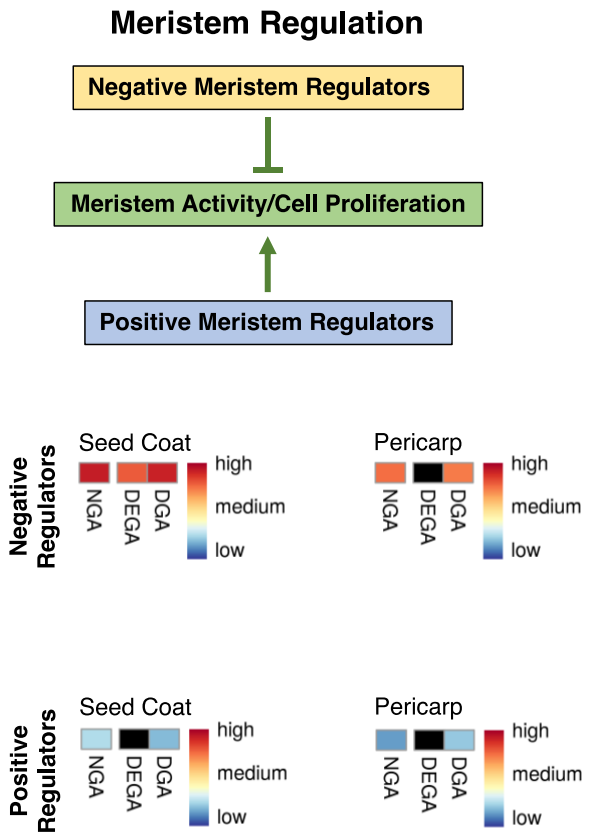

Figure 32. Differential expression of meristem genes during fruit growth arrest
Cell proliferation is controlled by regulatory proteins that positively and negatively regulate meristem activity in developing organs. Heatmap used to display collective changes in the expression of genes that control meristem activity in the seed coat and pericarp of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DEGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed.

Differential expression dormancy signaling genes during fruit growth arrest

Seed dormancy is a developmental transition induced during fruit maturation by signaling events in the maternal tissues, as well as the embryo (Iwasaki et al., 2022). Given the possibility that fruit growth arrest is mediated by a quasi-maturation process, we queried the gene expression data to determine if there was an alteration in signaling proteins associated with seed dormancy. Results showed that signaling proteins that negatively regulate dormancy in the seed coat were down-regulated, whereas signaling proteins that promote dormancy were up-regulated in the embryo (Figure 33). Thus, the pattern of dormancy signaling gene expression further supports our hypothesis that fruits undergo arrest by a quasi-maturation process.

Down-regulation of phase transition pathways are associated with growth arrest in maternal tissues

Plants undergo phase transitions during development (Samach and Smith, 2013; Zheng et al., 2019). Floral induction is a major phase transition that transforms vegetative meristems to flower producing meristems (Zhu et al., 2021). While a role for these pathways have yet to be identified during fruit development, the gene expression study showed that the expression two related floral repressor-like genes were induced during early and late stages of growth arrest in the seed coat and embryo (Figure 34). Taken together, we propose that these floral repressor-like genes were co-opted to promote (1) fruit maturation, (2) seed dormancy and/or (3) repress growth during growth cessation.

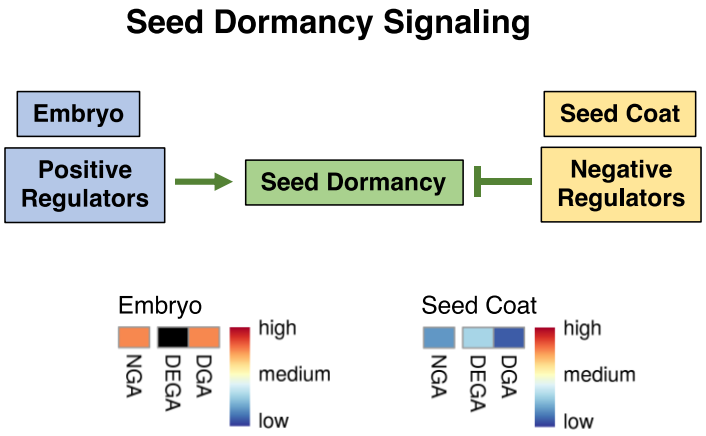

Figure 33. Differential expression of dormancy signaling genes during fruit growth arrest
Seed dormancy is regulated by signaling proteins that positively and negatively this process during maturation. Heatmap used to display collective changes in the expression of genes that control meristem activity in the seed coat and pericarp of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DEGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed.

The physiological mechanism of fruit growth arrest is not completely conserved between the early and summer fruit drop events

Up to ~60% of fruits abscise during early stages of development before fruits reach ~15 mm in diameter (Figure 1). To date, it is unclear as to whether the mechanism of early fruit drop is conserved with summer fruit drop. In our study, we harvested normal growing/control and arresting fruits during the early fruit abscission event when the average diameter was 12 mm. A sugar metabolite profiling experiment was performed to determine if the major physiological driver of early fruit abscission was associated with a depletion of sugar and starch in the seed coat and alteration in sugar and starch profiles in the pericarp and pedicel similar to summer fruit abscission (see Figure 15). Sugar metabolite analysis showed that there were no significant changes in the levels of sucrose, glucose, fructose, perseitol, mannoheptulose and starch in the seed coat of normal growing/control fruits and arresting fruits (Figure 35A-F). Therefore, early fruit growth arrest doesn't appear to involve a depletion of sugars and starch in seed coat. Interestingly, in the pericarp, a significant increase in the levels of sucrose, fructose, perseitol and mannoheptulose occurred in early arresting pericarp tissues compared to normal growing/control fruits (Figure 35A, C, D and E). The increase in sucrose and mannoheptulose in early arresting pericarp tissues is comparable to that which occurs during summer growth arrest (see Figure 15 for comparison). However, in contrast to summer growth arrest, the early arresting pericarp tissues also accumulated perseitol and displayed no changes in the levels of starch (Figure 35D and F). Taken together, our results suggest that the major physiological driver of early fruit abscission is not due to depletion of carbon in the seed coat due to decrease in carbohydrate supply and metabolism.

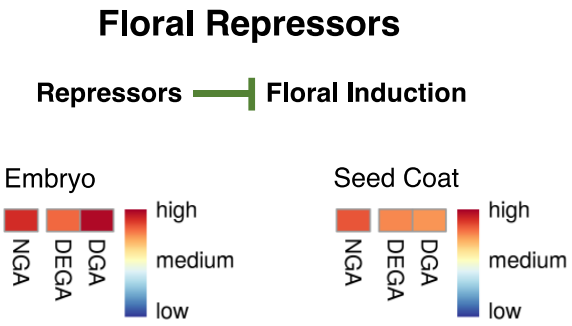

Figure 34. An increase in the expression of floral repressor during fruit growth arrest
Seed dormancy is regulated by signaling proteins that positively and negatively this process during maturation. Heatmap used to display collective changes in the expression of genes that control meristem activity in the seed coat and pericarp of fruits undergoing growth arrest naturally (NGA). Fruits arresting early (DPGA) and late (DEGA) in response to defoliation were used to examine the hierarchy of gene expression. The $\log_2$ fold scale for gene expression is displayed to the right of each heatmap. Black boxes indicate that a gene is not differentially expressed.

Arresting fruits display a significant decrease in aluminum levels during summer fruit abscission

Mineral analysis of arresting fruits indicates that fruit growth arrest is not associated with a deficiency in a macro- and micronutrient(s) (Figure 12). In this analysis, we discovered that fruits undergoing growth arrest displayed a significant reduction in aluminum compared to normal growing/control fruits in the seed coat, pericarp and embryo (Figure 36). However, in pedicels derived from normal growing control and arresting fruits, aluminum levels were similar (Figure 36). The significance in the relationship between fruit growth arrest and reduced levels of aluminum is not understood. While aluminum levels are significantly reduced during fruit growth arrest, **due to the toxicity of this metallic element, it not recommended to apply aluminum to limit fruit abscission.**

The impact of tree carbohydrate status on flowering and early fruit retention

In temperate regions, which experience relatively cool winter temperatures, avocado trees store starch in stem tissues (Scholefield et al., 1985; Whiley et al., 1996; Liu et al., 1999). During the spring and summer months, the decline in stem starch levels correlates with the onset and growth of vegetative flushes, flowering, fruit set and fruit development. Taken together, stem starch reserves appear to play a critical role in the growth and development of vegetative and reproductive units in the trees. To further validate this hypothesis, we utilize early and mid-season defoliation trials (DEF1 and DEF2, respectively), as well as a mid-season drought stress (DR) trial, to study the impact of stem carbohydrates on flowering and fruit set the following season.

Flowering was estimated using a visual scale of 1-5, where 1 and 5 represent as low and high level of flowering, respectively. In addition, flowering was measured by determining the average number of reproductive buds per shoot (Figure 37A and B). Results showed that the visual and reproductive buds per shoot measurement systems were highly comparable, with control and DEF2 tree exhibiting a similar level of flowering (Figure 37A and B). DEF1 trees exhibited the highest level of flowering while DR trees displayed a low level of flowering. As fruit set is not easily quantified without microscopic evaluation, we quantified the number of fruits per shoot at a late stage of flowering, as this likely reflects the rate of fruit set (Figure 37C, 11 December 2019 time point). In addition, first fruit abscission was also evaluated after the initial fruit drop event (Figure 37C, 16 January 2020 time point). Results showed DEF2 trees exhibited the highest number of fruits per shoot, followed by control trees (Figure 37C). In contrast, DEF1 and DR trees displayed a lower number of fruits per shoots (Figure 37C). Yield was estimated in the trial following the treatments in 2019. As expected DEF1 and DEF2 trees did not produce a crop, as defoliation causes >97% of the fruits to abscise (Figure 37D). In addition, compared to control trees, drought stress caused a significant reduction in yield, as this treatment caused a significant of fruit abscission event (Figure

37D). While differences in stem starch levels, as a results of the previous seasons treatments, defoliation and drought, impacted flowering, fruit retention and abscission, early- to mid-season, the final yield was similar across all treated trees in 2020 (Figure 37D), indicating that mid- to late-season fruit abscission must have reduced crop levels in control and DEF2 trees.

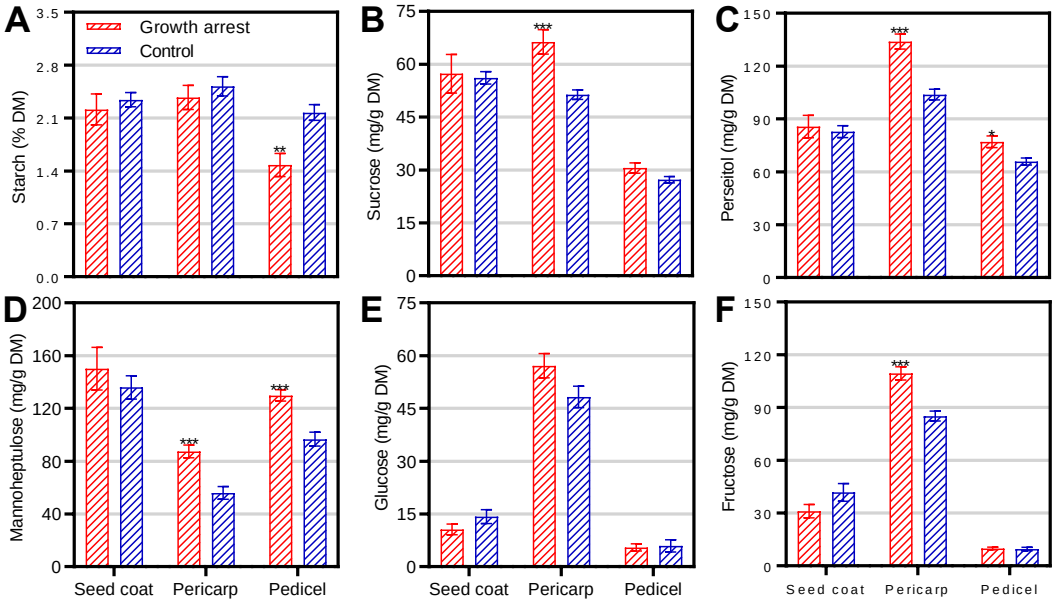

Figure 35. Quantification of sugar metabolites and starch in control and arresting fruit derived from the early fruit abscission event. The levels of (A) starch, (B) sucrose, (C) perseitol, (D) mannoheptulose, (E) glucose and (F) fructose were measured in normal growing/control and arresting fruits during the early fruit abscission event. Carbohydrates were measured in mg/g of dry mass (DM), except starch which is presented in % dry mass. Numbers are mean values derived from eight biological replicates (fruits), ± standard error of the mean (bars). Two-tailed Student's t-test was used to determine significant differences indicated by asterisks **p*=≤0.05, ***p*=≤0.01, ****p*=≤0.001.

After quantifying the effect of the above treatments on flowering, fruit set and early to mid-season fruit abscission, as well as yield, we evaluated stem carbohydrate levels at bud burst, peak flowering and the early fruit drop event to correlate these phenological events with tree carbohydrate status. At bud burst, stem starch levels were highest in DEF1 and DEF2 trees, while control and DR trees displayed lowest levels of starch (Figure 37A). At peak flowering, stem starch levels were highest in control, DEF2 and DR trees with starch levels lowest in DEF1 trees (Figure 38A). At the time in which trees advanced to the early fruit drop event, there was no significant differences in starch levels (Figure 38A). For the translocated sugars, sucrose, perseitol and mannoheptulose, the levels of these sugars varied between each treatment with no commonality for these sugars at bud burst and peak flowering (Figure 38B-D). Out of these three translocated sugars, perseitol appeared to display a similar pattern as starch across each treatment at bud burst and peak flowering (Compare Figure 38A with C). At the early fruit drop event, that accumulation pattern for sucrose, perseitol and mannoheptulose was similar (Figure 38B-D). The levels of glucose and fructose also varied across the treatment at each time point with no similarity with the pattern of starch levels across each treatment and time point (Figure 38E and F). Based on our study, the levels of stem starch and perseitol at bud burst and flowering appear to associate with the level of flowering and the number of fruits that are retained shortly after fruit set. Therefore, we propose that managing the levels of starch and perseitol from budburst to the early fruit abscission event, will be key to increasing fruit set and early fruit retention.

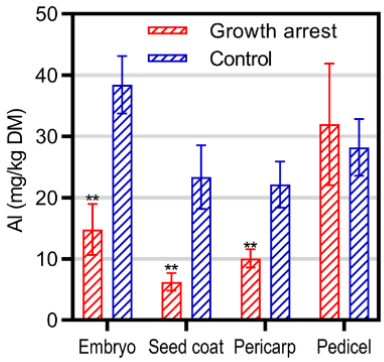

Figure 36. The levels of aluminum were reduced in fruit tissues undergoing growth arrest.
Aluminum levels in mg/kg dry mass (DM) were measured in the embryo, seed coat, pericarp and pedicle from normal growing (blue) and arresting (red) fruits with an average daily growth rate of 0.79 mm/day and 0.05 mm/day, respectively. Numbers are the mean values derived from 8 biological replicates (fruits), ± standard error of the mean (bars). Two-tailed Student's t-test was used to determine significant differences indicated by asterisks *p=≤0.05, **p=≤0.01, ***p=≤0.001.

Discussion

Summer fruit abscission is a target for management intervention

In avocado, variability in the pattern of fruit abscission has been described in different growing regions (Adato and Gazit, 1977; Slabbert, 1981; Davenport and Manners, 1982; Perez et al., 1988; Garner and Lovatt, 2008, 2016). Results from field trials in a coastal region in WA indicate that the initial fruit drop event is a major factor that impacts yield. However, a significant percentage of fruits, also abscise during the summer months. In the hot and dry climate of Riverland, SA, the average percentage of summer fruit drop can also be significantly high, particularly in years with high fruit set. Given that trees have invested up to 40% dry matter into fruits that drop during the summer (Whiley and Wolstenholme, 1990), mitigating fruit abscission during this period would have a considerable impact for maximizing the reproductive potential of the tree and increasing yield. We hypothesize that temporal fluctuations in the carbohydrate status of a tree combined with dominance interactions between growing vegetative shoots and developing fruits, as well as among fruits, causes a subset of fruits to abscise during the summer.

The impact of tree carbohydrate status and dominance interactions on fruit abscission

The impact of tree carbohydrate status on the level of fruit abscission was addressed by evaluating the effect of defoliation and shading on fruit drop. As fluctuations in stem starch levels correlates with the growth of vegetative shoots, flowering, fruit set and fruit growth (Scholefield et al., 1985; Whiley et al., 1996; Liu et al., 1999), we evaluated tree carbohydrate status by measuring stem starch levels in the treated and control trees in the defoliation and shading trials. We hypothesized that a reduction in tree carbohydrate status/stem starch levels in response to defoliation and shading would promote fruit abscission. Results from our study showed that 3-days before fruit abscission was initiated, stem starch levels were significantly reduced, as well as sucrose and perseitol levels, in defoliated trees. In response to shading, a reduction in stem starch levels was also associated with the onset of fruit abscission. However, in contrast to defoliation, the levels of sucrose and perseitol in stems were similar to control trees. In addition, the level of fruit abscission was highly variable between the shaded trees. Thus, shading is not as effective as defoliation at reducing stem carbohydrates and promoting a significant abscission event. Based on these results, we suggest that assessment of tree carbohydrate status during the summer months should include measuring starch, as well as perseitol and sucrose. Future R&D addressing the usage of NIR-reflectance spectroscopy should target starch, perseitol and sucrose for assessing tree carbohydrate status during the summer.

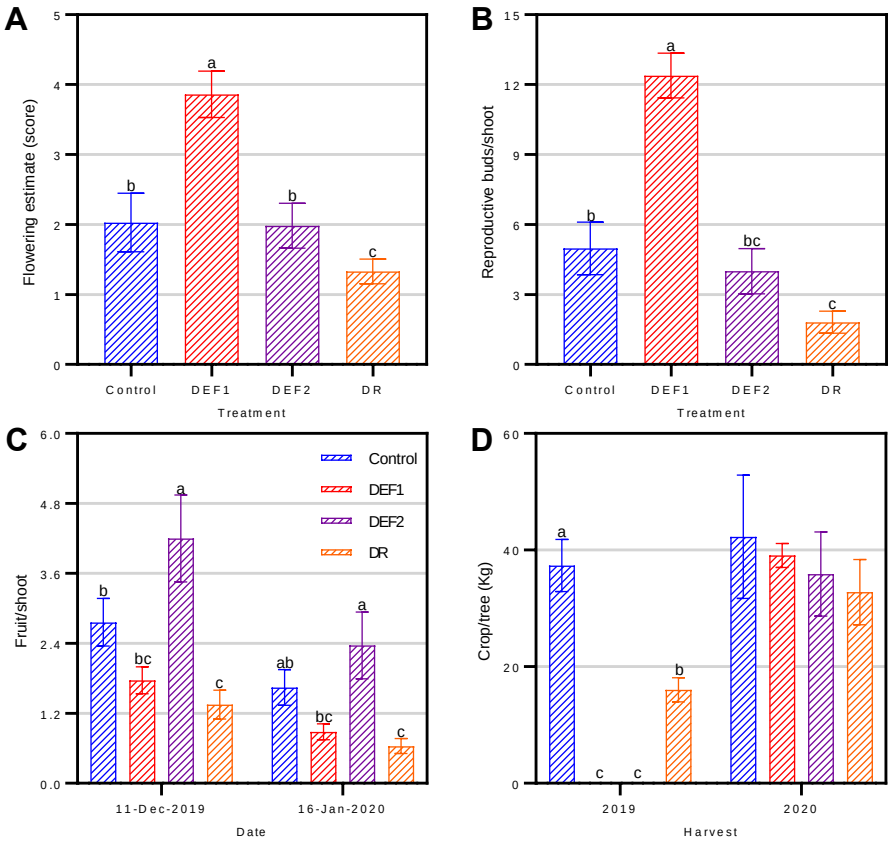

Figure 37. Relationship between flowering intensity, fruit abscission and yield.
In this study, early (DEF1) and mid-season (DEF2) defoliation, as well as drought stress (DR) were used to evaluate the impact of flowering on early fruit retention. The intensity of flowering was evaluated (A) visually and (B) by quantifying the number of reproductive buds per shoot. (C) The number of fruitlets retained per shoot was scored early in fruit development and after the initial fruit drop event. (D) Yield was determined in 2019 and 2020. (B and C) The number of reproductive buds and fruit per shoot was assessed using a subpopulation of 12 shoots selected before bud break in each tree. Numbers are mean values derived from 5-8 replicates (trees), ± standard error of the mean (bars). Lines with the same letter are not significantly different as determined by Analysis of Variance with post-hoc Tukey's Honestly Significant Difference test, $p \leq 0.05$.

The impact of dominance interactions on carbohydrate partitioning and fruit abscission was investigated in this project. It has been postulated that growth of vegetative flushes induces fruit abscission (Whiley and Wolstenholme, 1990; Salazar-García et al., 2013). In this project, we extended this hypothesis to address if carbohydrate availability may be a driver in dominance induced fruit abscission. First, we showed that tipped shoots retained more fruits. Furthermore, the levels of stem carbohydrate levels were similar between control and tipped shoots. These results indicate that excess carbohydrate that would normally be partitioned to vegetative shoots was supplied to support the development of additional fruits when the shoots were tipped. Taken together, our results provide evidence that growth of vegetative flushes diverts carbohydrates away from developing fruits, causing them to abscise.

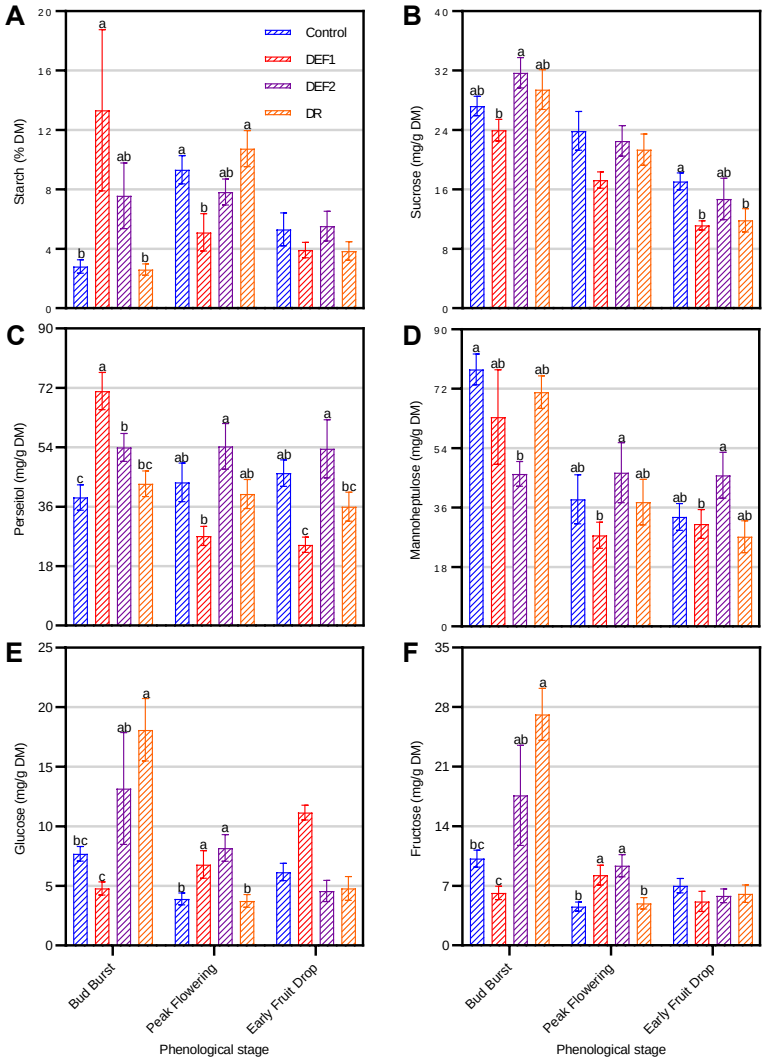

Figure 38. Evaluation of stem sugar metabolite and starch profiles during early stages of growth in trees with different rates of flowering and early fruit abscission.
(A) Starch, (B) sucrose, (C) perseitol, (D) mannoheptulose, (E) glucose, and (F) fructose were measured in one-year stems, as mg/g dry mass (DM) for sugars and % DM for starch. Stems were sampled at bud burst (3 July 2019), peak flowering (5 November 2019), and early fruit drop (3 December 2019) from DEF1, DEF2, DR and control trees. Numbers are mean values derived from 5-8 biological replicates (shoots), ± standard error of the mean (bars). Lines with the same letter are not significantly different as determined by Analysis of Variance with post-hoc Tukey's Honestly Significant Difference test, $p \leq 0.05$.

If 32 t/ha is a benchmark for production, then trees should be able to support summer fruit growth with limited summer fruit drop, particularly when a considerable amount of dry matter has been invested into the fruit (Whiley and Wolstenholme, 1990). A model that integrates tree carbohydrate status and dominance interactions is presented in figure 39. According to this model, carbohydrate supply to developing fruits during the summer months is altered by environmental events that impact tree carbohydrate status. For example, hot and dry conditions, cloudy days and a lack of appropriate management inputs, may temporarily reduce tree carbohydrate status, which reduces carbohydrate availability to support fruit growth and development. As a result of reduced carbohydrate availability, a subset of fruits in a tree will undergo growth arrest and abscise. While trees may fix and store sufficient carbon, dominance interactions are also expected to influence the flux of carbohydrates to developing fruits. According to this model, a higher flux of carbohydrates is partitioned to vegetative shoots and possibly roots during annual growth flushes. In addition, dominance interactions among fruits likely reduces partitioning to a subset of developing subordinate fruits. Further, dominance interaction among shoots and branches may also influence carbohydrate partitioning to fruits. Taken together, management of tree carbohydrate status (ie: canopy management for increased carbon assimilation) and dominance

relations have the potential to increase carbohydrate supply to a larger subset of fruits during the summer; thereby, increasing the reproductive and yield potential of the tree.

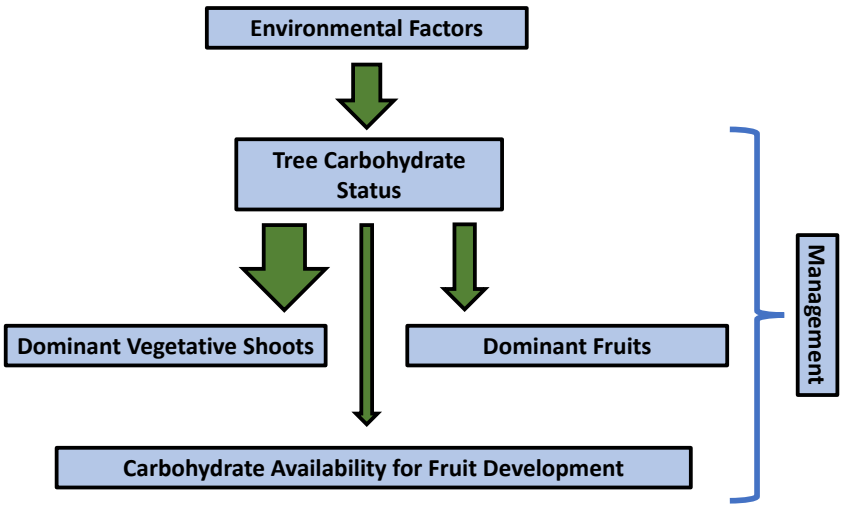

Figure 39. Impact of tree carbohydrate status and dominance interactions on carbohydrate availability for fruit development (retention).
Tree carbohydrate status is a reflection of net reserves and carbon assimilation capacity of a tree, which is influenced by environmental factors. While trees may accumulate and fix sufficient carbon to support a significant crop, dominance interactions influence the flux of carbohydrates to a subset of fruit in trees. According to this model, during periods of vegetative and possibly root growth (not shown in figure), a higher flux of carbohydrates is supplied to vegetative shoots. In addition, dominance interactions among fruits likely reduces partitioning to a subset of developing subordinate fruits. Not shown in this figure is the likelihood that dominance interaction among shoots may also influence carbohydrate partitioning to fruits. Management of tree carbohydrate (ie: canopy management) and dominance relations have the potential to increase carbohydrate partitioning to a larger subset of fruits; thereby, increasing the yield potential of the tree.

A developmental pathway for avocado fruit abscission

Avocado fruit abscission has been studied for >40 years, yet little progress has been made in understanding the developmental stages of this process. To effectively mitigate summer fruit abscission, the developmental process must be characterized in order to understand "how" fruit drop can be managed. Studies in apple and lichee suggest that fruit growth arrest is the first step in the abscission process (Greene et al., 2013; Zhao and Li, 2020). In addition, abscised avocado fruits tend to be smaller than persisting fruits (Perez et al., 1988; Garner and Lovatt, 2016). To extend these studies, we demonstrated that avocado fruits undergo growth arrest prior to abscission. In figure 40, we propose that fruit abscission is a multi-step developmental process initiated by growth inhibitory signals that promotes fruit growth arrest. We hypothesize that these growth inhibitory signals are taken up by all fruits, but only the ones with a relatively lower growth potential respond and undergo growth arrest. Once fruit growth arrest is completed or just after this process ceases, the seed coat undergoes senescence, which triggers abscission zone activation causing fruits to separate from the tree. Taken together, fruit growth arrest is the primary event in the avocado abscission process. Therefore, management intervention must target this step in the abscission process, either by: (1) preventing growth arrest and/or (2) reversing this process in order to allow fruits with a lower growth potential to persist and develop albeit at a slower rate. Understanding the physiological driver(s) of fruit abscission will help focus research efforts on how to management fruit growth arrest in order to reduce summer fruit abscission.

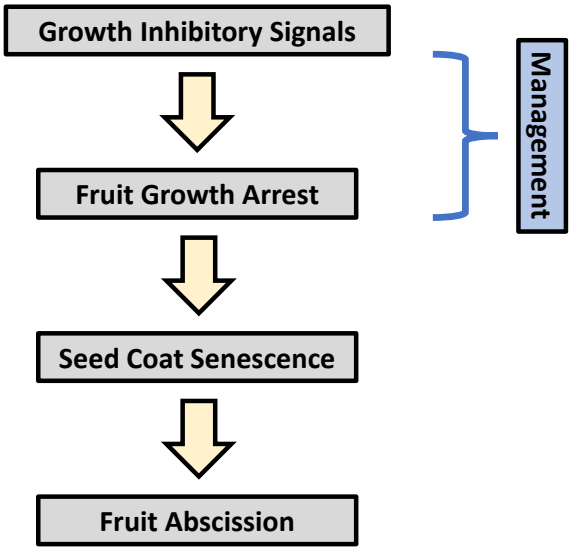

Figure 40. Developmental pathway for summer fruit abscission
According to this model, fruit growth arrest is the first step in the abscission process triggered by growth inhibitory signals. After fruit growth arrest is completed, seed coat senescence is triggered followed by fruit abscission. Management of fruit abscission must target methods to prevent or reverse growth arrest by the growth inhibitory signals.

Physiology and management of summer fruit abscission

In this project, we investigated whether the water potential of fruits was associated with summer fruit abscission. Our results showed that the water potential of fruits undergoing arrest was similar to fruits with a normal growth rate (Figure 11). To further examine water relations with summer fruit drop, we evaluate the impact of water stress on the fruitlet abscission. Results from our trial show that fruitlet abscission is only induced in severely water stressed trees, at a time when leaves exhibited a substantial amount of leaf burn and abscission. To validate these results, this trial should be repeated in another season. However, our results suggest that in a well-managed orchard with best practice irrigation in a coastal Mediterranean climate, water relations have little impact on fruit growth arrest and abscission. Mineral elements are critical in plant reproduction (Boldingh et al., 2016). Therefore, we investigated whether summer fruit abscission was associated with a deficiency in a mineral element(s), including nitrogen and boron. Our results showed arresting fruits typically had slightly higher levels of nitrogen and essential mineral elements, including boron (Figure 12). Thus, mineral element deficiency is not associated with fruit growth arrest. Further, at the tree level, experimental studies indicates that leaf nutrient concentration was not associated with the flower and fruitlet abscission events in southern California (Garner and Lovatt, 2008). Thus, in well managed orchards, optimizing tree nutrition programs may not be a viable pathway to mitigate summer fruit abscission. Taken together, our results suggest that in well managed orchards maintained in a favorable environment, improved irrigation and/or fertilization will not provide a viable pathway to mitigate summer fruit drop (Figure 41). However, in hot-climates and/or soils deficient in mineral elements, water and mineral element management may play a more critical role in regulating summer fruitlet abscission.

Tree carbohydrate status and dominance interactions influence the level of fruit growth arrest and abscission by reducing carbohydrate availability to a subset of developing fruits. Photosynthates including carbohydrates are supplied to fruits via the seed coat (Moore-Gordon et al., 1998; Robert, 2019); therefore, assessment of sugar metabolites in this maternal tissue of the seed provides insights into whether carbohydrates are limiting during fruit growth arrest. Our results showed that the translocated sugars, sucrose, perseitol and mannoheptulose, were reduced in the seed coat early in the growth arrest process indicating that carbohydrate availability is critical for

maintaining fruit growth. Further, during the later stage of growth arrest, the levels of these translocated sugars further declined, indicating the importance of carbohydrate availability for fruit growth.

Sucrose metabolism is a key enzymatic process necessary for the uptake of photosynthates (Ruan, 2012, 2014). To determine if sucrose metabolism is altered during fruit growth arrest, the levels of glucose and fructose were measured as these hexoses are produced when sucrose is catabolized. Our results show that glucose and fructose are significantly reduced in the seed coat, as well as the embryo and pericarp, in arresting fruits. The decline in glucose and fructose during early and later stages of fruit growth arrest, indicates that sucrose metabolism is targeted by growth inhibitory signals. The hypothesis that sucrose metabolism decreased in arresting fruits is supported by gene expression analyses, which shows that genes involved in sucrose metabolism are differentially expressed across all three fruit tissues. Therefore, our results suggest that the initiation and maintenance of fruit growth arrest involves a decrease in sucrose metabolism, which is essential for fruit development (Ruan, 2012; Robert, 2019).

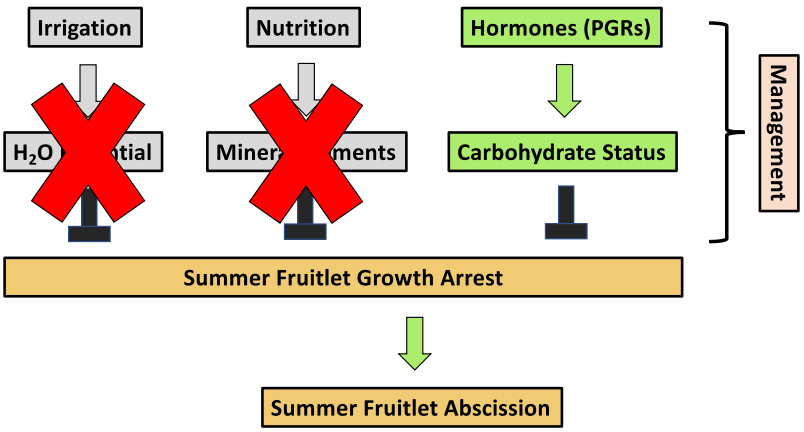

Figure 41. Management of summer fruit growth arrest and abscission.
In the AV16005 project, possible physiological processes were explored to identify an intervention pathway for management of fruit abscission. Results from the project suggest that in well-managed orchards in favorable environments irrigation and fertilization methods may not provide a viable pathway to limit summer fruit abscission. Given that fruit growth arrest involves significant changes in the hormones and genes involved the biosynthesis, signaling and transport of these signals suggests that the development of a plant growth regulator application(s) is the best option available to manage summer fruit abscission. However, in unfavorable climates (ie, hot-dry climates) and/or soils deficient in key mineral elements, including boron, water and/or plant nutrition will be important for maintaining tree health and fruitlet abscission.

Based on our findings, we propose that fruit growth arrest is initiated in the seed coat by the depletion of sugars and starch due to a reduced carbohydrate supply and sucrose metabolism. The down-regulation of sugar signaling genes that promote growth in the seed coat indicates that feedback between sugar signaling and carbohydrate uptake/metabolism may act to initiate and facilitate the growth arrest process. The sugar signaling and metabolic changes that occur in the seed coat in response to growth arrest, may transmit information to the pericarp and embryo to alter C7 metabolism and increase in starch biosynthesis, respectively. The increase in the starch levels in the embryo is significant in that the data suggests that the embryo transitions to a dormant-like state. Taken together, we conclude that fruit growth arrest is associated with a decrease and alteration in fruit carbohydrate status; therefore, management strategies must be focused on manipulating the growth potential and carbohydrate status of the fruit (Figure 41).

