Hort Innovation

Final Report

Inter-seasonal irregular bearing

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Inter-seasonal irregular bearing (AV23000)

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Public summary

Irregular bearing is a significant challenge that negatively impacts productivity and limits market development for the Australian avocado industry. The irregular bearing nature that underlies variation in annual yield is due to the fact that avocado experiences poor fruit set, high fruitlet abscission and biennial bearing, all of which are influenced by the environment. Currently, there are no effective tools to manage the drivers of irregular bearing, including the summer fruitlet abscission event.

Research from AV16005 demonstrated that fruitlets undergo growth arrest prior to abscission during the summer fruit drop event. At a late stage of fruitlet growth arrest, experimental studies suggest that a senescence program of development is induced in the seed coat that likely acts to trigger abscission. Interestingly, fruitlet growth arrest is associated with a carbohydrate starvation response that appears to change hormone activities that results in the activation of seed coat senescence. Thus, it has been speculated that a reduction in summer fruit abscission could be achieved by a plant growth regulator (PGR) application(s) that acts to maintain seed coat function and block senescence when carbohydrate availability becomes limiting.

The first objective of AV23000 was to develop an understanding of PGRs that act to reduce summer fruit abscission. To achieve this goal, PGRs that have the potential to maintain seed coat function and prevent senescence were identified and evaluated across two-seasons. Here, the project team identified working concentrations for two PGRs that showed potential to reduce summer fruit abscission. At the specified concentrations, these PGRs appear to have little impact on seed coat integrity and fruit development across two-seasons. The project team also investigated whether the outgrowth of the summer vegetative flush could be a factor that contributes to the summer fruit abscission event. In support of this hypothesis, experimental results indicate that the period in which the greatest number of shoots in the canopy undergo extension coincides with the greatest level of fruit drop. Thus, managing summer fruit drop may involve using PGRs that maintain fruit development and inhibit the growth potential of the summer flush. Together, this body of work lays the groundwork for developing a practical application to reduce summer fruit abscission.

The second objective of the project was to clarify the opportunity to limit irregular bearing using PGRs. Here, the project team published an article in Talking Avocados titled “Opportunities to manage irregular bearing in avocado” in 2024. In addition, the project leader utilized three speaking invitations at regional avocado forums to discuss the opportunity to manage irregular bearing using PGRs. These presentations also covered other technologies, including spray induced gene silencing and genome engineering, that may also have the potential to reduce the impacts of irregular bearing on productivity and market development.

Technical summary

Irregular bearing is a significant challenge that negatively impacts productivity and limits market development for the Australian avocado industry. The irregular bearing nature that underlies variation in annual yield is due to the fact that avocado experiences poor fruit set, high fruitlet abscission and biennial bearing, all of which are influenced by the environment. Currently, there are no effective tools to manage the drivers of irregular bearing, including the summer fruitlet abscission event.

Research from AV16005 demonstrated that fruitlets undergo growth arrest prior to abscission. Growth arrest is associated with a carbohydrate starvation response and a decrease in auxin activity that appears to induce a maturation phase of development, which likely allows 1-aminocyclopropane-1-carboxylic acid, the precursor of ethylene, and jasmonic acid to induce seed coat senescence. Further, we propose that seed coat senescence acts as a developmental switch that triggers fruitlet abscission. Based on this model, we propose that management of summer fruit abscission relies on the development of a practical method(s) that acts to maintain auxin activity and block senescence in the seed coat when carbohydrate availability becomes limiting.

The first objective of AV23000 was to develop an understanding of PGRs that act to reduce summer fruit abscission. To achieve this goal, PGRs that have the potential to maintain auxin activity and prevent seed coat senescence were identified and evaluated across two-seasons. Here, the project team identified working concentrations of NAA (5 mg/L) and 1-MCP (150 mg/L) that showed potential to reduce summer fruit abscission and increase yield. Results indicate that these working concentrations of NAA and 1-MCP have little impact on seed coat integrity and fruit development across two-seasons. The project team also investigated whether the outgrowth of the summer vegetative flush could be a factor that contributes to the summer fruit abscission event. In support of this hypothesis, experimental results indicate that the period in which the greatest number of shoots in the canopy undergo extension coincides with the greatest level of fruit drop. Thus, managing summer fruit drop may involve using PGRs that not only maintain seed coat integrity but also reduce the growth potential of the summer flush. Together, research derived from AV23000 lays the groundwork for developing a practical application to reduce summer fruit abscission in a future project.

The second objective of the project was to clarify the opportunity to limit irregular bearing using PGRs. Here, the project team published an article in Talking Avocados titled “Opportunities to manage irregular bearing in avocado” in 2024. In addition, the project leader utilized three speaking invitations at regional avocado forums to discuss the opportunity to manage irregular bearing using PGRs. These presentations also covered other technologies, including spray induced gene silencing and genome engineering, that may also have the potential to reduce the impacts of irregular bearing on productivity and market development.

Keywords

Avocado, fruit abscission, plant growth regulators, hormones

Introduction

Yield variability is a major challenge for domestic and export market development for the Australian Avocado Industry. In the 2022-2026 Avocado strategic plan, one of the key strategies to improve supply, productivity and sustainability is developing orchard management practices to increase production and yield consistency by leveraging knowledge from the underlying physiological processes that drive irregular bearing. The summer fruit drop event is a major factor that contributes to the low production potential of avocado, as well as fluctuations in annual yield. Therefore, developing management practices to reduce the summer fruit drop event has the potential to increase production and reduce yield variability in avocado. Moreover, reducing the impact of summer fruit abscission on yield variability would also be critical for domestic and export market development. Research in AV23000 builds on new knowledge that was developed in AV16005, which provided a framework for the physiological and developmental basis of summer fruit abscission. Here, experimental results from AV16005 indicate that fruitlet growth arrest is the primary step in the abscission process (Figure 1A; Smith and Goetz, 2023), which is initiated by a decrease in carbohydrate supply. Further growth arrest is coupled with the activation of a senescence program of development in the seed coat that likely acts to trigger abscission. As hormones, such as auxin, regulate the uptake and metabolism of carbohydrates (Eveland and Jackson, 2012), as well as the responses to sugar starvation and senescence, the hormonal control of seed coat senescence was investigated in AV16005. Experimental studies indicate that the senescence program of development is driven by a reduction in the main auxin, indole-3-acetic acid (IAA), followed by an increase in 1-aminocyclopropane-1-carboxylic acid (ACC) and jasmonic acid (JA) in the seed coat (Figure 1B; Smith and Goetz, 2023). Therefore, we speculate that managing summer fruitlet abscission will require a method to maintain seed coat function by sustaining auxin activity and inhibiting the action of ACC and/or JA. Therefore, a potential pathway for mitigating summer fruit abscission will require the usage of a plant growth regulator(s) that target auxin, ACC and/or JA.