A role for sugar signaling fruit growth arrest and abscission

While sugars serve as building blocks for primary metabolism and function as a source for cellular energy and reducing

power, a subset of these metabolites also act as signals, including glucose, trehalose-6-phosphate and sucrose (Eveland and Jackson, 2012). These signals act to coordinate metabolism in response to carbohydrate availability. Experimental studies in model plants show that levels of trehalose-6-phophate fluctuates with the levels of sucrose, but not other sugar metabolites (Figueroa and Lunn, 2016). Based on the apparent tracking of trehalose-6-phosphate with sucrose, it has been postulated that trehalose-6-phosphate acts as a signal that regulates growth and development of organs (ie: fruits) and meristems in response to sucrose availability and overall carbohydrate status of the plant (Figueroa and Lunn, 2016). While trehalose-6-phosphate was not measured in our studies, we propose that the levels of this sugar signal is reduced in the seed coat. First, we showed that the level of sucrose is significantly reduced in the seed coat and this decrease occurs early in the growth arrest process. Second, a number of *TPS-like* and *TPP-like* genes were differentially expressed in the seed coat, as well as the pericarp and embryo, suggesting that the synthesis of trehalose-6-phosphate is altered in these fruit tissues during growth arrest. Lastly, given that glucose is a substrate used to synthesize trehalose-6-phosphate, and this sugar metabolite is highly reduced across all fruit tissues, further suggests that the levels of trehalose-6-phosphate is reduced. As a decline in trehalose-6-phosphate is associated with the onset of sugar starvation, it is highly likely that this sugar signaling pathway is activated to promote fruit growth arrest. Thus, maintaining carbohydrate supply, which includes sucrose, is expected to maintain trehalose-6-phosphate levels in order to prevent the activation of sugar starvation linked to fruit growth arrest.

A role for phytohormone regulation of fruit growth arrest and abscission

Plant growth regulators (PGR) are used in horticulture cropping systems to modify plant architecture, increase fruit/seed quality and yield, provide resistance biotic pests/pathogens and tolerance to abiotic stress (Rademacher, 2015). Cross-talk between hormones and sugar signaling pathways function to mediate growth and development (Eveland and Jackson, 2012). For example, the interplay between sugar and hormone signaling affects the level of branching in plants (Barbier et al., 2019). Therefore, the ability to manage fruit growth arrest and abscission will likely require the development of a PGR based application(s) to allow fruits with a reduced growth potential and carbohydrate status to persist on a tree until they are harvested. As a first step towards developing a PGR based application, we utilized a multi-disciplinary approach in which genome wide gene expression analysis (transcriptome) was combined with hormone profiling to identify candidate hormones implicated in fruit growth arrest.

Auxin is critical for fruit development, as this hormone acts to coordinate growth of seeds and fruits, as well as promote the expansion of maternal tissues via cell division and/or cell expansion (Kumar et al., 2014; Figueiredo and Kohler, 2018; Robert, 2019; Fenn and Giovannoni, 2021). Results from the project show that the auxin biosynthesis genes are down-regulated in maternal tissues during growth arrest. In addition, genes that encode proteins that conjugate auxin with amino acids to inactivate this hormone are up-regulated in the maternal tissues during growth arrest. Consistent with these gene expression results, free IAA levels are significantly reduced in the maternal tissues during growth arrest. Furthermore, during growth arrest, a significant increase in IAA-Asp occurred in the seed coat. The early down-regulation of auxin biosynthesis genes combined with the up-regulation of auxin conjugation genes indicates that the growth arrest signals target these biochemical processes to regulate IAA levels during growth arrest in primarily in the seed coat. In addition, growth arrest was associated with an alteration in auxin transport and signaling gens in the maternal tissues, as well as the embryo but to a lesser extent. As auxin promotes cell division during leaf expansion (Du et al., 2018), we hypothesized that the interplay between auxin and adaxial/abaxial polarity regulators will be essential for maintaining the expansion of the seed coat and pericarp. In support of this hypothesis, we showed that genes, which encode regulators of adaxial/abaxial polarity, were generally down-regulated during growth arrest. Further, as the interplay between sugar signaling/metabolism and auxin regulates growth in response to carbohydrate availability (Eveland and Jackson, 2012), maintaining auxin levels in fruits fated to abscise will be critical for retention. Lastly, as genetic studies show that auxin represses leaf senescence (Kim et al., 2011), this hormone will be essential for repressing senescence in the seed coat, which is induced at or just after fruit growth arrest.

During the growing phase of fruit development, auxin and gibberellin act to regulate cell expansion with auxin (Kumar et al., 2014; Fenn and Giovannoni, 2021). However, as avocado fruit growth is primarily mediated by cell division, a role for gibberellin in fruit development is not clear. In our study, we showed that GA_1 is detected in the pericarp and embryo suggesting that this active form of GA may play a role in these tissues during fruit development. In contrast, GA_4 was not detected in the pericarp and embryo and the levels of this hormone was quite low in the seed coat of normal growing fruits. Therefore, GA_4 doesn't appear to play a role in avocado fruit development. Interestingly, a significant increase in the levels of GA_1 and GA_4 occurred in the seed coat during fruit growth arrest. The increase in the levels of GA_1 and GA_4 is consistent with an increase in the expression of GA biosynthesis genes in the arresting seed coat. The increase in GA_1 and GA_4 in the seed coat during growth arrest is interesting. However, given that avocado fruit development is primarily mediated by cell division, it would seem highly probable that in order to maintain a mode of growth primarily mediated by cell proliferation, a mechanism(s) must exist to limit GA-mediated cell expansion. Therefore, the increase in GA_1 and GA_4 in the seed coat may be a secondary event associated with growth arrest, rather than a primary driver that suppresses growth.

As cytokinin is positive regulator of cell division, this hormone is implicated in mediating cell proliferation during the growth phase of fruit development (Kumar et al., 2014; Fenn and Giovannoni, 2021). While cytokinin biosynthesis and/or deactivation genes were differentially expressed in the seed coat, pericarp and embryo, respectively, the levels tZ, as well as cZ and iP, were not significantly altered in response to growth arrest. The increase in expression of cytokinin response factors that negatively regulate cytokinin-signaling suggests that cytokinin activity is regulated at the response level. At this time, it is unclear how these cytokinin response factors are regulated.

It is well known that ethylene is a positive regulator of senescence and abscission in plants (Sawicki et al., 2015; Botton and Ruperti, 2019). However, the impact of this hormone on these two processes that occur late in development is age dependent (Dubois et al., 2018; Koyama, 2018). For example, during the early stage of leaf development, ethylene inhibits growth but is unable to promote senescence and abscission until a leaf undergoes the maturation transition. Further, ethylene activity is negatively regulated by sugar signals (Moore et al., 2003; Fu et al., 2021), including glucose, a product of sucrose catabolism. In addition to sugars, antagonistic interactions between auxin and ethylene regulates growth, senescence and abscission of fruits (Iqbal et al., 2017). For example, in climacteric fruits, including avocado, ripening is a process controlled in part by ethylene (Salazar-García et al., 2013; Kumar et al., 2014; Fenn and Giovannoni, 2021). The ripening phase of fruit development is mediated by the maturation transition, a process that is dependent upon a reduction in auxin activity (Chirinos et al., 2023). Thus, the ability of ethylene to induce ripening is dependent upon a decline in auxin. Taken together, during the growing phase of fruit development, both sugar signaling and auxin repress the maturation transition, which allows fruits to attain their final size before undergo senescence and abscission at a late stage of development. In our research project, we showed glucose levels are reduced early in the fruit growth arrest process. Further, auxin biosynthesis genes and auxin conjugation genes are down- and up-regulated, respectively, during early fruit growth arrest in the seed coat. In addition, free auxin levels are significantly reduced in the maternal tissues at a late stage of growth arrest. At this time and later in the growth arrest process, a number of ethylene biosynthesis and signaling genes were up-regulated in the seed coat, pericarp and embryo during growth arrest. Due to the gaseous nature, it is difficult to quantify ethylene in arresting and normal growth fruits derived orchards at distance from the laboratory. To overcome this barrier, we quantified the levels of ACC, the precursor of ethylene (Adams and Yang, 1979). We show that an increase in ACC levels was only apparent in the seed coat of arresting fruits. Given that ACC levels increased in the seed coat but not the pericarp and embryo, indicates that the ethylene precursor, ACC, may be primarily produced in the seed coat during growth arrest. Subsequently, ACC may move to the other tissues where this precursor is converted to ethylene by ACC oxidase. Thus, according to this model, the growth arrest signal(s) promotes ACC production in the seed coat, which may be converted in this tissue as well as the pericarp and embryo.

JA is a lipid-derived hormone that activates defense responses against pests and pathogens (Guo et al., 2018). As plant defense is an energy consuming process, JA functions to shift carbohydrate supply from vegetative and/or

reproductive growing units to the carbon demanding-metabolic pathways associated with defense (Guo et al., 2018; Nguyen et al., 2022). As a result, JA is an effective repressor leaf, petal and fruit growth and increased levels of this hormone reduces yield (Huang et al., 2017; Guo et al., 2022). In addition, JA promotes senescence in mature organs (Huang et al., 2017). Experimental studies indicate that JA acts in part to inhibit growth by repressing cell division (Noir et al., 2013), a process that mediates fruit growth in avocado (Schroeder, 1953; Cowan et al., 2001; Dahan et al., 2010). Results from our study showing that JA is induced in the seed coat during growth arrest, suggests that JA is acting to restrict growth. Given that JA and ethylene act to inhibit growth suggests that these hormones cooperatively act to promote growth arrest in the seed coat. Further, as JA and ethylene promote senescence in mature organs indicates that at a late stage of growth arrest, these hormones cooperatively function to induce seed coat senescence. Lastly, activation of JA in the seed coat may function to reduce carbohydrate uptake, therefore, targeting this hormone may be key to maintaining carbohydrate supply to fruits.

While ABA is associated with immature fruit abscission (Botton et al., 2011; Eccher et al., 2013), the role of this hormone in this developmental process is unclear (Botton and Ruperti, 2019). Insights into ABA function during immature fruit abscission may be leveraged by through a known role of this hormone during fruit and seed development. For example, an increase in the levels of ABA is associated with the maturation transition (Kumar et al., 2014; Fenn and Giovannoni, 2021). In addition, applications of ABA hastens the maturation transition (Gupta et al., 2022), indicating that this hormone is involved in the switch from growth to ripening. Further, maternally derived ABA plays a role in positively regulating seed maturation and dormancy (Iwasaki et al., 2022), processes that occur before the fruit ripens (McAtee et al., 2013). In our research project, we showed that a significant increase in ABA occurred in the pericarp at a late stage of growth arrest. Further, a subset of ABA biosynthesis genes were up-regulated in the pericarp, seed coat, and embryo, during early and late stages of growth cessation. The accumulation of the ABA catabolites in the seed coat and embryo indicates that ABA is tightly regulated in the seed tissues. This is supported by the fact that ABA deactivation genes are induced during early and late stages of growth arrest. Thus, we propose that the growth arrest signal(s) promotes ABA production to accelerate the maturation transition to promote seed dormancy, inhibit growth and allow fruits to be competent for abscission

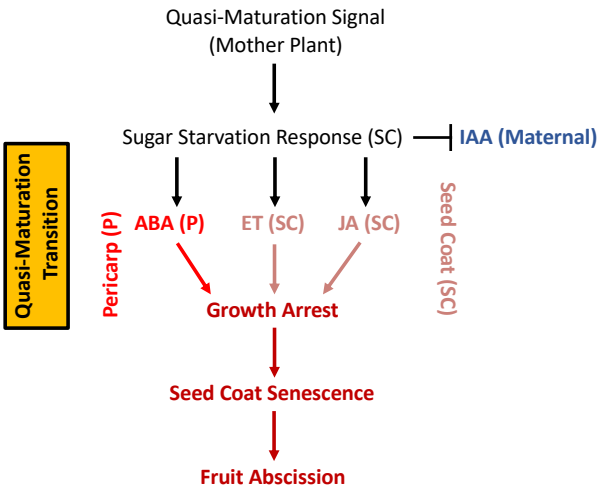

Figure 42. Physiological basis of immature fruit abscission.
In this model, a quasi-maturation like signal is taken up by developing fruitlets. In immature fruits with a lower carbohydrate potential, a sugar starvation response is initiated which reduces free IAA (auxin) levels and promotes the biosynthesis of ethylene (ET) and jasmonic acid (JA) in the seed coat and abscisic acid (ABA) in the pericarp. While ethylene promotes growth arrest, low IAA levels combined with an increase in JA and ABA promotes a quasi-maturation transition which is required for seed coat senescence and fruit abscission. To manage summer fruit abscission, strategies should be focused on reducing growth arrest in part by limiting the quasi-maturation transition initiated in fruits fated to abscise.

In apple, experimental evidence suggests that immature fruit abscission is caused by a 'nutritional stress' signal that induces ethylene in the cortex, which moves to the seed to induce embryo abortion. As a result, these immature fruits fated to abscise undergo developmental arrest, which results in abscission due to an impairment of auxin transport through the pedicel. Based on our study in avocado, we propose an alternative model (Figure 42). According to this model, avocado fruit abscission is mediated by a carbohydrate signaling event that induces a sugar starvation response due to a reduced uptake and metabolism of soluble sugars. The sugar starvation response acts to promote the quasi-maturation transition by reducing free IAA levels via and suppression of auxin biosynthesis genes and up-regulation of auxin conjugation genes. As the levels of sugar signals and auxin decrease in the seed coat, ethylene is produced in the seed coat, which acts to inhibit growth. In addition, the sugar starvation response promotes the biosynthesis of JA and ABA in the seed coat and pericarp, respectively. An increase in JA may function in part to reduce carbohydrate uptake in the seed coat. Further, as JA and ABA have been linked to seed maturation processes (Li et al., 2004; Iqbal et al., 2017; Iwasaki et al., 2022), the production of these hormones act to promote a quasi-maturation transition, which limits growth and increases the competency for abscission. In addition, ABA may act as a signal that induces seed dormancy, which is supported by the fact that embryos accumulate starch at the later stage of growth arrest. Therefore, managing immature fruit abscission in avocado should be focused on suppressing the quasi-maturation transition in fruits fated to abscise.

Understanding the hierarchy of hormones, including auxin, ethylene and JA, during early to late stages of growth arrest in the seed coat is a logical step towards developing a PGR based practical application to limit summer fruit abscission. Therefore, additional research aimed at identifying the causal hormones involved in summer fruit growth arrest is a logical step to take for developing of a PGR based application(s) to manage summer fruit abscission.

Developmental pathways associated with a quasi-maturation transition for growth arrest

Fruits undergo sequential phases of development after fruit set (Kumar et al., 2014; Fenn and Giovannoni, 2021). During the growth phase of development, auxin plays a fundamental role in mediating cell division and expansion. Before fruits ripen or senescence, these reproductive structures must undergo the maturation transition, which is necessary for abscission to occur (McAtee et al., 2013; Kumar et al., 2014). Based on studies in apple, it was proposed that immature fruits exit the growth phase via developmental arrest, prior to abscission (Sawicki et al., 2015; Botton and Ruperti, 2019). That is, according to this model, fruits fated to abscise bypass the maturation transition during the abscission process. Based on the results from AV16005, we propose an alternative model in which fruits undergo a quasi-maturation transition during growth arrest for the acquisition of abscission competence. To better understand the developmental mechanisms that drive this quasi-maturation transition, we queried the gene expression profiling database to identify pathways related to auxin activity that are targeted by the growth arrest signal(s).

According to recent models, adaxial/abaxial polarity is essential for maintaining the expansion of leaves, floral organs and integuments by restricting auxin activity to the boundary or middle domain of the developing organ (Du et al., 2018; Heisler, 2021; Nakayama et al., 2022). Given that integuments and the ovary wall of the carpel give rise to the seed coat and pericarp after fertilization, suggests that adaxial/abaxial polarity genes may play a role in the expansion of maternal tissues during fruit development. Our results show that an overall down-regulation of adaxial polarity genes is associated with fruit growth arrest. In addition, abaxial polarity genes are differentially expressed during the growth arrest. Thus, our results suggest that the growth of maternal tissues is mediated adaxial/abaxial polarity genes. Further, adaxial/abaxial polarity is targeted directly or indirectly via auxin by the quasi-maturation signal to inhibit growth arrest.

During the growth phase of development, cell proliferation is mediated by leaf meristem located at the margins and inner tissues of the leaf (Tsukaya, 2021). As avocado fruit growth is primarily mediated by cell proliferation, we hypothesized that a leaf-like meristem controls fruit growth. Based on the gene expression profiling study, we propose that the quasi-maturation signal(s) induces the expression of meristem regulator genes that negatively regulate cell proliferation to suppress fruit growth. Moreover, in leaves, negative regulators of meristem activity promote the transition from cell proliferation to cell expansion and differentiation. As the latter processes are associated with maturation, we propose that

up-regulation of meristem regulator genes that negatively regulate cell proliferation function to initiate the quasi-maturation transition.

Results from AV16005 support a model that growth arrest is mediated in part by a quasi-maturation transition. As seed maturation and embryo dormancy are induced prior to ripening (McAtee et al., 2013), we provide experimental evidence that these processes are associated with growth arrest. First, at a late stage of growth arrest, the embryo accumulates starch, a carbohydrate reserve that accumulates in seeds during dormancy. Second, ABA and JA produced in the maternal tissues may act to induce seed maturation and dormancy. Third, we show that the pattern of expression of dormancy signaling genes in the seed coat and embryo is consistent with a dormancy response initiated in the seed. Fourth, the increase in expression of floral repressors in response to growth arrest may act to promote seed dormancy during growth arrest. Together, our data support an alternative hypothesis that fruits fated to abscise undergo a quasi-maturation process, which is necessary for promote seed coat senescence and abscission. Further, the quasi-maturation transition induces a dormancy-like response to arrest embryo growth.

Physiology of early fruit abscission

Avocado fruit abscission occurs throughout the growing season and based on our study, as well as the work of others (Salazar-García et al., 2013), a massive fruit drop event occurs during early stages of fruit development. Fruit drop persists at a lower but significant level in a continuous manner or in waves throughout the remainder of the growing season. While our work showed that avocado trees drop 24% or more fruit during the summer, at a time when fruits accumulate up to 40% dry matter (Whiley and Wolstenholme, 1990), we believe that this period of abscission should be targeted to improve yield and reduce irregular bearing. Our study provides evidence that carbohydrate supply and metabolism in the seed coat are key factors for fruit retention and a reduction in the processes is a primary driver of fruit abscission. During the course of our work, we harvested normal growing/control fruits and arresting fruits during the early fruit drop event. We utilized these samples to determine if the physiological drivers of fruit abscission are conserved between the early and summer fruit drop events. To our surprise, and in contrast to summer arresting fruits, fruits undergoing growth cessation during the early fruit drop event, displayed normal levels of sugars and starch in the seed coat. These results suggest that carbohydrate supply and metabolism in the seed coat are not targeted for growth arrest. Interestingly, the pericarp displayed an increase in transport sugars suggesting that carbohydrates are not being utilized for growth. Currently, the lack of conservation between early and summer fruit abscission with regards to carbohydrate status of the seed coat, as well as the pericarp, is not understood. Therefore, the development of applications targeting summer fruit abscission may not be successful in retaining more crop when applied during the early abscission event.

Role of tree carbohydrate status in flowering and fruit set

Annual avocado yields are well below the theoretical production potential due to irregular bearing, which is mediated by poor fruit set and/or high fruit abscission. Fluctuations of stem starch levels correlate with the onset and growth reproductive and vegetative structures in the tree (Scholefield et al., 1985; Whiley et al., 1996; Liu et al., 1999). Therefore, the timing and degree of starch accumulation in stems, as well as mobilization, appears to be an indicator of tree carbohydrate status, which is predicted to influence the reproductive potential of a tree (Whiley and Wolstenholme, 1990; DeJong, 2019). To further validate this hypothesis, we utilize trees derived from fruit abscission trials to correlate stem starch levels at bud burst, flowering and early fruit abscission, as these treatments effected the carbohydrate status during these phenological events. While flowering and fruit retention are easy to quantify, fruit set is difficult to measure without the usage of invasive methods. To estimate fruit set, we scored the number of fruitlets per shoot at a late stage of flowering with the idea that fruit set would correlate with the initial crop load early in fruit development. Based on the results from this trial, we developed the following conclusions.

1. Stem starch and perseitol levels at bud burst can be used as a predictive tool to estimate the level of flowering.

2. Trees with a high level of flowering retain fewer fruitlets per shoot than trees with a moderate level of flowering.

The depletion of stem starch and perseitol in high flowering trees is associated with a reduced number of retained fruitlets. Thus, we propose that maintaining sufficient stem starch and perseitol levels at flowering is key to increase fruit set. Methods to increase the levels of starch and perseitol in stems during the winter and maintain the levels of these carbohydrates during flowering is predicted to increase fruit set and the number of retained fruitlets early in fruit development.

3. Trees with different rates of fruit set/early fruit retention produce similar yields. We propose that summer fruit abscission functions to adjust crop levels retained early in fruit development. Therefore, managing summer fruit abscission the first step toward increasing yield and managing irregular bearing.

Outputs

Table 1. Output summary

Output	Description	Detail
Project Management	M&E plan developed	Program logic & monitoring and evaluation completed Project risk management completed Stakeholder engagement/communication plant developed
Desktop Study	A comprehensive review on horticulture reproductive biology was completed	This review covered key plant reproductive topics that impact yield in horticulture tree crops including flowering, fruit set, fruit development and fruit abscission. Current knowledge for avocado reproduction was included and knowledge gaps were identified for future research. The section reviewed on fruit abscission was utilized to construct a framework on how to better approach and study avocado fruit drop to develop a new knowledge base. This step is essential toward the development of new innovative tools to limit irregular bearing
New Knowledge on summer fruit abscission in avocado	Characterized fruit drop over the course of the growing season	The seasonal pattern of fruit drop was characterized in south-west coastal region in WA and Riverland in SA, in which fruit abscission is a major irregular bearing factor that limits production. Summer fruit drop was identified as a key fruit abscission event to manage
New Knowledge on summer fruit abscission in avocado	Systems developed to study fruit abscission	Defoliation trials result in a significant fruit drop event which causes trees to drop on average ≥97% of the crop. This effective treatment was used to study fruit abscission A shading trial gave rise to a highly variable and extended fruit drop event in which trees abscised 30-80% of their crop. As a results, it was concluded that this treatment was not an effective method for studying fruit abscission.
New Knowledge on summer fruit abscission in avocado	A role for tree carbohydrate status on summer fruit abscission	Demonstrated the association between tree carbohydrate status and fruit drop using defoliation and shading trials. Stem starch levels are a good indicator of tree carbohydrate status Stem sucrose and perseitol levels are also good indicators of tree carbohydrate status
New Knowledge on summer fruit abscission in avocado	Developmental pathway for summer fruit abscission determined	Fruit abscission is a multi-step process Fruit growth arrest is the initial step in the fruit abscission developmental pathway or process Seed coat senescence likely follows fruit grow arrest The physical separation of fruits from the tree is the last step in the fruit abscission developmental pathway or process Management of fruit abscission must target fruit growth arrest, either by preventing or reversing growth cessation
New Knowledge on summer fruit abscission	Physiology of summer fruit abscission	In well managed orchards in a favorable environment, water relations only impact fruit abscission when the trees exhibit extreme water stress. Further, the water potential doesn't

in avocado		appear to be a major driver of fruitlet growth arrest.
New Knowledge on summer fruit abscission in avocado	Physiology of summer fruit abscission	Nitrogen and/or mineral element deficiency are not associated with summer fruit growth arrest and abscission in well managed orchards
New Knowledge on summer fruit abscission in avocado	Physiology of summer fruit abscission	An alteration in carbohydrate status in the fruit tissues is associated with the early and later stages of summer fruit growth arrest and abscission Fruit growth arrest is associated with: -Depletion of sugar metabolites and starch in seed coat -Hyperaccumulation of starch in embryo -Increase and decrease in perseitol and mannoheptulose in pericarp, as well as a decrease in starch levels
New Knowledge on summer fruit abscission in avocado	Transcriptome analysis determined for arresting and control fruits	Changes in the expression of genes involved in carbohydrate uptake, metabolism and catabolism is associated with summer fruit growth arrest and abscission Changes in the expression of genes involved in sugar signaling associated with summer fruit growth arrest and abscission Gene expression analysis supports the hypothesis that sugar starvation induced in the seed coat drives summer fruit growth arrest and abscission
New Knowledge on summer fruit abscission in avocado	Transcriptome analysis determined for arresting and control fruits	Candidate hormones implicated in summer fruit growth arrest abscission identified Data suggests that summer fruit growth arrest and abscission is associated with: 1. Changes in the activities of hormones that promote growth, including auxin, gibberellin, cytokinin and brassinosteroid 2. Increase in the activities of ethylene, abscisic acid, and jasmonic acid activities
New Knowledge on summer fruit abscission in avocado	Physiology of summer fruit abscission	Fruit growth arrest targets auxin activity to: 1. Suppress auxin biosynthesis 2. Increase auxin conjugation to inactivate this hormone
New Knowledge on summer fruit abscission in avocado	Physiology of summer fruit abscission	Fruit growth arrest targets ethylene, ABA and JA 1. Increase in the levels of ACC (precursor of ethylene) in the seed coat 2. Increase in the levels of JA in the seed coat 3. Increase in the levels of ABA in the pericarp
New Knowledge on summer fruit abscission in avocado	Transcriptome analysis determined for arresting and control fruits	Experimental results suggest that fruit growth arrest signals target adaxial and abaxial polarity to suppress the expansion of maternal tissues (seed coat and pericarp).
New Knowledge on summer fruit abscission in avocado	Transcriptome analysis determined for arresting and control fruits	Experimental results suggest that fruit growth arrest signals target meristem activity to suppress cell proliferation in the maternal tissues (seed coat and pericarp). Repression of meristem activity may be linked to the induction of a quasi-maturation transition required for abscission.

New Knowledge on summer fruit abscission in avocado	Transcriptome analysis determined for arresting and control fruits	Experimental results suggest that the fruit growth arrest signal(s) promotes dormancy signaling in the seed to arrest embryo growth. The up-regulation of floral repressors may act to mediate the dormancy-like response initiated in the seed.
New Knowledge on summer fruit abscission in avocado	Integrated model for immature fruit abscission	Collectively, experimental data suggests that the growth arrest signal(s) promotes a quasi-maturation transition that: 1. Acts in part to promote growth arrest 2. Acquires the competence to abscise
New Knowledge on early fruit abscission in avocado	Physiology of early fruit abscission	Fruit growth arrest occurs during the early fruit drop event In contrast to the summer fruit drop event, results show that fruit growth arrest is not associated with a depletion of carbohydrates in the seed coat via reduced metabolism and uptake. Therefore, the physiological driver of fruit growth arrest that occurs during the early fruit drop event is not known and is distinct from the summer growth arrest. Managing the early fruit drop event will likely require a different practical application than mitigating summer fruit abscission.
New Knowledge on the relationship between tree carbohydrate status with early reproductive events	Physiological drivers of flowering and fruit set	Stem starch and perseitol levels at bud burst and flowering correlate with the levels of flowering and early fruit retention, which likely reflects fruit set. While increasing stem starch and perseitol levels during the winter is key for flowering, the levels of these storage carbohydrates must be maintained for increasing fruit set and early fruit retention. Therefore, manipulation of flowering may provide a pathway to increase fruit set. Trees with varying levels of early fruit retention/fruit set, produce similar yields. Therefore, summer fruit abscission is a key factor the adjusts crop levels when trees set a high crop.
Extension	Dissemination of research results from AV16005 and presentations on irregular bearing	Presentations were given at Avocado Regional forums (AV17005) to update industry on the progress of project (see below and appendix E) Presentations were given at Avocado Regional forums (AV17005) on fruit set to disseminate current thinking on this major driver of irregular bearing (see below and appendix E) A presentation given at an Avocado Regional forum (AV17005) on plant growth regulators (see below and appendix E)
Extension	Dissemination of research results from AV16005	Two industry articles published in Talking Avocados (AV18003; see below and appendix C)
Industry impact	Linkages established with Hort Innovation funded projects	The project developed linkages with AV19006, AV19005, AV17006 and AS17000 to increase industry impact (see below)

Extension Activities

Research results were disseminated to industry at Avocado Regional Forums (AV18003; National avocado industry communications program) and through publications of research articles in Talking Avocados (AV17005; Avocado industry development and extension). Industry publications are displayed in Appendix C.

A list of presentations given at Avocado Regional Forums over the course of the project (see forum announcements in Appendix E)

- 2017: Tristate Regional Forum – Overview of AV16005 project
- 2019: Tristate Regional Forum – Dissemination of research from AV16005 project & presentation of the use of plant growth regulators
- 2019: Pemberton Regional Forum – Discussion led on scoping the need for canopy management
- 2020: Western Australia (Manjimup) Forum - Presentation on research results from AV16005 & presentation on avocado fruit set
- 2021: Tristate Regional Forum – Presentation on the connection between nutrition and tree carbohydrate status Key research results from AV16005 were integrated with this presentation
- 2021: Avogrow Seminar – Online presentation primarily on research from AV19006. However, key research results from AV16005 were integrated into presentation
- 2022: North Queensland Forum – Presentation on research results from AV16005 & presentation on avocado fruit set
- 2022: South Queensland Forum – Presentation on research results from AV16005 & presentation on avocado fruit set

Industry Impact

To increase industry impact, AV16005 established linkages with to AV19006 (Carbohydrate monitoring to predict yield), AV19005 (Understanding the mode of action of phosphite in avocado for enhanced management of Phytophthora root rot), AV17006 (Avocado industry Capacity Building-WA), AS17000 (National tree genomics program). Field trials used to study fruit abscission were also utilized by AV19006 to preliminary assess NIR reflectance spectroscopy as a non-destructive method to estimate carbohydrates in avocado. We collaborated with Drs Elizabeth Dann and Kaylene Bransgrove of AV19006 to investigate the possibility that fruit abscission is associated with fungal pathogenesis. In addition, we performed sugar metabolite analysis capability for this project. Declan MacCauley of AV17006 provided technical capability for field trial evaluation in WA. In return, we provided field trial training and development.

Outcomes

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
< List the outcome (e.g. knowledge, awareness, practice change, commercialization, availability of new knowledge for next phase project) >	< Align to the relevant Fund outcome, strategy and KPI >	< Describe and define the outcome in terms of how it was realized by the target stakeholder group(s). Explain how the outcome is relevant at the Fund level >	< What forms of evidence were collected to identify and understand the outcome (e.g. survey, observation, feedback) >
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy 2: Develop improved orchard management practices to increase productivity, yield consistency and fruit quality based on improved knowledge of tree physiology.	New knowledge for managing fruit abscission. Determine the impact of summer fruit abscission on the reproductive potential of the tree, as trees have invested up to 40% dry matter into fruits during the period of the growing season.	Field trials established and monitored in WA (south-west coast) and SA (Riverland). Summer fruit drop significantly reduces the reproductive potential of the tree. For details, refer to pages 12-13 and 50 in the results and discussion sections, respectively, regarding the seasonal patterns of fruit abscission.
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy 2: Develop improved orchard management practices to increase productivity, yield consistency and fruit quality based on improved knowledge of tree physiology.	New knowledge for managing fruit abscission & tree physiology. Tree carbohydrate management has been proposed as a method for increasing yield. Determine role of tree carbohydrate status on summer fruit abscission	Trials developed to negatively impact tree carbohydrate status (defoliation and shading) When trees are defoliated or shaded, onset of fruit drop is associated with a decline in stem starch levels, as well as perseitol and sucrose under severe carbohydrate limiting conditions. For details, refer to pages 12-16 and 50-53 in the results and discussion sections, respectively.
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy	New knowledge for managing fruit abscission & tree development. Developmental constraints (ie: dominance interactions) limit carbohydrate availability to	Trials developed in WA and SA to understand the impact of vegetative shoot growth on carbohydrate availability to developing fruits. An increase in fruit retention by shoot tipping

	2: Develop improved orchard management practices to increase productivity, yield consistency and fruit quality based on improved knowledge of tree physiology.	developing fruits. Determine if dominance interactions between vegetative shoots and fruits limits carbohydrate availability to developing fruits.	is associated with an apparent increase in carbohydrate availability as determined by stem carbohydrate levels. For details, refer to pages 16-18 and 50-53 in the results and discussion sections, respectively.
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy 2: Develop improved orchard management practices to increase productivity, yield consistency and fruit quality based on improved knowledge of tree physiology.	New knowledge for managing fruit abscission. Determine the developmental pathway for fruit abscission to identify what step in this process should be targeted for management intervention.	Trials developed in WA and SA to characterize the developmental pathway of summer fruit abscission. Summer fruit abscission must be managed by targeting fruit growth arrest, the first step in this developmental process. For details, refer to pages 18-20 and 53 in the results and discussion sections, respectively.
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy 2: Develop improved orchard management practices to increase productivity, yield consistency and fruit quality based on improved knowledge of tree physiology.	New knowledge for managing fruit abscission. Determine the physiological basis of fruit abscission and how this correlates with summer growth arrest and abscission for management intervention.	Trials developed in WA and SA to evaluate starch and sugar metabolite profiles in developing and arresting fruits. Summer fruit abscission must be managed by maintaining the carbohydrate status of the fruits (seed coat). For details, refer to pages 22-26 and 54-56 in the results and discussion sections, respectively.
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy 2: Develop improved	New knowledge for managing fruit abscission. Determine the physiological basis of fruit abscission and how this correlates with summer growth arrest and abscission for management intervention.	Trials developed in WA to evaluate fruit water potential, water stress and nitrogen/mineral elements in arresting and developing fruits. Fruit growth arrest is not associated with a change in water potential and

	orchard management practices to increase productivity, yield consistency and fruit quality based on improved knowledge of tree physiology.		mineral element deficiency. Severe water stress induces fruitlet abscission, which is reversed by appropriate levels of irrigation. Results from Garner and Lovatt, 2008 indicates that leaf nutrients were not associated with fruitlet abscission. Therefore, in a well-managed orchard in a favorable environment, irrigation and/or nutrition may not be key pathways for mitigating summer fruit abscission. For details, refer to pages 15 and 29 in the results and discussion sections, respectively.
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy 2: Develop improved orchard management practices to increase productivity, yield consistency and fruit quality based on improved knowledge of tree physiology.	New knowledge for managing fruit abscission. Gain insight into the possible role of sugar signaling is fruit growth arrest. Gain insight into the hormonal basis of summer fruit abscission for the development of plant growth regulator (PGR) based management application(s).	Trials developed in WA to capture gene expression profiles between arresting and developing fruits. Sugar signaling-starvation response in seed coat implicated in fruit growth arrest Candidate hormones implicated in fruit growth arrest identified. New integrative model that describes the developmental basis of fruit growth arrest. For details, refer to pages 28-41 and 55-59 in the results and discussion sections.
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy 2: Develop improved orchard management practices to increase productivity, yield consistency and fruit	New knowledge for managing fruit abscission. Determine if fruit growth arrest is associated with the early fruit drop event. Determine if an alteration in carbohydrate availability and metabolism is associated with the early fruit drop event.	Trials developed in WA to examine sugar metabolite profiles between arresting and developing fruits harvested during the early fruit drop event. Fruit growth arrest is the initial step in fruit abscission during the early fruit drop event. In contrast to summer fruit arrest, the carbohydrate

	quality based on improved knowledge of tree physiology.		status of the seed coat is not altered during early fruit growth arrest. The physiological mechanism of the early fruit drop event is not conserved with the summer fruit drop event. Management applications developed for summer fruit abscission may not be applicable for mitigating the early fruit drop event. For details, refer to pages 47 and 60 in the results and discussion sections, respectively.
Knowledge for irregular bearing management intervention.	AV16005 aligns with the Avocado Strategic Investment Plant 2022-2025 – Availability of new knowledge for growers to enable orchard yield consistency. KPI – Outcome 2, Strategy 2: Develop improved orchard management practices to increase productivity, yield consistency and fruit quality based on improved knowledge of tree physiology.	New knowledge for managing fruit abscission. Determine if tree carbohydrate status is associated with flowering and fruit set.	Trials developed in WA to examine the impact of stem carbohydrates on flowering and fruit set. Stem starch and perseitol levels at bud burst and peak flowering may be used as predictors for flowering and fruit set/early fruit retention, respectively. Trees that set and retain and more crop early in fruit development display similar yields as trees with that set and retain a lower crop level. Thus, summer fruit abscission is a key irregular bearing factor that adjusts crop levels. Targeting the summer fruit abscission is the first step in mitigating irregular bearing in avocado.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
< Refer to the M&E Plan >	< Identify aspects of project performance that address the Key Evaluation Questions >	< List opportunities for improvement and future development >
Are key reproductive events identified for improving avocado production?	The desktop analysis identified two major reproductive events for improving avocado production: (1) poor fruit set and (2) high fruit abscission. Both of these factors are the major drivers of irregular bearing and the environment in each growing region determines which factor is the predominating limitation for yield.	A new review should be developed based on publications derived from AV16005. This review should integrate new research findings with previous studies and develop a new model of fruit abscission in avocado.
Are top candidates appointed for avocado research positions?	Top candidates were appointed to the two positions for this project: (1) Amnon Haberman-postdoctoral fellow and (2) Marc Goetz-research technician. Amnon has extensive research experience in apple and olive. In addition, he completed a one-year postdoctoral position working with avocado rootstocks. Marc Goetz has extensive research experience in plant reproductive biology and acquired bioinformatic skills required for the transcriptome analysis.	The project appointed highly experienced post-doctoral fellow and research technician.
Is high nitrogen treatment an effective method to induce fruit abscission?	Pruning and/or high nitrogen levels did not induce a significant fruit drop event. Therefore, defoliation and shading trails were performed and both of these methods induced a significant wave of fruit drop. Defoliation had the greatest impact with minimal variability, while shading trees displayed a high degree variation.	Methods to induce fruit abscission have been developed in AV16005 and should be leveraged in a future project to determine the hierarchy of the hormonal control of fruit abscission. Utilization of these fruit inducible systems will be essential for development of PGR based application to reduce summer fruit abscission. Further, the methods that induce fruit abscission can also be used to evaluate the efficacy of the PGR based application(s).
Are all trial sites well established and being maintained?	The orchards selected for field trials were well maintained and provided excellent support for the project.	Fruit abscission trials should be performed at well managed orchards in which trees display a high propensity of fruit set. To study fruit set, trials should be