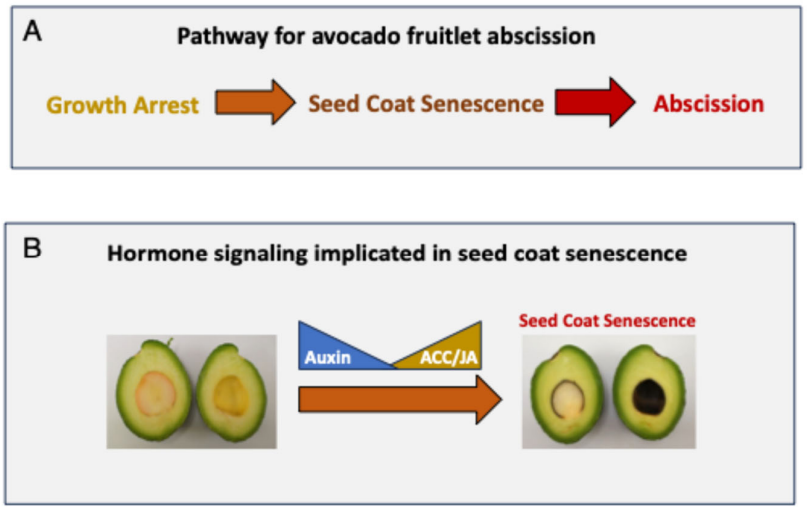

Figure 1. (A) Avocado fruit abscission is initiated by growth arrest followed by seed coat senescence, which triggers abscission. (B) Diagram of hormonal control of seed coat senescence. While auxin inhibits seed coat senescence, ACC and JA are hormones that promote this degenerative program of development.

Methodology

One of the objectives of AV23000 is to develop an understanding of PGRs for limiting summer fruitlet abscission. Here, we proposed that management of summer fruitlet abscission could be achieved by maintaining auxin activity to suppress the activation of the seed coat senescence. Here, we used NAA-20, which contains the highly stable synthetic auxin, 1-naphthaleneacetic acid (NAA; Figure 2A). In our trials, NAA was applied to trees at 5, 15 and/or 25 mg/L. We also speculated that inhibiting ethylene signaling would suppress seed coat senescence and abscission. As ACC derived-ethylene likely promotes senescence (Gepstein and Thimann, 1981; Woodson *et al.*, 1985; Skirycz *et al.*, 2011; Vanderstraeten *et al.*, 2019), we utilized Harvista, which contains the active ingredient, 1-methylcyclopropene (1-MCP), which inhibits ethylene signaling (Figure 2B). In our trials, 1-MCP was applied at 25 and 150 mg/L. As JA also functions with ethylene to regulate senescence (Koyama, 2018; Ryun Woo et al., 2019), we proposed that inhibiting JA biosynthesis could also be used to suppress seed coat senescence (Figure 2C). The JA biosynthesis inhibitor, diethyldithiocarbamate (DIECA), was applied at 25 and 100 µM (note: there are no commercial products for DIECA). To evaluate the impact of the above PGRs at the specified concentrations, seven shoots per tree were tagged and the number of fruitlets per shoot were recorded on a regular basis. For each treatment, four trees were selected. All applications were applied every two to three weeks using a backpack sprayer in which the PGRs were applied to a point of run-off. Personal protection equipment, including respirators and coveralls, was worn to limit exposure to the PGRs.

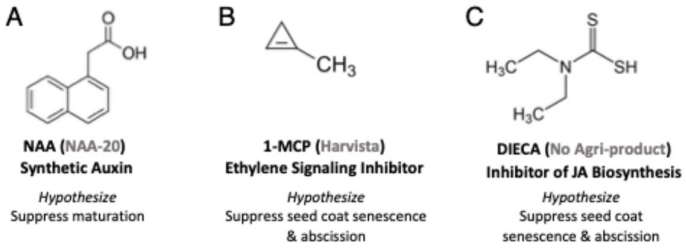

Figure 2. Evaluation of plant growth regulators to reduce summer fruit abscission. (A) 1-naphthaleneacetic acid (NAA) is a synthetic auxin. (B) 1-methylcyclopropene (1-MCP) is an inhibitor ethylene signaling. (C) diethyldithiocarbamate (DIECA) is an inhibitor of JA biosynthesis.

To determine if the NAA, 1-MCP and/or DIECA treatments impacted fruit development, the levels of auxin (indole-3-acetic acid (IAA)) and ACC were quantified in the seed coat. We also quantified IAA conjugated with aspartic acid (Asp), as results from AV16005 showed that IAA-Asp accumulated in the seed coat prior to senescence; thereby, reducing the concentration of free, active IAA (Smith and Goetz, 2023). In addition, we evaluated whether the NAA, 1-MCP and/or DIECA treatments impacted sugar levels in the seed coat to determine if these PGRs altered carbohydrate supply and/or metabolism in the seed coat. For the sugar assessment of the seed coat, we quantified sucrose, glucose, fructose, perseitol and mannoheptulose. The expectation is that if any of the PGRs used in our trials negatively impact fruit development, we would observe a decline in IAA and increase in IAA-Asp and/or ACC, as well as decrease in sugar levels in the seed coat. The hormone metabolites, IAA, IAA-Asp and ACC were extracted from cryogenically ground 50 mg seed coat samples and quantified using liquid chromatography with tandem mass spectroscopy as previously described (Clayton-Cuch et al., 2021). Soluble sugars were quantified from cryogenically ground 100 mg seed coat samples using high performance liquid chromatography as previously described (Goetz et al., 2021).

It has been speculated that summer fruitlet abscission is induced by the expansion of the summer vegetative flush, which acts by diverting carbohydrates away from developing fruits. As management might involve the use of plant growth retardants to reduce the growth potential of the summer flush, we sought to better examine the timing of the summer vegetative flush in relation to the summer fruit drop. Two methods were used to examine the outgrowth of the summer vegetative flush. The first method involved tagging and measuring the length of selected shoots, 10 shoots per tree. While measuring the length of shoots is a quantitative approach to examine the timing of the summer flush, we found that many of the selected shoots failed to initiate a summer flush. The inability to quantify the timing of the summer flush led us to image trees at regular time intervals to examine the growth of the canopy in the second year of the project. This method

provides a better picture of the timing and duration of the flush, but also the peak period of the flush.

The second objective of this project was aimed at clarifying the opportunity to manage irregular bearing. This activity was addressed via extension and communication activities. This included giving presentations at regional avocado forums and publishing an article in Talking Avocados. In these activities, the usage of plant growth regulators to manage irregular bearing was not only addressed but other technologies including spray induced gene silencing and genome engineering was also presented as alternatives to manage irregular bearing.

Results and discussion

Objective 1: Develop an understanding of PGRs for limiting summer fruitlet abscission

Evaluating the impact of NAA, 1-MCP and DIECA on summer fruit abscission

To explore the feasibility for using PGRs to reduce summer fruit abscission, NAA, 1-MCP and DIECA were trialed with the goal of maintaining auxin activity, suppressing ethylene/ACC signaling and inhibiting JA biosynthesis in the seed coat. In 2024, trees were treated with NAA at 5 and 15 mg/L, 1-MCP at 25 and 150 mg/L, and DIECA at 25 and 100 µM. All the above PGRs were applied on day 1, 15 and 38 of the trial. This trial was initiated when the average diameter of the fruitlets was 51.1 mm. Results showed that the average percentage of summer fruit abscission was only 3.5% in the control trees at the end of the trial (Figure 3A), which was likely due to the lower-than-expected crop load for the 2023-24 season. Due to the low rate of fruitlet abscission, we were unable to evaluate the impact of NAA, 1-MCP and DIECA on reducing the summer fruit drop (Figure 3A). Results also showed that yield, quantified as kg/tree and the number of fruit/tree, was not significantly different between the PGR treated and control trees (Figure 3B and C). In addition, none of the PGRs significantly impacted the average mass of the fruit (Figure 3D). Taken together, due to the lower-than-expected crop load for the 2023-24 season, we were unable to evaluate the impact of NAA, 1-MCP and DIECA on reducing summer fruit abscission and increasing yield.

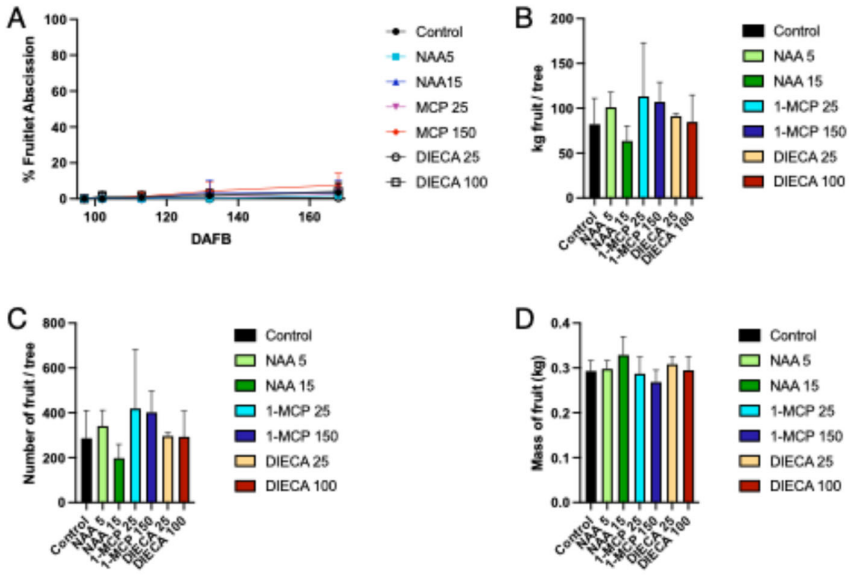

Figure 3. Evaluation of NAA, 1-MCP and DIECA on summer fruit abscission and yield in 2024. (A) Percent fruit abscission in response to NAA, 1-MCP and DIECA treatments. Impact of NAA, 1-MCP and DIECA on (B) yield (kg/tree), (C) number of fruits/tree and (D) average mass of fruit (kg).

In 2025, we established a new PGR assessment trial to evaluate the impact of NAA, 1-MCP and DIECA on summer fruit abscission. Due to the low crop load in the previous season combined with ideal conditions at flowering, many of the trees were carrying a high crop load (Figure 4A and B). Note: the canopy in the upper part of the trees was reduced due to a high number of determinate inflorescences produced during flowering (Figure 4A and B). In contrast, trees with a relatively low crop load displayed a more complete canopy (Figure 4C). In this PGR trial, the NAA (5, 15 and 25 mg/L), 1-MCP (25 and 150 mg/L) and DIECA (25 and 100 µM) treatments were applied at 86, 98, 112, 133 days after full bloom (DAFB). At 86 DAFB, the average diameter of the fruitlets was 34 mm. In the first 28-days of the trial, control trees dropped an average 70% of the crop, while trees treated with NAA at 5 mg/L dropped 50% of their crop (Figure 5A). Trees treated with NAA at 15 and 25 mg/L dropped an average 61 and 66% of their crop, respectively on day 28. At this time point, average fruitlet abscission in trees treated with 25 and 150 mg/L of 1-MCP was 49 and 59%, respectively (Figure 5B). In addition, average fruit drop in trees treated with 25 and 100 µM DIECA was 61 and 71% on day 28 (Figure 5C). At the end of the trial, control trees dropped an average 74% of their crop, while trees treated with NAA at 5, 15 and 25 mg/L dropped 55, 69 and 77% of their crop, respectively (Figure 5A). In addition, average abscission in trees treated with 1-MCP at 25 and 150 mg/L was 66 and 61%,

respectively, at the end of trial (Figure 5B). Lastly, average fruit drop at the end of the trial in trees treated with DIECA at 25 and 100 µM was 66 and 73%, respectively, (Figure 5C). ***Although the average percent of fruitlet abscission in trees treated with the NAA, 1-MCP and DIECA applications were not significantly different from the control, the overall trend in fruit drop displayed in NAA at 5 mg/L and 1-MCP at 150 mg/L is promising and warrants further evaluation.***

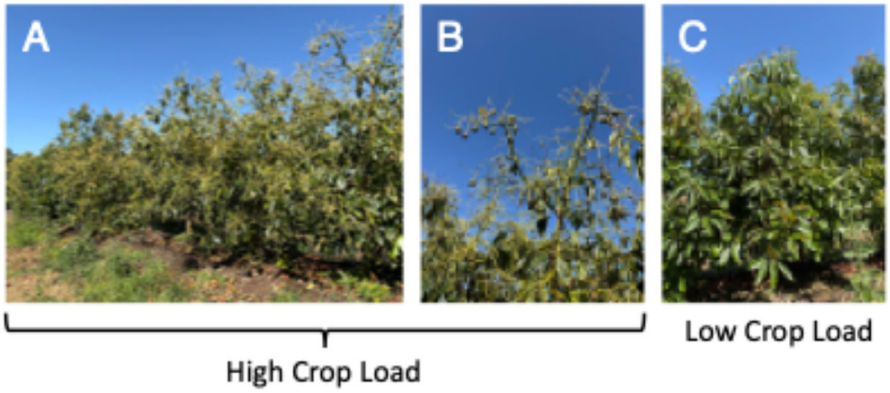

Figure 4. Images of trees with (A and B) a high crop load and (C) a low crop load.