		performed in a growth cabinet with potted trees to examine how cool temperature and/or low humidity impacts fruit set. In parallel, trials should be performed in regions that experience cooler spring time temperatures (ie: Pemberton/Manjimup, WA and Toowoomba, QLD) to identify the impact of cool temperature and/or low humidity on fruit set.
Are measurements and record keeping consistent between trials?	All methods and record keeping used to evaluate and monitor field trials were consistent across different trials.	The usage of digital based tools such as fruit dendrometers improve measurement and record keeping via data transfer. During COVID, the project team was unable to perform trials in WA; therefore, the team established a fruitful working relationship with a well-managed orchard in the Riverland, SA.
Are climate factors altering the outputs from the field trials?	There was no apparent impact of climate factors on field trials. That is, we were fortunate that weather events didn't destroy our trials.	Preliminary results suggest that the usage of dendrometers to monitor fruit growth arrest are useful for understanding the climate factors that influence growth arrest.
Are analyzed results and outputs being distributed to the Project Reference Group Committee in a timely manner?	For each Project Reference Group (PRG) meeting, a draft of the milestone report was distributed to before each meeting. This provided each committee member with an opportunity to provide input into each milestone report and the research direction. In addition, a PRG-presentation was provided to each committee member prior to the meeting.	To improve M&E and allow the PRG members to have input into the field trials, PRG meetings should be held at an earlier time point to allow the committee to have more input into the project.
Are key hormones, sugars and/or signaling genes identified in the fruit abscission induction trials?	This was achieved by first developing trial systems to induce fruit abscission in association with a decline in tree carbohydrate status. Next, the developmental pathway of fruit abscission was characterized in which we showed that fruit growth arrest is the initial step in the abscission process, which should be targeted for management intervention. Next, the physiology of fruit growth arrest was evaluated and results showed that this process	We developed a pathway on how to develop a project aimed at understanding reproductive based problems that impact yield. This systematic systems-based approach can be adopted to address other drivers of irregular bearing. The next phase of the project should determine the hierarchy of hormone signaling and the extent in which these hormones influence the growth potential and carbohydrate

	is associated with a decline in the carbohydrate status in the seed coat. Finally, a transcriptome analysis was performed and candidate hormones and sugar-signaling genes associated with fruit growth arrest were identified.	status of the fruit. This step is essential for targeting a specific hormone(s) for a PBR based application to limit fruit abscission.
Are stored carbohydrates the key factor for determining the degree of shoot and fruit competition?	Experimental results from trials addressing dominance interactions between vegetative shoots and developing fruits indicates that stored carbohydrates are leveraged to support growth of these sinks. Further, results support a model in which the dominant interactions divert carbohydrates derived from stem reserves away from fruits to support vegetative shoot growth.	The results from AV16005 addressed dominance interactions between the spring flush and developing fruits. A complementary study addressing dominance interactions between the summer flush and developing fruits to shed light on whether growth of summer flushes diverts carbohydrates away from fruits.
Are trials well maintained and is the genetic and/or environment the main driver in the regulation of productivity via the rootstock?	This research activity was dropped from the project. Based rootstock evaluation on yield in at the trial in Waikerie, none of the rootstocks in the trial were able to overcome the biennial bearing phenotype displayed in this trial. Therefore, we were unable to identify a rootstock(s) that promoted high annual yields. It was recommended by the PRG committee to cease this activity and focus research efforts on fruit abscission.	M&E through the PRG committee provided the project with flexibility to increase impact to address the role of tree carbohydrate status on flowering and fruit set/early fruit retention.
Were the methods and analysis appropriate for the research?	A multi-disciplinary approach was taken to study and analysis the results from the fruit abscission trials. Our methodical approach combined with multi-disciplinary methods was essential for the success of the project.	This project utilized a systems-based approach to study fruit abscission in which physiology and genomic based research was integrated to better understand the biological and developmental basis of this problem. This system based/multi-disciplinary approach must be adopted for continued research in fruit abscission, as well as fruit set, the two major drivers of irregular bearing.
What was the reach of extension events and project communications?	Extension for the project was mediated through project presentations at avocado regional forums (AV17005). In addition, two industry articles were published in Talking avocados (AV18003).	The project team is extremely grateful for support from AV17005 and AV18003. This support allowed the team to give project updates to growers at the avocado regional forums across Australia. In addition,

		we also were given opportunities to give talks on the problems associated with poor fruit set and the use of plant growth regulators in avocado and other horticultural tree crops to increase production. The mode of dissemination should be continued in the future.
Are growth hormones targeted by fruit abscission signals?	Based on our results, we propose that fruit abscission is mediated by a signal(s) that functions to inhibit growth based on the carbohydrate status of the fruit. We believe this growth inhibitory signal(s) acts directly and indirectly on at least 7 hormones. Further, the data indicates that the inhibitor signal(s) target key developmental genes that integrate hormone and sugar signaling to induce growth arrest.	The major challenge moving forward is to identify the hormone(s) to target for management intervention. This can be achieved in part by characterizing the hierarchy of hormone signaling by measuring each of these hormones at early and later stages of fruit growth arrest.
To what extent can stored starch levels be manipulated?	Stems accumulate starch in the winter months. Experimental studies indicate that carbohydrate reserves are used to support vegetative and reproductive development in the spring and early summer. Stored stem starch levels appear to manipulated by the timing of harvest, such that late harvests that negatively impact stem starch reserve accumulation. In addition, a heavy flowering event, which depletes stem starch reserves, is associated with a lower rate of fruit set/early fruit retention.	Winter fertilization may have the potential to increase stem starch reserves but needs to be validated. It is expected that work from Inaki Hormaza's team will address the impact of winter fertilization. When a publication(s) arises from this work, recommendations can be made to industry regarding winter fertilization. A major challenge for manipulating carbohydrate reserves is to limit the extent of flowering, which depletes stem starch reserves. We hypothesize that limiting flowering may provide a pathway to maintain sufficient reserves to support a higher level of fruit set/early fruit retention. However, mitigation of summer fruit abscission is critical as this irregular component is a major factor that adjusts crop levels after a high level of fruit set/early fruit retention.
Can the hormone, carbohydrate and gene expression/signals be integrated to identify key intervention points?	Through the sugar metabolite and transcriptome analysis, key developmental regulators were identified that may function to	The usage of fruit dendrometers combined with defoliation will provide a pathway to better understanding of how sugar and

	integrate sugar and hormone signaling to promote or inhibit growth.	hormone pathways are integrated. In addition, the development of a rapid in vitro system should be explored in the next project. If an in vitro system can be developed, these potential integrator genes can be used as markers assess fruit growth arrest in order to develop a PGR based applications to limit fruit drop in avocado.

Project Variations as the result of "Monitoring & Evaluation" with consultation and input from the Project Reference Group Committee and approval by Hort Innovation are listed below.

2017 Project Variation:
Based on "Monitoring & Evaluation" from the Project Reference Group Committee and approval from Hort Innovation, the project end date was extended 8-months to 31-08-2021 to enable the appointment of preferred postdoctoral candidate, Dr Amnon Haberman, to a 3-year position. This was a revenue neutral variation.

2020 Project Variation:
Based on "Monitoring & Evaluation" from the Project Reference Group Committee, it was decided that the milestones on: (1) manipulating stored carbohydrates and (2) evaluating high productivity (rootstock x environment) were not achievable. Because of the opportunity created from the fruit abscission trials, resources for these milestone objectives have been shifted to evaluate how tree carbohydrate status effects flowering and fruit set using trees subjected to defoliation and drought stress. The proposed changes are supported by the Project Reference Group and the variation is revenue neutral.

2021 Project Variation:
Based on "Monitoring & Evaluation" from the Project Reference Group Committee, we requested to extend the end date of project by 7-months, 31 March 2022. This was a revenue neutral extension that allowed the team to perform a deep dive of the avocado gene expression data to identify candidate hormones involved in fruit growth arrest and abscission. It also allowed the team to establish a collaboration with Dr. Christine Beveridge (AS17000) to initiate experiments to quantify hormones in arresting and control fruit samples to better understand the hormonal control of fruit growth arrest and abscission.

2022 Project Variation:
The project was extended to 31 May 2023, as requested by Adrian Hunt at Hort Innovation, to continue R&D on avocado irregular bearing and engage with an independent evaluator, who will evaluate project performance and make recommendations about future research as a 'where to next' strategy recommendation aimed at managing irregular bearing. The evaluator/strategy developer will be engaged directly by Hort Innovation.

Recommendations

Recommendation for future R&D investment aimed at managing summer fruit abscission are provided below.

In the major avocado growing regions of central QLD and coastal regions of WA, as well as Tristate (when fruit set isn't impacted by high or low temperatures), immature fruit abscission is the limiting factor for production. Based on our study, we recommend that summer fruit abscission should be targeted for management intervention given that this event substantially reduces the reproductive potential of the tree. Given that trees have invested up to 40% dry matter into fruits during the summer (Wolstenholme et al., 1990), managing abscission during this period will help growers limit "wasted carbohydrates" in the form of abscised fruits for increasing the reproductive potential of the tree.

Results from our study support the hypothesis that tree carbohydrate status and dominance interactions impact fruit abscission. Dominance interactions likely play a major role in fruit abscission, as avocados trees display a bias for vegetative growth (Wolstenholme et al., 1990; Salazar-García et al., 2013). Experimental evidence suggests that growth of vegetative shoots (spring and summer flushes) diverts carbohydrates away from developing fruitlets. In addition, the outgrowth of vegetative shoots shades actively photosynthesizing leaves, and as a result, carbon fixation is predicted to temporarily decrease until the leaves of new flush mature. Further, dominance interactions are likely enhanced when trees experience a temporary reduction in carbohydrates due to climate events. Thus, the ability to overcome a temporary decline in carbon fixation and/or supply, may provide a pathway to reduce summer fruitlet abscission. While the industry utilizes paclobutrazol and/or uniconazole to limit the extension of vegetative shoots, additional management solutions should be developed to limit the dominance of vegetative shoots in order to increase carbohydrate supply to developing flowers and fruits.

Our work demonstrated that fruit growth arrest is the primary development event of early and summer fruit abscission. Therefore, management intervention must target growth arrest, rather than abscission. Growth of organs is often controlled by signals produced outside the organ. For example, floral induction is induced by the integration of environment cues (temperature and light) as well as endogenous cues (sugar signaling and hormones including gibberellin) in leaves. This signals act to regulate the expression of *FT*, which moves to the shoot apex to increase the organogenic potential of the meristem and to induce flowering. In addition, strigolactone produced in roots acts a mobile signal to negatively regulate the outgrowth of axillary buds. While the nature of the signal that induces growth arrest during the early stage of fruitlet abscission is unknown, our results suggest that this signal initiates a maturation phase of growth, which is necessary for abscission competence. During organ maturation, cells transition from a proliferation to expansion and differentiation. Given that differentiation is often a one-way street that is irreversible, the success of a management tool(s) will depend on the ability to block or limit differentiation during an early stage of growth arrest.

Fruits undergo maturation and become competent to abscise when auxin activity declines in the maternal tissues of the fruit (McAtee et al., 2013; Kumar et al., 2014; Chirinos et al., 2023). Results from our project suggest that the seed coat is the major site of auxin biosynthesis. Further, auxin levels are significantly reduced in the seed coat during early and late stages of growth arrest. In addition, the expression of auxin transport and signaling are altered during early and late stages of growth arrest. The seed coat is the organ in the fruit responsible for the uptake and distribution of carbohydrates derived from photosynthesis and carbon reserves (Radchuk and Borisjuk, 2014). Results from AV16005 show that carbohydrate levels are significant reduced in the seed coat during early and late stages of growth arrest. Given that the interplay between auxin and sugar signaling/metabolism is critical for growth (Wang and Ruan, 2013), it is recommended that future R&D develop methods to maintain auxin activity in order to maintain growth and prevent maturation.

Ripening is a process of senescence that occurs after fruits undergo maturation (McAtee et al., 2013; Kumar et al., 2014). Competence to abscise is dependent upon maturation and the ripening transition. Experimental studies indicate that ethylene and jasmonic acid play a synergistic role in regulating organ senescence and abscission (Kim, 2014; Kim et al., 2015). In contrast, auxin activity suppresses organ senescence and abscission (Kim et al., 2011; Sawicki et al., 2015; Botton and Ruperti, 2019). Results from AV16005 show that ethylene and jasmonic acid are induced during growth arrest. It is highly likely that these hormones act to suppress cell proliferation in the seed coat during growth arrest. Further, the suppression of cell proliferation by ethylene and jasmonic acid may involve the activation of fruit maturation. We also propose that at a late stage of growth arrest, ethylene and jasmonic acid may act synergistically to promote seed coat

senescence. As ethylene and jasmonic acid are induced during growth arrest in the seed coat, it is also recommended that future R&D develop methods to suppress the activity of these hormones.

While this research project was focused on understanding the physiological and developmental basis of summer fruitlet abscission, it also recommended that future R&D focus on "what makes a fruit resistant to abscission". For example, while shading induced fruit abscission, a substantial number of fruits grew and developed under the low light conditions. An understanding of "what" allowed the persisting fruits to grow and development, could also reveal new insights for managing summer fruitlet abscission.

In addition to fruit abscission, poor fruit set is a major factor of irregular bearing which reduces annual yields well below the theoretical production potential in avocado regions of Pemberton/Manjimup, Toowoomba and Tablelands regions. Experimental studies show that pistil starch content at the female phase of avocado flower development positively correlates with fruit set and retention (Alcaraz and Hormaza, 2009). Starch accumulation during female flower development is highly variable and the factors that determine starch accumulation in these female reproductive structures are not understood. Based on researcher performed in AV16005, we hypothesize that carbohydrate availability required for pistil starch accumulation is negatively impacted by excessive inflorescence/flower production, dominance interactions with the vegetative spring flush and harvesting mature crops at or after flowering. In addition, experimental studies provide evidence that cool temperature and/or low humidity may impair starch accumulation in pistils during flower development; thereby, reducing the number of high-quality flowers available for fruit set (Alcaraz and Hormaza, 2009). Therefore, understanding the physiological drivers that control pistil starch accumulation and how patterns of reproductive and vegetative development, environmental factors (temperature x humidity) and harvest practices impact these drivers will provide a knowledge base framework required to develop new management tools to increase the yield potential of orchards in avocado growing regions where fruit set is limiting. It is recommended that a future project address the above questions addressing poor fruit set for better crop management.

Refereed scientific publications

Journal articles

A draft of a refereed journal article for publication has been attached as a confident document until published (see Appendix F).

Industry articles

Böttcher, C., Shaw, L., Haberman, A., Goetz, M., Beveridge, C., Smith, H., 2022. Developing a physiological framework for managing summer fruit abscission in avocado. Talking avocados 33, vol 3, 57-60. (See appendix B for the article)

Haberman, A., Goetz, M., Smith, H., 2022. Understanding avocado fruit abscission for improved management intervention. Talking Avocados 33, vol 1, 57-62. (See appendix B for the article)

Haberman, A., Smith, H., 2019. Positioning for better management of avocado fruit drop. Talking Avocados 30, vol 3, 60-62. (See appendix B for the article).

Conference proceedings

Haberman, A., Smith, H., 2019. Toward an understanding of avocado fruit abscission for increasing production. In: Proceedings IX World Avocado Congress. World Avocado Congress, 2019, 1-6. (See appendix C for proceeding).

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Intellectual property

No project IP or commercialisation to report.

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Appendices

Contents

Appendix A: Desktop Study

Stage I Report for Hort Innovation AV16005

Concepts and knowledge of reproductive development in horticultural cropping systems

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October 5, 2017

Summary

Avocado production is dependent upon a number of key events that occur during reproductive growth. For the stage 1 Hort Innovation project, AV16005, a review on plant reproductive biology was developed which covers key concepts in the physiology and genetics of pollination, fertilization, fruit set and fruit abscission. In addition, a brief summary of floral bud initiation and methods for carbohydrate determination has been included as requested by the Avocado Strategic Investment Advisory Panel. In this review, key concepts in plant reproduction derived from research in model plant systems are integrated with the current knowledge leveraged from avocado research. A common theme has been identified in model plant systems, in which plant reproduction is regulated by the interplay between hormone/plant growth regulators and carbon signaling and metabolism. By understanding how reproduction events (ie: pollination, fertilization, fruit set and abscission) are regulated and the physiological mechanism of resource competition, key intervention points can be identified in order to develop effective management tools to enhance growth and development as a means to increase production. At the end of the review, a list of key knowledge gaps and research areas have been proposed for fruit abscission, pollination and fruit set. While this list is not complete, it serves as a foundation to not only further develop the project plan for AV16005 on fruit abscission, but also serve to lay the groundwork for future projects aimed at maximizing yield and reducing seasonal variation in avocado.

Floral bud initiation and development

Flower bud initiation is a key event triggered by endogenous and environment signals that promote the floral transition at the shoot apical meristem (Samach and Smith, 2013; Wilkie et al., 2008). Studies in annual and perennial plant species demonstrate that FLOWERING LOCUS T (FT) functions as a phloem mobile proteinaceous signal that promotes the floral transition. In response to a favorable light duration, temperature, hormones and

nutrient signaling, FT is synthesized in the leaves and transported to the meristem via the phloem. In shoot apex, FT functions with numerous meristem regulators to switch the fate of the vegetative meristem to one with inflorescence or flower identity depending on the levels of FT. Once the floral transition is completed, floral bud formation commences (Samach and Smith, 2013).

In horticultural tree crops, experimental studies show that the expression of *FT* is induced in late autumn and early winter in mango, citrus, avocado and olive (Haberman et al., 2017; Munoz-Fambuena et al., 2011; Nakagawa et al., 2012; Ziv et al., 2014). A significant reduction in *FT* expression occurs in response to heavy fruit load and gibberellin (Haberman et al., 2017; Munoz-Fambuena et al., 2011; Nakagawa et al., 2012; Ziv et al., 2014). Research in olive indicates that cold temperature is the key environmental inductive signal that triggers *FT* expression in late autumn early winter (Haberman et al., 2017). Olive trees that fail to experience cold temperatures produce vegetative buds due to a lack of FT induction. Moreover, floral buds can be produced during the summer months by placing trees in a cold temperature chamber for several weeks (Haberman et al., 2017). Currently, there is growing concern that evergreen tree crops grown in many subtropical regions may not experience the necessary cold temperature regime required for flowering due to climate change. The ability to manipulate flowering in these conditions will be key to maintaining production regions impacted by climate change.

Pollen development and fertilization

Pollination is a key event in reproduction and occurs when pollen grains are transferred to the receptive stigma at the apical end of the pistil (Lora et al., 2016). In a compatible interaction between the pollen and stigma, pollen grains will undergo rehydration and become metabolically active. After germination of the pollen grain, the pollen tube grows through the stigma, penetrates the style and migrates toward the ovule on the transmitting tract. Chemicals secreted by synergids in the ovule function to chemically guide the pollen tube as it migrates down the transmitting track. Double fertilization occurs when the pollen tube penetrates and releases the sperm cells inside the ovule resulting in the formation of the embryo and the endosperm (Lora et al., 2016).

Self-incompatibility is a strategy that a subset of plants have evolved in order to eliminate self-fertilization (Doucet et al., 2016). In compatible selection systems, successful fertilization is dependent upon pollen performance, pollen-pistil interactions and climate factors, such as temperature and humidity (Lora et al., 2016). During pollen development, the accumulation of nutrient reserves provides energy and resources for pollen grain survival and germination (Reinders, 2016). However, pollen tube growth is dependent upon nutrient reserves supplied by the style (Herrero and Dickinson, 1979). In addition to nutrients, gibberellin is a key hormone regulator of pollen performance and anther development (Jacobsen et al., 1996; Koornneef and Vanderveen, 1980). Plants with reduced levels of gibberellin display a significant decrease in pollen viability, germination and tube growth (Chhun et al., 2007; Goto and Pharis, 1999; Singh et al., 2002).

Pollen density is another key factor in pollen performance, as high pollen density stimulates germination and tube growth (Boavida and McCormick, 2007; Brewbaker and Majumder, 1961). The 'pollen competition' hypothesis predicts that increasing pollen density on the stigma results in competition such that highly viable and larger sized pollen grains are more likely to fertilize the egg cell (Lora et al., 2016). Moreover, pollen competition leads to an increase in offspring fitness with progeny that display vigorous growth, which augments sink strength (Hormaza and Herrero, 1996). Consistent with this hypothesis, high pollen load resulting from hand pollination increases fruit size in pear (Zhang et al., 2010). In addition to pollen viability and size, pollen-pistil interactions are also critical for modeling competitive effects, such that pollen with high compatibility with the pistil will have a selective advantage over pollen with less compatibility.

Pollen viability, germination and tube growth is affected by temperature (Sharma and Nayyar, 2016). Exposure to cold temperatures during flowering leads to an increase in ABA levels in the pollen, which reduces carbohydrate metabolism and leads to male sterility. Experimental studies indicate that an increase in ABA negatively regulates cell wall invertases, sugar transporters and gibberellin biosynthesis, which are all critical for maintaining optimal metabolism, viability and growth (Sharma and Nayyar, 2016). In tobacco, a decrease in a cell wall invertase gene expression in pollen grains results in male sterility and this phenotype is associated with a loss of starch and cell wall integrity (Goetz et al., 2001). Interestingly, in cold tolerant plants, cell wall invertase gene expression is not repressed and ABA levels fail to accumulate in response to cold temperature (Sharma and Nayyar, 2016). As a result, cold tolerant pollen grains can maintain their metabolism for development, germination and tube growth. It should also be pointed out that applications of gibberellin reduce male sterility in response to cold stress (Sakata et al., 2014). Taken together, plants with reduced levels of ABA that maintain gibberellin biosynthesis and carbohydrate transport/metabolism in the anther and pollen during cold temperatures will likely display a high degree of cold tolerance.

Avocado pollination, pollen performance and pollen-pistil interactions

Avocado is characterized as a fruit tree crop with low fruit set, ranging from 0.23% to 0.001%, despite producing numerous flowers (Sedgley, 1980). Poor pollination is a key factor that contributes to low fruit set. A recent study in California showed that on average only two pollen grains with no pollen tube growth were attached to the stigma of abscised flowers (Garner and Lovatt, 2016). In addition, increasing pollen density via hand pollination significantly increases fruit set (Alcaraz and Hormaza, 2014; Alcaraz et al., 2013). The protogynous dichogamy nature of avocado flowers in which the pistil is receptive before the pollen grains are dispersed favors cross-pollination (Davenport, 1986). Honeybees, which are inefficient and non-native pollinators of avocado, are used in production. Together, these two factors have a negative impact on pollination and production (Salazar-Garcia and Lovatt, 2013).

Avocado cultivars are separated into two classes based on the relative timing of the protogynous dichogamy flower opening system (Davenport, 1986). In type-A cultivars, including Hass, the flower opens for a few hours in the morning with a receptive pistil before closing. Subsequently, pollen shedding takes place during the second

flower-opening phase, which occurs the following afternoon. In type-B cultivars, the female and male flowering opening periods occur in the afternoon and following morning, respectively. Therefore, B-type cultivars, including Bacon, Ettinger, Fuerte and Zutano, which shed pollen in the morning, are often used as pollinizers for cultivars including Hass, as long as the bloom period overlaps. In addition, other compatibility factors contribute to pollination, as increased pollen load with B-type pollinizers, Marvel and Nobel, showed a significant increase in fruit set compared to Fuerte (Alcaraz and Hormaza, 2014). While outcrossing has been observed in California, Israel, Spain, Australia and Florida (Salazar-Garcia and Lovatt, 2013), the rates of outcrossing are typically higher in trees planted in close proximity to the pollinizer (Alcaraz and Hormaza, 2011; Degani et al., 1997; Garner et al., 2008; Kobayashi et al., 2000; Vrecenargadus and Ellstrand, 1985). Interestingly, at 30 meters, the outcrossing rates for Ettinger and Ardith was 0.91 and 0.82 respectively, indicating that there is variation in pollen viability between avocado pollinizers (Degani et al., 1997). Fuerte fruits derived from outcrossing events with Teague, Topa-Topa or Ettinger were typically larger than fruits produced from self-fertilization events (Degani et al., 1990). The major question remains, does outcrossing improve fruit set and overall yield? Experimental studies in Israel indicate that ovaries fertilized with pollen derived from an outcrossing event are less likely to abscise than fruit/seed from self-fertilization (Degani et al., 1997; Degani et al., 1986). However, correlation between outcrossing rates and yield were slight to not significant in Hass orchards in California and Spain (Alcaraz and Hormaza, 2011; Kobayashi et al., 2000; Vrecenargadus and Ellstrand, 1985). The conflicting reports on the impact of outcrossing on avocado yield are likely due to climatic differences. Compared to Israel, Spain and California have lower springtime temperatures and higher relative humidity during the blooming period, which may extend the receptivity of the stigma. In addition, climatic differences between trial sites may also influence pollen performance. While self-pollination occurs, other compatibility mechanisms may reduce fertilization.

Temperature influences pollen performance and fertilization in avocado. In experiments in which Hass pistils were pollinated with Fuerte pollen, the optimal day and night temperatures for pollen tube growth and fertilization were 25^0C and 20^0C, respectively (Sedgley and Annells, 1981). However, when day and night temperatures were reduced to 17^0C day and 12^0C night, a marked decline in pollen tube growth, fertilization and ovary development occurred (Sedgley and Annells, 1981). Based on research in model plant systems, the reproductive decline in response to low temperatures may be linked to elevated levels of ABA and a reduction in carbohydrate metabolism. At high temperatures, 33^0C day and 28^0C night, the rate of pollen tube growth and embryo growth was accelerated but by 14 days after fertilization, all fruits set at this temperature regime abscised (Sedgley and Annells, 1981). This high temperature effect on early embryo and fruit development may disrupt the ability of the fruit/seed to effectively take up the required resources needed to sustain growth and maintain sufficient sink strength.

Is there variation in pollen performance between cultivars under different temperature regimes? Pollen tube performance was examined in 9 cultivars under a 17^0C day/12^0C night cycle (Sedgley and Grant, 1982). In this experiment, pollen tube growth and the percentage of ovules penetrated by the pollen tube were examined in hand self-pollinated experiments. Results showed the rate of pollen tube growth and ovule penetration by pollen

tube was the highest in Wurtz and Reed, two type-A cultivars. Interestingly, all 5 B-type cultivars, including Edranol, had minimal tube growth and penetration ≤1 at this low temperature regime. It would be interesting to examine pollen tube growth and penetration on Hass pistils with each of these cultivars to determine if the tolerance is due to the pollen and/or pistil. In addition, germplasm and breeding populations produced in California, Florida, Israel and Mexico, may possess genotypes that produce pollen with good performance under cool and/or warm temperature regimes. In vitro pollen germination and growth protocols have been developed in avocado (Alcaraz and Hormaza, 2014; Loupassaki et al., 1997); therefore, these systems could be used to effectively screen genotypes and identify individuals that produce pollen grains, which germinate and grow under cool (17^0C day/12^0C night cycle) and/or warm (33^0C day/28^0C night cycle) temperatures. Once identified, field trials could be used to further evaluate pollen grain performance, as well as determine the relative timing and duration of bloom.

As mentioned above, the style is essential for providing the pollen tube with nutrients required for growth. Interestingly, studies in avocado show that starch accumulates in the ovaries during flowering (Sedgley, 1979) and carbohydrate content is highly variable among flowers (Alcaraz et al., 2010; Boldingh et al., 2016). Recent studies indicate that flowers/pistils with high starch content are more likely to set and retain fruit than flowers/pistils with low starch content (Alcaraz et al., 2013). It would be interesting to understand the timing of starch deposition. Does starch deposition occur during or after carpel development? Also, is there a positive correlation between starch and gibberellin levels and does this hormone promote starch accumulation in the styles and ovaries of avocado pistils? If this is the case, starch content may be modified by applications gibberellin during the appropriate stage of inflorescence and flower development.

Fruit set and development

Fruits develop from pistils, which consist of one or more carpels that are fused or develop freely. Carpels, which are modified leaves produced during flower development (Mathews and Kramer, 2012), undergo specific patterning events and tissue differentiation that involve the integration of hormones including auxin, cytokinin and gibberellin with cell specific transcriptional networks (Marsch-Martinez and de Folter, 2016). Once carpel development is completed, cell division and differentiation ceases due to an increase in ABA and upregulation of negative growth regulators including IAA9, ARF7 and PROLIFERA (SIDELLA) (Vriezen et al., 2008). A fundamental unit of a carpel is the ovary, which contains one or more ovules (Cucinotta et al., 2014; Reyes-Olalde et al., 2013). Upon fertilization, the ovule(s) will develop into the seed, while the ovary wall will give rise to the fleshy fruit.

Successful fruit set and development is critical for production in horticultural fruit crops. Fruit and seed set is initiated during pollination and fertilization and marks the transition from ovary to fruit development. In most fruit crops, a fruit will undergo two successive stages of growth before reaching maturity (Crane, 1964; Gillaspy et al., 1993). The first stage of growth is characterized by a rapid phase of cell division, which functions to increase the number of cells in the pericarp or ovary wall. In the second phase of development, cell expansion is the main

driver of growth, which primarily occurs in the mesocarp, the middle layer of cells in the pericarp. During the second stage of growth, fruits accumulate high levels of carbohydrates (Gillaspy et al., 1993). In most plants, once a fruit reaches its final size, growth ceases and ripening occurs.

Hormonal control of fruit set and development

The seed control hypothesis predicts that hormones synthesized and secreted by seeds functions to promote growth of the pericarp as well as synchronize fruit and seed development (Crane, 1964; Gillaspy et al., 1993; McAtee et al., 2013). This hypothesis is supported in part by the fact that final fruit size is dependent upon the number of seeds (Gillaspy et al., 1993). In addition, fruits with multiple ovules that display incomplete fertilization or seed development will give rise to irregular shaped fruit in which the majority of growth occurs in regions surrounding the fertilized ovaries. Auxin, gibberellin and cytokinin are key hormones that promote fruit set and development (de Jong et al., 2009; Kumar et al., 2014; McAtee et al., 2013). Experimental studies indicate that these hormones are synthesized in seeds and transported or secreted to the surrounding tissue to regulate growth. Moreover, these hormones function cooperatively in a spatial and temporal manner to regulate the cell division and expansion stages during fruit development. In support of this hypothesis, single applications of auxin, cytokinin or gibberellin stimulate a limited level of fruit growth in the absence of fertilization and the degree of growth varies depending on hormone treatment and species (Crane, 1964). However, the combined application of these plant hormones to unfertilized ovaries promotes parthenocarpic fruit development (de Jong et al., 2009; Kumar et al., 2014; McAtee et al., 2013).

Experimental evidence indicates that auxin acts to positively regulate cell division as applications of auxin to unfertilized ovaries undergo a high rate of cell division (Bungerkibler and Bangerth, 1983; Pattison et al., 2014). Interestingly, auxin controls cell expansion in part by promoting gibberellin biosynthesis in the seed and surrounding tissues (Ozga et al., 2009; Pattison et al., 2015). In contrast to auxin, gibberellin functions primarily to control cell expansion (de Jong et al., 2009; Kumar et al., 2014). For example, tomato ovules treated with gibberellin show an increase in pericarp volume due to cell enlargement (Bungerkibler and Bangerth, 1983; Serrani et al., 2007). In addition, application of the gibberellin inhibitor, paclobutrazol, reduces tomato fruit set and size, but can be reversed by gibberellin treatment (Serrani et al., 2007). While auxin is known to regulate gibberellin biosynthesis in fruits (Nadeau et al., 2011; Ozga et al., 2009; Pattison et al., 2015; Serrani et al., 2008), experimental studies show that gibberellin regulates auxin transport in roots (Willige et al., 2011). Therefore, the interplay between auxin and gibberellin may function to regulate the temporal and spatial dynamics of fruit growth. In actively dividing tissues including meristems and developing organs, cytokinin functions to promote cell division (Perilli et al., 2010; Schaller et al., 2014). Experimental evidence indicates that developing seeds synthesize trans-Zeatin (tZ), an abundant and active cytokinin that is key to promoting cell division (Jameson and Song, 2016; Matsuo et al., 2012). Consistent with this hypothesis tZ levels peak during the cell division stage of fruit development. In addition, transcripts for genes that encode enzymes required to synthesize this active form of cytokinin accumulate during the first phase of fruit growth. The biosynthesis of tZ in the tomato seed is not only necessary for embryo and endosperm development, but is likely transported or secreted to the pericarp to

regulate the cell division in the pericarp (Gillaspy et al., 1993; Matsuo et al., 2012). Applications of the synthetic cytokinin, 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) increases the levels of auxin and gibberellin, as well as the biosynthetic genes that produce these hormones (Ding et al., 2013). It would be interesting to determine if cytokinin regulates gibberellin biosynthesis via auxin. In addition to regulating IAA biosynthesis, cytokinin also regulates auxin transport during fruit development (Schaller et al., 2015). Collectively, crosstalk between cytokinin, auxin and gibberellin ensures that fruit development is maintained and coordinated in a temporal and spatial manner. Understanding the temporal and spatial dynamics of hormone crosstalk and how they function to regulate the two stages of growth is critical for developing new methods to modify fruit set and growth.

Negative regulators of fruit set and development

Tomato is a model system to study the mechanisms that control fleshy fruit development (Kumar et al., 2014). Genetic manipulation of auxin and gibberellin signaling also supports a role for these hormones in regulating fruit set and development. Auxin signaling genes *IAA9* and *ARF7* are expressed at high levels in unpollinated ovaries where they function to inhibit growth (de Jong et al., 2009; Wang et al., 2009). Plants genetically manipulated to reduce the levels of IAA9 and ARF7 display parthenocarpic fruit development (de Jong et al., 2009; de Jong et al., 2011; Wang et al., 2005). Further, ARF7 appears to be a key factor in mediating the cross-talk between auxin and gibberellin (de Jong et al., 2011). Loss of function of the *ARF7* ortholog in Arabidopsis called *ARF8* also leads to parthenocarpy (Goetz et al., 2006), indicating that the function of this ARF is conserved in plants. Tomato plants that have a mutation in a DELLA gene called *procera* display a gibberellin constitutive response phenotype in which these plants produce parthenocarpic fruit (Carrera et al., 2012). Collectively, these results show that IAA9, ARF7 and PROCERA act as repressors of growth in unfertilized ovaries and upon fertilization auxin acts to reduce the activity and expression of IAA9 and ARF7, while gibberellin promotes the degradation and transcriptional inhibition of PROCERA Therefore, current models predict that the interplay between auxin and gibberellin function to synergistically promote fruit set and growth by de-repressing the transcription and activity of these negative growth regulators as well as other hormone signaling proteins yet to be identified. Consistent with this hypothesis, numerous auxin signaling and transport genes have been identified in transcriptomic studies and appear to function at discrete stages of development to regulate fruit growth and carbohydrate metabolism (Lemaire-Chamley et al., 2005; Pattison et al., 2014; Pattison et al., 2015; Wang et al., 2009). Moreover, gene expression profiling suggest that the derepression of growth also involves the down-regulation of ABA and ethylene biosynthesis and signaling genes, which function to negatively regulate fruit growth in the absence of pollination and fertilization (Nitsch et al., 2009; Vriezen et al., 2008). Functional characterization of these newly identified hormone signaling genes will provide a better understanding the interplay between auxin, gibberellin, ABA and ethylene in regulating fruit set and growth.

Interestingly, numerous photosynthetic genes are also up-regulated during tomato fruit growth (Lemaire-Chamley et al., 2005; Wang et al., 2009). These photosynthetic genes are mainly expressed in the placenta and septum tissues of tomato fruits (Pattison et al., 2015). It has been speculated that elevated levels of these photosynthesis related genes function to fix CO_2 released during respiration in the actively dividing and

expanding fruit tissues (Pattison et al., 2015).

Integration of hormone and sugar signaling for fruit growth

Carbohydrates provide an essential energy source required for growth and provide the building blocks to synthesize cell wall components during cell expansion. Therefore, growth is regulated by the integration of sugar and hormone signaling events to ensure developmental processes are initiated and maintained when carbohydrates are available (Lastdrager et al., 2014). As a result, the transport of sucrose and possibly other sugars to fruits provides an essential energy source required to sustain and regulate growth in response to carbohydrate availability, as well as modulate sink strength.

Cell wall invertases play a critical role in establishing sink strength by degrading sucrose to glucose and fructose in seeds (Cheng and Chourey, 1999; Cheng et al., 1996) and fruits (Zanor et al., 2009). In tomato, the cell wall invertase, *Lycopersicum Invertase5* (*LIN5*), is expressed in ovaries and young fruits, as well as other floral organs (Fridman and Zamir, 2003; Godt and Roitsch, 1997). In young fruits, mRNA transcripts for *LIN5* localize to vascular tissue (Fridman et al., 2004) and plants with reduced *LIN5* gene expression produce small fruit and show an increase in fruit abscission (Zanor et al., 2009). In maize, the *Minature1* (*Mn1*) gene encodes a cell wall invertase that localizes to the endosperm transfer layer in the seed (Cheng et al., 1996). Mutations in *Mn1* fail to cleave sucrose entering the endosperm leading to decreased levels of glucose and fructose in the seed. As a result, the deficiency in sucrose uptake and degradation results in a decrease in seed size (Cheng et al., 1996). Interestingly, a ten-fold decrease in auxin is also observed in *mn1* mutant kernals (LeClere et al., 2008). Further the *ZmYUCCA* auxin biosynthetic gene was dramatically reduced in the basal region of *mn1* kernals (LeClere et al., 2010). Applications of 50 mM glucose, a concentration equivalent to that found in the basal region of normal maize kernals, induced *ZmYUCCA* expression by 10-fold. Taken together, these results indicate that cleavage of incoming sucrose to glucose and fructose not only provides energy required for growth, but this is key to maintaining high auxin levels. In addition to crosstalk between invertase and auxin, cytokinin appears to increase sink strength by positively regulating cell wall invertases (Ehness and Roitsch, 1997). In pea, gibberellin produced in the seed coat correlates with the sucrose uptake and starch accumulation in the seed (Nadeau et al., 2011). Taken together, fruit growth is a highly regulated process that involves the interplay between sucrose metabolism (invertase activity), glucose signaling, and hormone activities (cytokinin, auxin and gibberellin). Understanding the temporal and spatial dynamics of this sugar-hormone interaction will be important to better understand how to manipulate sink strength. Moreover, key 'hubs' in this sugar-hormone crosstalk may be negatively targeted under nutrient, biotic and abiotic stresses as a means to modulate or induce developmental arrest and fruit abscission.

Avocado fruit set and development

The seed coat plays a major role in avocado fruit development. After fruit set, the seed coat develops quickly as this is a major site for hormone biosynthesis (Bower and Cutting, 1988). During early stages of fruit and seed development, the seed coat, endosperm and embryo contain high levels of active cytokinin(s) (Blumenfeld and

Gazit, 1970), while the mesocarp appears to contain measureable levels of an inactive form of cytokinin (Gazit and Blumenfeld, 1970). Gibberellin and auxin activities are also detected in the avocado seed coat and endosperm during early stages of growth (Blumenfeld and Gazit, 1972; Gazit and Blumenfeld, 1972). In contrast, gibberellin and auxin activities were not detected in avocado mesocarp extracts, indicating that these hormones are highly regulated in the fleshy fruit. Hormones produced in the endosperm and embryo promotes seed development, while hormones synthesized in the seed coat likely function to regulate growth of the mesocarp. At this time, it is unclear if these growth-promoting hormones diffuse or are actively transported from the seed coat to the mesocarp tissue. As seeds and fruits mature and growth slows, the activity of cytokinin, gibberellin and auxin decline (Blumenfeld and Gazit, 1970, 1972; Gazit and Blumenfeld, 1970, 1972), while ABA levels increase (Bower and Cutting, 1988). The loss of auxin and gibberellin activity in mature fruits corresponds to the time when fruits accumulate oil and the seed coat darkens due to cell death. While cytokinin, gibberellin and auxin activities have been identified in seed tissues, the type or isoforms for each of these hormones has yet to be identified.

A negative regulator of cytokinin activity was identified in the mesocarp, which accumulated to the highest levels in mature fruits (Gazit and Blumenfeld, 1970). The inhibitor appears to be 1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene, which prevents cytokinin induced growth in soy bean callus and wheat coleoptiles (Bittner et al., 1971). At this time, it is unclear as to whether this molecule regulates fruit growth by inhibiting cytokinin-induced cell division in the mesocarp of avocado.

The primary photosynthetic sugar produced in avocado is D-mannoheptulose (Liu et al., 2002), which may be converted to perseitol via transaldolase activity (Tesfay et al., 2012). In addition to sucrose, D-mannoheptulose and perseitol are mobile sugars transported to sinks including developing fruits (Liu et al., 2002). In avocado, early fruit growth is marked by an increase in soluble sugars, including D-mannoheptulose and perseitol (Liu et al., 1999b). Glucose and fructose are produced but at much lower levels, indicating that hexoses likely function as signals required for fruit development in cooperation with auxin, cytokinin and gibberellin. During the autumn, when fruit growth slows and oil production increases in the mesocarp, there is a decline in D-mannoheptulose, while the levels of perseitol increase slightly. It has been postulated that D-mannoheptulose functions as an energy source to maintain respiration and cell growth processes, while perseitol acts as a storage carbohydrate during fruit development (Cowan, 2004; Tesfay et al., 2012). Interestingly, D-mannoheptulose and perseitol may function to inhibit ripening, as the levels of the sugars rapidly decline when the fruit is removed from the tree (Liu et al., 2002).

In addition to accumulating sugars and proteins, the developing avocado mesocarp tissue accumulates oleic acid as well as other fatty acids, which is a unique feature of avocado fruit development compared to other fruits (Davenport and Ellis, 1959; Eaks, 1990; Kikuta and Erickson, 1968). Ultrastructural studies indicate that oil cells develop in the mesocarp when oil bodies bud from the smooth endoplasmic reticulum, migrate to the vacuole and eventually fuse (Plattaloia et al., 1983). At the time of maturity, the oil cell is nearly filled with lipids, which

displaces water from the cells. Gene expression profiling demonstrates that transcripts involved in sucrose degradation and fatty acid biosynthesis accumulate at significant levels in the mesocarp during fruit development (Kilaru et al., 2015). Moreover, high transcripts levels were detected for *WRINKLED1*, a key regulator of fatty acid biosynthesis in plants.

Fruit abscission

As described above, fruit development is a highly regulated process that begins at the time of pollination and fertilization and ceases at maturity (Gillaspy et al., 1993). Fruit trees regulate crop load in response to endogenous signals and environmental triggers (Ito and Nakanot, 2015; Sawicki et al., 2015). As a result, not all fruit that set attain maturity. Further, the number of fruits that abscise varies depending on timing of fruit set, the amount of shoot growth, the number of fruits that set and climate extremes the trees experience during the growing season.

While fruit tree crops typically experience two waves of fruit abscission (Sawicki et al., 2015), the developmental timing of these waves can differ between species. For example, in apple, the first wave of fruit abscission occurs during the cell division phase and is referred to as the 'physiological fruit drop', which is mediated by a hierarchical dominance system (Arseneault and Cline, 2016). The second wave of fruit drop, known as the 'preharvest fruit drop' occurs during ripening, four weeks before harvest and is distinct from the physiological fruit drop. In citrus, the first wave occurs early during the cell division phase and the second phase, known as the 'June Drop' in the northern hemisphere, occurs during the expansion phase (Mehouachi et al., 1995). Evaluation of citrus fruit growth rates indicates that slow growing fruit are more likely to abscise than the fast growing fruits (Ruiz et al., 2001; Zacarias et al., 1995). The percentage of fruit abscission that occurs during these waves of fruit drop can be increased if trees experience unfavorable climatic conditions (Sawicki et al., 2015).

In most fruit tree crops, including avocado, apple and citrus an inhibition or reduction of fruit growth precedes fruit drop (Bangerth, 2000; Garner and Lovatt, 2016; Ruiz et al., 2001; Sedgley, 1980; Zacarias et al., 1995). It was estimated that avocado fruit abscission occurs seven days after growth cessation (Sedgley, 1980). Therefore, the abscission event that occurs during fruit abscission is likely a secondary affect of developmental arrest. Understanding how the internal and external signals target a subset of fruit and switch the fate from active to limited growth is key to developing new management approaches to increase production.

Two hypotheses to explain fruit abscission have been developed. The first hypothesis predicts that carbohydrate availability and the nutritional status of the tree controls the severity of fruit abscission (Goldschmidt, 1999; Sawicki et al., 2015). This is supported by the fact that defoliation and shading trees, which decrease carbohydrate levels, results in an increase fruit abscission. In contrast, increasing carbohydrate levels in shoots via girdling decreases fruit abscission (Iglesias et al., 2006). The second hypothesis referred to as the correlative dominance model predicts that hormones alone or in conjunction with assimilate limiting conditions function to

regulate fruit abscission (Bangerth, 1989; Li and Bangerth, 1999). For example, fruits that set early act as the dominant sink because they maintain a higher rate of auxin transport out of the fruit than the younger dominated fruit. In the shoot where these auxin transport pathways converge, an auto inhibitory signal is generated to reduce auxin synthesis and transport from the younger dominated fruit. According to this model, fruit abscission can also be induced under conditions that favor shoot growth (Bangerth, 1989; Li and Bangerth, 1999). For example, dominant shoots that display a high rate of auxin transport out of the shoot apex compared to the basal developing fruit, induces developmental arrest and abscission.

The abscission zone

Fruits separate from the plant via the abscission zone (AZ), which is located at specific sites in the pedicel, peduncle and/or the base of the fruit (Estornell et al., 2013). The abscission zone can be specified early in development or after fruit development. Activation of the abscission zone is the final step for the separation of the fruit from the tree. Auxin and ethylene are two key hormones that regulate AZ activation. In actively growing fruits, a high rate of auxin transport out of the fruit and through the pedicel to the stem prevents AZ activation by making these cells insensitive to ethylene. After fruit growth cessation, the decrease in auxin transport and signaling in the pedicel increases ethylene sensitivity in the AZ. As a result, ethylene levels increase due to the upregulation of ethylene biosynthetic genes. Finally, genes that encode proteins involved in programed cell death and cell wall modification rapidly increase, which allows the fruit to physically separate from the tree. Interestingly mutations in ethylene perception fail to prevent abscission. Instead, abscission is delayed in plants having mutations in ethylene perception, indicating that ethylene dependent and independent pathways cooperatively function to activate the AZ following developmental arrest (Butenko et al., 2006; Patterson and Bleecker, 2004).

In temperate regions of the world, vegetative shoot growth coincides with fruit development, which often results in sink competition for carbohydrates, minerals and hormones necessary for growth. Vigorous vegetative shoot growth induces fruit abscission (Bangerth, 2000; Sawicki et al., 2015), while the bending and girdling of shoots appears to increase fruit set, by reducing shoot growth (Bangerth, 2000). Application of cytokinin induces fruit abscission by promoting the growth of lateral shoots (Elfving and Cline, 1993). Methods aimed at increasing the sink strength of shoots in order to induce fruit abscission will allow researchers to identify key factors that trigger developmental arrest across fruit tree crops.

Fruit abscission in apple

Apple has been used as a model system to study fruit growth and abscission (Botton et al., 2011; Dal Cin et al., 2005). Apples are produced on terminal corymb inflorescences, which typically produce five flowers. In this inflorescence, the apical flower gives rise to the central fruitlet, while the lateral fruitlets are initiated from the basal set of flowers. A gradient of dominance occurs in the apple cluster such that the central fruit, which is initiated first, is the dominant fruit while last fruit to develop in the basal position is the least dominant fruit. As

a result, the abscission rate for the youngest lateral fruit (L1) is high, while the opposite is true for the dominant central and L3 fruits at the apex of the inflorescence. Further, the degree of dominance can be manipulated by shading or applications with cytokinin, which reduce carbohydrate levels or enhance the sink strength of shoots, respectively (Bangerth, 2000; Beruter and Droz, 1991; Green, 2002).

A possible role for ethylene in developmental fruit arrest and abscission has been postulated (Dennis, 2002). To better understand the temporal and spatial dynamics of ethylene in fruit drop, a fruit abscission induction system was developed that increases competition between the dominant sinks (central and L3 fruitlets) and the weak L1 fruitlet (Botton et al., 2011; Dal Cin et al., 2005). Experimental studies showed that ethylene accumulates at high levels 4-days after induction in abscising fruitlets compared to the dominant fruitlets (Dal Cin et al., 2005). Moreover, transcripts that encode the rate limiting ethylene biosynthetic enzyme, 1-aminocyclopropane-1-carboxylate synthase (*MdACS5B*), peak early in the fruit cortex, 3-days after induction (Dal Cin et al., 2005). Subsequently, the mRNA levels for *MdACS5B* increase in the seed and peduncle. Interestingly, four ethylene receptors are expressed highly in the seed (Eccher et al., 2013). Botton et al., 2011 proposed that ethylene produced in the cortex of abscising fruit would act as a mobile signal that would diffuse into the seed and induce embryo abortion. In support of this hypothesis, fruit abscission appears to be dependent upon the levels of ethylene produced in the cortex and the number of ethylene receptors translated in the seed (Eccher et al., 2015). In this model, high levels of ethylene produced in the L1 fruit cortex diffuse into the seed and saturate the low number of ethylene receptors, which would generate an amplified signal to induce embryo abortion. However, in the central or L3 fruitlet, the high number of receptors in the seed is not saturated when a low amount of ethylene is produced in the cortex, as a result, fruit growth and maturation continues (Eccher et al., 2015).

Currently, the identity of the fruit abscission signal(s) that promotes developmental arrest is not clear in apple or other fruit crops. A resource competition model has been proposed to explain fruit abscission in apple. In this model, the nutritional requirements for fruit development become limiting in a tree after fruit set, due to fruit-fruit and shoot-fruit competition (Botton et al., 2011). The expectation from this model is that weaker sinks, such as the L1 fruitlets, will display a sugar starvation phenotype. Consistent with this model, results showed that high levels of soluble sugars including sucrose were found in abscising L1 fruitlets compared to the central and L3 dominant fruitlets (Abruzzese et al., 1995; Botton et al., 2011). It was proposed that the high sucrose levels in the abscising fruit triggers a sugar starvation response event that leads to growth arrest and abscission (Botton et al., 2011). However, the sampled fruits in these experiments were not of the same age; therefore, the differences in sucrose levels may reflect a difference in the developmental sugar profiles between these fruits (Ackerman and Samach, 2015). To address this, soluble sugars including sucrose were examined in the central and L1 fruitlets during flower and early fruit development. Results showed that the levels of sucrose and other soluble sugars accumulated at similar levels over the course of development in the central and L1 fruitlets. These results indicate that L1 fruitlet abscission is not mediated by a sucrose induced nutritional deficit signaling event and the factors that promote fruit drop have yet to be identified (Ackerman and Samach, 2015).

Fruit abscission in avocado

Avocado is characterized by having a low rate of fruit set (<0.1%) together with high rate of flower and fruit abscission (Davenport, 1982; Garner and Lovatt, 2008; Whiley and Schaffer, 1994). In orchards that experience biennial bearing, the previous year's crop load has little impact on the fruit abscission (Garner and Lovatt, 2008). Taken together, these observations lead researchers to speculate that fruit abscission is not entirely driven by a limitation of assimilates (Garner and Lovatt, 2008).

Avocado typically has two waves of fruit drop (Adato and Gazit, 1977); however, in some environments fruit drop is a continuous event that occurs gradually over the growing season. During the first wave of avocado fruit drop, histological analysis was performed to characterize the anatomy of the abscised fruitlets over a 28-day period South Australia (Sedgley 1980). Results showed that 92.5% of the fruitlets that abscised during the first week we unfertilized. However, from 8 to 28 days, the majority of abscised fruitlets were fertilized (68.1%), and had a normal pattern of embryo development (Sedgley, 1980). The lack of fertilization as well as seed degeneration also contributes to fruit abscission in Australia, California, Israel and South Africa (Garner and Lovatt, 2016; Sedgley, 1980; Steyn et al., 1993; Tomer and Gazit, 1979).

The relationship between abscising fruitlets and plant hormones, ethylene and ABA, was examined in avocado. Results indicate that small fruitlets with deteriorated seeds have higher levels of ethylene than larger sized fruitlets with normal seeds (Adato and Gazit, 1977). The majority of the ethylene produced was derived from the seed. Compared to persisting fruitlets, weakly attached fruitlets accumulated higher levels of ethylene (Adato and Gazit, 1977) and ABA (Adato and Gazit, 1977; Garner and Lovatt, 2016). Davenport and Manners, 1982, also found that fruitlets abscising from excised de-leafed branches also accumulated high levels of ethylene, most of which was produced from the seed coat. In summary, avocado fruits that have undergone developmental arrest accumulate high levels of ethylene and ABA, which likely functions to maintain growth suppression in abscising fruit. This mechanism would ensure that assimilate uptake is prevented in developmentally arrested fruits.

The avocado small fruit phenotype and its relationship with abscission

Hass avocado trees produce normal and small sized fruit (Zilkah and Klein, 1987). The premature cessation of growth that occurs in the small fruits is associated with seed coat senescence and a reduction of cell division in the mesocarp leading to developmental arrest (Cowan et al., 2001). The small fruit phenotype is initiated between 60 to 90 DAFB (days after full bloom) in association with a rapid 3.5^0C increase in canopy temperature (Cowan et al., 2005). 3-hydroxy-3-methylglutaryl-coenzyme a reductase (HMGR) is a key enzyme for the biosynthesis of isoprenoids used for the production of thousands of compounds including plant hormones such as cytokinin, brassinosteriod, gibberellin and ABA (Cowan et al., 2001). Experimental studies in tomato and avocado show that HMGR activity is required for fruit growth (Cowan et al., 1997; Narita and Gruissem, 1989).

Further, avocado small fruits have reduced HMGR activity, which is likely due to increased levels of ABA (Cowan et al., 1997), which is known to inhibit HMGR activity in pea (Russell and Davidson, 1982). Moreover, auxin biosynthesis in the seeds of small fruits appears to be impaired (Cowan et al., 2005). Consistent with a decrease in cell number, small fruits are associated with a decrease and increase in the expression of cell cycle regulators that activate and repress cell division, respectively (Dahan et al., 2010; Sabag et al., 2013).

The correlation of fruit growth cessation with increased levels of ABA and seed coat senescence is associated with both fruit abscission and the small fruit phenotype. In addition, as fruits grow and mature the levels of cytokinin and auxin activity decline, while ABA levels rise (Bower and Cutting, 1988). Similar to fruit abscission and the small fruit phenotype, an increase in ABA correlates with the decline in cell division and seed coat senescence in mature fruit (Cowan et al., 1997). A pressing question is what allows mature and small fruits to stay attached to the tree after a slowing or cessation of growth, respectively? Is the small fruit phenotype due to the precocious maturation of fruit development rather than an immature abortive event? The negative correlation of ABA with growth and its association with fruit abscission, maturation and the small fruit phenotype indicates that ABA may not be the primary causal signal but rather a response factor that maintains the inhibitory growth state.

New methods to measure carbohydrates for effective tree management

The "carbohydrate and phenological cycling" hypothesis predicts that productivity is dependent upon carbohydrate levels in trees (Whiley and Wolstenholme, 1990; Wolstenholme and Whiley, 1989). The ability to determine carbohydrate levels, such as stored starch, will help drive effective management decisions and inputs to increase productivity. Further, the ability to manipulate carbohydrate partitioning into flowers and fruits, while maintaining a sufficient level of shoot growth will allow growers to increase yields and stabilize annual production.

In avocado, starch is stored in the stems of shoots during the winter when growth is limiting (Liu et al., 1999a; Scholefield et al., 1985). The ability to measure stored starch in the winter and spring may allow growers to predict yield and implement the necessary management tools to increase or maintain yield if stored starch levels are low. Traditional methods for measuring starch utilize a laborious and expensive enzymatic assay system that is not suitable for commercial adoption. Fourier transform infrared spectroscopy (ATR-FT-IR) was recently used to measure starch and nitrogen levels in grapevine (Schmidtke et al., 2012). In addition, a Fourier transform near infrared spectroscopy (FT-NIRS) system has been developed to determine dry matter content in Hass avocado fruit (Wedding et al., 2013). The advantage of using these infrared spectroscopy systems is that minimal preparation is needed for each measurement. However, calibration and statistical models must be implemented before these systems can be used. The CSIRO Land & Water unit at the Waite Campus in Adelaide has a FT-NIR spectroscopy system, which can be accessed by the Agriculture and Food – Horticulture unit. Therefore, the possibility exists to develop a FT-NIR system to measure stored starch in the stems of avocado trees during winter and growing seasons.

Key Knowledge Gaps and research areas in avocado reproductive development

1. In response to competition with vegetative shoot growth and dominant fruit development, an understanding of the signal(s) that triggers fruit growth cessation during abscission is required in order to develop methods to reduce abscission. Also, does this fruit growth cessation signal(s) function to trigger fruit abscission during abiotic stress?
2. Understanding key physiological and molecular events that inhibit pollen germination, growth and fertilization in response to low temperatures.
3. Are there cold or heat tolerant avocado genotypes that could be used as pollinizers in regions that experience cold or hot day and night temperatures during flowering, respectively?
4. Can the selection of new pollinizers with high pollen viability and vigorous growth be used to increase fruit set and reduce abscission? Has there been a concentrated effort to identify the optimal pollinizers for Hass under the different climatic conditions?
5. Understanding the physiological and molecular events that regulate starch deposition during pistil development in order to develop methods to increase fruit set.
6. What are the physiology and molecular events that prevent abscission of small fruits?
7. Understanding the effect of humidity on pollen viability in Hass. Research in Fuerte pollen showed pollen viability was severely compromised at 40%.
8. While this is beyond the scope of this proposal, new funding aimed at modeling light interception and canopy architecture may also drive new canopy management methods in order to maximize photosynthesis required for carbohydrate production. In turn, this would have the potential to reduce resource competition in order to maximize production.

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To whom it may concern,

I have read with interest the "Stage I Report for Hort Innovation AV16005 Concepts and knowledge of reproductive development in horticultural cropping systems" prepared by Harley Smith for HAL funding. In my opinion, the content and the concepts presented in the review are relevant for optimizing avocado production and the review highlights key areas of reproductive development for avocado research of interest not only for Australia but elsewhere for other avocado producing areas.

Sincerely

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האוניברסיטה העברית בירושלים
THE HEBREW UNIVERSITY OF JERUSALEM
الجامعة العبرية في اورشليم القدس

הפקולטה לחקלאות
מזון וסביבה

September 16th 2017

Regarding: *Stage I Report for Hort Innovation AV16005 Concepts and knowledge of reproductive development in horticultural cropping systems*.
By Dr. Harley Smith, CSIRO.

To The Avocado Strategic Invest Panel

I have carefully read the abovementioned report. I found it excellent.

Dr. Smith has an excellent understanding of the Avocado tree and Avocado industry from years of research in UC Riverside. Dr. Smith also has a broad and deep understanding of developmental processes in plants, based on his early career and present work.

In addition, Dr. Smith has shown here the rare ability to go over a huge amount of literature, identify what is interesting and connected. It would have been easier for him to cite all literature and throw into the report all the conflicting results from years and years of good and sometimes mediocre research. Dr. Smith read the literature, and clarified for the reader an underlying pattern of findings in different plant species. These findings suggest a clear path of research that should be currently conducted in Avocado.

To summarize, the content and the concepts presented in this review are relevant and the review highlights key areas of reproductive development for avocado research.

Alon Samach

Appendix B: Industry and Technical Journal Articles

Positioning for better management of avocado fruit drop

Amnon Haberman and Harley M. Smith, CSIRO Agriculture and Food

Avocado is a low yielding tree crop with average annual production levels equivalent to approximately 10t/ha, which is considerably lower than the theoretical value of 32.5t/ha, as estimated by Wolstenholme 1987. Low yields are attributed to the semi-domesticated nature of avocado[1], due to unfavourable traits including vigorous shoot growth, excessive flowering, low fruit set and high fruit abscission[2,3]. In addition, avocado has a high propensity for alternate (biennial) bearing[4]. The predicted rise in global temperatures will likely enhance these traits and further reduce annual yields[5]. Together, the additive effects of these yield-associated traits limit production and present a major challenge for Australian orchard management for maximising yields and reducing seasonal variation.

Challenges in Australian avocado production

Yield associated traits are controlled by the interaction between the genetics, climate and management inputs[6], as well as the age of the tree. To increase Hass yields, new management tools must be developed to reduce the impact of excessive vigour, low fruit set, high fruit abscission and biennial bearing. To achieve this, it is necessary to have a basic understanding of the physiology that drives these yield associated traits. Fruit abscission is a central component controlling fruit production and this process is poorly understood in avocado, as well as other fruit tree crops. Therefore, this article is focused on fruit drop, which is the major aim of the Hort Innovation funded project, AV16005.

Early fruit abscission

During the early fruit abscission event, a majority of fruitlets abscise within the first five weeks after fruit set[7,8]. The initial phase of the early fruit drop event is due to the abscission of unfertilised fruitlets[7]. The later phase of this fruit abscission event involves the abscission of fertilised fruitlets, typically between 6-10mm in size. Growers estimate that approximately 30-50% of the fertilised fruitlets drop during the early fruit abscission event.

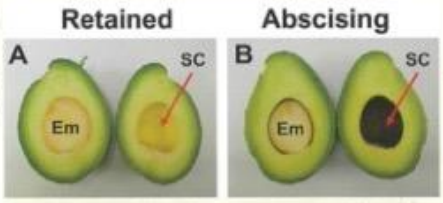

Figure 1. *Seed coat senescence is associated with fruit drop. Fruits firmly attached to the tree (A) display little seed coat senescence compared to fruits undergoing fruit abscission (B). Embryo, Em; seed coat, SC*

Interaction between the spring flush and developing fruitlets

Due to the coincidence of vegetative and reproductive growth in the spring, it has been proposed that the early fruit abscission event is mediated in part by the growing spring flush, which competes with the developing fruitlet for photosynthates and nutrients (reviewed by Salazar-García et al. 2013). In support of this hypothesis, Salazar-García and Lovatt (1998) reported that 'functionally determinate' inflorescences are three times more productive than indeterminate inflorescence shoots. Paclobutrazol and uniconazole are growth retardants that reduce stem elongation and leaf expansion via inhibition of gibberellin biosynthesis. As elongating stems have a high growth potential, an increase in yield by applications of paclobutrazol at flowering was associated with an augmentation in the number of fruits[11,12]. Applications of paclobutrazol also increased fruit size, which also contributes to higher yields[13]. However, other reports showed that applications of paclobutrazol, as well as uniconazole, at flowering did not increase yield[14-16]. In addition, studies showed that removal of the spring flush or applications of paclobutrazol increased fruit set; however, yield was not increased due to a heavy fruit drop during the summer in the treated trees[17,18]. The discrepancy of the effect of paclobutrazol and/or uniconazole on fruit drop and yield demonstrates the underlying complexity of the early fruit abscission event and mechanism(s) that mediates resource (carbohydrates) distribution to actively growing tissues in the tree.

Challenges for studying the early fruit abscission event

One of the major challenges for studying the early fruit abscission event is the low rate of fruit set followed by a high rate of fruitlet drop (reviewed by Salazar-García et al. 2013). The combined effect of these two traits severely reduces the ability to directly compare developmental profiles between retained and abscising fruitlets. This is extremely important, as studies in model plant systems show that early fruit development is marked by massive changes in fruit physiology, including hormone signalling and gene expression[19,20]. Therefore, if retained and abscising fruits are not collected at the same developmental age, then it becomes extremely difficult to compare the physiological differences in order to identify the key factors that mediate abscission. Moreover, this hindrance also obstructs the ability to effectively study the interaction

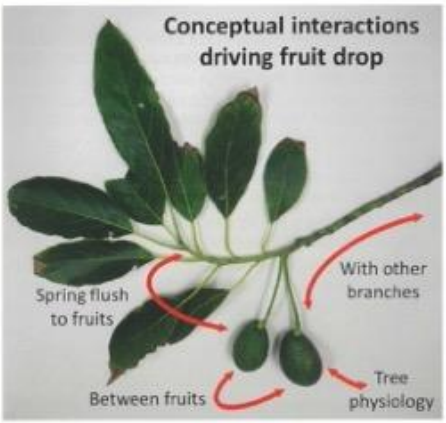

Figure 2. *An illustration of resource competition based on an avocado branch with two developing fruits and a vegetative spring flush. Red arrows indicate conceptual interactions between growing vegetative and reproductive units of the shoot that are implicated in the regulation of fruit abscission.*

between the vegetative flushes and developing fruits. However, a basic understanding of the physiological basis of the summer fruit drop event will likely apply to the early fruit abscission event.

Summer fruit drop

The integration of genetic determinants, climatic events and management practices has impact on tree physiology and resource (carbohydrates) availability. As tree carbohydrate levels are essential for growth, the adjustment of crop load in response to resource availability is hypothesized to be a major factor that regulates the summer fruit drop event [3,21]. Therefore, understanding how tree crop load is adjusted in response to resource availability and the physiological mechanism(s) that mediate fruit abscission may provide the knowledge required to develop new management tools to reduce fruit drop and increase production. Moreover, new management tools aimed at reducing the summer fruit drop will likely be effective for reducing the early fruit drop event.

Role of seed coat in fruit abscission

Experimental studies demonstrate that seed development is required for fruit retention and development[7,8]. The seed coat is the maternal component of the seed, which functions to provide the embryo with photosynthates and nutrients required for growth[22]. Moreover, the seed coat also synthesises plant growth regulators/hormones critical for regulating embryo development (reviewed by Bower and Cutting 1988; Robert et al. 2018). Interestingly, seed coat senescence is an observable characteristic associated with abscising fruits[8,25] (*Figure 1*). Therefore, the seed coat function appears to be a critical tissue that determines the fate of a fruit, retained versus abscised.

Model of fruit abscission

We have developed a model to explain fruit abscission in avocado. In this model, a subset of fruit in a tree undergoes abscission in response to a resource availability signal(s). As pointed out above, the physiology of the tree is speculated to be a key determinant of fruit drop (illustrated in *Figure 2*). In addition, competition for resources between fruits and with shoots also drive fruit drop. At this time, the nature of this resource availability signal(s) is unknown. The fruit abscission event is viewed as a multistep process in which the resource availability signal(s) mediate fruit growth cessation.

Given that the seed coat plays a major role in fruit development and senescence of this tissue is associated with abscission, it is highly likely that seed coat mediates the cessation of fruit growth. After growth cessation, the seed coat undergoes senescence and the abscission zone is activated in the pedicel, which leads to the physical separation of the fruit from the tree. Therefore, the primary event of fruit abscission is fruit growth cessation, while the secondary event involves the process that mediates fruit drop. Based on this model, fruit abscission can only be reversed during fruit growth cessation. Once seed coat senescence is initiated, the cessation of fruit growth cannot be reversed. Therefore, in order to develop new tools to limit fruit abscission, an understanding of the physiological basis of fruit growth cessation is required.

The AV16005 Hort Innovation funded project

The primary aim of the AV16005 Hort Innovation funded project is to study the physiology of fruit growth cessation, as well as seed coat senescence and fruit abscission. However, we are lacking the capability to distinguish fruits fate to develop to maturity from fruits targeted for abscission, during early stages of fruit growth cessation. To overcome this problem, trials were performed to identify treatments that would induce a massive fruit drop event by limiting carbohydrate availability. Results from these trials showed that extensive removal of new vegetative growth promotes a massive fruit drop event. Using this approach, different fruit tissues, as well as pedicels and stems, were collected at regular time intervals from treated and control trees.

We are currently analysing the tissues using analytical and molecular methods to identify candidate hormones, metabolites, carbohydrates and genes that correlate with fruit growth cessation. This information will be integrated and used to construct the physiological and developmental pathways that mediate fruit growth cessation. Finally, these pathways will be incorporated into the model above, which will serve as a knowledge base for developing new management tools to limit fruit abscission.

Research and Development

Positioning for better management of avocado fruit drop continued

We acknowledge and thank the contribution of Jasper Farms (WA), Delroy Orchards (WA), Chinoola Orchards (SA) and Thiel Orchards (SA) to the project and technical assistance from Jacinta Foley (Jasper Farms) and Declan McCauley (WA DPIRD).

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Understanding avocado fruit abscission for improved management intervention

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Abstract

The Australian avocado industry is aiming to improve production and profitability in order to build a sustainable and competitive supply of avocados to meet consumer requirements. Poor fruit set, high fruit abscission, as well as biennial bearing, are the major drivers of irregular bearing, which is a significant challenge to the Australian avocado industry. The Hort Innovation funded-irregular bearing project, AV16005, is aimed at characterizing the steps involved in the fruit abscission process and identifying the physiological factors that control this developmental pathway. Results from this research project are being used to establish a knowledge platform to be leveraged in future projects to develop pathways for creating new innovative management tools to mitigate fruit abscission. Moreover, it is envisioned that this knowledge platform relating to immature avocado fruit abscission will be applied to manage fruit drop in other tree crops.

Avocado production is heavily influenced by a high rate of immature fruit abscission (Figure 1)[1-4]. It has been postulated that fruit drop is induced, in part, by a dominance interaction in which growth of vegetative flushes diverts carbohydrates away from developing fruits[5,6]. In addition, avocado fruit drop is likely influenced by dominance interaction among fruitlets, in which fruits with a high growth potential cause the abscission of fruits with a low growth potential. Dominance interaction among fruits is known to occur in other tree crops[7]. While avocado fruit abscission has been studied for over 40 years[3], little progress has been made in describing and understanding the drivers of this developmental process. Previous studies collectively identified differences in size, integrity and hormones produced between persisting

Figure 1. After fruit set (top panel), avocado trees undergo a fruit abscission throughout the growing season (bottom panel), which is a major challenge for avocado production.

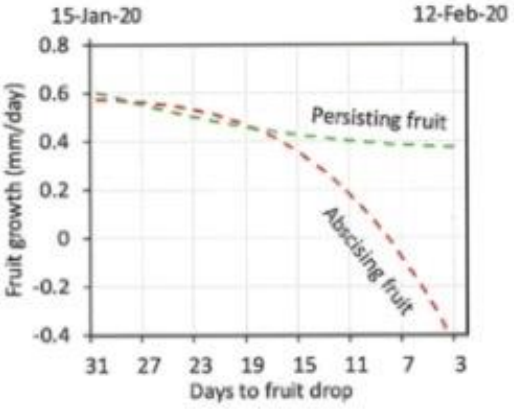

Figure 3. Growth rates of persisting and abscising fruits. Persisting fruits maintain a relatively high growth rate, between 0.4 to 0.6 mm/day, from 15 January to 12 February (top x-axis). Over the period of the trial, fruits that switch from a high to a low growth rate abscise after growth arrest (bottom x-axis).

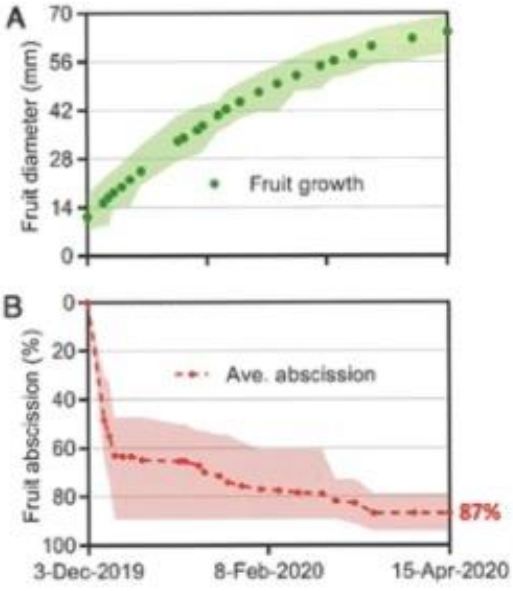

Figure 2. Seasonal pattern of avocado fruit growth and abscission. The mean diameter of fruit growth plotted over the course of the season (top panel). Pattern of fruit abscission over the course of a growing season (bottom panel). In this trial, 87% of the fruit that set abscised over the course of the growing season.

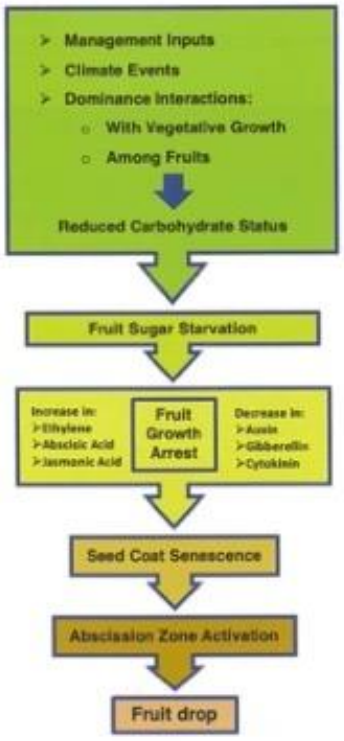

Figure 4. An integrated model of the fruit abscission process and the physiological drivers that mediate growth arrest.

and abscised fruit[1-3,8,9]. However, an understanding of the abscission process and early physiological events is necessary for the development of new management systems to effectively mitigate fruit drop.

Seasonal pattern of fruit abscission

Seasonal trends in fruit abscission appear to vary, with one to three waves of abscission occurring over the course of the growing season[1-4,8,9]. In order to identify key intervention junctures that could be targeted for mitigating immature fruit abscission, it is necessary to characterize the seasonal pattern of fruit drop. As seed development is critical for avocado fruit development and most immature fruits that abscise in the first two weeks are unfertilized[3,10], this trial was initiated when the average diameter of fruits was approximately 12 mm to ensure all fruits were fertilized (Figure 2A). Over the course of the growing season, a steady increase in the average diameter of fruits was observed (Figure 2A). A significant fruit abscission event was observed early in fruit development, in which trees dropped approximately 63% of the crop (Figure 2B). After this initial fruit abscission event, approximately 24% of fruits dropped across the period from mid-December to the beginning of April (Figure 2B). These results show that an initial wave of fruit drop significantly reduces crop load. However, the further abscission of immature fruits, after the initial fruit drop event, also has a considerable impact on yield. Therefore, developing management tools to mitigate fruit abscission after the initial wave of fruit drop is predicted to have a significant impact for increasing yield in avocado.