We also examined fruitlet abscission by scoring the average number of fruits per shoot. At the start of the trial, shoots selected to evaluate abscission in the control and treated trees had approximately 7 fruits per shoot (Figure 5D-F). At the end of the trial, control trees retained approximately 1.6 fruitlets per shoot, while trees treated with NAA at 5, 15 and 25 mg/L retained an average 3.4, 2.1 and 1.5 fruitlets per shoot, respectively (Figure 5D). In trees treated with 1-MCP at 25 and 150 mg/L, the average number of fruitlets per shoot was 2.4 and 3.2, respectively (Figure 5E). The average number of fruitlets per shoot at the end of the trial for trees treated with DIECA at 25 and 100 µM was 2.7 and 1.9, respectively (Figure 5F). ***Although the average number of fruits per shoot were not significantly different when comparing the treatments with the control, there appeared to be a general trend in which trees treated with NAA and 1-MCP at 5 mg/L and 150 mg/L, respectively, displayed an increase in fruit retention.***

In addition to scoring abscission, we also evaluated the impact of NAA, 1-MCP and DIECA on yield, by scoring the average weight of fruit (kg) per tree, average number of fruits per tree and average weight of fruit (kg). The average yield in control trees was 19.8 kg/tree, while trees treated with NAA at 5, 15 and 25 mg/L had an average yield of 35.6, 17.2 and 12.4 kg/tree, respectively (Figure 6A). Trees treated with 1-MCP at 25 and 150 mg/L had an average yield of 15.5 and 29.9 kg/tree, respectively (Figure 6B). The average yield observed in trees treated with DIECA at 25 and 100 µM was 20.5 and 19.4 kg/tree, respectively (Figure 6C).

In control trees, the average number of fruits per tree was 84.5, while trees treated with NAA at 5, 15 and 25 mg/L had an average 145.5, 77 and 53.5 fruit per tree, respectively (Figure 6D). The average number of fruits per tree in trees treated with 1-MCP was 59.8 and 120, respectively (Figure 6E). Trees treated with DIECA at 25 and 100 µM had an average 85.3 and 73.3 fruit per tree, respectively (Figure 6F).

The average mass of fruit produced in control trees was 0.24 kg, while fruit produced in trees treated with NAA at 5, 15 and 25 mg/L had an average weight of 0.25, 0.22 and 0.24 kg, respectively (Figure 6G). Trees treated with 1-MCP at 25 and 150 mg/L had an average fruit weight of 0.26 and 0.25 kg, respectively (Figure 6H). The average weight of fruit produced in trees treated with DIECA at 25 and 100 µM was 0.24 and 0.27 kg, respectively (Figure 6I). ***While yield (kg of fruit/tree and number of fruit per tree) measured in 2025 wasn't significantly different between the treated and control trees, application of NAA and 1-MCP at 5 and 150 mg/L, respectively, displayed an apparent higher level of productivity compared to control trees, which closely aligns with a trend of reduced abscission in trees treated with these PGRs.***

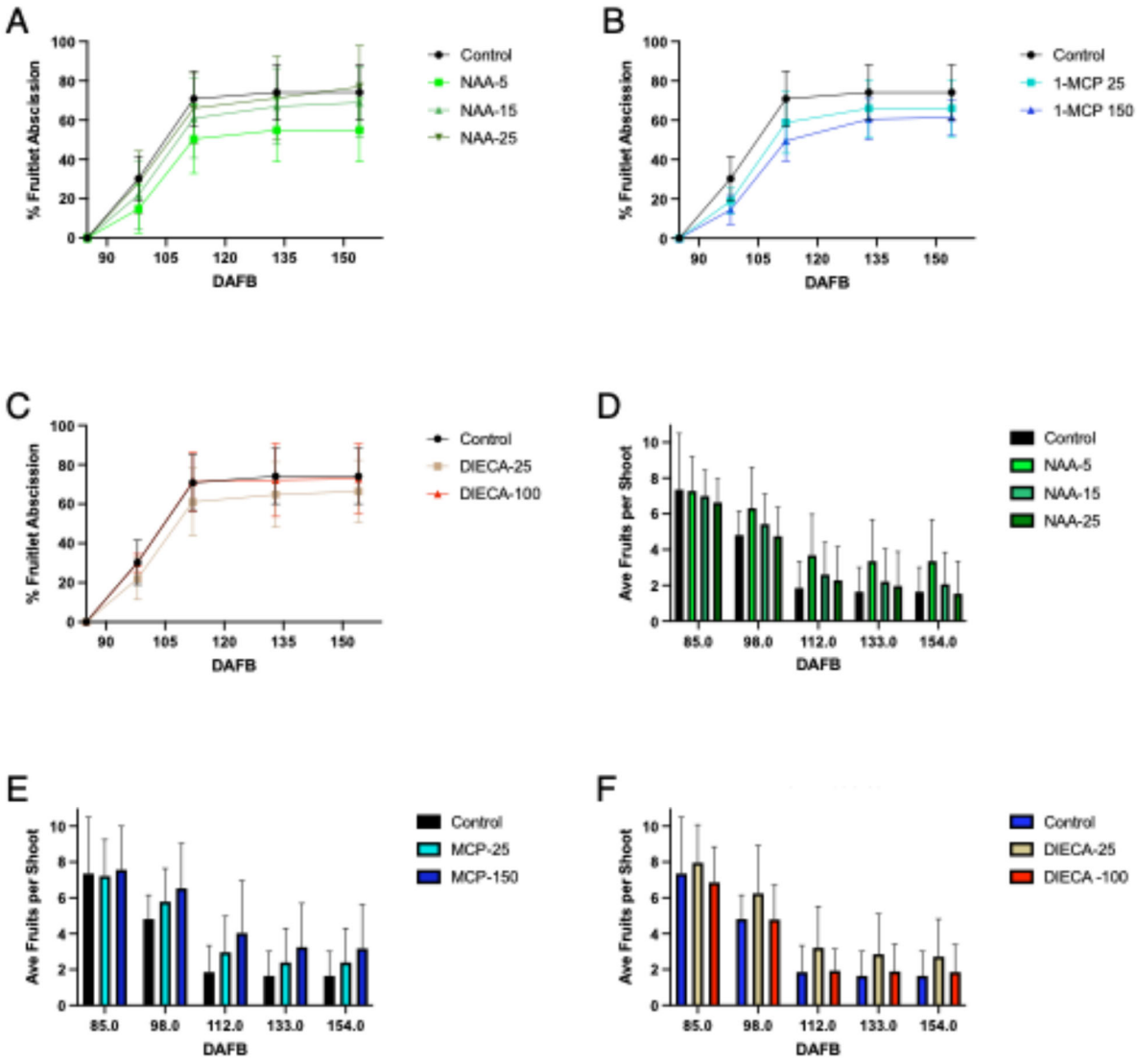

Figure 5. Evaluation of NAA, 1-MCP and DIECA on summer fruit abscission in 2025. (A) Percent fruit abscission in response to (A) NAA (5 and 15, 25 mg/L), (B) 1-MCP (25 and 150 mg/L) and DIECA (25 and 100 µm) treatments. Impact of (D) NAA (5, 15 and 25 mg/L), (E) 1-MCP (25 and 150 mg/L) and (F) DIECA (25 and 100 µm) on the number of fruits per shoot.