Impact of tree carbohydrate status on fruit abscission

It has been hypothesized that fruit trees adjust their crop load based on the carbohydrate status of the tree[11,12]. However, methods to estimate tree carbohydrate status throughout the growing season are not currently available[13], making it difficult to determine if carbon availability influences immature fruit drop in avocado. To overcome this challenge, the AV16005 project utilized defoliation and shading trials to better understand whether tree carbohydrate status influences immature fruit abscission. Results from these trials showed defoliation and shading induced fruit abscission, with the former method causing trees to drop nearly 100% of the crop in a relatively short period of time. Moreover, a marked reduction in stem carbohydrate levels preceded the fruit abscission event indicating that tree carbohydrate status influences immature fruit abscission in avocado.

Fruit growth cessation is an early step in the immature fruit abscission process

In order to study the early physiological drivers of fruit abscission, it was necessary to develop a method to identify fruits at an early stage of the abscission process. At the time the project commenced, methods to capture fruits early in the abscission process were lacking, due to the fact that it

was not possible to identify, prior to an abscission event, which fruits were going to persist and which were going to abscise. To overcome this challenge, we hypothesized that inherent changes in fruit growth may mediate the immature fruit abscission process. To test this hypothesis, >1000 fruits were tagged and the diameter measured at regular time intervals across six different trials in three seasons. Figure 3 displays the results from one trial used to characterize the fruit abscission process under natural conditions without any manipulations used to induce fruit drop. The results show that persisting fruits that continue to develop over the course of the trial display a relatively high growth rate (0.4 to 0.6 mm/day increase in fruit diameter). In contrast, fruits that switch from a high to a low growth rate undergo growth arrest before abscission. Therefore, the first step in the fruit abscission process involves growth arrest which is initiated approximately 15 days before abscission (Figure 3). The arrest of fruit growth followed by fruit abscission was also found to occur under conditions used to induce fruit drop, such as defoliation. Further, we also found that seed coat senescence occurred late in the growth arrest process. Taken together, these results strongly suggest that fruit growth arrest is the initial step in the fruit abscission process. Therefore, abscission management tools need to address fruit growth rather than delaying the formation of the abscission zone, which mediates the physical separation of fruits from the trees.

Fruit growth cessation is associated with a decline in carbohydrate status and metabolism

Carbohydrates are key metabolites used to drive growth[14]. Further, experimental studies suggest that dominance interactions between shoots and fruits, as well as among fruits, involves sugar signaling[15,16]. To better address whether changes in carbohydrate status and metabolism are associated with fruit growth arrest, sugar metabolites were compared between fruits with a high growth rate and fruits undergoing growth arrest. Results showed that fruits undergoing growth arrest displayed a marked reduction in the carbohydrate status and metabolism in the seed. Examination of key genes involved in sugar metabolism and signaling also support the model that fruit growth arrest is associated with a significant reduction in carbohydrate status and metabolism. It should be noted that nitrogen and mineral nutrient deficiency were not associated with fruit growth cessation in well-managed orchards. In addition, a change in fruit water potential did not occur during growth cessation indicating that water availability is not a driver of growth cessation. Taken together, research results from the AV16005 project indicates that a decrease in the carbohydrate status of fruits is a key physiological driver of immature fruit abscission. Therefore, reducing immature fruit abscission will require new management tools aimed at maintaining fruit carbohydrate status, which is essential for growth.

Hormones implicated in fruit growth cessation

Previous studies in avocado indicate that fruit growth is mediated by the production of auxin, cytokinin and gibberellin derived from developing seeds[17-19]. As plant hormones interact and influence sugar signaling and metabolism[20,21], a gene expression analysis was performed to examine the regulation of key hormone and developmental pathways implicated in fruit growth arrest. Results showed that fruits undergoing growth arrest display significant reduction in the expression of genes involved in auxin and gibberellin biosynthesis in the seeds, as well as the pericarp. In addition, gene expression analysis also suggests that auxin transport and signaling is altered during growth arrest. At the same time, there is an increase in cytokinin catabolism genes which suggests that this hormone is degraded during the growth arrest. Previous studies in apples indicates that ethylene and abscisic acid play a role in immature fruit abscission[15,22,23]. Furthermore, abscised avocado fruits produce high levels of abscisic acid[1]. The gene expression analysis undertaken as part of project AV16005 also shows that key genes involved in ethylene, abscisic acid and jasmonic acid biosynthesis and signaling were significantly up-regulated during fruit growth arrest. Based on the gene expression analysis, we propose that the increase in ethylene, abscisic acid and jasmonic acid acts collectively to mediate growth arrest and seed coat senescence. Taken together, the results of our gene expression study suggests that fruits undergoing growth arrest display a significant shift in hormone homeostasis. To confirm this hypothesis, we are now collaborating with Prof. Christine Beveridge and Dr Lindsay Shaw at the University of Queensland, to directly measure the levels of key hormones implicated in fruit growth arrest. This collaboration links AV16005 with AS17000 (National Tree Genomics Program) to create synergy between these two projects. Results from the hormone analysis will further provide fundamental knowledge that will support the development of new methods used to mitigate immature fruit abscission in avocado.

Integrated model of fruit abscission

An integrated model of fruit abscission is presented in Figure 4, which summarizes the key achievements of the AV16005 project. Our work supports a model in which tree carbohydrate status is the primary physiological driver that impacts carbohydrate availability to developing fruit. Tree carbohydrate status is determined by orchard management inputs, climate events and/or dominance interactions between vegetative and reproductive growth, as well as among fruits. As a result, the carbon status of a subset of fruits decreases, altering hormone levels and signaling. Reduced sugar signaling and metabolism, together with the decline and increase in hormones that promote and inhibit growth, respectively, induces fruit growth arrest. At the time of growth arrest, seed coat senescence occurs followed by the activation of the abscission zone. The final step in the abscission process occurs when the fruit separates and drops from the tree.

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Hort Innovation
Strategic levy investment
AVOCADO FUND

Developing a physiological framework for managing summer fruit abscission in avocado

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Avocado is a semi-domesticated tree crop with a theoretical yield potential of 32 t/ha1. Due to the irregular bearing behaviour of avocado, which is primarily driven by low levels of fruit set and high rates of immature fruit abscission, as well as biennial bearing, average annual production levels across Australia and the world are well below the theoretical yield potential. Moreover, establishing overseas export markets to manage current and future oversupply issues will require effective mitigation tools that limit irregular bearing. Methods to sufficiently improve avocado fruit set and reduce immature fruit abscission are currently lacking due to a limited understanding of the physiological and developmental basis of immature fruit abscission and fruit set. Therefore, to effectively mitigate irregular bearing, an in-depth understanding of immature fruit abscission and fruit set is required, which can be leveraged to identify potential intervention strategies in order to develop new methods to maximize yield and reduce seasonal variation.

Summer Fruit Abscission is a major target for management intervention

Fruit abscission is the major factor that negatively impacts production in coastal regions of south western, WA, Tristate area (Riverland) and central QLD. In these regions, 20% or more of the crop will drop during the summer, which is further increased by hot and dry conditions2,3. Given that trees have invested up to 40% dry matter into fruits that drop during the summer2, mitigating summer fruit abscission would have a considerable outcome for mitigating irregular bearing. Research derived from AV16005 supports the hypothesis that seasonal and temporal fluctuations in the carbohydrate status of a tree combined with dominance interactions between fruits and vegetative flushes, as well as among fruits, limits carbohydrate availability to a subset of fruits, resulting in abscission. Further, crop load, environmental conditions and management inputs, which influence tree carbohydrate status and dominance interactions, also impact fruit abscission.

Fruit growth arrest is the primary developmental event of summer fruit abscission

In Hass, experimental studies showed that abscised fruits were statistically smaller than retained fruits, during the summer fruit abscission event in California4. Further, in Fuerte, smaller sized fruits have a higher tendency to abscise compared to larger sized fruits, after the first wave of fruit drop5. Based on these studies, we investigated the possibility that fruits under growth arrest prior to abscission. To test this hypothesis, the diameter of developing fruits was measured at regular time intervals using hand-held digital callipers, as well as fruit dendrometers. Subsequently, these measurements were used to calculate the average growth rates of persisting and abscising fruits. At the start of the trial, the average growth rate of persisting fruits was approximately 0.6 mm/day, which declined to 0.36 mm/day at the end of the trial (Figure 1A). Growth rates for individual fruits that abscised were calculated and collated to better understand if a change in growth rate precedes abscission. Results from this analysis showed that fruits underwent growth arrest prior to abscission during the summer growing period (Figure 1B). Therefore, to mitigate summer fruit abscission, practical strategies must be aimed at managing fruit growth arrest, rather than abscission.

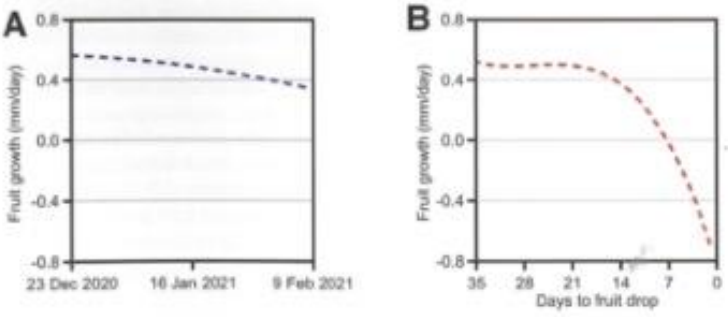

Figure 1.

Physiological drivers of fruit growth arrest

The AV16005 project developed hypotheses aimed at predicting the physiological drivers of summer fruit growth arrest and abscission. The first two of the hypotheses tested had the potential to have an immediate impact for the development of a practical application(s), while the remaining hypotheses require additional basic research due to the lack of understanding of immature fruit abscission.

A change in water potential is not the primary driver of summer fruit growth arrest and abscission

As water stress conditions reduce yields, irrigation strategies were proposed as a means to increase the reproductive potential of a tree 6. Therefore, we addressed whether a change in fruit water potential is a driver of summer fruit growth arrest and abscission. If so, new irrigation management strategies could be developed to manage summer fruit growth arrest and abscission. However, our results showed that summer fruit arrest is not associated with changes in fruit water potential. Therefore, modified irrigation regimes in well managed orchards don't appear to be a feasible solution to manage summer fruit abscission (Figure 2).

A deficiency in a mineral element(s) is not a key driver of summer fruit growth arrest and abscission

Mineral elements acquired from the soil are essential for plant growth and development, as well as yield7,8. A previous study examined whether the nutrient status of the tree influences avocado fruit abscission9. Tree nutrient status was determined by measuring nitrogen and other essential mineral elements in leaves. Results from this study indicate fruit abscission was not associated with changes in leaf nutrient status9. To further determine whether nutrient deficiency may be involved in summer fruit abscission, the levels of key mineral elements were measured in arresting and control fruits with a normal growth rate. Results indicate that mineral element deficiency is not a physiological driver of summer fruit abscission in well-managed orchards (Figure 2). Therefore, new plant nutrition strategies do not appear to be a feasible solution to mitigate summer fruit abscission in well managed orchards.

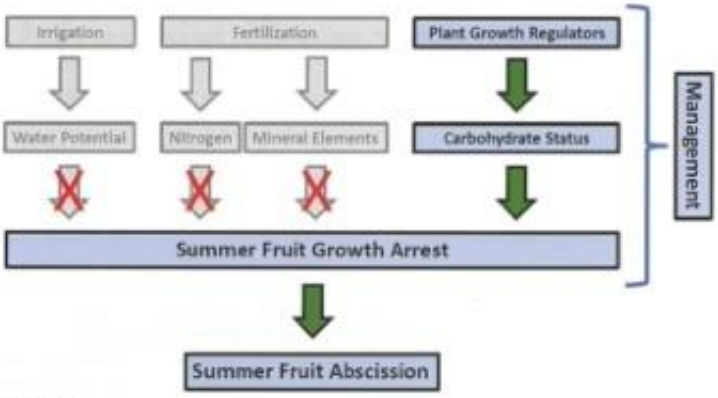

Figure 2.

A change in the carbohydrate status of the fruit is associated with summer growth arrest and abscission

Experimental studies indicate that fruit abscission is mediated by reduced carbohydrate availability, signaling and availability in citrus10,11 and apple12. Given that carbohydrates are key substances required for growth, we hypothesized that reduced carbohydrate status of the fruit would be associated with fruit growth arrest during summer fruit abscission. To test this hypothesis, sugar metabolites and starch were measured in the seed and pericarp of normal growing and arresting fruits during the summer. In addition, gene expression studies were also performed to further examine the impact of growth arrest on carbohydrate metabolism. Results from our studies indicate that carbohydrate supply and metabolism is severely reduced in the seed. In addition, carbohydrate metabolism is also altered in the pericarp. Taken together, a decrease in the carbohydrate status of the fruit appears to be the primary driver of summer fruit growth arrest and abscission (Figure 2). Given the interplay between hormones and carbohydrate signaling and metabolism, managing fruit growth arrest will require an understanding of the hormonal control of fruit growth arrest, which can be leveraged to develop a plant growth regulator (PGR) application(s) to limit summer fruit growth cessation and abscission (Figure 2).

A change in the hormone status of the seed is associated with summer growth arrest and abscission

To understand the hormonal changes associated with fruit growth arrest, key hormone profiles were determined for arresting and persisting fruits (note: the hormone profiles in arresting fruits shown were derived from a late stage of fruit growth arrest; Figure 3). Auxin is a fundamental growth promoting hormone that acts to coordinate the growth of seed and fruit tissues during development13-15. In persisting fruits, preliminary results indicate that auxin levels were high in seeds relative to the pericarp. However, during growth arrest, the levels of auxin in the seed and pericarp was significantly reduced (Figure 3). Cytokinin is another hormone implicated in fruit growth and development13. Preliminary results suggest that the levels of cytokinin were similar between persisting and arresting fruits. However, gene expression studies indicated that growth arrest involved changes in cytokinin response rather than mechanisms that control the levels of this hormone (Figure 3). Currently, we are evaluating hormone profiles for active gibberellins and brassinosteroids to determine if these hormones change in response to summer fruit growth arrest.

In apple, experimental studies indicate that abscisic acid and ethylene mediate immature fruit abscission12,16-18. Consistent with studies in apple, preliminary results indicate that abscisic acid levels primarily increased in the pericarp during avocado fruit growth arrest (Figure 3). In addition, gene expression studies suggest that ethylene plays a role in avocado summer fruit growth arrest and abscission (Figure 3), as the expression of ethylene biosynthesis and signaling genes were induced in the seed and pericarp during growth arrest. We are currently developing a method to quantify aminocyclopropane-1-carboxylic acid (ACC), the metabolite converted to ethylene by ACC oxidase19, in order to validate a role for ethylene in summer fruit growth arrest.

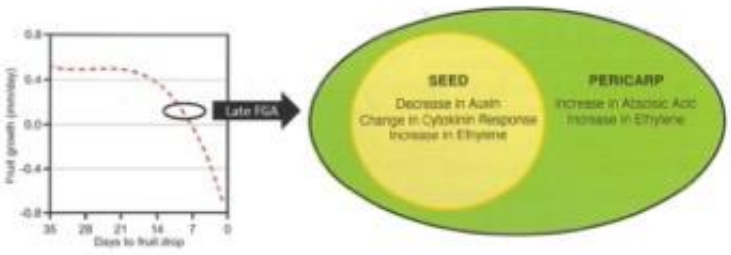

Figure 3.

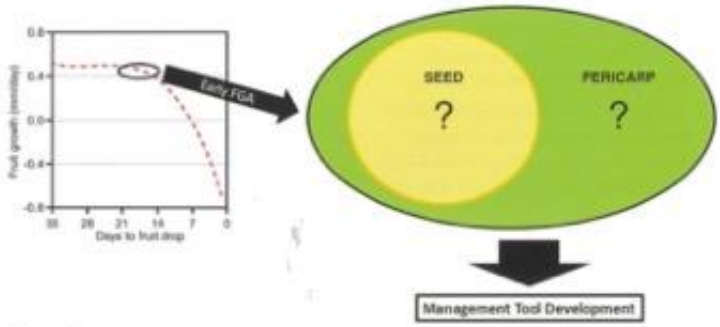

Figure 4.

Summary

Managing irregular bearing is a major challenge for the Australian Avocado Industry as this impacts supply and the ability to establish overseas export markets required to increase profitability in years when there is an oversupply of avocados. Research from AV16005 shows that mitigation of summer fruit abscission requires the ability to manage fruit growth arrest in order to reduce fruit drop. As summer fruit growth arrest is not associated with a reduction in fruit water potential or mineral element deficiency, modification of irrigation and fertilization practices will likely not provide a feasible pathway to manage summer fruit drop. Our results showing that the carbohydrate potential of arresting fruits is reduced indicate that growth arrest must be managed via methods to alter carbohydrate supply and metabolism, which is regulated in part via plant hormones. Preliminary hormone quantification studies indicate that changes in the levels of auxin, abscisic acid and ethylene, as well as cytokinin response, are associated with the later stage of growth arrest. Current research is focused on validating the preliminary studies. To build a pathway for impact, the next step of the research project is to identify hormonal changes associated with early growth arrest (Figure 4). This step is absolutely key for developing plant growth regulator application(s) to manipulate hormonal changes in order to allow fruits with a lower growth and carbohydrate potential to persist on the tree until maturity is reached.

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Appendix C: Proceedings Abstract – IX World Avocado Congress

Toward an understanding of avocado fruit abscission for increasing production

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Abstract
Average avocado yields are far below the estimated potential. Low yields are attributed to yield-associated traits that include extensive vegetative shoot growth, excessive flowering, low fruit set, high fruit abscission and a propensity for biennial bearing. Environmental conditions and management inputs influence the impact of these traits on yield. Climate models predict that the frequency of extreme climatic events will increase in the future, and as a result will impact yields. Therefore, breeding elite varieties and developing new management tools are essential for increasing production. The development of new management tools depends on the understanding of the physiology and regulation of yield-associated traits. Fruit abscission is a yield-associated trait that greatly contributes to irregular production patterns in avocado trees and orchards. Moreover, fruit abscission limits production in a diverse set of environments, including subtropical, Mediterranean, and cool climates. Therefore, research and development outcomes aimed at reducing fruit abscission will have a major impact on avocado industries throughout the world.

Introduction
The potential for avocado annual fruit production is estimated at 32 t/ha (Wolstenholme, 1987). However, actual average annual yields are approximately 10 t/ha, substantially lower than the estimated potential. Poor production levels are attributed to the semi-domesticated nature of avocado (Gama-Campillo and Gomez-Pompa, 1992), which includes several counter-productive traits such as vigorous vegetative growth, excessive flowering, biennial bearing, low fruit set and a high rate of fruit abscission (Goldschmidt, 2013; Lahav and Lavi, 2009). Moreover, the effects of climate change will further enhance these traits resulting in even more variations in annual yields. The additive effects of these yield-associated traits together with climate change are a major challenge for avocado production throughout the world.

Improving orchard performance through the development of new varieties and innovative management tools
Yield is a function of the interaction between the genetics, environment and management inputs (Hatfield and Walthall, 2015). In terms of avocado genetics, the dominant variety used in production throughout the world is ‘Hass’, which is a chance seedling identified in the mid 1920’s (Chen et al., 2009). While ‘Hass’ produces good quality fruit, it is not well adapted to many of the production areas around the world (Wolstenholme, 2013). Climate models predict that the environment will have a negative impact on production in many parts of the world (Howden et al., 2005); therefore, there is pressing need to develop new varieties for increasing orchard performance in the future. However, due to the long juvenile phase of avocado, breeding and evaluating new varieties takes a considerable amount of time.

To increase the yields of Hass and other varieties used in current production, new management tools must be developed to reduce the impact of yield-associated traits. In order to achieve this outcome, it is essential to develop an integrative knowledge base platform derived from a basic understanding of the physiological drivers that mediate these yield-associated traits. In Australia, avocados are produced in a wide range of environments including the subtropical regions of northern Queensland, to the hot and dry regions along the Murray River and the cool Mediterranean climate in the Pemberton region of Western Australia (www.avocado.org.au). While each region has its specific problems that affect production, such as the six-spotted mite in the southwest region of Western Australia, fruit abscission is a universal factor that limits production across all environments in Australia (Newett, 2013). Therefore, research and development outcomes aimed at reducing fruit abscission will have a major impact on avocado industries throughout the world including Colombia, Kenya and Tanzania.

Fruit abscission in avocado

Fruit abscission occurs throughout the growing season with a peak period of fruit drop occurring within the first five weeks after fruit set (Garner and Lovatt, 2016; Sedgley, 1980). During this high period of fruit drop, unfertilized fruits abscise within the first two weeks, while fertilized fruits ranging from 6-12 mm in size abscise for the remaining three-weeks (Sedgley, 1980). Growers estimate that 30-50% of the fertilized fruitlets that set abscise within this five-week period. The remaining fruit persisting on the tree have two developmental fates. A small subset of these fruits will continue to develop over the course of the growing season until the reach maturity for havest. The second set of fruits will develop and at some point, undergo fruit abscission. This later phase of fruit abscission can progress in a continuous manner or in a large wave based on the tree physiology, which is heavily influenced by the orchard environment and stress. The ability to identify key intervention time points throughout the growing season in order to minimize fruit abscission has been extremely difficult, as the factors that mediate fruit abscission are unknown.

Interaction between young fruitlets and the growing spring flush

The growth potential of avocado fruit increases over the course of the growing season, which is likely attributed to the development of the seed (Cowan et al., 2001). During the early stages of development, growth of the young fruitlets overlaps with the extension of the vegetative spring flush. Therefore, it has been hypothesized that the abscission of young fruitlets at early stages results from resource competition with the growing spring flush (Figure 1; Whiley et al., 2013). In support of this hypothesis, functionally determinate inflorescences are up to three times more productive than indeterminate inflorescences (Salazar-Garcia et al., 1998). In addition, indeterminate inflorescences retain more fruit when the terminal vegetative bud is removed (Lahav and Lavi, 2009).

The developing stems and leaves create a high growth potential for the spring flush. The high rate of cell wall biosynthesis in the growing stems results in a significant demand for carbohydrates for maintaining structural integrity of the vegetative shoots (Kebrom, 2017).

Growth retardants, such as paclobutrazole and uniconazole, are used in avocado industries to reduce the growth potential of shoots, as these plant growth regulators inhibit gibberellin biosynthesis required for stem elongation (Whiley et al., 2013). It has been hypothesized that reducing the growth potential of the vegetative spring flush will increase carbohydrate and nutrient availability required for fruit development, thereby reducing fruit abscission. In support of this hypothesis, experimental trials showed that applications of paclobutrazol at flowering reduced early fruit abscission (Adato, 1990; Kohne and Kremer-Kohne, 1987; Wolstenholme et al., 1990), increased fruit size (Whiley et al., 1992), as well as augmented yield (Adato, 1990; Kohne and Kremer-Kohne, 1987). However, other reports indicate that applications of paclobutrazol had little or no effect on yield (Penter et al., 2000; Stassen et al., 1999; Symons and Wolstenholme, 1990). In one study, applications of paclobutrazol reduced early fruit abscission; however, there was no increase in yield due to a heavy summer fruit drop event (Wolstenholme et al., 1990). The authors speculated that the paclobutrazol induced reduction in leaf expansion in spring flush limited the photosynthetic capability of the leaves. Therefore, the smaller leaf area was not able to supply the increased crop with photosynthates, which resulted in fruit abscission during the summer. Taken together, paclobutrazol is extremely effective at reducing the growth potential of the spring flush; however, variation in the effect on yield demonstrates the complexity of the mechanism(s) that regulate resource (carbohydrates) distribution during the spring and early summer.

The high volume of flowering and low rate of fruit set presents a major challenge for studying the physiological mechanism(s) that mediate the early fruit abscission (reviewed by Salazar-García et al. 2013). Creating a condition that increases fruit set and decreases fruit abscission would allow researchers to directly compare developmental profiles between developing (retained) and abscising fruitlets. Creating these conditions for profiling developing and abscising fruits is crucial, as studies in model plant systems demonstrate that early fruit development is marked by extensive changes in fruit physiology (Kang et al., 2013; Kumar et al., 2014). The inability to profile developing and abscising fruits at the same developmental stage will introduce artifacts that could obscure the ability to identify the key factors that mediate abscission.

The abscission of fruitlets during the summer

While a wave of fruit abscission is often mediated by stress-related events, little is known about the mechanism of the summer fruit drop that occurs in a continuous manner or in a wave independent of stress. It has been speculated that the summer fruit drop is mediated in part by tree resource levels, such as carbohydrates, which are essential for growth. If resource availability is reduced, the crop load is adjusted to an appropriate level that can be supported (Goldschmidt, 2013; Sawicki et al., 2015). Therefore, understanding how tree crop load is adjusted in response to resource availability and the physiological mechanism(s) that control fruit abscission may provide the knowledge necessary to develop advanced management tools aimed at reducing fruit abscission. Moreover, management tools aimed at reducing the summer fruit drop will likely be effective for reducing the early fruit drop event.

Role of seed coat in fruit abscission

Seed development is critical for fruit development and retention (Garner and Lovatt, 2016; Sedgley, 1980). The seed is composed of the embryo, endosperm and seed coat. While the embryo and endosperm are products of double fertilization, the seed coat is derived from the maternal integuments. Studies in model plant system show that the seed coat functions to provide the embryo with photosynthates and nutrients required for growth (Costa et al., 2012). Moreover, the seed coat also produces hormones critical for regulating embryo development (Bower and Cutting 1988; Robert et al. 2018). During the summer fruit drop, avocado seed coat senescence is associated with abscising fruits (Blumenfeld and Gazit, 1974; Garner and Lovatt, 2016; Figure 2). Therefore, the maternally derived seed coat appears to be a functional determinate that controls the fruit fate, to develop versus to abscise.

Model of fruit abscission

We propose a model that describes fruit abscission in avocado (Figure 3). According to this model, the physiology of the tree is a key determinate of fruit abscission. Tree carbohydrate availability and stress influence fruit abscission. Furthermore, mechanisms that control resource distribution target a subset of fruit to undergo abscission. While the mechanisms are not understood, competition for resources between fruits and with shoots can induce fruit drop. Furthermore, seed coat senescence is a key tissue that regulates fruit abscission. It should be pointed out inhibition of the summer/fall flush by high crop load likely involve a similar resource distribution mechanism. Therefore, understanding the mechanisms that mediate fruit abscission may also be applied to better understand biennial bearing.

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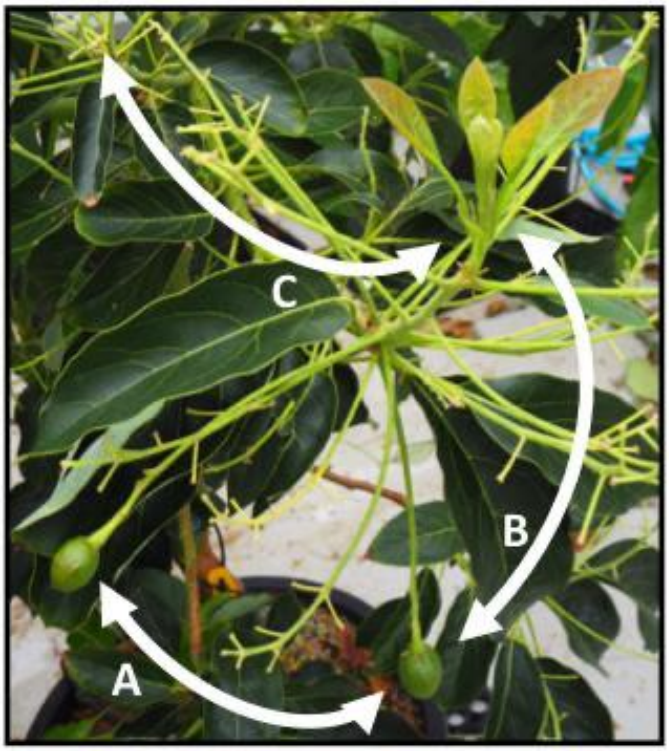

Figure 1: An illustration displaying the competition between the spring and developing fruitlets. The high rate of fruit abscission within the first five-weeks is mediated by three competition events: (Arrow A) fruitlet to fruitlet, (Arrow B) spring flush to fruitlet, and (Arrow C) branch – branch. All of these competition events results in the diversion of carbohydrates released from reserves away from developing fruitlets; thereby causing fruit abscission.

Figure 2: Abscising fruits display seed coat senescence. (A and B) Normal seed coat development is associated with developing fruits firmly attached to the tree. (C and D) Abscising fruits display varying levels of seed coat senescence. Note, the embryo has been removed from the fruits to visualize the seed coat with is marked with an asterisk (*).

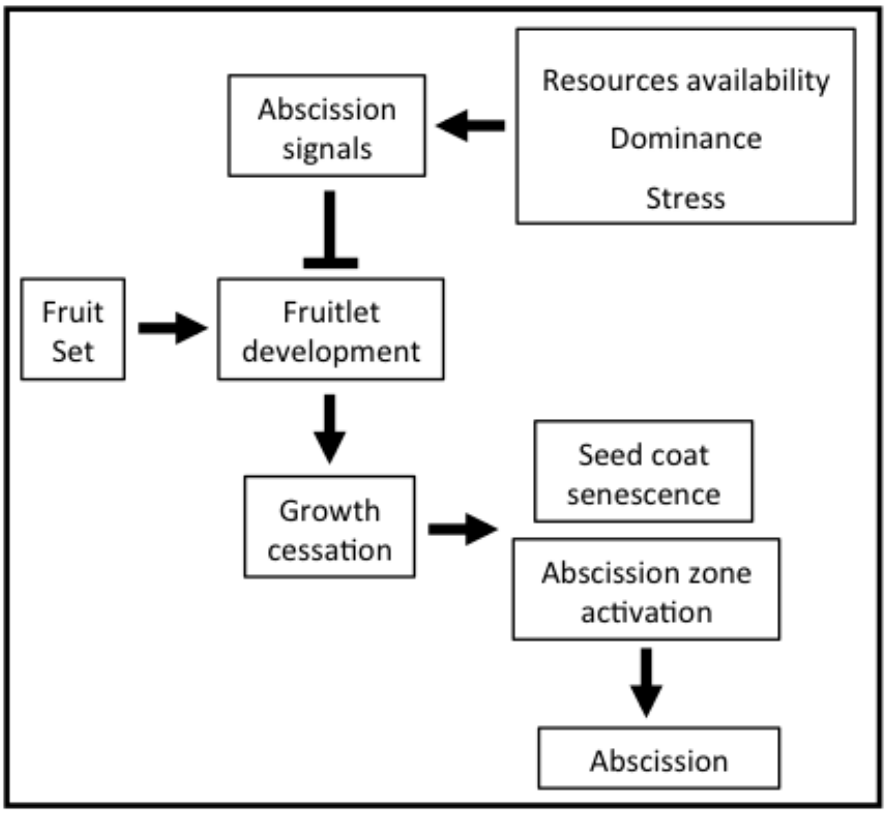

Figure 3: A model of the sequence of events for fruit abscission abscission. Abscission signals are produced in resonse to resource availability, dominance and stress, which target a subset of developing fruitlets in the tree. In response to the abscission signals, fruitlets undergo growth cessation. The senescence of the seed coat and activation of the abscission zone result in fruit drop.

Appendix D: Extension Activities

AGENDA FOR SOUTH AUSTRALIAN AVOCADO STUDY GROUP MEETING
Wednesday 26 July 2017
Starting at the Costa Exchange Training Room, 260 Chowilla St, Renmark then later at Nick Hobbs' Chinoola orchard, Chino St, Renmark

10:00 am	**MORNING TEA**
10:30 am	Welcome & introductions – Simon Newett, Department of Agriculture and Fisheries, Nambour, Queensland
10:35 am	Industry update – information from AAL – Kym Thiel, Director, AAL
10:45 am	Managing frosts on avocado orchards – Lisa Martin, Ripe Horticulture
11:15 am	10 mins discussion on frost management - all
11.25 am	Outline of the new irregular bearing project – Dr Harley Smith, CSIRO, Adelaide
12:00 pm	Managing heat waves on avocado orchards – Lisa Martin, Ripe Horticulture
12:30 am	10 mins discussion on management during heat waves - all
12:40 pm	**LUNCH**
1:25 pm	New video "How to plant an avocado tree"
1:30 pm	Management of Phytophthora root rot - Simon Newett, Queensland Department of Agriculture & Fisheries
2:00 pm	Complete evaluation forms and discuss the next avocado extension project
2:15 pm	Background to the orchard walk – Nick Hobbs
2:25 pm	**FARM WALK – focus on management of heat and cold**
3:30 pm	Depart

Morning tea and lunch will be provided but for catering purposes please **RSVP** by **9 am Monday 24 July** with names attending to simon.newett@daf.qld.gov.au or send a text with names attending to **0400 565 784** (or leave a message). Please let us know if you have special dietary requirements.

We look forward to seeing you there.

Horticulture Innovation Australia

Avocados Australia

Queensland Government

PTO for directions

AGENDA FOR VICTORIAN AVOCADO STUDY GROUP MEETING
Thursday 27 July 2017
At Glenn Goldup's orchard, Lot 7, Sculthorpe Rd, Nangiloc, Victoria

10:00 am	**MORNING TEA**
10:30 am	Welcome & introductions – Simon Newett, Department of Agriculture and Fisheries, Nambour, Queensland
10:35 am	Industry update – information from AAL delivered by …….
10:45 am	Managing frosts on avocado orchards – Lisa Martin, Ripe Horticulture
11:15 am	10 mins discussion on frost management - all
11.25 am	Outline of the new irregular bearing project – Dr Harley Smith, CSIRO, Adelaide
12:00 pm	Managing heat waves on avocado orchards – Lisa Martin, Ripe Horticulture
12:30 am	10 mins discussion on management during heat waves - all
12:40 pm	**LUNCH**
1:25 pm	New video "How to plant an avocado tree"
1:30 pm	Management of Phytophthora root rot - Simon Newett, Queensland Department of Agriculture & Fisheries
2:00 pm	Complete evaluation forms and discuss the next avocado extension project
2:15 pm	Background to the orchard walk – Glenn Goldup
2:25 pm	**FARM WALK – focus on management of heat and cold**
3:30 pm	Depart

Morning tea and lunch will be provided but for catering purposes please **RSVP** by **9 am Monday 24 July** with names attending to simon.newett@daf.qld.gov.au or send a text with names attending to **0400 565 784** (or leave a message). Please let us know if you have special dietary requirements.

We look forward to seeing you there.

Horticulture Innovation Australia

Avocados Australia

Queensland Government

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Avocado Regional Forum

Pemberton Regional Forum

Tuesday June 4n

Presentatio s: **Pemberton Sports Club, 1 Club Road, Pemberton**

Orchard walk: Shane Bendotti - 243 Golf Links Road, Pemberton

Agenda

Time	Item	Presenter
8:45am - 9:15am	***Registration***	***Tea & Coffee Available***
9:15am - 10:00am	Industry Update	**John Tyas** - Avocados Australia (AAL)
10:00am - 10:15am	New fruit management and handling project - AV18000	**Declan McCauley** - Department of Primary Industries and Regional Development
10:15am - 10:45am	***Morning Tea***	
10:45am - 11:30am	***Canopy management fundamentals and the evolution towards higher density plantings***	**Dudley Mitchell** - Nuffield Scholar
11:30am - 12:15pm	Canopy management - Grower experiences	**Panel discussion**
12:15pm - 12:30pm	Scoping the need for a canopy management project	**Harley Smith** - CSIRO
12:30pm - 1:15pm	***Lunch***	
1:30pm - 3:00pm	Orchard Walk	Shane Bendotti
End		

Queensland Government | Hort Innovation Strategic levy investment | AVOCADO FUND | Avocados Australia | Department of Primary Industries and Regional Development, Government of Western Australia

This event is part of the strategic levy investment project, Avocado industry development and extension *(AV17005).*

This project has been funded by Hort Innovation, using the Hort Innovation avocado research and development levy, co-investment from the Queensland Department of Agriculture and Fisheries, and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Avocado Regional Forum

TriState Regional Forum

Wednesday August 14 2019

Presentations: **Renmark Club,** 160 Murray Avenue, Renmark

Orchard walk: **Nick Hobbs - Chinoola Orchards,** 160 Tarcoola Street, Renmark

Agenda

Time	Item	Presenter
8:45am - 9:15am	***Registration***	***Tea & Coffee Available***
9:15am - 10:00am	Industry Update	**John Tyas** - Avocados Australia (AAL)
10:00am - 10:15am	New fruit management and handling project - AV18000	**Bridie Carr** - Department of Agriculture & Fisheries
10:15am - 10:45am	***Morning Tea***	
10:45am - 11:30am	***Using Plant Growth Regulators (PGRs) in Avocados***	**Harley Smith** - CSIRO
11:30am - 11:45am	Maximising yield and reducing seasonal variation - AV16005 (project update)	**TBA** - CSIRO
11:45am - 12:30pm	Managing canopies through PGRs and nitrogen	**Grower Experiences**
12:30pm - 12:40pm	Q&A session	**Panel Discussion**
12:40pm - 1:30pm	***Lunch***	
1:05pm - 1:30pm	***General Meeting***	**South Australian Avocado Growers Association (SAAGA)**
1:30pm - 3:00pm	Orchard Walk	**Nick Hobbs & Sebastian Recabarren (IMTRADE Australia)** - PGR trial
	End	*PGR - Plant Growth Regulator*

Queensland Government

Hort Innovation Strategic levy investment

AVOCADO FUND

Avocados Australia

This event is part of the strategic levy investment project, Avocado industry development and extension *(AV17005).*

Avocado Regional Forum

Western Australia - Manjimup

Thursday 12 March 2020

Presentations: Manjimup Wellness Centre, 1a Edwards St, Manjimup WA 6258.