Evaluating the impact of NAA, 1-MCP and DIECA on the physiology of the seed coat

The seed coat is a critical organ that regulates both seed and pericarp development (Radchuk and Borisjuk, 2014). For example, during fruit and seed development, the seed coat is responsible for the uptake of sugars, amino acids and mineral elements. In addition, experimental studies in model plant species indicate that auxin produced in the seed coat is critical for regulating embryo development (Figueiredo and Köhler, 2018; Robert, 2019; Guo *et al.*, 2022). In AV16005, we showed that free IAA was 10- to 11-times higher in the seed coat than in the embryo and pericarp, indicating that the seed coat plays a fundamental role in fruit development (Smith and Goetz, 2023). In addition, results from AV16005 suggest that a 7.2-fold decrease in seed coat IAA levels together with a 2.6-fold increase in ACC levels initiate seed coat senescence to activate fruitlet abscission when carbohydrate supply to developing fruitlets is reduced. Therefore, to evaluate if NAA, 1-MCP and DIECA impacted the viability of the seed coat, we quantified the levels of IAA, IAA-Asp (inactive form of IAA), ACC (proxy for ethylene) and soluble sugars, including glucose, fructose, sucrose, perseitol and mannoheptulose, in the seed

coat derived from fruitlets treated with a single application of NAA at 5, 15 and/or 25 mg/L, 1-MCP at 25 and 150 mg/L, and DIECA at 25 and 100 µM in 2024 and 2025.

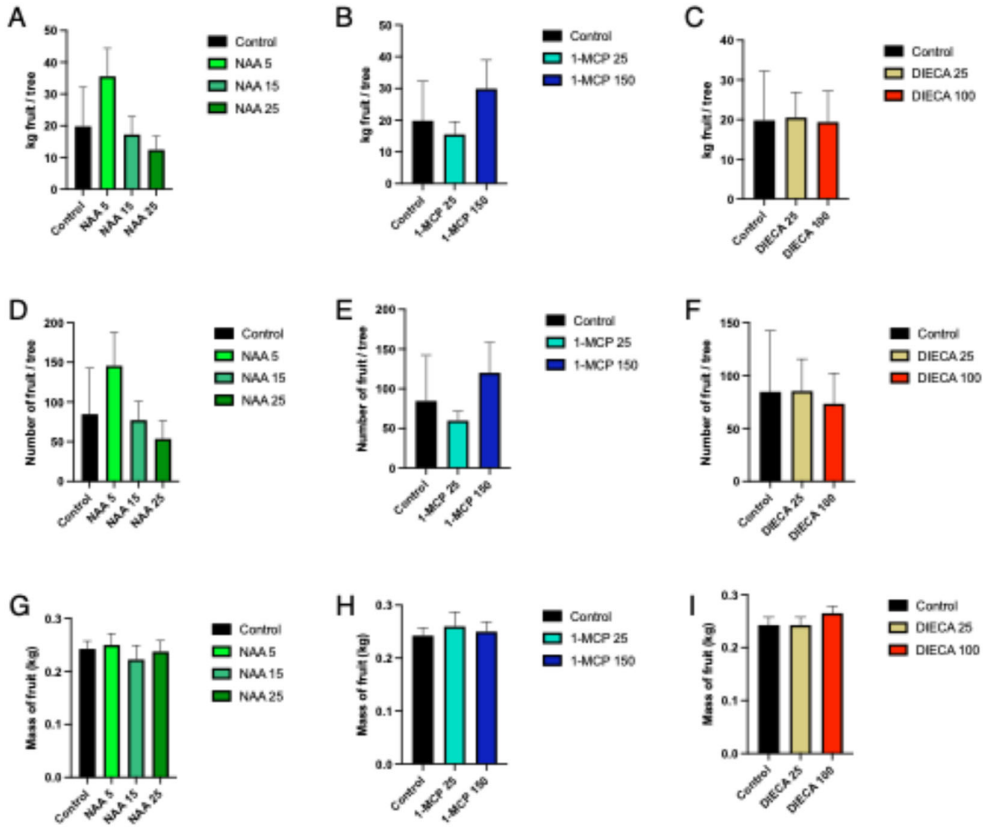

Figure 6. Evaluation of NAA, 1-MCP and DIECA on yield in 2025. Impact of (A) NAA (5, 15 and 25 mg/L), (B) 1-MCP (25 and 150 mg/L) and (C) DIECA (25 and 100 um) on yield (kg/tree). Assessment of (D) NAA (5, 15 and 25 mg/L), (E) 1-MCP (25 and 150 mg/L) and (F) DIECA (25 and 100 um) on number of fruit per tree. Impact of (G) NAA (5, 15 and 25 mg/L), (H) 1-MCP (25 and 150 mg/L) and (I) DIECA (25 and 100 um) on average mass of fruit (kg).

In 2024, this trial was initiated when the average diameter of the fruitlets was 51.1 mm. After treatment with the specified concentrations of NAA, 1-MCP and DIECA, the seed coat was isolated from fruitlets on day 5, 13 and 34 in PGR treated and control trees. Five-days after treatment, seed coat IAA levels derived from the PGR treatments were not significantly different from the control (Figure 7A). However, 13-days after trees were treated with NAA at 5 or 15 mg/L or 1-MCP at 150 mg/L a significant decrease in seed coat IAA levels was observed (Figure 7A; NAA 5 mg/L, p=0.004; NAA 15 mg/l, p=0.009; 1-MCP 150 mg/L, p=0.01). Here, treatment of trees with NAA at 5 mg/L and 15 mg/L showed a 1.8- and 1.7-fold reduction in seed coat IAA levels 13-days after the trial was initiated. At this time point, a 1.6-fold reduction in seed coat IAA levels was observed in fruitlets harvested from trees treated with 1-MCP at 150 mg/L. Thirteen-days after the trial was initiated, seed coat IAA levels were not significantly different for 1-MCP 25 mg/L, as well as the DIECA treatments, compared with the control (Figure 7A). Thirty-four-days after the PGRs were applied, only NAA at 15 mg/L caused an apparent and significant decline in IAA levels in the seed coat (Figure 7A, p=0.02). In response to this treatment at 34-days, seed coat IAA levels were reduced by 1.2-fold compared to the control seed coat samples.