The Centre is down the road from the Police station and near Timber Park.

Orchard walk: Vic Grozotis, 'Applewood', Lot 99 Ipsen St, Manjimup

Time	Item	Presenter
8:00am - 8:30am	***Registration***	***Tea & Coffee Available***
8:30am - 9:15am	Industry Update	**John Tyas** - Avocados Australia (AAL)
9:15am - 9:30am	California Avocado Tour & WAC highlights	**Liz Singh** - Avocados Australia (AAL)
9:30am - 10:00am	***Morning Tea***	
10:00am - 10:10am	Overview of new avocado IPM project - AV19001	**Alison Matthews** - DPIRD
10:10am - 10:50am	***Avocado fruit set - limiting factors and knowledge gaps***	**Harley Smith** - CSIRO
10:50am - 11:15am	Positioning for better management of Avocado fruit abscission - AV16005 project overview	**Amnon Haberman** - CSIRO
11:15am - 11:45am	Grower experiences - Pollination, fruit set and fruit retention	
11:45am - 12:00pm	Supply chain project results - AV18000	**Declan McCauley** - DPIRD
12:00pm - 1:00pm	***Lunch***	
1:00pm - 1:30pm	Phosphonates, field trials and flower blight - AV16007 project update	**Liz Dann** - Queensland Alliance for Agriculture and Food Innovation (QAAFI)
1:30pm - 1:45pm	Orchard Overview and Day Review	
2:00pm - 4:00pm	Orchard Walk	**Vic Grozotis -** Applewood
End		

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Avocado Regional Forum

TriState - Mildura

Wednesday, May 5 2021

Presentations: The Setts, 110-114 Eighth St, Mildura, VIC

Orchard walk: Gill Farms, Kulkyne Way, Iraak, VIC

Time	Item	Presenter
8:30am - 9:00am	***Registration***	
9:00am - 9:05am	Welcome	**Kym Thiel** - Avocados Australia (AAL) TriState Director
9:05am - 9:35am	Industry Update	**John Tyas** - Avocados Australia (AAL)
9:35am - 9:50am	Fruit quality in the supply chain	**John Agnew** - Department of Agriculture & Fisheries (DAF)
9:50am - 10:05am	Fruit quality at retail	**Adam Goldwater** - Applied Horticultural Research (AHR)
10:05am - 10:45am	***Morning Tea***	
10:45am - 11:15am	***Improving avocado nutrition***	***Lisa Fyffe*** - *Ripe Hortic lture*
11:15am - 11:40am	Connection between nutritio and tree carbohydrates	**Harley Smith** - CSIRO
11:40am - 11:50am	Nutritio and fruit robustness	**Simon Newett** - Department of Agriculture & Fisheries (DAF)
11:50am - 12:20pm	Avocado nutritio nfertilisers, fertigatio , salt	**Grower Experiences**
12:20pm - 1:20pm	***Lunch***	
1:20pm - 1:40pm	Orchard Overview & Forum Review	Hardeep Singh - Gill Farms
2:30pm - 3:30pm	Orchard Walk	
End		

Queensland Government

Hort Innovation Strategic levy investment

AVOCADO FUND

Avocados Australia

This event is part of the strategic levy investment project, Avocado industry development and extension *(AV17005).*

Research Update

Thursday August 12

Presentations: Conducted via **ZOOM**

Bookings - https://www.trybooking.com/BTBPG

Note: Starting times will be as follows: QLD, NSW, VIC - 1pm, SA - 12:30pm, WA - 11am.

Agenda

Time	Item	Presenter
1:00pm - 1:02pm	Introduction	**Simon Newett** - Department of Agriculture & Fisheries (DAF)
1:02pm - 1:16pm	Estimating carbohydrate levels in avocado using non-destructive methods for commercial development (AV19006)	**Harley Smith** - CSIRO
1:16pm - 1:30pm	Managing flies for crop pollination (PH16002)	**David Cook** - Department of Primary Industries and Regional Development (DPIRD)
1:30pm - 1:44pm	Is fruit quality meeting consumer expectations? (AV18000 and AV19003)	**Noel Ainsworth** - DAF and **Adam Goldwater** - Applied Horticnltural Research (AHR)
1:44pm - 1:58pm	Avocado Irrigation Summit Outcomes (AV17005)	**Liz Singh** - Avocados Australia
1:58pm - 2:00pm	Conclusion	**Simon Newett** - Department of Agriculture & Fisheries (DAF)
End		

Queensland Government | Hort Innovation Strategic levy investment | AVOCADO FUND | Avocados Australia

This event is part of the strategic levy investment project, Avocado industry development and extension *(AV17005).*

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Avocado Regional Forum

North Queensland - Atherton

Wednesday, August 25 2021

Presentations: Atherton Hotel "The Stump", 90 Main Street QLD 4883

Orchard walk: Coming soon!

Time	Item	Presenter
8:30am - 9:00am	***Registration***	
9:00am - 9:10am	Welcome	**Jim Kochi** - Avocados Australia Chair
9:10am - 9:40am	Industry Update	**John Tyas** - Avocados Australia (AAL)
9:40am - 9:55am	Managing Avocado Pests: Current Threats & Future Directio s (AV19001)	**Ian Newton** - Department of Agriculture & Fisheries (DAF)
9:55am - 10:10am	New Phenological Cycles	**Ebony Faichney** - Department of Agriculture & Fisheries (DAF)
10:10am - 10:50am	***Morning Tea***	
10:50am - 11:20am	***Avocado fruit set - limiting factors and knowledge gaps in avocado***	***Harley Smith*** - *CSIRO*
11:20am - 11:50am	Positioning for better management of avocado fruit abscission	**Amnon Haberman** - CSIRO
11:50am - 12:10pm	Plant Growth Regulators	**Grower Experiences**
12:10pm - 12:20pm	Fruit quality in the supply chain	**Noel Ainsworth** - Department of Agriculture & Fisheries (DAF)
12:20pm - 1:20pm	***Lunch***	
1:20pm - 1:40pm	Orchard Overview & Forum Review	
1:40pm - 3:30pm	Orchard Walk	
End		

Queensland Government · Hort Innovation Strategic levy investment · AVOCADO FUND · Avocados Australia

This event is part of the strategic levy investment project, Avocado industry development and extension *(AV17005).*

This project has been funded by Hort Innovation, using the Hort Innovation avocado research and development levy, co-investment from the Queensland Department of Agriculture and Fisheries, and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Avocado Regional Forum

South Queensland - Crows Nest

Wednesday, February 23 2022

Presentations: Crows Nest RSL and Community Centre – 28-30 Williams Street, Crows Nest 4355
Orchard walk: Bill and Kathy Kereczko – 46 Blanck Rd, Ravensbourne 4352

Time	Item	Presenter
8:30am - 9:00am	***Registration***	
9:00am - 9:05am	Welcome	**Daryl Boardman** - Avocados Australia (AAL)
9:05am - 9:35am	Industry Update	**John Tyas** - Avocados Australia (AAL)
9:35am - 9:50am	Managing Avocado Pests: Current Threats & Future Directions (AV19001)	**Ebony Faichney / Ian Newton** - Dept of Agriculture & Fisheries (DAF
9:50am - 10:05am	Supply chain feedback (AS18000) update	**Noel Ainsworth** - Dept of Agriculture & Fisheries (DAF)
10:05am - 10:45am	***Morning Tea***	
10:45am - 12:15am	**The Challenges of Avocado Flowering, Pollination and Fruit Set**	***Harley Smith*** **-** CSIRO *(30min)* ***Amnon Haberman*** **-** CSIRO *(30min)* (DAF) *(30min)* ***Q & A session*** **-** Chaired by **Bridie Carr** - Dept of Agriculture & Fisheries (DAF)
12:15pm - 1:15pm	***Lunch***	
1:15pm - 1:30pm	Forum Review & Orchard Overview	
1:30pm - 2:00pm	Travel to orchard	
2:00pm - 3:00pm	Orchard Walk	Bill and Kathy Kereczko 46 Blanck Rd, Ravensbourne 4352
End		

This event is part of the strategic levy investment project, Avocado industry development and extension *(AV17005).*

This project has been funded by Hort Innovation, using the Hort Innovation avocado research and development levy, co-investment from the Queensland Department of Agriculture and Fisheries, and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Appendix E: Presentations at International Conferences

Avocado Brainstorming 2018

Towards a sustainable future

30 March 2018

Dear colleagues,

We have confirmed participants that will attend Brainstorming. All of you have been identified as Chairs/Co-Chairs of different sessions as outlined in the table below. There will be eight "brainstorming" workshop sessions, each 2 hours. As with previous Brainstorming meetings we will also be having 2 poster sessions on Tuesday and Thursday afternoon to promote networking among the participants.

You will recall that the objective of Avocado Brainstorming is to review the status of our scientific knowledge through in-depth discussions and networking to formulate an integrated future vision for the worldwide industry. The following includes some information and suggestions on how you may wish to organize an oral session to ensure it helps meet these objectives.

1. We suggest that each session includes an informal keynote presentation by each of the two Co-Chairs for that session to provide background to each session. We ask that you make contact with your Co-Chair before the meeting so that you can coordinate the scope of your presentations to avoid too much unnecessary overlap.
2. We ask that you limit these presentations to 20 to 30 minutes each so that there will be sufficient time (say 30 to 45 minutes) for interactive discussions around the session topics. In this context we hope you will be able to lead discussion on the topic and to encourage the participating scientists to share their latest research discoveries.
3. Please leave at least 30 minutes of the 2 hours for discussion. How you place the discussion within the 2 hours we will leave to you.
4. We have also attached a list of meeting participants which may help you identify people with relevant experience who can provide comment during the session. This may help you encourage contributions from participants who might be nervous about speaking up in this type of workshop session.
5. At the end of your session we ask that you consider providing a brief "wrap up" to highlight future directions for research and development relevant to the session discussions.

For the 2 poster sessions we ask the Chairs to consider the following:

1. We will make a call for posters in early April that will include the final size of the poster.
2. Review all abstracts submitted (will require a 10 May deadline) and organize the posters into groupings.
3. Consider organizing 2 – 3 minute presentations for each poster and developing the outline for the schedule for each poster session. The poster sessions will be held in conjunction with the afternoon social. The Organizing committee will work with you on this.

Finally, one of your important duties as a session chair is to provide us with a session summary submitted no later than July 1, 2018. Several of our sponsors are requiring a summary of the outcomes of the meeting to justify the sponsorship. We have attached the final report from the 2015 meeting to provide a guideline for this report.

Best wishes,

Mary Lu Arpaia
mlarpaia@ucanr.edu

Zelda Van Rooyen
zelda@westfaliafruit.co.za

Respond to: mlarpaia@ucanr.edu

Organizing Committee:

M. L. Arpaia, A. Barrientos-Priego, I. Hormaza, F. Mena, R. Ploetz, G. Thorp, Z. Van Rooyen

Avocado Brainstorming 2018

Session	Session Title	Session Co-Chairs	Potential topics (speakers)
Day 1	Providing for the consumer: Health, Safety, Flavor	Nikki Ford Lise Korsten David Obenland	
Day 1	New Technology to improve avocado production	Nicki Taylor Mark Buhl	Howard Blight – education in the cloud; www.agricolleges.com Zander Ernst – trellising and intense canopy management of avocado Cyropreservation/Tissue Culture - Mitter
Day 2	Challenges to Productivity: Diseases	Randy Ploetz Kerry Everett	Root diseases Fruit diseases Foliar diseases
Day 2	Challenges to Productivity – how the tree regulates return bloom and crop load	Harley Smith Rodrigo Iturrieta Vered Irihimovitch	Factors controlling flower induction – Smith Developing a common language for avocado – Iturrieta Vered Irihimovitch Flowering and Fruit Set – Pattermore Flowering biology – Hormaza
Day 2	Poster Session 1	Neena Mitter Noelani van den Berg	
Day 2	Where Theory Meets Practice	Guy Witney Francisco Mena Ben Faber	Avner Silber Tatiana Cantuarias-Avilés
Day 3	FIELD DAY		
Day 4	Challenges to Productivity – Genetics, Genomics and Biotechnology	Aureliano Bombareley Alejandro Barrientos	The importance of Persea/wild germplasm – Barrientos Transformation and Genomics – Mitter, Pliego The Persea genome – Bombareley Breeding tools for the future?
Day 4	Meeting the challenges of the future	Mary Lu Arpaia Zelda Van Rooyen Tim Spann	Interactive Workshop
Day 4	Poster Session 2	Neena Mitter Noelani van den Berg	
Day 4	Tying the loose pieces together – planning for the future	Jose Chaparro Nigel Wolstenholme	The future avocado tree? Keeping the big picture in mind

WAC
IX World Avocado Congress
- Colombia -

September 23 to 27·2019
Medellín - Colombia

IX Congreso Mundial de Aguacate"WAC-2019"
Medellín-Colombia

Medellín, December 30, 2018

Doctor
Harley Smith
CSIRO Agriculture & Food
Waite Campus. Locked Bag 2
Glen Osmond, SA 5064 Australia

Dear Dr. Smith. Receive a warm greeting.

On September 24 to 26 at Plaza Mayor, Convention and Business Center in Medellin, the **IX World Avocado Congress**, academic and business event that gathers around 1300 leaders of the avocado industry, will be held. This year, the congress will have around 10 speakers and 30 experts from different countries that will discuss the global context of the avocado, technological developments, market trends and the challenges that the field has for the future.

The Academic Committee of the event is honored to invite you as a Symposium speaker at the IX World Avocado Congress to address the topic of PRODUCTION & PRODUCTIVITY, with the subjects, " Alternating and erratic productions in avocado". We are aware that your contributions will be of high value to the audience and for the growth of the avocado industry globally.

Corpohass · www.WorldAvocadoCongress.co

WAC
IX World Avocado Congress
- Colombia -

September 23 to 27·2019
Medellín - Colombia

We would like to inform you that the Organizing Committee has granted you the following benefits as a Symposium lecturer during the academic days of the congress:

- "All Access" participation ticket worth US $ 1,600, with which you will be accredited to WAC-2019, in order to fulfill your commitment and the other activities of the congress during the three event days.
- Economy class flight ticket.

We kindly request that you express your acceptance in writing. In the same way, please send us the complete curriculum vitae, short curriculum (bio) and photo with at least 1800x1800 pixels, by email to academicwac@corpohass.co, before Dicember 21, 2018.

Date: September 24 to 26, 2019
Location: Plaza Mayor, International Convention and Business Center Medellin, Colombia.

Cordially,
Martha E. Londoño Z., MSc
Academic Committee Coordinator
World Avocado Congress-2019

Corpohass · www.WorldAvocadoCongress.co

Avocado Brainstorming – 2023

Maroochydore, Queensland, Australia
27 – 31 March 2023

Greetings colleagues,

We are writing to extend an invitation to attend the upcoming Avocado Brainstorming meeting which will be held from 27 March–31 March in Maroochydore (north of Brisbane), Australia. This will be the seventh Avocado Brainstorming Meeting which began with the first meeting in Riverside, California in 1999.

For those of you who have not participated before, a little background information. The meeting's primary objective is to share knowledge with the express purpose of stimulating discussion, communication and collaboration among scientists with the belief that this will result in enhanced long-term sustainability of the world avocado industry. Research collaborations that have resulted from previous meetings include collaboration on rootstock breeding, collaborative work on avocado genomics, discussion on postharvest disorders and work on avocado water relations.

The goals of the meeting are three-fold: build research networks, new relationships and collaborations among international science groups; encourage upcoming early career scientists to make a career in avocado research; and discuss and share ideas about specific industrywide topics of interest that will enhance long-term viability of the international industry including improved cultural and postharvest practices that optimize output while minimizing resource utilization.

To ensure your participation in Avocado Brainstorming 2023, we will be able to cover your expenses related to your participation (accommodation and meals in Maroochydore, the field trip on the way back to Brisbane and bus transportation to/from Brisbane) with a registration fee of US$275 per person due to generous sponsorship we have received. Once you confirm participation, we will provide a link to make the payment by credit card. Please note this may mean having a roommate at the meeting venue. Liz Dann has agreed to be the on-site coordinator of the meeting and we have selected the Surf Air resort hotel in Maroochydore as the meeting venue which has a combination of suites and private rooms.

You will need to provide your own transportation to/from Brisbane, Australia. Full details will be provided in near future correspondence but plan to fly into Brisbane. Maroochydore does have an airport if you wish to go that route. Most major airlines service Brisbane, Australia routes and it is an approximate 2.5 hour drive to Maroochydore from Brisbane. Transportation to Maroochydore will be by bus and covered within the registration price.

The Maroochydore area is the epicenter of the Australian avocado industry, and we will have the opportunity on the final day to visit the Maroochy Horticultural Center, a nursery and a commercial grove on our return to Brisbane.

As with previous Brainstorming meetings there will be poster sessions and other planned activities to promote networking among the participants.

Please let us know if you have ideas for sessions or people who should be invited (send name, e-mail and general area of expertise as soon as possible). As in the past, we want to include graduate students and post-doctoral researchers who conduct research on avocado. Time, funds, space, and the desire to promote networking dictate that the number of participants must be limited to ~75.

Please let us know by **25 February** whether you plan to attend the meeting by answering the attached form.

We hope to see you in Australia!

2023 Avocado Brainstorming Meeting

27 March (Monday)

8:30 AM – 12:00 PM – Tour to the University of Queensland – Prof. Neena Mitter

8:30 am – 9:00 am – Meet at bus, check-in and loading of luggage

9:00 am - 9:30 am – Bus to Longpocket UQ from group hotel
9:35 am - 10:00 am – Introduction to the research at QAAFI that impacts avocado – N. Mitter
10:00 am -10:30 am – Morning tea plus show and tell tissue cultures from the lab, acclimatized TC plants, and networking
10:30 am - 11:00 am – Tour of the lab in small groups
11:00 am - 12:30 pm – UQ/QUT researchers present 15 min snapshots (to be confirmed)

12:30 pm – Depart for Surfair Beach Hotel; Box Lunch enroute

~3:00 PM - Arrive via bus to Surf Air Conference Venue

6:00 – 6:30 PM – Welcome Get Together

6:30 PM – Dinner

28 March (Tuesday)

7:00 AM – Breakfast buffet

8:30 – 10:30 AM - Session 1 – What will the future avocado look like? – Hormaza, Smith

What growers, consumers and researchers need in avocado improvement

10:30 – 11:00 AM - Break

11:00 AM – 1:00 PM - Session 2 – Tools for the future – Mitter, Fernandez

Technologies to accelerate breeding, tissue culture, new varieties and rootstocks

1:00 – 2:00 PM – Lunch Buffet-style

4:00 – 6:00 PM – Poster Session 1

6:00 – 8:00 PM – Session 3 - Managing the grove to optimize for productivity for the future – Mauk, Haberman

Irrigation, nutrition, planting densities, pruning, weeds, pests, disease

8:15 PM –Dinner

29 March (Wednesday)

7:00 AM – Breakfast buffet

8:30 – 10:30 AM - Session 4 - Avocado flowering, fruit set and fruit retention and how it is managed – Faber, Gould

Pollinators, Pollinizers, Habitat improvement, Flowering Genes, Alternate Bearing

10:30 – 11:00 AM - Break

11:00 AM – 1:00 PM - Session 5 – Crop Management in a Changing World – Spann, Chaparro

Climate predictions, temperature modification, light control, Varietal differences, Reflective materials

1:00 – 2:00 PM – Lunch Buffet-style

4:00 – 6:00 PM – Poster Session 2

6:00 – 8:00 PM – Session 6 – Breakout Group Discussions – Challenges going forward – Van Rooyen, Spann, Arpaia

What is needed for the future market, research, to address current and future issues?

8:15 PM –Dinner

30 March (Thursday)

7:00 AM – Breakfast buffet

8:30 – 10:30 AM - Session 7 – Technology for the Future – Solares, Focht

Robotics, Gene Bank & Phenology Data Base

10:30 – 11:00 AM - Break

11:00 AM – 1:00 PM - Session 8 Summarizing and thinking about challenges going forward – Mena

1:00 – 2:00 PM – Lunch Buffet-style

2:00 – 6:00 PM – Free time/Networking

6:30 PM –Dinner

31 March (Friday)

7:00 AM – Breakfast buffet

8:00 AM – Depart on bus tour enroute to Brisbane; Dropoff at Courtyard Hotel in Southbank (expected arrival around 5:00 PM). Tour includes Maroochy Horticultural Research Station; Flemings' Nursery and orchard stop (Glasshouse Mountains).

Academic programme for the 2023 10th World Avocado Congress in New Zealand

Refer to https://www.wacnz2023.com/programme/academic-programme

Time	Breeding		Times	Plant nutrient management		Time	Pests		Time	Fruit rots	
Chair	Mary Lu Arpaia			Jonathan Dixon			Matt Dyck			Elizabeth Dann	
11.35 - 11.50	Peggy Mauk Field evaluation of five rootstocks developed by the rootstock breeding program at the University of California, Riverside (UCR).		11.35 - 11.50	Luis Montgomery Nutrient standards by age of leaf in 'Hass' avocado (Persea americana Mill.) in the north coast of Peru	Spa	11.35 -11.50	Christina Hoddle Oviposition Biology and Behavior of the Large Avocado Seed Weevil, *Heilipus lauri* (Coleoptera: Curculionidae)	Eng	11.35 - 11.50	Kerry Everett Temperature and seasonality of infection of 'Hass' avocados by fruit rot pathogens	Eng
11.55 - 12.10	Therese Bruwer Screening of cultivars for pre-harvest orchard cold tolerance	Eng	11.55 - 12.10	Marcelo Brossi Santoro Optimum leaf sample size for nutritional diagnosis of subtropical rainfed Hass orchards	Eng	11.55 - 12.10	Edith Estrada-Venegas Rearing the large avocado seed borer *Heilipus lauri* (Coleoptera:Curculionidae) in México.		11.55 - 12.10	Anita Boyum Evaluation of alternative spray programs for the control of Pre- and Post-harvest diseases on Fuerte and Hass in South Africa	Eng
12.15 - 12.30	Zander Ernst Breeding and selecting cultivars and rootstocks set for the future of the avocado industry.	Eng	12.15 - 12.30	Francisco Mena Völker Nitrogen distribution and it´s effect on productivity in avocado trees var. Hass in Chile.	Spa	12.15 - 12.30	Maxwell Kibor Abundance and diversity of thrips (Thysanoptera) on avocados and macadamias in the Levubu region of Limpopo Province, South Africa	Eng	12.15 - 12.30	Philip Elmer Field testing bio-fungicides for efficacy against postharvest rots in New Zealand 'Hass' avocados	Eng
12.35 - 12.50	Juan Sebastián Arias García Hass avocado phenology in the andean tropics of Caldas, Colombia		12.35 - 12.50	Luis Montgomery Nutrient standards by reproductive stage development in 'Hass' avocado (Persea americana Mill.) in the north coast of Peru	Spa	12.35 - 12.50	Edith Estrada-Venegas Mites associated to ambrosia beetles (*Curculionidae: Scolytinae*) on avocado (*Persea americana*) in Mexico.		12.35 - 12.50	Lucia Ramos Romero The relationship between postharvest rots and orchard variables in 'Hass' avocado orchards in New Zealand: The Avovantage project.	Eng
12.55 - 1.45 Lunch											
Time	**Breeding**		**Time**	**Pollination/fruit set**		**Time**	**Pests**		**Time**	**Postharvest**	
	Wendy Petrie			Jonathan Dixon			Brad Siebert			Jem Burdon	
1.50 - 2.05	Neena Mitter Innovating avocado - Lab to orchard and beyond	Eng	1.50 - 2.05	Andres Bascope Use of natural bioactive compounds to mitigate oxidative stress and increase fruit set in avocados.	Eng	1.50 - 2.05	Paul Pidakala Effect of ethyl formate and phosphine fumigant as a disinfestation treatment on 'Hass' avocado fruit quality and target pest mortality	Eng	1.50 - 2.05	Robert Valkenburg Comparison of methods for rapid non-destructive and destructive measurement of dry matter, firmness, and rots in Hass Avocados	Eng
2.05 - 2.20	Theo Bekker Westfalia launches two new co-owned avocado rootstocks from its breeding and selection program	Eng	2.05 - 2.20	Harley Smith Physiological basis of summer fruit abscission in avocado.	Eng	2.05 - 2.20	Armando Equihua-martinez The avocado stem borer *Heilipus cruciatus* (Coleoptera:Curculionidae) in Veracruz México.		2.05 - 2.20	Ryan Fink Unlocking ripeness and category growth through advanced imaging, machine learning and smarter sorting	
2.20 - 2.35	Eric Focht Eating quality, sensory profile, volatile analysis, and postharvest evaluation of two early season Hass-like mutants: Flavia and Eugenin	Eng	2.20 - 2.35	Juan Sebastián Arias García Fruit retention in Hass avocado by cardinal point in two contrasting zones of the Andean tropics in Colombia	Spa				2.20 - 2.35	Gonzalo Martinez Hermosilla Prediction of packed avocado porosity and its application on rapid optimisation of packaging design	
2.35 - 3.05 Afternoon tea											
Kiri Te Kanawa Theatre											
Times	**Closing ceremony**										
Chairs	Wendy Petrie Jen Scoular								Eng		
3.10 - 4.00	Vote for next host country Closing of the 10th World Avocado Congress								Eng		

Appendix F: Draft of Technical Manuscript

Avocado fruitlet abscission is initiated by a maturation phase of development that permits abscission

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Abstract:
Fruits undergo sequential phases of development after fruit set. During the growth phase of fruit development, auxin promotes cell division and expansion. However, at the end of the growth phase, the transition to ripening (senescence) and abscission, is mediated by fruit maturation, which is triggered by a decline in auxin activity. In many fruit tree crops of economic importance, the abscission of immature fruits by correlative dominance interactions and climate events negatively impacts yield. While a mechanistic understanding of cell separation in the abscission zone is well understood, how immature fruits prematurely exit the growth phase of development and undergo abscission is poorly understood. Here, we show that avocado fruitlets fated to abscise undergo growth arrest prior to abscission. Transcriptomic reprogramming displayed in arresting fruit tissues indicates that growth inhibition is initiated in the seed coat by a decrease in auxin activity. The overall suppression of growth in maternal tissues is associated with the differential expression of adaxial/abaxial polarity genes together with the upregulation of genes that negatively regulate meristem activity. In addition, the embryo and seed coat derived from arresting fruitlets exhibit changes in the expression of genes associated with seed dormancy. While the current hypothesis predicts that seed abortion induces fruitlet abscission, we propose an alternative hypothesis in which the growth arrest signal(s) acts to promote the maturation of maternal tissues to induce seed dormancy, which suppresses growth and allows fruitlets to transition to a ripening phase that permits seed coat senescence and abscission.

Keywords: Fruit development, auxin, abscission, leaf polarity, meristem, seed dormancy

Introduction

Fruit development is initiated upon fruit set, a process that typically involves fertilization (Kumar et al., 2014; Fenn and Giovannoni, 2021). Once initiated, fruits undergo a phase of growth mediated by cell division and/or expansion. At a late stage of growth when the seeds and maternal tissues reach maturity, fruits transition to a ripening or senescence phase of development (McAtee et al., 2013; Kumar et al., 2014). This transition is not only essential to for ripening, but it is also required for organ abscission (Kim, 2014; Ma et al., 2021).

Auxin is a fundamental hormone that regulates fruit development (Pattison et al., 2014). Auxin activity promotes fruit set, as well as the cell division and cell expansion stages during growth (Kumar et al., 2014; Fenn and Giovannoni, 2021). Moreover, auxin functions as a key signal coordinating growth and development of the seed and fruit tissues (Pattison et al., 2014; Figueiredo and Kohler, 2018; Robert, 2019; Guo et al., 2022). For example, upon fertilization of the central cell, auxin produced in the endosperm promotes seed coat development (Figueiredo et al., 2016), as well as stimulates growth of the fleshy part of the fruit (Guo et al., 2022). In addition, auxin produced in the seed coat drives early embryo development and positively regulates seed size (Robert et al., 2018; Li et al., 2021). Taken together, auxin biosynthesis and transport are essential processes for coordinating the growth of filial and maternal tissues during seed and fruit development (Figueiredo and Kohler, 2018; Robert, 2019; Fenn and Giovannoni, 2021; Guo et al., 2022).

At the end of the growth phase, the decline in auxin activity is required for fruit maturation and the transition to ripening and abscission (McAtee et al., 2013; Kumar et al., 2014; Pattison et al., 2014). For example, in the non-ripening *rin* mutant identified in tomato (*Solanum lycopersicum*), auxin response fails to decrease in the pericarp at a time when the wild-type cultivar ripens (Shin et al., 2019). In addition, applications of auxin to tomato fruits at early and late stages of maturation delays the ripening transition (Su et al., 2015; Li et al., 2016; Tobaruela et al., 2021; Chirinos et al., 2023). Lastly, competence to ripen is associated with an increase in the levels of inactive auxin conjugates, including the amino acid aspartic acid (Asp) (Böttcher et al., 2010; Chirinos et al., 2023). Taken together, physiological and genetic studies suggest that fruit maturation and the competence to ripen and abscise requires a decrease in auxin levels either through the downregulation of auxin biosynthesis genes and/or increase in expression auxin conjugation genes.

In tomato, experimental studies indicate that transition to ripening is initiated in the locule gel, before expanding to the outer pericarp (Chirinos et al., 2023). Further, an increase in locule number as displayed in the *faciated* (*fas*) and *locule number* (*lc*) mutants increases fruit size and alters fruit shape (Lippman and Tanksley, 2001; van der Knaap et al., 2014; Chu et al., 2019). As seed derived auxin plays a fundamental role in coordinating fruit growth (Figueiredo and Kohler, 2018; Robert, 2019; Guo et al., 2022), it is highly probably that the maturing seeds

encased in the locules may be influencing the transition to ripening and abscission initiated in the locule gel.

In many fruit tree crops of economic importance, a significant number of fruits that set will undergo immature fruit abscission (IFA) during the growing phase of fruit development (Sawicki et al., 2015). It has been hypothesized that correlative dominance interactions between expanding vegetative shoots and developing fruits, as well as among fruitlets, induces IFA (Bangerth, 2000; Sadka et al., 2023). According to this model, auxin produced by the dominant vegetative shoot or fruitlet prevents auxin export from the dominated immature fruits, which results in the activation of cell separation in the abscission zone (AZ). Interestingly, results from shading and applications of plant growth regulators that induce IFA showed that apple fruitlets undergo growth arrest prior to abscission (Ward and Marini, 1999; Greene et al., 2013). Therefore, it appears that the primary effect of correlative dominance interactions in fruitlets fated to abscise is a cessation of growth followed by abscission.

Hormone signaling plays a fundamental role in mediating fruit abscission including the regulation of cell separation via antagonistic interactions between auxin and ethylene (Sawicki et al., 2015; Botton and Ruperti, 2019). According to this model, transport of auxin through the AZ prevents ethylene from inducing mechanisms that mediate cell separation. However, a decline in auxin levels from reduced auxin transport sensitizes cells in the AZ to ethylene, which functions to initiate the metabolic processes associated with abscission. It has been hypothesized that IFA is initiated by mobile signals produced in fruitlets fated to abscise (Sawicki et al., 2015; Botton and Ruperti, 2019). Once initiated, the signal(s) move to the AZ to activate cell separation. Evidence that supports this hypothesis was primarily derived from studies performed in apple (*Malus* x *domestica*) using a fruit thinning agent that induces IFA (Bangerth, 2000; Dal Cin et al., 2005; Botton et al., 2011). Using this inducible system, experimental evidence suggests that ethylene produced in the cortex acts as a mobile signal that initiates IFA prior to AZ activation (Botton et al., 2011; Eccher et al., 2015). According to this model, ethylene diffuses from the cortex to the seed to promote embryo abortion, which would likely reduce auxin transport through the AZ to induce cell separation. Using an inducible system that involves the girdling and defoliation of shoots bearing fruits, molecular studies indicate that ethylene also plays a role in intra-organ signaling prior to IFA in litchi (*Litchi chinensis*) (Li et al., 2015; Zhao and Li, 2020). In addition, auxin levels decline in litchi fruitlets undergoing IFA in response to girdling and defoliation (Kuang et al., 2012). While these hormone studies shed light on intra-organ signaling associated with IFA, the early developmental mechanism(s) that act in the fruitlet to initiate abscission is poorly understood.

In this manuscript, we address the developmental basis of IFA in avocado, as this tree crop displays a high rate of fruitlet abscission that negatively impacts yield (Salazar-García et al., 2013). Our results show that the fruitlet growth arrest is a primary event of IFA, which is

initiated in the seed coat. At the time of fruitlet growth arrest or thereafter, the seed coat undergoes senescence. Fruitlet growth arrest is associated with a decrease in free auxin levels in the maternal tissues and an alteration in the expression of polar auxin transport genes in the seed coat, pericarp and embryo. In response to growth arrest, the transcriptome of maternal tissues, including the seed coat and pericarp, is associated with the differential expression of genes involved in adaxial/abaxial polarity, which is necessary for regulating auxin activity and cell proliferation during organ expansion (Heisler, 2021; Nakayama et al., 2022). The transcriptome of maternal tissues also exhibits changes in the expression of meristem related genes, which regulate the switch from cell proliferation to differentiation during organ expansion (Tsukaya, 2021). Moreover, the seed coat and embryo display changes in the expression of genes that encode dormancy signaling proteins. Taken together, our results suggest that growth arrest is mediated by the maturation of maternal tissues that suppresses growth and induces a seed dormancy-like response. The induction of a maturation phase of development allows the arresting fruitlets to transition to a ripening phase that initiates seed coat senescence and abscission.

Results:

Fruits undergo growth arrest prior to abscission

To better understand how immature fruits transition to abscission, we investigated the possibility that avocado fruits undergo growth arrest prior to abscission. While fruitlet growth rates can be determined using calipers (Greene et al., 2013), here, fruit dendrometers were utilized to characterized the daily growth patterns when the average diameter of fruitlets was 36.5 mm. (Figure 1A). Over the course of the trial, the average diameter increased at a steady rate until the average fruitlet diameter reached approximately 52 mm. During this period the average growth rate of immature fruitlets was 0.53 mm/day (Figure 1B). After this time point, the average increase in the diameter and growth rate of the developing fruitlets was slightly reduced such that by the end of the trial, the average growth rate was 0.41 mm/day (Figure 1A and B).

Immature fruits abscised throughout this trial with a peak in daily abscission occurring when the diameter was approximately 48 mm, just prior to the slight decrease in the daily growth rate of developing fruitlets (Figure 1C). The growth rate of fruitlets that abscised over the course of this trial was collated and displayed in Figure 1D. Results showed that fruitlets fated to abscise underwent growth arrest before they shrunk and separated from the tree. Based on these results, the process of growth arrest takes approximately 6-days and another 4- to 6-days to abscise. Seed coat senescence is a prominent phenotype of recently abscised avocado fruitlets (Salazar-García et al., 2013). Therefore, we examined the seed coat from fruitlets undergoing arrest, as well as during the shrinkage stage, to determine the timing of seed coat senescence. Based on these results, early signs of seed coat senescence were typically apparent during the shrinkage phase that occurred after growth arrest (Figure 1E). Taken together, we propose that IFA is initiated by signals that cause a subset of fruitlets to

undergo growth arrest. During a late stage of growth arrest or thereafter, the seed coat undergoes senescence, which become visible during the shrinkage phase of IFA. Lastly, the physical separation of the fruitlets from the tree is the final step in the IFA pathway.

The transcriptome of maternal tissues is highly responsive to growth arrest

To better understand the intra-organ developmental events that are spatially and temporally associated with growth arrest, we harvested actively growing/persisting fruitlets, as well as immature fruits that switched to low growth rate under natural conditions. In a second trial, defoliation was used as a system to induce IFA, as this treatment caused an average 97% of fruitlets to abscise (Supplemental Figure 1). In order to temporally capture fruitlets during an early and late stage of growth arrest, immature fruits with a normal growth rate and arresting fruitlets were harvest 6-days after defoliation. In addition, persisting fruitlets with a normal growth rate were harvested from untreated trees and used to compare with normal growing and arresting fruits harvested from the defoliation trial. The spatial determinants for growth arrest were ascertained by separating the fruit samples into seed coat, embryo and pericarp. The pericarp tissue was primarily comprised of mesocarp tissues with limited endocarp tissues and no exocarp tissue. To identify changes in the transcriptome associated with and induced by fruitlet growth arrest, RNA-sequencing was performed.