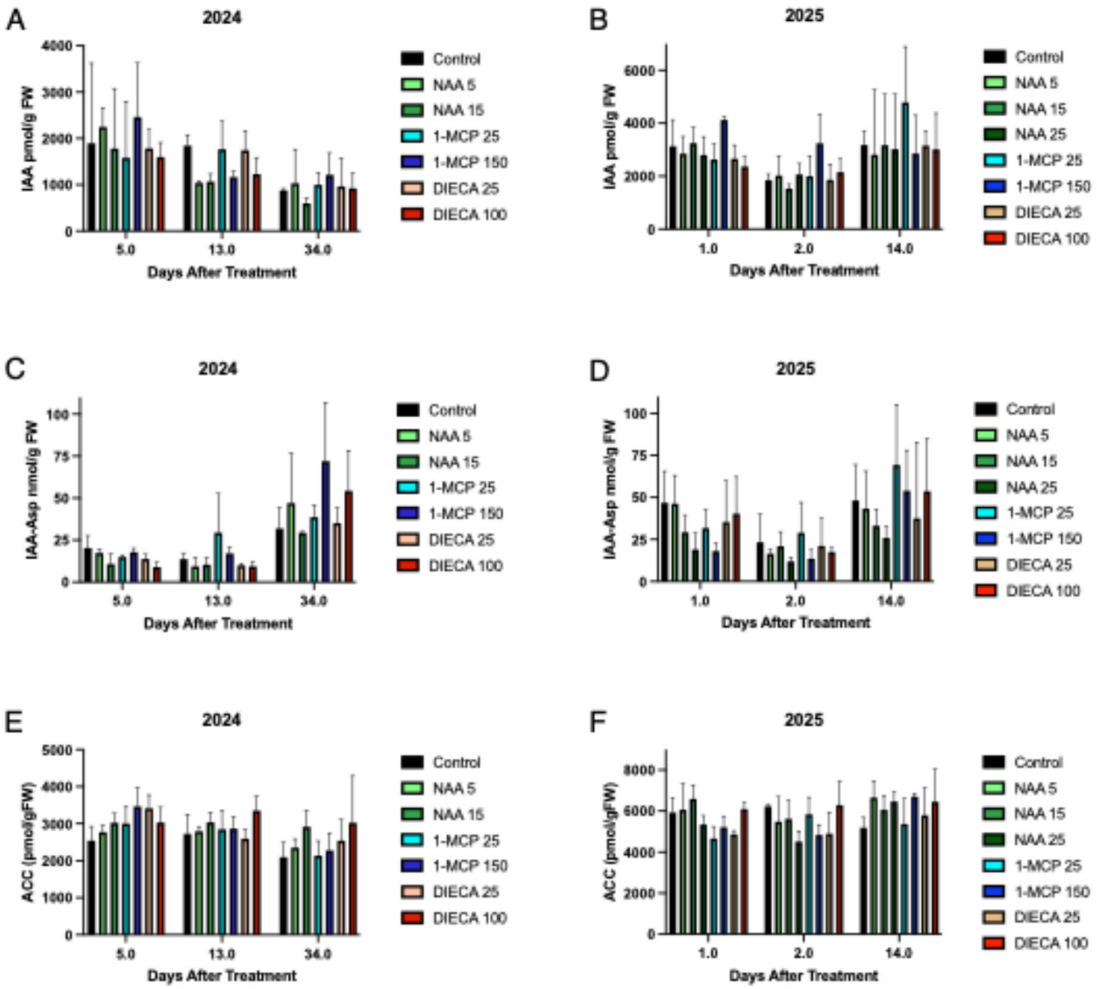

Figure 7. Evaluation of NAA, 1-MCP and DIECA treatments on the levels of auxin (IAA), IAA-Asp and ACC in the seed coat in 2024 and 2025. Impact of NAA (5, 15 and/or 25 mg/L), 1-MCP (25 and 150 mg/L) and DIECA (25 and 100 um) on (A and B) IAA, (C and D) IAA-Asp and (E and F) ACC in the seed coat in (A, C and E) 2024 and (B, D and F) 2025.

In 2025, a single application of NAA (5 mg/L, 15 mg/L and 25 mg/L), 1-MCP (25 and 150 mg/L) and DIECA (25 and 100 µM) was applied to trees when the average diameter of fruitlets was 34 mm. Fruitlets were harvested and the seed coat was isolated 1-, 2- and 14-days after treatment. Results showed that none of the PGRs treatments yielded in a significant decrease or increase in IAA levels in the seed coat 1, 2 and 14 days after treatment (Figure 7B). ***It is interesting to point out that the tress in 2024 had a more extensive canopy at the start of the trial compared to trees in 2025. In addition, the average size of fruitlets at the start of the trial in 2024 and 2025 differed. Together, these differences may have influenced the impact of the NAA at 5 and 15 mg/L treatments on seed coat IAA levels in 2024 and 2025. Based on our results, we suggest that NAA applications may reduce free IAA levels in the seed coat, but this will likely vary from year to year depending on canopy coverage and the developmental stage of the fruitlets. Further, the reduction in seed coat IAA levels may be attributed to the fact that IAA levels are highly regulated in the plants. Thus, increasing auxin activity via NAA application may feedback and temporally reduce free IAA levels in the seed coat. However, the reduction in seed coat free IAA levels in response to NAA applications is not as substantial as the decrease that occurs during fruitlet growth arrest and abscission. Therefore, based on the above results, we speculate that trees treated with NAA levels ≤ 15 mg/L will have little impact on avocado fruit development when applied after the diameter of fruitlets reach 34 mm.***