Principle component analysis (PCA) of fruit samples derived from the natural and defoliation induced abscission trials showed that normal growing and arresting tissue samples were primarily separated by the first two principial components, which explained 52 and 23% of the variance in the dataset (Figure 2A). The variance between the arresting and normal growing seed coat and pericarp transcriptomes under natural conditions and in response to defoliation was substantially higher compared to the variance in the embryo samples. This is supported by the fact that the overall number of differentially expressed genes in the seed coat and pericarp was higher compared to the embryo (Figure 2B-D). Therefore, the transcriptomes of maternal tissues were highly responsive to fruit growth arrest. Although defoliation is an artificial treatment used to induce fruit drop, the arresting fruit samples derived from natural and defoliation induced IFA clustered together (Figure 2A). Thus, PCA demonstrates that defoliation induced growth arrest is valid method to experimental study the temporal events of IFA. In the maternal tissues, particularly the seed coat, PCA showed that harvested fruits with a normal growth rate from defoliated trees clustered along an axis that was more closely positioned to the normal growing control samples than the arresting samples in the seed coat and pericarp. As a result, we reasoned that comparing normal growing fruits from defoliated and control trees is a valid approach to uncover transcriptomic changes associated with an early stage of fruit growth cessation.

A reduction in maternal auxin levels is associated with growth arrest

Indole-3-acetic acid (IAA) is the major form of auxin that regulates fruit development (Kumar et al., 2014; Fenn and Giovannoni, 2021). Therefore, we investigated whether growth arrest

was associated with changes in the expression of genes that regulate IAA homeostasis in the seed coat, pericarp and embryo. In the two-step IAA biosynthesis pathway, tryptophan is converted to indole-3-pyruvic acid (IPA) via TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA) (Mashiguchi et al., 2011; Won et al., 2011). In the second step, the rate limiting YUCCA (YUC) flavin monooxygenase-like enzymes catalyse the conversion of IPA to IAA. The tissue and cellular pool of free IAA is also controlled in part by GRETCHEN HAGEN 3 (GH3.1) acyl acid amido synthetases, which conjugate IAA with aspartic acid (Asp), as well as other amino acids, to store, transport or inactivate this hormone (Staswick et al., 2005). Taken together, free IAA levels are regulated by biosynthesis and conjugation (Figure 3A).

The transcriptome profiles for the seed coat, pericarp and embryo were queried for the differential expression of genes that encode enzymes involved in IAA biosynthesis and GH3-conjugation during growth arrest. Results showed that *YUC4-like* and *YUC6-like* genes were downregulated in the seed coat and pericarp during fruit growth cessation (Figure 3B). Moreover, in the seed coat, the levels of *YUC4-like* and *YUC6-like* were repressed early in the growth arrest process (Figure 3B). In fruits undergoing growth arrest, two *GH3.1-like* genes were upregulated in the seed coat (Figure 3C). Further, in response to defoliation, *GH3.1-like_1* was induced in the seed coat during early growth arrest. In the pericarp, the expression of *GH3.1-like_1* was significantly increased during late growth cessation (Figure 3C). In addition, to changes in the expression of genes involved in IAA homeostasis in the seed coat and pericarp, transcription factors that mediate auxin response were differentially expressed in the maternal tissues and to a lesser extent in the embryo (Supplementary Figure 2). Thus, the maternal tissues undergo changes in the expression of IAA biosynthesis and conjugation genes, which suggest that free IAA levels are reduced during growth arrest.

To further address a role of auxin in IFA, the levels of IAA were quantified in the seed coat, pericarp and embryo of arresting and normal growing fruitlets. Results showed that in normal growing fruits, IAA was 11.4- and 10.2-fold higher in the seed coat than in the pericarp and embryo, respectively (Figure 3D), indicating that the seed coat is a major site of auxin biosynthesis during this period of fruitlet development. During fruitlet growth arrest, IAA levels were significantly reduced in the seed coat, as well as pericarp (Figure 3D). To determine if auxin conjugation was associated with the decrease in free IAA levels during fruit growth arrest, the levels of IAA-Asp were quantified in the seed coat and pericarp, and embryo in normal growing and arresting fruitlets (Figure 3E). Results showed that level of IAA-Asp significantly increased in the seed coat 7.3-fold during growth arrest. While the average level of IAA-Asp appeared to increase in the pericarp during growth arrest, this trend was not significant (p=0.13). In the embryo, there were no significant changes in the levels of IAA and IAA-Asp between normal growing and arresting fruitlets (Figure 3D and E). Taken together, these results suggest that growth arrest signal(s) targets auxin biosynthesis and/or conjugation in the maternal tissues to promote the cessation of fruitlet growth during IFA.

Polar auxin transport genes are differentially expressed in response to growth arrest

Experimental studies suggest polar auxin transport is essential for coordinating the growth of the embryo and expansion of maternal tissues during seed and fruit development (Pattison et al., 2014; Figueiredo and Kohler, 2018; Robert, 2019). In the arresting seed coat, pericarp and embryo, a number of genes that encode IAA influx and efflux carriers, as well as cellular transporters, were differentially expressed during growth arrest suggesting that the polar transport of auxin in the fruitlet was altered during growth cessation (Figure 4A-C). Indeed, a number of *PIN1-like* genes were downregulated in the seed coat, pericarp and embryo of arresting fruitlets compared to immature fruits with a normal growth rate (Figure 4B). Further, transcript abundance for a subset of these *PIN1-like* genes was suppressed early in the growth arrest process (Figure 4B). In contrast, transcript abundance for *AUX1-like*, *ABCB19-like_3*, and *ABCB19-like_4* increased in the seed coat and pericarp during fruit growth arrest (Figure 4A and B). In addition, a *PIN3-like* gene was upregulated in all three tissues of arresting fruits (Figure 4B). The upregulation of *PIN3-like*, *AUX1-like* and *ABCB19-like* genes may be part of a stress response to maintain polar auxin transport when IAA levels become limiting (Zhang et al., 2012; Wang et al., 2019).

Organ polarity genes are differentially expressed during seed coat and pericarp arrest

Auxin plays a critical role in mediating the expansion of organs during the proliferative phase of development (Heisler, 2021; Tsukaya, 2021; Nakayama et al., 2022). This is achieved in part by the juxtaposition of adaxial and abaxial domains, which confines auxin activity to the margins and middle domain of the leaf (Wang et al., 2011; Zhang et al., 2020). Organ polarity genes that define the adaxial and abaxial domains are critical for expansion of the leaves, floral organs and integuments (Figure 5A) (Kelley and Gasser, 2009; Nole-Wilson et al., 2010; Nakayama et al., 2022). As organ polarity factors have been implicated in the regulation of fruit development (Clevenger et al., 2015), we hypothesized that the arrest of maternal tissues during IFA would involve an alteration in the expression of genes that specify adaxial and abaxial identity. Consistent with this hypothesis, a number of class III *HD-ZIP-like* genes, including *CORONA-like* (*CNA-like*), *PHAVOLUTA-like* (*PHB-like*) and *PHABULOSA-like* (*PHB-like*), which act to specify adaxial identity (Merelo et al., 2017), were downregulated during growth arrest in the seed coat and pericarp (Figure 5B). Further, transcript abundance for *AGO10-like* genes, which function to sequester *miR165/166* in order to limit post-transcriptional regulation of *class III HD-ZIP-like* genes (Figure 5A) (Zhang and Zhang, 2012), were reduced in the seed coat and pericarp during growth arrest (Figure 5B). In the seed coat, a subset of *class III HD-ZIP-like* and *AGO10-like* genes were downregulated early in the growth arrest process indicating that these genes may be targeted by the growth cessation signal(s). In addition, *AS1-like* genes were downregulated in the seed coat and pericarp but only during late growth arrest (Figure 5B). In contrast, *AS2-like* genes were upregulated during late growth arrest in the seed coat (Figure 5B). Thus, the differential expression of *AS1-like* and/or *AS2-like* genes in the maternal tissues may be part of a response that acts late in the growth arrest process.

Organ polarity is also controlled by transcription factors that specify abaxial identity, including KANADI (KAN) and AUXIN RESPONSE FACTOR 3 (ARF3)/ETTIN (ETT) (Figure 5A) (Merelo et al., 2017). Transcriptome analysis showed that *KAN1-like* genes were differentially expressed in maternal tissues during growth arrest (Figure 5C). In the seed coat, *KAN1-like_1* and *KAN1-like_3* were upregulated, while *KAN1-like_2* was repressed during growth arrest (Figure 5C). In the pericarp of fruits undergoing growth cessation, only *KAN1-like_1* was upregulated; whereas, transcript abundance for *KAN4-like_1* and *KAN4-like_2* was reduced. Overall, *ARF3/ETT-like* genes were downregulated in the seed coat and pericarp in response to growth arrest. Moreover, in the seed coat, the increase and decrease in the expression of *KAN1-like_3* and *ARF3/ETT-like_2*, respectively, occurred during early growth arrest (Figure 5C) indicating that these abaxial genes are highly responsive to the growth arrest signal(s) (Figure 5C). Taken together, results show that growth arrest of the maternal tissues is associated with significant changes in the expression of genes that specify adaxial and abaxial identities. As a result, failure to maintain organ polarity is predicted to negatively impact the expansion of maternal tissues during IFA.

Fruit growth arrest is associated with an overall decrease in the expression of genes that control the cell cycle

During the growth phase of fruit development, auxin plays a fundamental role in promoting cell division (Kumar et al., 2014; Fenn and Giovannoni, 2021). As avocado fruit growth is primarily mediated by cell division (Schroeder, 1953; Cowan et al., 2001; Dahan et al., 2010), the transcriptome data was interrogated to determine if the expression of cyclins (CYCs) and cyclin dependent kinases (CDKs), which control cell cycle phase transitions (De Veylder et al., 2007), were altered during growth arrest. In general, CYCD and CYCB control the G1 to S and G2 to M transitions, respectively, while CYCA regulates the S to G2 and G2 to M transitions (Figure 6A). In addition, CDKB primarily regulates the G2 to M phase transition (De Veylder et al., 2007). Results showed that a substantial number of *CYCD-like*, *CYCA-like* and *CYCB-like* genes, as well as *CDK-like* genes, were downregulated in the maternal and embryo tissues of the fruitlets during growth arrest (Figure 6B and C). The early decrease in expression of *CYCB-like* and *CYCA-like* genes, as well as *CDKB-like* genes, indicates that the actively dividing cells primarily arrest in the G2 phase, as well as the S phase, of the cell cycle. In the maternal tissues, not all D-cyclins were repressed, as *CYCD6-1-like_2* was upregulated late in the growth arrest process (Figure 6B). In addition, the expression of *CYCD5-1-like* increased during early growth arrest but was suppressed later in this process (Figure 6B). Taken together, these results suggest that the fruit growth arrest signal(s) targets cell cycle machinery to inhibit cell proliferation.

Maternal tissues display an alteration in the expression of genes that control meristem activity during fruit growth arrest

Leaf blade expansion is driven in part by the activity of the margin and plate meristems, which promote cell proliferation during development (Tsukaya, 2021). Meristem activity is negatively regulated by the cell proliferation arrest front that forms in the distal tip of the leaf and moves in a wave like manner towards the base during leaf expansion. Experimental studies show that class II TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTORs (TCPs), NGATHA (NGA), BLADE ON PETIOLE 1 (BOP1) and BOP2 transcription factors function at the arrest front to repress leaf meristem activity and cell proliferation during leaf expansion (Tsukaya, 2021). In addition, the ubiquitin receptor, DA1, also negatively regulates cell division at the arrest front in leaves (Vanhaeren et al., 2017). On the flipside, the AINTEGUMENTA (ANT) and ANGUSTIFOLIA3 (AN3) transcription factors act to positively regulate leaf meristem activity and cell proliferation (Tsukaya, 2021). The interplay between these positive and negative regulators of leaf meristem activity controls the timing of the transition from cell proliferation to differentiation, in which the former developmental process is associated with cell expansion (Figure 7A) (Sarvepalli and Nath, 2018; Tsukaya, 2021). As organs differentiate, they undergo a maturation phase to achieve their final form and function. Given the homology between the maternal tissues of the fruit with leaves (Mathews and Kramer, 2012; Liu et al., 2023), we speculated that leaf meristem determinants play a role in repressing the expansion of the seed coat and pericarp during fruit growth arrest. Consistent with this hypothesis, results showed that the arresting maternal tissues were associated with an increase in expression of the class II TCP genes, *CYCL2* and *TCP2-like*, as well as *NGA1-like1*, *BOP1/2-like* and *DA1-like* (Figure 7B). In the seed coat, *CYCL2*, *NGA1-like_1*, *BOP2-like* and *DA1-like* were induced early in the growth arrest process. However, transcript levels for *BOP1-like* and *TCP2-like* only increased in the seed coat during late growth arrest (Figure 7B). In contrast, a small subset of transcription factors that negatively regulate meristem activity, including *TCP3-like*, *TCP13-like* and *NGA1-like_2*, were downregulated at a later stage of growth arrest in the seed coat (Figure 6B). Results from the transcriptome analysis also showed that *ANT-like* genes and *AN3-like_2*, were downregulated in maternal tissues at a late stage of growth arrest (Figure 7B). In contrast, *AN3-like_1* was upregulated at a late stage in the arresting seed coat. Taken together, our results suggest that genes controlling meristem activity are targeted by the growth arrest signal(s) to suppress cell proliferation and promote differentiation in maternal tissues.

A dormancy-like response is induced in the seeds of fruitlets undergoing arrest

After a phase of growth, seeds undergo a maturation phase which ends in dormancy (Mathews and Kramer, 2012; Liu et al., 2023). Interestingly, the dormancy potential of a seed is influenced by signals produced in the seed coat (Iwasaki et al., 2022). Therefore, if the suppression of meristem activity in the maternal tissues of arresting fruits involves the activation of differentiation, a process associated with maturation, then a dormancy-like signaling pathway(s) may be activated in the seed coat and embryo. FLOWERING LOCUS T (FT), and MOTHER OF FT (MFT) signaling proteins play critical and opposing roles in mediating dormancy during seed maturation (Figure 8A) (Footitt et al., 2011; Nakamura et al., 2011;

Chen et al., 2014). Therefore, we examined the expression levels of these genes in maternal tissues and embryo. Results showed that the expression of *FT-like_1/2* were downregulated during growth arrest in the maternal tissues (Figure 8B). Further, in the seed coat, transcript levels for *FT-like_1/2* decreased early in the growth arrest process. The expression of *FT-like_1* was also suppressed early in the arresting pericarp. Together, the early downregulation of *FT-like* genes in the maternal tissues indicates that these signaling factors may be targeted by the growth arrest signal(s) to induce seed dormancy. In the maternal tissues, *MFT-like* was downregulated during early and late stages of growth arrest (Figure 8B). In contrast, the expression of *MFT-like* increased during late growth arrest in the embryo (Figure 8B). Together, the decrease in *FT-like_1/*2 expression in maternal tissues combined with an increase in *MFT-like* transcript levels in the embryo supports a hypothesis that a maturation phase of development is induced during growth arrest.

Similar to seed dormancy, the floral transition is regulated by environmental cues and internal signals (Freytes et al., 2021). As FT is a key integrator of the floral transition, the expression of this signaling factor is highly regulated to ensure that plants undergo flowering at a time that will ensure reproductive success (Osnato et al., 2021; Zhu et al., 2021). *TEMPRANILLO 1* (*TEM1*) and *TEM2* (also known as *RAV2*) encode transcription factors that directly repress *FT* in leaves (Figure 8C) (Castillejo and Pelaz, 2008; Hu et al., 2021). Overexpression of *TEM1* not only delays flowering but also promotes leaf senescence, as well as inhibits seed germination under saline conditions (Castillejo and Pelaz, 2008; Osnato et al., 2012; Fu et al., 2014). Further, experimental studies suggest that genes related to *TEM1/2* negatively regulate the growth of ovaries and seeds in rice (Osnato et al., 2020). In line with the above studies, results showed that *TEM1/2-like* genes were induced in the seed coat, pericarp and embryo during growth arrest (Figure 8D). In the seed coat and embryo, the *TEM1/2* genes were induced early in the growth arrest process. In addition, an increase in the expression of *TEM2-like_2* was associated with early growth cessation in the pericarp (Figure 8D). Thus, the upregulation of *TEM1/2-like* genes in the maternal tissues indicates these transcription factors may play a key role in repressing growth and promoting seed dormancy by down-regulating *FT-like* genes during the growth arrest process.

Discussion

Fruits undergo sequential phases of development, starting with growth and ending with ripening and abscission (Kumar et al., 2014; Fenn and Giovannoni, 2021). Fruit and seed maturation is essential for initiating the transition to ripening/senescence and abscission (McAtee et al., 2013; Kim, 2014; Kumar et al., 2014; Ma et al., 2021; Iwasaki et al., 2022). While it has been speculated that IFA is caused by an abortive event that induces developmental arrest (Sawicki et al., 2015; Botton and Ruperti, 2019), we propose an alternative hypothesis in which fruitlet abscission is induced by a signal(s) that triggers a maturation phase of development in the maternal tissues to induce growth arrest and seed dormancy. Further, the maturation of maternal tissues in arresting fruitlets promotes a

ripening transition that initiates seed coat senescence and permits fruitlets to undergo abscission.

Maternal control of fruit growth arrest

Maternal tissues play a critical role in regulating seed development (Iwakawa et al., 2007; Radchuk and Borisjuk, 2014). For example, the seed coat plays a fundamental role in initiating embryo dormancy during maturation (Iwasaki et al., 2022). Moreover, transcriptome studies showed that the cortex tissue of apple fruitlets undergoing IFA was more responsive than the seed (Botton et al., 2011). In our study, we showed that the transcriptomic changes induced during growth arrest were substantially greater in the seed coat and pericarp compared to the embryo. Further, principal component analysis showed that the transcriptome of seed coat and pericarp was more closely related to each other than the differentially expressed genes of the embryo during growth arrest, indicating that the mechanism of growth arrest is conserved in the maternal tissues. In the maternal tissues of tomato fruits, experimental results suggest that the transition to ripening is initiated in the locular tissues, which surrounds the seeds, before expanding to the pericarp (Chirinos et al., 2023). At an early stage of growth arrest induced by defoliation, our results show that the avocado seed coat exhibited a greater number of differentially expressed genes than the pericarp suggesting that the seed coat is the maternal tissue that transmits information to induce growth arrest in the pericarp and embryo.

A role for auxin in fruit development, growth arrest and abscission

Auxin plays a fundamental role in mediating the cell division and expansion during growth (Kumar et al., 2014; Fenn and Giovannoni, 2021). Further, a decrease in auxin activity appears to be an essential step in promoting fruit maturation (McAtee et al., 2013; Kumar et al., 2014). Here, our results suggest that the fruit growth arrest signal(s) acts to reduce free IAA by negatively regulating *YUC4/6-like* genes in the seed coat and pericarp. In addition, the increase in the level of IAA-Asp and upregulation of *GH3.1-like* indicates that IAA conjugation acts to inactivate and reduce free IAA levels in the seed coat. The early down- and upregulation of *YUC4/6-like* and *GH3.1-like*, respectively in the seed coat indicates that the fruit growth arrest signal(s) targets auxin at an initial stage of growth cessation. In addition to the reduction in maternal IAA levels, genes that encode auxin efflux, influx and intercellular transporters, were differentially expressed, including the downregulation of *PIN1-like* genes, in the arresting maternal tissues and embryo. Interestingly, the reduction in IAA levels combined with an increase in IAA-Asp in the maternal tissues of tomato fruits is associated with maturation and the competence for ripening (Chirinos et al., 2023). Therefore, we speculate that the decrease in auxin activity in the maternal tissues initiates phase of development that suppress fruit growth and promotes a ripening transition, which permits seed coat senescence and abscission.

Organ polarity factors are targets of the IFA signal(s) that promotes growth arrest

While a number of genes that control fruit size and shape by altering the pattern and rate of cell division have been identified (van der Knaap and Ostergaard, 2018; Snouffer et al., 2020), the developmental basis of auxin mediated expansion of the maternal tissues of the fruit is not well understood. Prior to fertilization, polarity genes that specify the adaxial and abaxial domains in carpels and integuments are critical for the expansion of these determinant organs (Bowman et al., 2002; Kelley and Gasser, 2009; Turchi et al., 2015). In addition, organ polarity genes are implicated in the expansion of pericarp tissues after fertilization in tomato (Clevenger et al., 2015). In this manuscript, results show that the expression of adaxial, *HD-Zip III-like*, *AGO10-like, AS1-like*, as well as abaxial, *ARF3/ETT-like*, genes are downregulated at early and/or later stages of fruit growth arrest in the seed coat. Given that AGO10 functions to sequester *miR166* in the adaxial domain of leaves (Zhu et al., 2010; Yu et al., 2017), the decrease in *AGO10-like* expression in response to growth arrest may result in the cleavage of *HD-ZIP III* transcripts due to an increase in the levels of this microRNA. Further, as *AGO10* is positively regulated by auxin (Zhang et al., 2020), the decline auxin activity may account for reduced *AGO10-like* expression in the maternal tissues during growth arrest. In addition to the decrease in transcript abundance for *HD-ZIP III-like*, *AGO10-like*, *AS1-like* and *ARF3*/*ETT-like* genes, the expression of *KAN1*-like and *AS2-like* genes increased during growth arrest in the seed coat. In Arabidopsis, studies suggest that KAN1 negatively regulates auxin activity in order to specify and maintain the boundary between the adaxial and abaxial domain (Merelo et al., 2013; Xie et al., 2015; Huang et al., 2021). In addition, experimental studies showed that increased levels of *AS2* in leaves negatively regulates cell proliferation, which reduces leaf expansion (Iwakawa et al., 2007). Thus, the increased expression of *KAN1-like* and *AS2-like* may act in the maternal tissues to inhibit auxin activity and expansion of maternal tissues.

The differential expression of adaxial and abaxial genes described above is likely linked to changes in auxin homeostasis and transport induced by the growth arrest signal(s) in the maternal tissues. This is supported by experimental studies indicating that the juxtaposition of the adaxial and abaxial domains forms a narrow boundary that restricts auxin activity to the leaf margin to promote blade expansion (Caggiano et al., 2017; Ram et al., 2020; Heisler, 2021). Further, auxin biosynthesis is required for leaf margin identity and blade expansion (Wang et al., 2011; Martinez et al., 2021). Therefore, we hypothesis that the inhibition of auxin biosynthesis and transport via the growth arrest signal(s) in regions of the seed coat and pericarp that exhibit margin meristem activity perturbs the expression the adaxial and abaxial polarity genes and microRNAs. As result of this inhibitory auxin cascade, the maternal tissues of the fruit undergo growth arrest.

The growth arrest signal targets meristem activity in maternal tissues of fruit

Expansion of leaves and fruits is typically mediated by a phase of cell division followed by a period of cell expansion before these determinate organs reach their final size (Fenn and Giovannoni, 2021; Nakayama et al., 2022). During leaf development, meristem activity is regulated by the formation and migration of the arrest front, which plays a fundamental role

in the determining leaf size and the timing of maturation (Tsukaya, 2021; Nakayama et al., 2022). In contrast to leaf development, the molecular mechanisms that control meristem activity and cell proliferation during fleshy fruit development is poorly understood. The ovary wall and seed coat are derived from the pistil and integument, respectively. Given that integuments and pistils are evolutionarily related to leaves (Mathews and Kramer, 2012; Liu et al., 2023), the transcription factors and networks that control cell proliferation and meristem activity in leaves likely perform a conserved function during the cell division phase of fruit growth. As avocado fruit development is primarily mediated by cell division, we explored the possibility that fruit growth arrest is mediated by key factors that control meristem activity and cell proliferation during leaf development. This hypothesis is supported by the fact that a substantial number of cell cycle factors were downregulated during early and late stages of growth arrest. Moreover, the expression of *ANT-like* and *AN3-like* genes, which act to promote meristem activity and cell proliferation in leaves (Tsukaya, 2021), were differentially expressed during growth arrest in the maternal tissues. Further, results showed that a number of genes, which negatively regulate meristem activity and/or cell proliferation in leaves and/or petals of other species (Hileman, 2014; Tsukaya, 2021), were upregulated during growth cessation, including *CYCL2*, *TCP2-like*, *NGA1-like_1 BOP1/2-like* and *DA1-like*. The early induced expression of *CYCL2* and *BOP2-like* in the seed coat during early growth arrest, indicates that these transcription factors may act to initiate IFA in response to the growth arrest signal(s). Further, the upregulation of *DA1-like* indicates that this F-box protein may play a role in protein ubiquitination and degradation required to suppress meristem activity in response to the growth arrest signa(s).

Class II CYC/TB1 TCP transcription factors are key transcription factors that control leaf and petal expansion (Hileman, 2014). For example, in *Iberis amara*, increased expression of *IaTCP1* (CYC) represses the expansion of adaxial petals via suppression of cell proliferation resulting in the formation of bilateral or monosymmetric flowers (Busch and Zachgo, 2007). In *Gerbera*, functional studies showed that overexpression of *GhCYC2* reduces the growth of leaves, stamens and the petals of ray flowers (Broholm et al., 2008). In contrast, increased expression of *GhCYC2* increased petal size in disk flowers. The opposing functions of *GhCYC2* in ray and disc flowers is not restricted to *Gerbera*, as *CYC* acts to repress and promote the growth of the dorsal stamens and petals in *Antirrhinum*, respectively (Luo et al., 1996). In magnoliid species, including *Aristolochia arborea* and *Persea americana*, *CYC2-like* (*CYCL2*) genes were identified and functional studies were performed in *Arabidopsis* (Horn et al., 2015). In contrast to *IaTCP1*, increased expression of *AarCYCL2* did not alter petal size (Busch and Zachgo, 2007; Horn et al., 2015). Thus, ability of CYC2 and CYCL2 to repress or promote growth is likely influenced by tissue specific factors, which modify the activity of these class II TCP transcription factors. Given that *CYCL2* is highly induced during early and a later stage of avocado fruit growth arrest, we propose that this class II TCP transcription factor represses meristem activity in the seed coat and pericarp in association with another unknown transcription factor(s).

During leaf development, BOP1/2 transcription factors control proximal-distal patterning of leaves (Ha et al., 2003; Ha et al., 2007). In the proximal domain of the leaves, BOP1/2 functions to suppress meristem activity (Ha et al., 2007), as well as negatively regulates blade formation during juvenile development in *Oryza longistaminata* (Toriba et al., 2020). Further, experimental studies indicate that BOP1/2 suppresses meristem activity by activating *AS2* (Jun et al., 2010). In light of these studies, it has been proposed that BOP1/2 act as negative regulators of meristem activity by promoting differentiation during leaf development (Jun et al., 2010; Tsukaya, 2021). Given that BOP1/2 and AS2 are upregulated during growth arrest suggests that this regulatory module also acts to suppress meristem activity in the maternal tissues during IFA. Further, this regulatory module may suppress growth by promoting a maturation phase of development.

IFA induces seed dormancy determinants during a late stage of growth arrest

After the growing phase of development, seeds undergo maturation, initiated by signals in the maternal tissues, including FT, as well as MFT in the embryo (Iwasaki et al., 2022). Further, during the seed maturation, FT and MFT also act to suppress and promote dormancy in the seed coat and embryo, respectively (Footitt et al., 2011; Nakamura et al., 2011; Chen et al., 2014). In our study, we showed that *FT-like_1* and *FT-like_2* are downregulated during an early and late stage of growth arrest in the seed coat. *FT-like_1* and *FT-like_2* are also downregulated in the pericarp. While *MFT-like* is repressed in the maternal tissues, this dormancy signaling gene is induced at a later stage of embryo arrest. The regulation of *FT-like_1/2* and *MFT-like* in the seed coat and embryo, respectively, suggests that the seeds in arresting fruits transition to a dormant-like state rather undergo an abortive process due to developmental arrest. This hypothesis is further supported by the fact that growth arrest is associated with an increase in the levels of ABA the pericarp, as well as starch accumulation in the embryo (Böttcher et al., in preparation; Haberman et al., in preparation). Lastly, it is tempting to speculate that the induction of a dormant-like state in the seed is mediated by *TEM1/2*, which are induced during early and late stages of growth arrest in the seed coat and embryo. As TEM1/2 acts to repress flowering by negatively regulating *FT* in leaves (Castillejo and Pelaz, 2008; Hu et al., 2021), these transcription factors may carry out a similar function to down-regulate *FT-like_1* and *FT-like_2* in maternal tissues as a means to induce a dormant-like state in the embryo during growth arrest. Further, in the embryo, TEM1/2 may also act to inhibit growth as part of the dormancy-like response induced by the growth arrest signal(s).

Conclusion

Based on our study, we propose that IFA is mediated by an unknown signal(s) that promotes a fruit maturation-like phase of development that is generated in the seed coat before expanding to the pericarp and embryo to induce growth arrest. The proposed shift from growth to the maturation is mediated by a decrease in auxin activity, which results in the differential expression of adaxial/abaxial polarity genes in the seed coat and pericarp. Further,

the significant increase in the expression of *CYCL2* and *BOP1/2* suggests that these transcription factors may play a key role in mediating the switch from meristem mediated growth to maturation. In addition, the induction of *TEM1/2-like* expression indicates that these transcription factors act to promote seed dormancy via downregulation of *FT-like* genes in the maternal tissues. Together, the maturation signaling cascade initiated in the seed coat and transmitted to the pericarp and embryo induces a ripening transition, which promotes seed coat senescence and permits the arrested fruitlets to undergo abscission.

Materials and Methods

Plant material and fruit growth rate determination

Three to four-year old avocado, *Persea americana* cv. 'Hass', trees maintained at a well-managed orchards in south-western Western Australia (-33.63557, 115.46191) and in the Riverland of South Australia (-34.070312, 140.818789) were selected for this study. To determine if fruits undergo growth arrest prior to abscission, 52 Phytech fruit dendrometers (https://www.phytech.com) were utilized to capture daily changes in the diameter of randomly developing fruitlets derived from 'Hass' trees maintained in the Riverland, South Australia (-34.070312, 140.818789). The diameter measurements were utilized to determine the average growth rate (mm/d) of persisting fruitlets over a 50-day period. The average growth rate (mm/d) of fruitlets that abscised was determined by collating the daily changes in the diameter of fruitlets prior to abscission. Cumulative abscission was determined by scoring the percentage of fruitlets that abscised over the course of the trial. From this data, the percentage of fruits that abscised per day was determined.

Natural fruitlet abscission trial

To identify developmental pathways and genes involved in fruitlet growth arrest, two independent trials were established at an orchard in south-western Western Australia (-33.63557, 115.46191). In the natural fruitlet abscission trial, approximately 37 fruits were randomly tagged in six trees (222 fruits total). Each fruitlet was assigned a number and marked with a permanent pen on opposite sides of the fruit. At regular time intervals, the diameter of each fruitlet was measured by placing the calipers (Kincrome K11105 Digital, Melbourne, Australia) on the markings and recording the value. The fruitlet diameter measurements were used to calculate the growth rate (mm/d) for each fruitlet over the course of the trial. When fruits reached an average diameter of 43.8 mm, five fruits that maintained a normal growth rate of 0.49 mm/d were harvested. At the same time, five fruits that switched from a normal to low growth rate of 0.03 mm/day where collected. After harvesting the normal growing and arresting fruitlets, the embryo, seed coat and pericarp tissues were separated, frozen on dry ice and shipped to the CSIRO laboratory at the Waite Campus for RNA extraction, IAA and IAA-Asp quantification.

Defoliation induced fruitlet abscission trial

In the second trial, 100 fruits were randomly tagged across three trees before they were defoliated. In addition, 80 fruits were randomly tagged in untreated/control trees. This trial was initiated when the average diameter was 36 mm. Each tagged fruitlet was assigned a number. After marking the opposites sides of each fruitlet with a permeant pen, the diameter was measured using a Kincrome K1105 digital caliper over the course of the trial. Fruitlet diameter measurements were performed 1, 4, 6 and 12 days after defoliation in the treated and control trees. Six-days after defoliation, five fruits with a normal growth rate in the untreated control and defoliated trees, which displayed average growth rates of 0.65 and 0.60 mm/d, respectively, were harvested. In addition, five fruits that switched to an average growth rate of 0.10 mm/day were also harvested from defoliated trees at this time point. All harvested fruitlets were separated into embryo, seed coat and pericarp samples, frozen on dry ice and shipped to the CSIRO laboratory at the Waite Campus for RNA extraction.

Extraction of RNA from fruit tissues

Total RNA was extracted using the Sigma Spectrum Plant Total RNA kit (Sigma-Aldrich Pty. Ltd., Sydney, NSW, Australia, #STRN250) according to the manufacturer's instructions. RNA was eluted once with 50µl of Elution Buffer and DNA was digested with the TURBO DNA-free kit (ThermoFisher Scientific, Adelaide, SA, Australia, #AM1907) in a total volume of 60µl following the manufacturer's instructions. Library preparation and sequencing were performed using the Australian Genome Research Facility Ltd (AGRF, Melbourne, Australia) RNA sequencing service. Libraries were sequenced on an Illumina NovaSeq 6000 system (Illumina Inc, San Diego, CA, USA). Sequenced reads were 150 bp long with paired-ends (PEs).

Transcriptomic analysis

In total, 105 RNA-seq libraries were sequenced and a total of ~4.44 billion PE raw reads were obtained with an average of 42 million reads per library. The overall quality of the sequencing data was assessed using FastQC v0.11 (Andrews, 2010). Reads were trimmed using Trim Galore v0.6.6 in paired-end mode to remove adapters and low-quality sequences (Krueger, 2021). After trimming the reads, transcripts were assembled using Cufflinks v2.2.1 (Trapnell et al., 2010) and the STAR v2.7 package (Dobin et al., 2013) was utilized to align the transcripts to the *Persea americana* cv. 'Hass' genome containing 8,135 contigs and 25,211 predicted genes (Rendon-Anaya et al., 2019). The number of reads per gene were counted using the HTSeq package with default parameters (Putri et al., 2022). All sequenced transcripts were queried with the Basic Local Alignment Search Tool X (BLASTX) (Altschul et al., 1990), using ncbi-blast[8] v2.10.1+ against the non-redundant protein sequence database (nr) and the Swiss-Prot protein sequence database (sp) in BLASTDB v5. Additional BLASTX runs were performed against the same databases restricting the results by Taxonomy ID: 3702 to *Arabidopsis thaliana*. Based on these BLASTX runs, the 25,211 predicted genes were annotated using the top hits by BLASTX identity less than 1e-3, prioritizing Swiss-Prot accession numbers and gene descriptions for *A. thaliana* wherever present.

The read count matrix from the RNA-Seq analysis was subjected to differential gene expression analysis using the Bioconductor R (v. 4.0.2) package DESeq2 v.1.28.1 (Love et al., 2014). The DESeq2 package, based on the negative binomial distribution, was used to normalize the RNA-seq data and to identify differentially expressed genes with an adjusted *p*-value of 0.05 (Likelihood Ratio Test, sample size $n = 5$) and a fold-change of more than 2x (i.e. log2FC ≥ 1). Prior to data visualization, the variance stabilizing transformation was used within the DESeq2 package to remove the dependence of the variance on the mean.

IAA and IAA-Asp quantification

To quantify IAA and IAA-Asp, 50 mg of seed coat, pericarp and embryo samples derived from normal growing and arresting fruits was ground to a fine powder in liquid nitrogen using the Cryomill cryogenic grinder (Retsch®, Haan, Germany) and extracted in 1.0 mL of ice-cold 50% (v/v) aqueous acetonitrile containing 500 pmol of deuterated IAA-d5 (Cambridge Isotope Laboratories, Andover, USA) and IAA-Asp-d5 (Böttcher et al., 2010) as previously described (Clayton-Cuch et al., 2021). Briefly, after vortexing and sonicating the samples at 4^0C, the samples were centrifuged at 14,000xg and the supernatant from each sample was loaded onto a HLB-SPE cartridge (30 mg, Waters, Wexford, Ireland) pre-conditioned with methanol, nanopore water and 50% (*v*/*v*) acetonitrile. After collecting the flow through, the cartridge was rinsed with 1.0 mL 30% (*v*/*v*) acetonitrile and the flow through and eluate was combined and dried at 50^0C in a vacuum concentrator (SP Genevac miVac, Ipswich, United Kingdom). Dried samples were resuspended in 50 µL 30% (*v*/*v*) acetonitrile

IAA and IAA-Asp were analyzed by liquid chromatography with tandem mass spectroscopy (LC-MS/MS) as previously described (Böttcher et al., 2010; Clayton-Cuch et al., 2021). Briefly, samples were analyzed on the Agilent 1260 Infinity II HPLC (Agilent, Santa Clara, CA, USA) with an Agilent 6470 Triple Quad mass spectrometer equipped with a jet stream ionization source. A Luna C18 column (75 × 4.6 mm, 5 µm; Phenomenex, Torrance, CA, USA) was used to separate metabolites after injecting 10 µL of each sample. IAA and IAA-Asp were eluted with a gradient of 0.01% formic acid in water and 0.01% formic acid in 90% (v/c) acetonitrile with a flow rate of 0.35 mL/min. Multiple reaction monitoring-mass spectrometry was used to detect IAA and IAA-Asp.

Statistics

Measurements were taken from multiple biological samples in which $n=5$. Statistical differences were determined using the two-tailed Student's t-test. Comparisons with statistically significant *p*-values were provided.