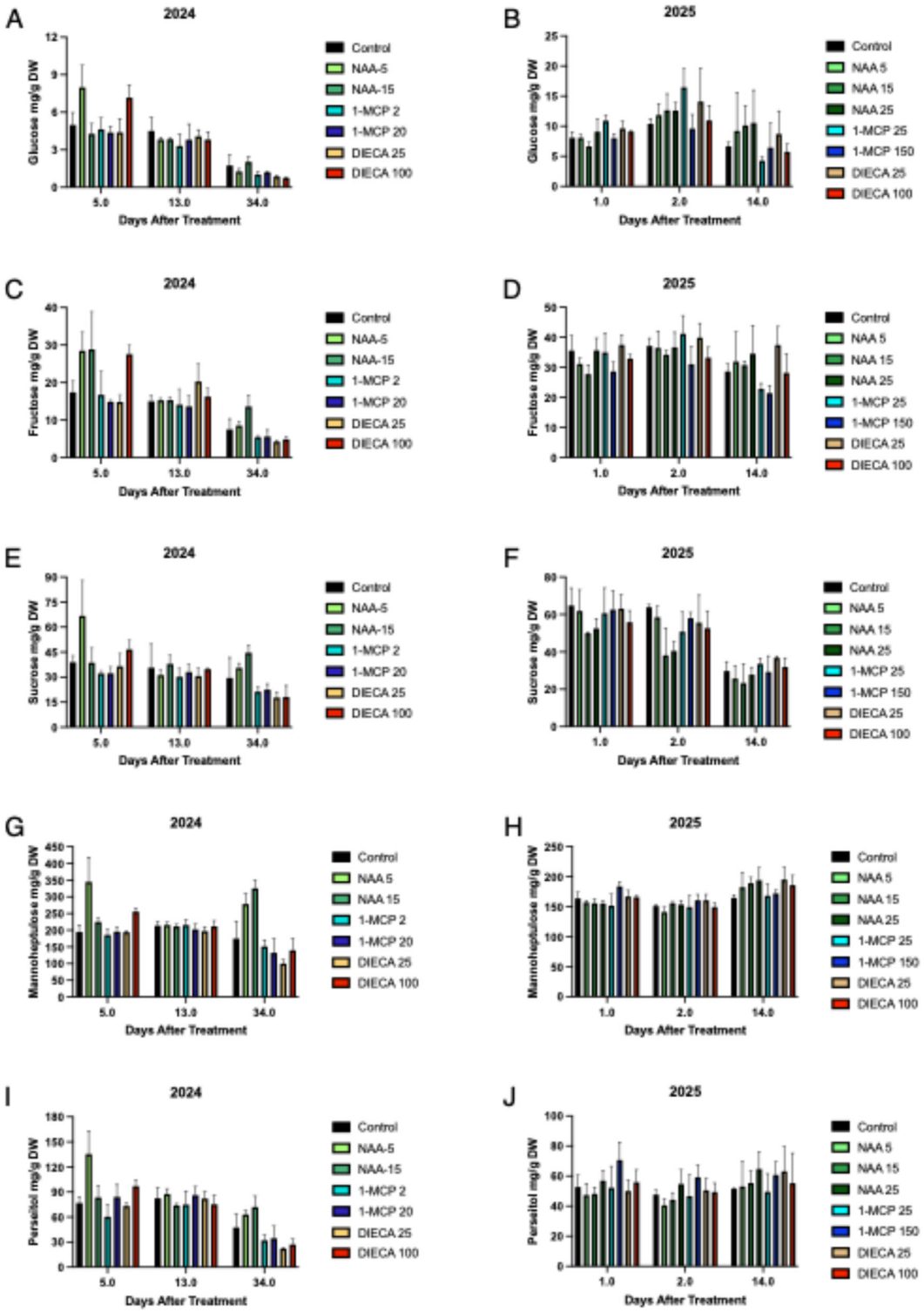

Figure 8. Evaluation of NAA, 1-MCP and DIECA treatments on the levels of soluble sugars in the seed coat in 2024 and 2025. Impact of NAA (5, 15 and/or 25 mg/L), 1-MCP (25 and 150 mg/L) and DIECA (25 and 100 um) on (A and B) glucose, (C and D) fructose, (E and F) sucrose, (G and H) mannoheptulose and (I and J) perseitol in the seed coat in (A, C, E, G and I) 2024 and (B, D, F, H and J) 2025.

Experimental studies indicate that free IAA levels are regulated in part by conjugation with amino acids including aspartic acid (Asp) (Staswick *et al.*, 2005). During avocado fruitlet growth arrest and abscission, we reported that the decrease in free IAA levels was accompanied with a significant increase in IAA-Asp levels in the seed coat (Smith and Goetz, 2023). The impact of NAA, 1-MCP and DIECA on IAA-Asp levels is displayed in Figure 7C and D from trials performed in 2024 and 2025, respectively. While the levels of IAA-Asp appeared to vary with the seed coat sampling dates in 2024 and 2025, we did not observe a significant increase in IAA-Asp that also corresponded with a decrease in IAA in the seed coat in response to the PGR applications (Figure 7C and D). ***Based on these results, we suggest that the concentrations of the PGRs used in this study, do not appear to activate the conjugation of IAA with Asp that would result in a decrease in free IAA levels in the seed coat.***

In 2024, the levels of ACC were quantified in the seed coat of fruitlets harvested from trees treated with NAA (5 and 15 mg/L), 1-MCP (25 and 150 mg/L) and DIECA (25 and 100 µM), as well as from fruitlets derived from untreated control trees. Results showed that there was not a significant increase in the levels of ACC in the seed coat from fruitlets harvested 5-, 13- and 34-days after trees were treated with a single application of NAA, 1-MCP and DIECA (Figure 7E). In 2025, there was no significant increase or decrease in seed coat ACC levels one-day after trees were treated with NAA, 1-MCP and DIECA (Figure 7F). A significant reduction in seed coat ACC levels occurred two-days after treatment with NAA at 25 mg/L (p=0.005) and 1-MCP at 150 mg/L (p=0.009; Figure 7F). In contrast, 14-days after the above PGRs were applied to trees there was a significant increase in seed coat ACC levels in trees treated with NAA at 25 mg/L (p=0.03) and 1-MCP at 150 mg/L (p=0.007). This increase in seed coat ACC levels in response to NAA at 25 mg/L and 1-MCP at 150 mg/L corresponded to a 1.2 and 1.3-fold increase, respectively, which is below the level of ACC produced in the seed coat during abscission. ***Together, the results indicate that the NAA, 1-MCP and DIECA treatments did not induce ACC production to a level that could impair fruit development.***

We also examined the impact of NAA, 1-MCP and DIECA treatments on soluble sugars in the seed coat. First, we examined the levels of glucose and fructose, to assess whether the NAA, 1-MCP and DIECA treatments potentially impacted the breakdown of sucrose, which is critical for fruit growth and development (Yong-Ling et al., 2010). As sucrose, mannoheptulose and perseitol are sugars that are likely transported from leaves to fruits in avocado (Liu et al., 2002), we also evaluated these sugars to determine if the PGR treatments potentially impacted carbohydrate supply to developing fruitlets. In 2024, results showed that treatments of trees with NAA at 5 mg/L was associated with an apparent increase in glucose, fructose, sucrose, mannoheptulose and perseitol in the seed coat 5-days after this PGR was applied to trees (Figure 8A, C, E, G, I). The increase in these soluble sugars in response to the NAA at 5 mg/L application was only significant for fructose (p=0.032), mannoheptulose (p=0.028) and perseitol (p=0.023), but not for glucose (p=0.062) and sucrose (p=0.091). At this time point, there was also a significant increase in fructose (p=0.013), mannoheptulose (p=0.01), and perseitol (p=0.025) in the seed coat 5-days after trees were treated with 100 µM DIECA (Figure 8C, G and I). In 2024, there was no significant difference in seed coat soluble sugars 13-days after the NAA, 1-MCP and DIECA treatments were applied to the trees (Figure 8A, C, E, G, I). However, 34-days after treatment, there was a significant increase in seed coat mannoheptulose levels in trees treated with NAA at 5 and 15 mg/L (Figure 8G; 5 mg/L, p=0.042; 15 mg/L, p=0.01).