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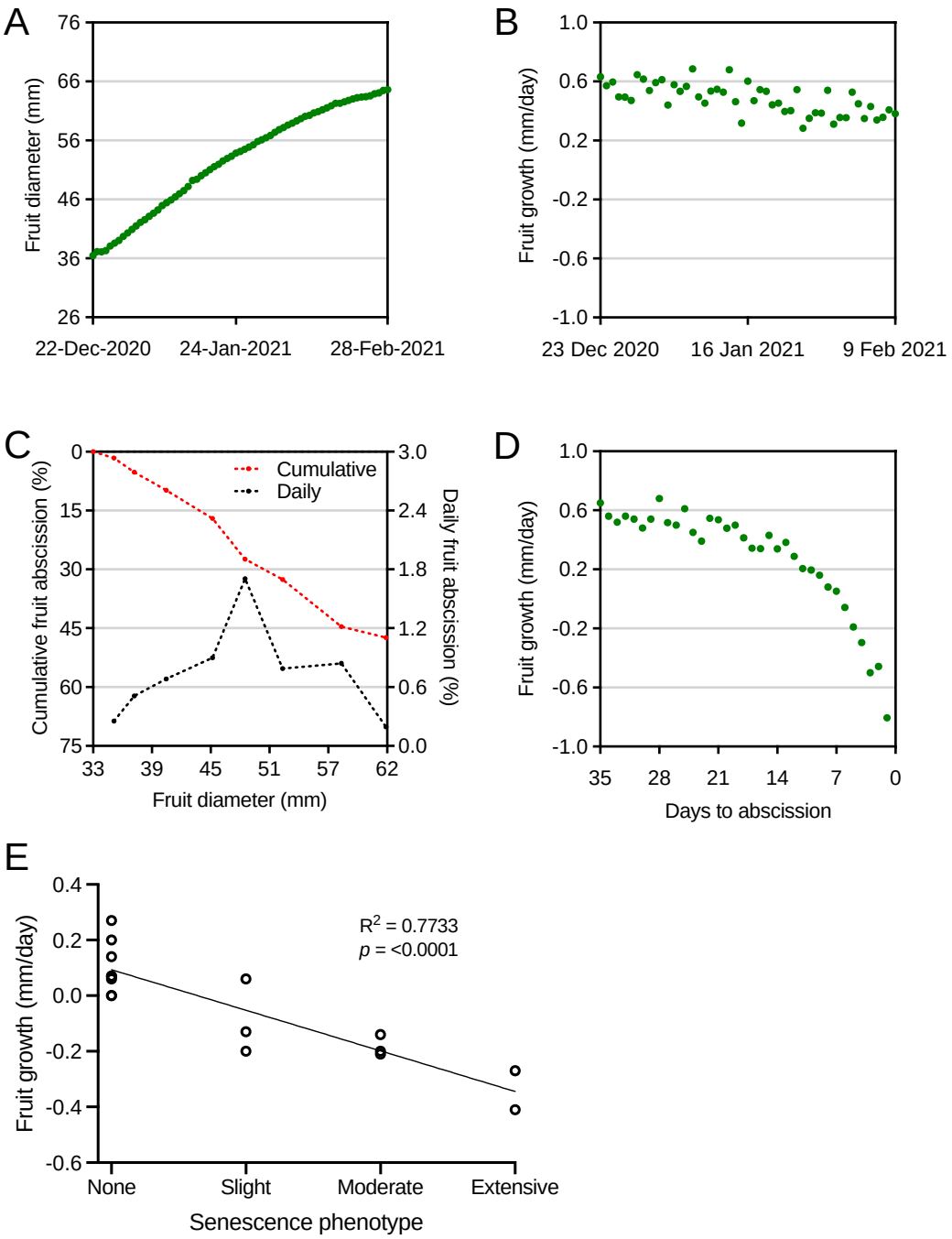


Figure 1. Fruit growth arrest is a primary event of IFA
(A) A steady increase in the diameter of immature fruits occurs during the growing phase of fruitlet development. (B) The growth rate (mm/d) is displayed for persisting fruitlets. (C) Cumulative (red) and daily (black) percentage of fruitlet abscission during the growing phase of the trial. (D) The growth rate of fruitlets that abscised during the trial was collated and displayed. (E) Correlation of seed coat senescence during late growth arrest and shrinkage stages of IFA.

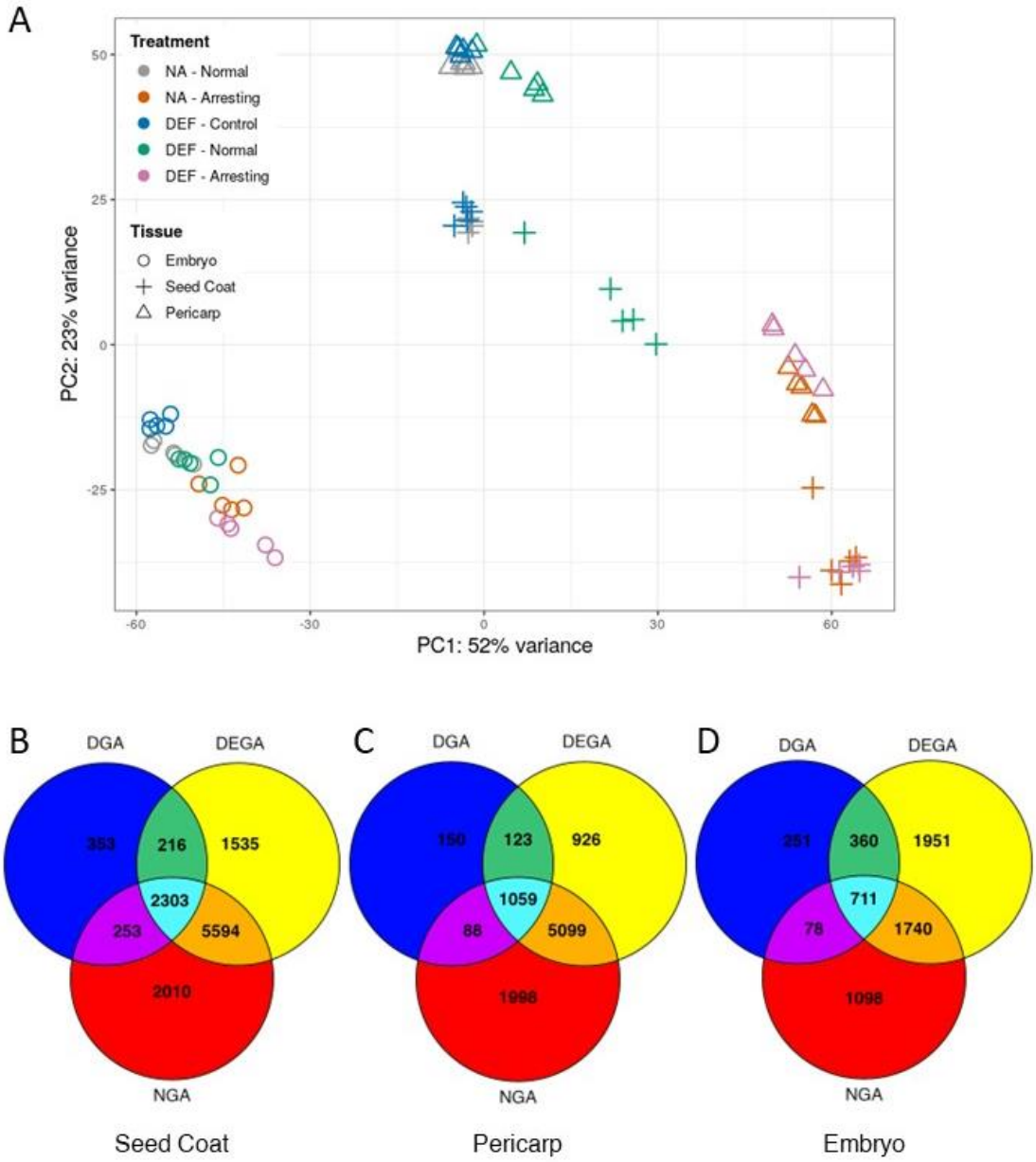


Figure 2: The maternal tissues are highly responsive to the growth arrest signal(s)
(A) Principal component analysis for the transcriptomes of the seed coat, pericarp and embryo derived from fruitlets undergoing arrest naturally (NA) and in response defoliation (DEF). Variance is displayed as a percentage for principal component 1 and 2. The transcriptomes of the seed coat, pericarp and embryo derived from normal growing and arresting fruitlets, NA-Normal and NA-Arresting, respectively, are shown. In addition, the transcriptomes of the seed coat, pericarp and embryo derived from normal growing (DEF-Normal) and arresting (DEF-Arresting) fruitlets harvested from defoliated trees were compared to normal growing fruitlets collected from untreated control trees (DEF-Control). (B) Venn diagrams displaying shared and uniquely differentially expressed genes between the seed coat, pericarp and embryo of arresting and normal growing fruitlets that undergo IFA naturally and in response to defoliation (DEF).

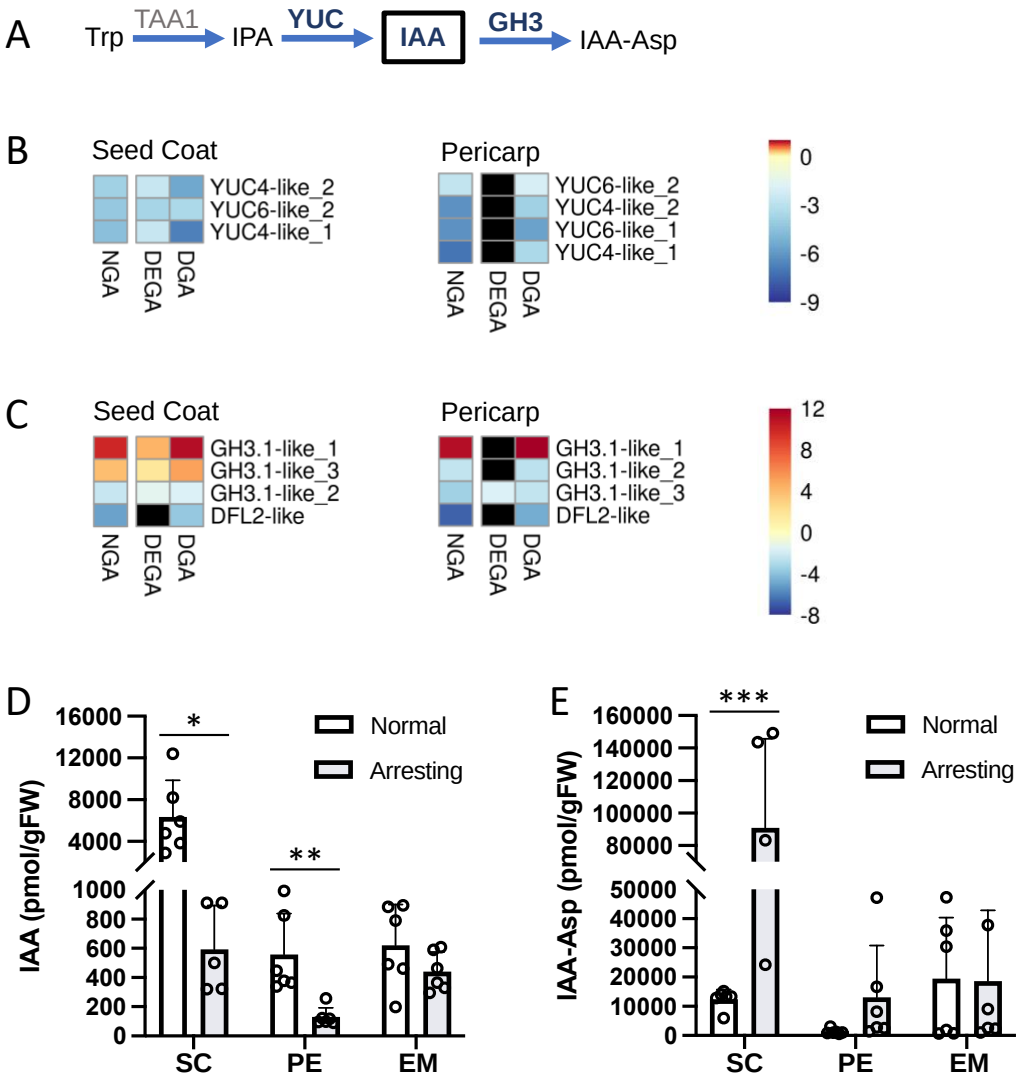


Figure 3. Regulation of auxin levels in response to fruit growth arrest
(A) Free indole-3-acetic acid (IAA; blue box) is regulated by the TAA1/YUC biosynthesis pathway, as well as GH3-conjugation with aspartic acid (Asp) and/or other amino acids. Differential expression of (B) *YUC4/6-like* and (C) *GH3.1-like*/*DFL2-like* in the seed coat and pericarp of fruits that underwent growth arrest naturally (NGA; *n*=5). Defoliation was utilized to harvest fruitlets at an early (DEGA) and late (DGA) stage of growth cessation (*n*=5). Log_2 fold change of expression is displayed by a gradient color scale. (D) Free IAA and (E) IAA-Asp levels were quantified in the seed coat (SC), pericarp (PE) and embryo (EM) in normal growing (dark bars) and arresting fruits (light bars). The levels of IAA and IAA-Asp are displayed in pmole/g of fresh weight (FW) with means ±SD calculated from six biological replicates for all samples except the arresting seed coat (SC), which was derived from five biological replicates. **p*=0.0055, ***p*=0.0044, ****p*=0.0064.

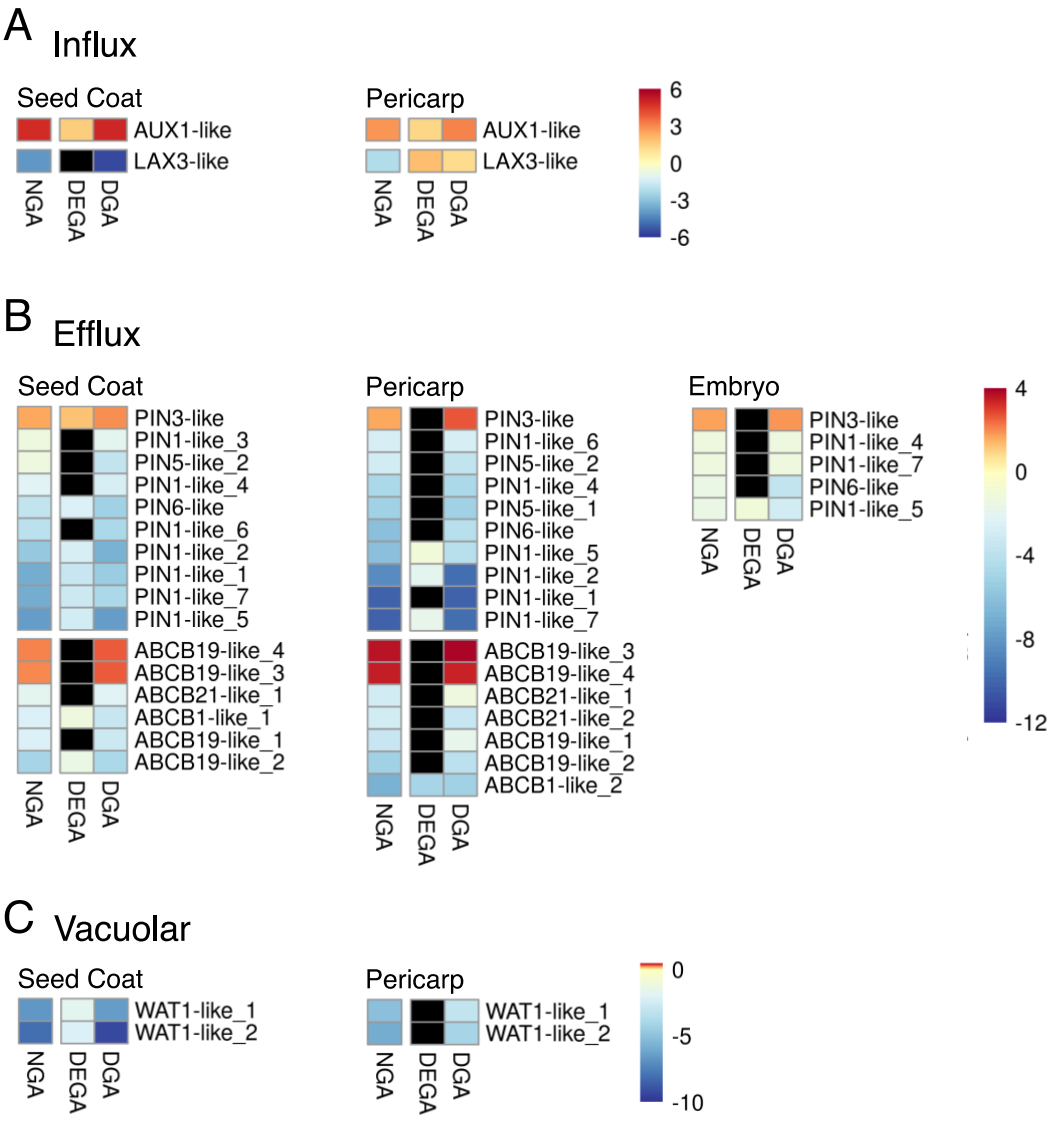


Figure 4. Growth arrest is associated with the differential expression of genes that regulate auxin transport.

Differential expression of genes involved in auxin (A) influx, (B) efflux and (C) transport across the tonoplast of the vacuole in the seed coat, pericarp during fruit growth arrest. (B) Genes involved in auxin efflux were also differentially expressed in the embryo. Differential expression of genes that occurred during natural growth arrest (NGA) is shown (*n*=5). Defoliation was utilized to harvest fruitlets at an early (DEGA) and late (DGA) stage of growth cessation (*n*=5). Log_2 fold change of expression is displayed by a gradient color scale.

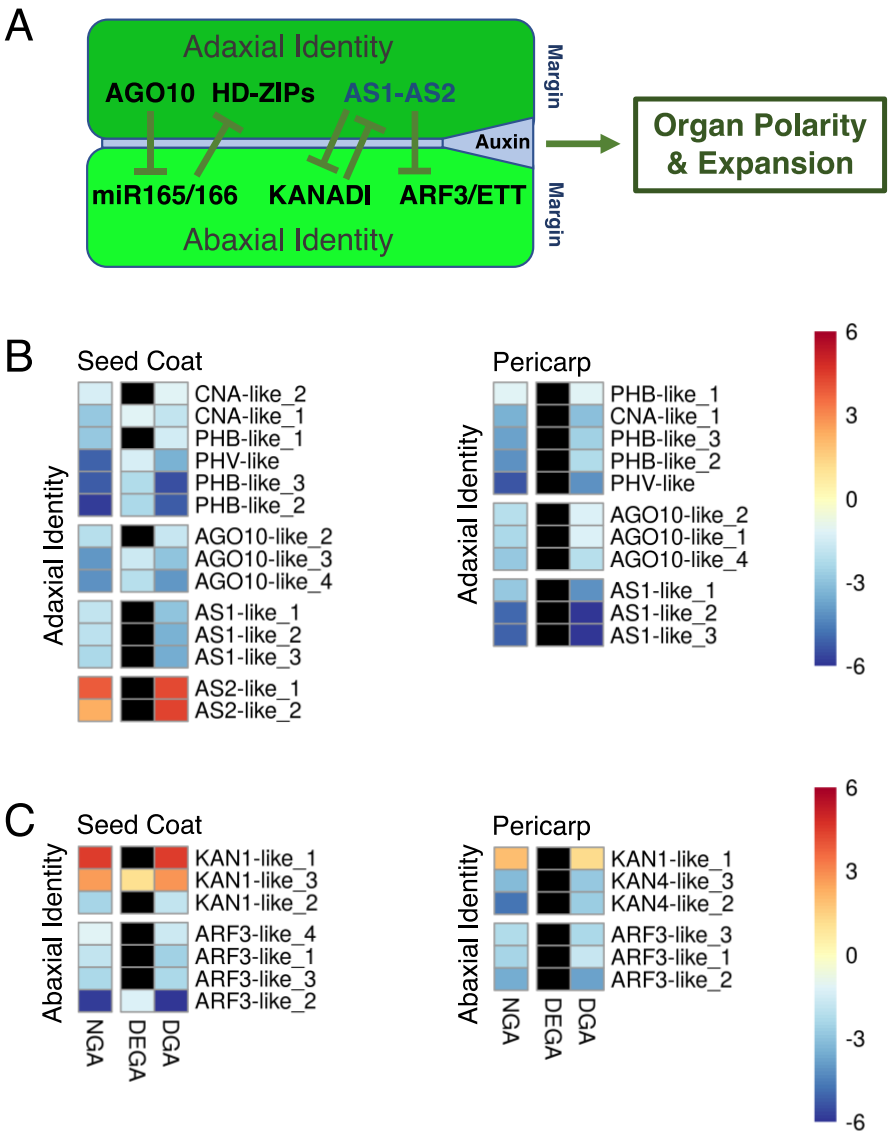


Figure 5. Differential expression of adaxial/abaxial polarity genes is associated with fruit growth arrest

(A) Interplay between adaxial and abaxial factors regulates blade expansion in leaves by confining auxin activity to boundary between these domains. Differential expression of genes involved in specifying (B) adaxial and (c) abaxial domains of determinant organs. Differential expression of genes that occurred during natural growth arrest (NGA) is shown (*n*=5). Defoliation was utilized to harvest fruitlets at an early (DEGA) and late (DGA) stage of growth cessation (*n*=5). Log_2 fold change of expression is displayed by a gradient color scale.

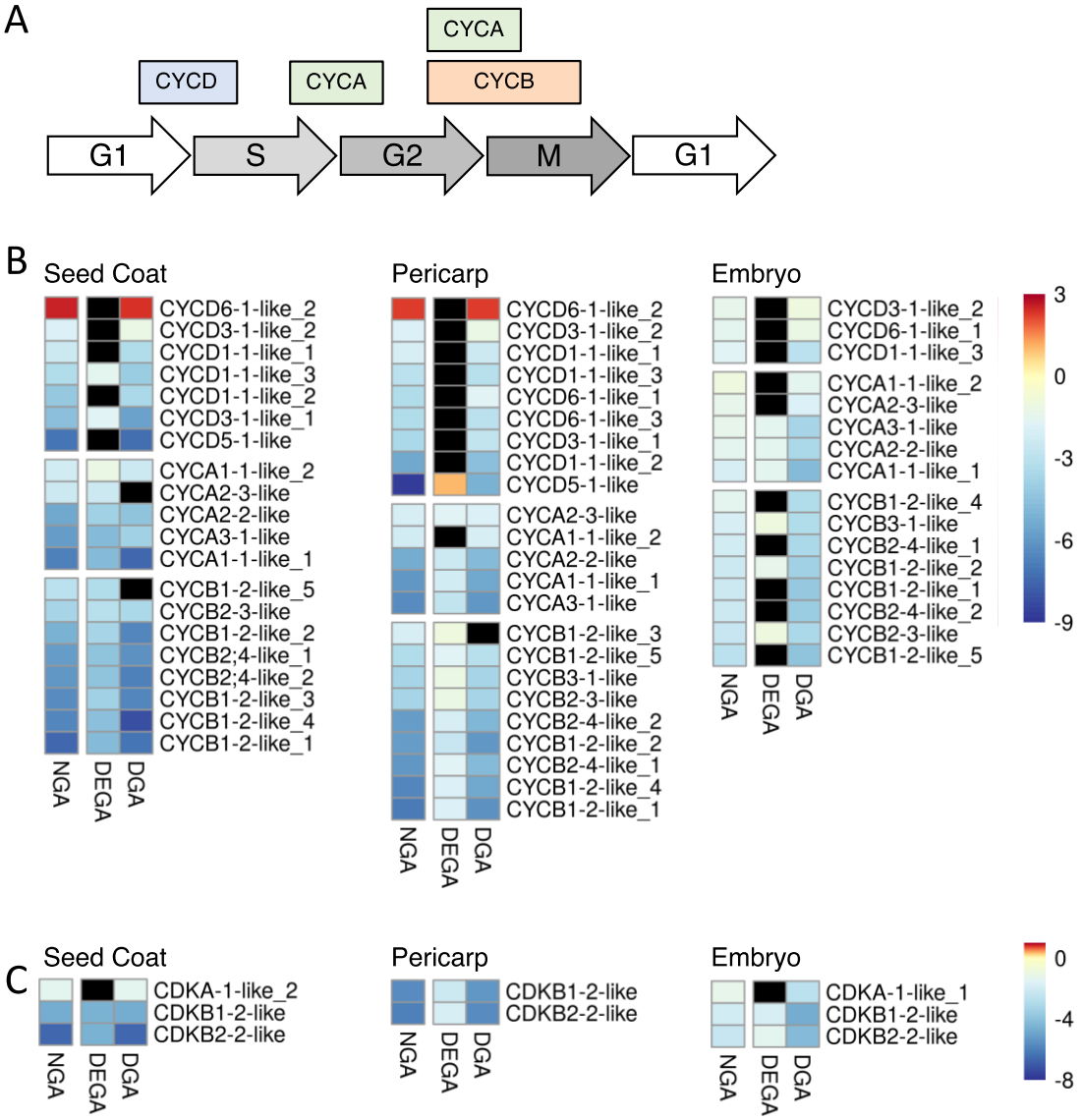


Figure 6. Fruit growth arrest is associated with the differential expression of genes involved in regulating the cell cycle

(A) Phases of the cell cycle from G1 to M are displayed with cyclins (CYC) that regulate the growth (G), DNA-synthesis (S) and Mitotic (M) phase transitions. Differential expression of (B) CYCD, CYCA and CYCB and (C) cyclin dependent kinases (CDK) genes involved in regulating the cell cycle phase transitions. Differential expression of genes that occurred during natural growth arrest (NGA) is shown (*n*=5). Defoliation was utilized to harvest fruitlets at an early (DEGA) and late (DGA) stage of growth cessation (*n*=5). Log_2 fold change of expression is displayed by a gradient color scale.

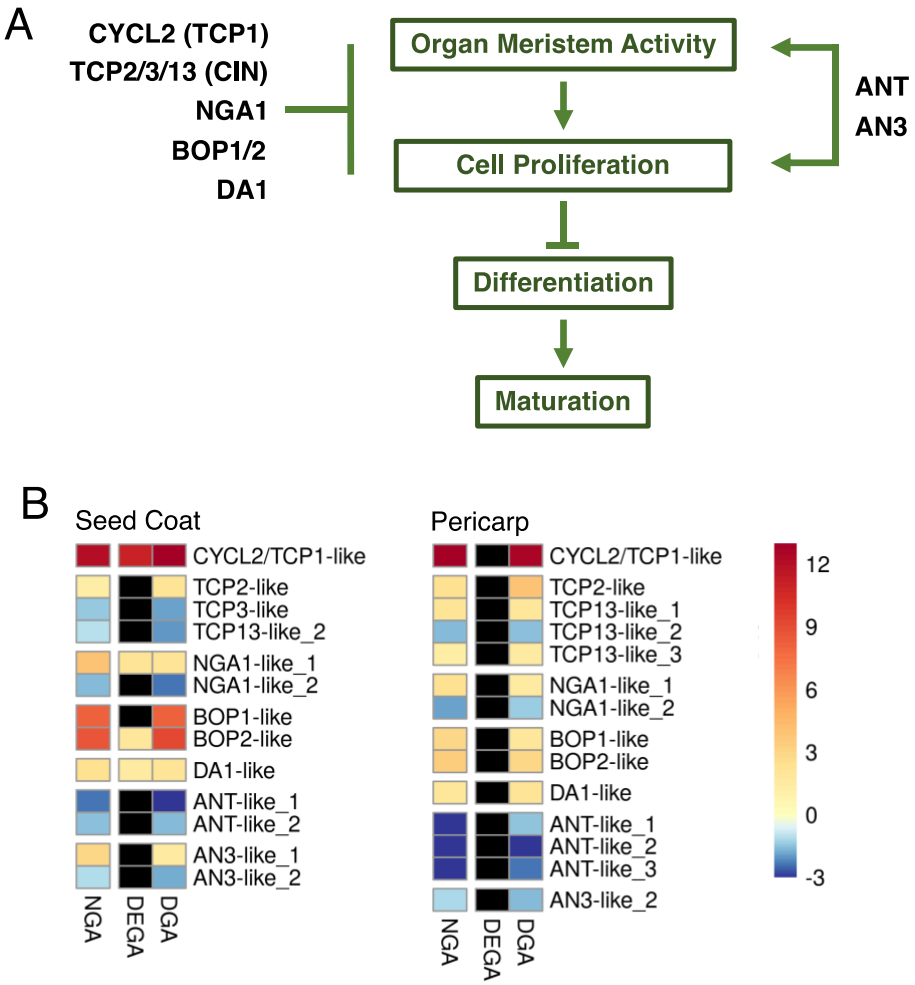


Figure 7. Differential expression of genes that regulate meristem activity and cell proliferation during growth arrest.

(A) Organ meristem activity promotes cell proliferation during organ expansion. Meristem activity and cell proliferation is controlled by genes that encode regulatory proteins that promote or suppress cell proliferation during organ development. The suppression of meristem activity mediates the transition from cell proliferation to differentiation and maturation. (B) Differential expression of genes involved in regulating the meristem activity and transition from cell proliferation to differentiation and maturation. Differential expression of genes that occurred during natural growth arrest (NGA) is shown (*n*=5). Defoliation was utilized to harvest fruitlets at an early (DEGA) and late (DGA) stage of growth cessation (*n*=5). Log_2 fold change of expression is displayed by a gradient color scale.

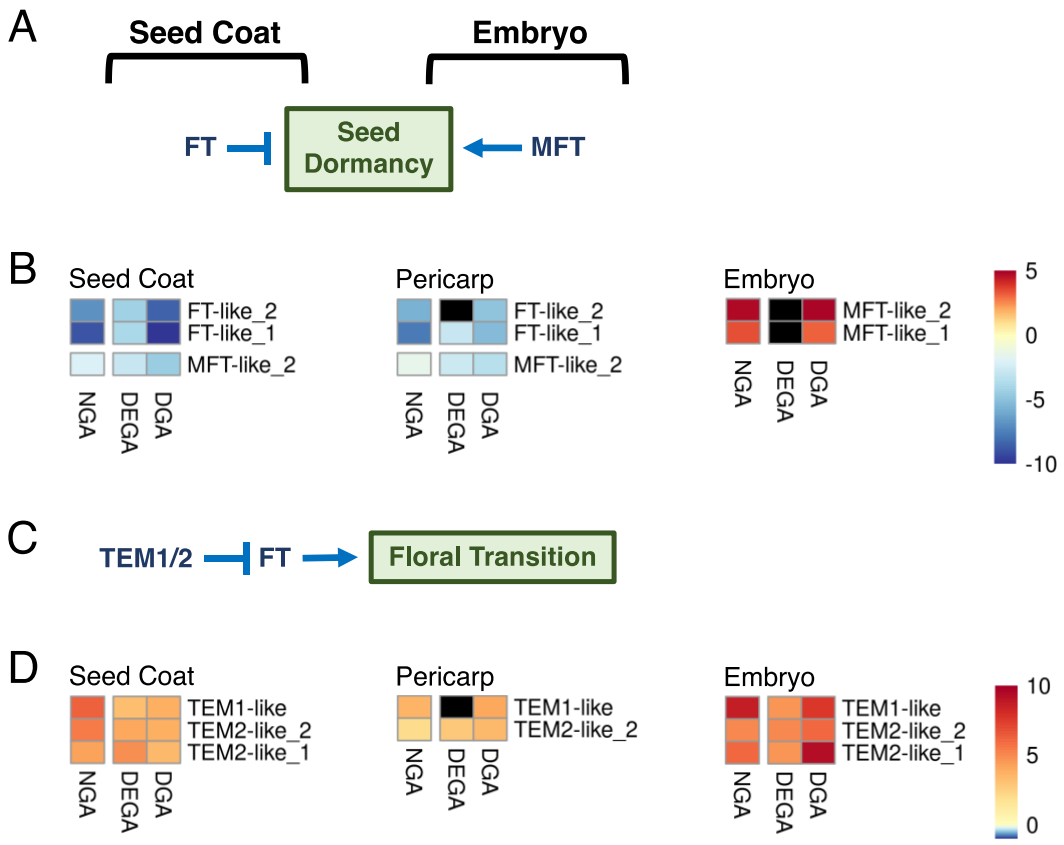
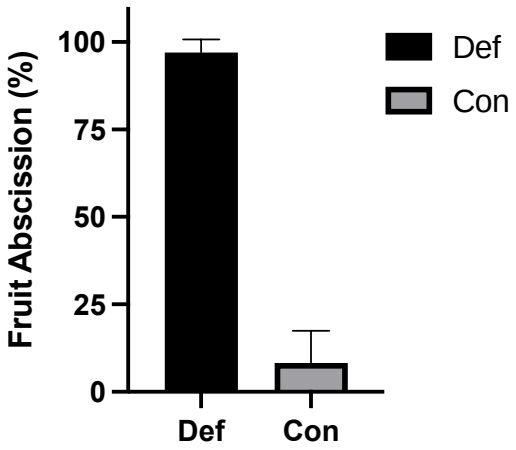
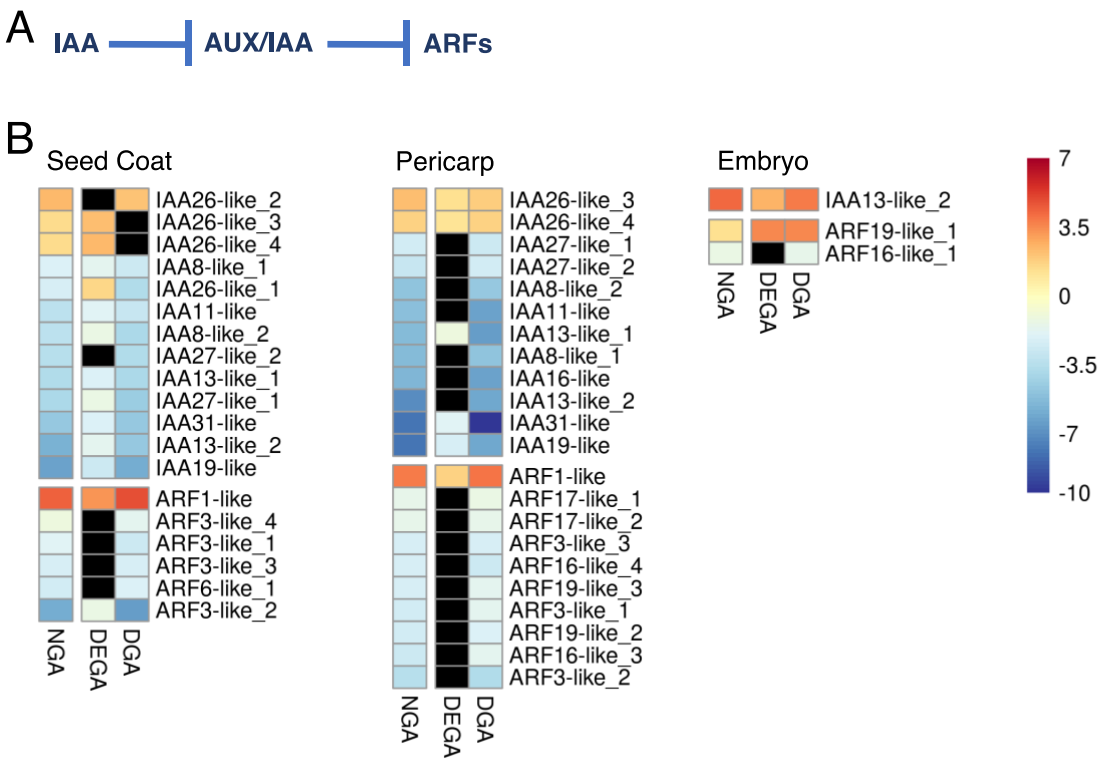


Figure 9. Differential expression of seed dormancy signaling proteins during growth arrest
(A) In the seed coat, FT acts to suppress the dormancy potential of the seed. In contrast, MFT acts in the embryo to promote seed dormancy. (B) In the seed coat, *FT-like_1/2* and *MFT-like* were down-regulated in response to growth arrest. Transcript abundance for *MFT-like* increased in embryo during late growth arrest. (C) TEM1/2 suppress the floral transition via down-regulation of *FT*. (D) In the seed coat, pericarp and embryo *TEM1/2-like* genes increased during growth arrest. Differential expression of genes that occurred during natural growth arrest (NGA) is shown (*n*=5). Defoliation was utilized to harvest fruitlets at an early (DEGA) and late (DGA) stage of growth cessation (*n*=5). Log_2 fold change of expression is displayed by a gradient color scale.



Supplementary Figure 1. Defoliation is an effective treatment to induce immature fruit abscission. Fruit abscission (%) was evaluated in defoliated (Def) and untreated control (Con) trees. A subset of fruits tagged at the start of the trial was scored for abscission six weeks after the treated trees were defoliated (*n*=6).



Supplementary Figure 2: Differential expression of transcription factors that mediate auxin response during growth arrest.
(A). A general pathway for auxin mediated response in plants. (B) Differential expression of Aux/IAA proteins (IAAs) and Auxin Response Factors (ARFs) during growth arrest. Differential expression of genes that occurred during natural growth arrest (NGA) is shown (*n*=5). Defoliation was utilized to harvest fruitlets at an early (DEGA) and late (DGA) stage of growth cessation (*n*=5). Log_2 fold change of expression is displayed by a gradient color scale.