In 2025, a single application of NAA (5, 15 and 25 mg/L), 1-MCP (25 and 150 mg/L) and DIECA (25 and 100 µM) was applied to trees when the average diameter of fruitlets was 34 mm. Fruitlets were harvested and the seed coat was isolated 1-, 2- and 14-days after the PGR treatments. In trees treated with 1-MCP at 25 mg/L, there was a significant increase in seed coat glucose levels 1- and 2-days after treatment (Figure 8B; 1-day, p=0.02; 2-day, p=0.03). Fourteen-days after treatment with 1-MCP at 25 mg/L, there was a significant decrease in seed coat glucose levels (Figure 8B, p=0.01). Based on our results, the other PGR treatments had little impact on seed coat glucose levels (Figure 8B). Out of the PGRs applied in this trial, only 1-MCP at 25 and 150 mg/L had an impact on seed coat fructose levels 14-days after treatment (Figure 8D; p=0.03 for both 1-MCP treatments). Seed coat sucrose levels were significantly reduced by NAA at 15 mg/L (p=0.04) and 25 mg/L (p=0.001) 2-days after treatment (Figure 8F). Although not statistically significant, there was apparent reduction in seed coat sucrose levels 1-day after application with NAA at 15 mg/L and 25 mg/L (Figure 8F). Based on our results, the other PGR treatments had little impact on seed coat sucrose levels (Figure 8F). Out of all the PGR treatments, only NAA at 15 mg/L resulted in a

significant increase in seed coat mannoheptulose levels 14-days after treatment (Figure 8H). For perseitol, none of the treatments significantly impacted the levels of the 7-carbon sugar in the seed coat (Figure 8J).

As seed coat function is critical for seed and fruit development (Radchuk and Borisjuk, 2014; Smith and Goetz, 2023), the project team evaluated whether any of the PGR treatments impacted the physiology of the seed coat. In avocado, experimental studies suggest that seed coat senescence is initiated by a decrease in sugar uptake and metabolism, as well as a decrease in IAA and increase in ACC/ethylene (Smith and Goetz, 2023). Therefore, to evaluate the impact of NAA, 1-MCP and DIECA on the physiology of the seed coat, we quantified IAA, IAA-Asp, ACC and soluble sugars in the seed coat derived from PGR treated and control trees. Results from above indicate that the PGR treatments may have transiently changed IAA or sugar levels after treatment in 2024 or 2025. However, none of the PGR treatments collectively reduced sugar and IAA levels, as well as increased ACC, in the seed coat. ***Therefore, based on the results presented, we speculate that none of the PGR treatments, including NAA and 1-MCP at 5 and 150 mg/L, respectively, resulted in collective changes in sugar and hormone metabolites that would negatively impact seed coat function.***

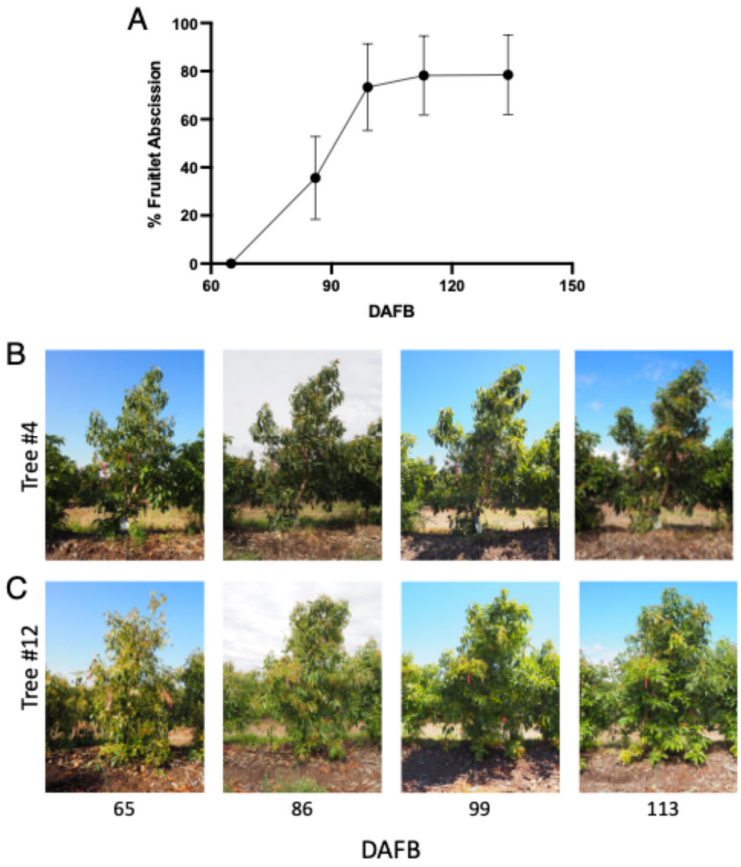

Figure 9. Association of the summer vegetative flush with summer fruit drop. (A) Average percent fruitlet abscission. Images of tree (B) #4 and (C) #12 over the course of the abscission trial.

Association of the summer vegetative flush with fruitlet abscission

It has been postulated that the outgrowth of the summer flush is a major factor that induces fruitlet abscission. Therefore, summer fruit abscission trials were performed in 2024 and 2025 to determine if the outgrowth of the summer flush was associated with the peak summer fruitlet abscission event. As summer fruitlet abscission was minimal in 2024, we were unable to evaluate whether the summer flush overlapped with the peak summer fruitlet abscission event. In 2024, we

learned that attempting to quantify the summer flush via tagging and measuring the length of vegetative shoots is not a useful method to quantify the summer flush unless hundreds of shoots are labeled/per tree. This was due to the fact that many of the tagged shoots did not produce a summer flush.

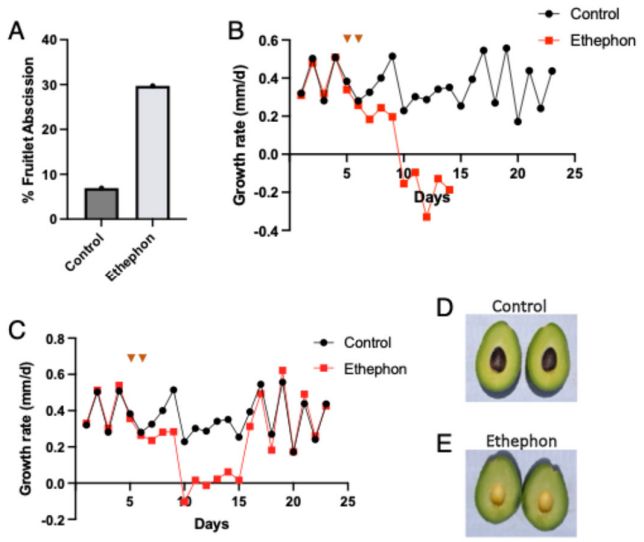

Figure 10. Ethephon induces fruitlet abscission in a pathway distinct from natural fruitlet abscission. (A) Percent fruitlet abscission in control and Ethephon treated trees. (B and C) Comparison of fruitlet growth rates from control and Ethephon treated trees. (B) Fruitlet growth rates compared between control and ones that abscised in response to Ethephon. (C) Fruitlet growth rates compared between control and ones that persisted in response to Ethephon. Image of seed coat derived from a fruitlet that abscised from a (D) control and (E) Ethephon treated tree. Note: seed coat senescence was only apparent in fruits that abscised from control trees.

In 2025, we quantified fruitlet abscission in 15 trees. This trial was initiated when the average diameter of the fruitlets was 34 mm. We evaluated the outgrowth of the summer flush by taking images of trees on the days we quantified fruitlet abscission, which corresponded to 65, 86, 99 and 113 days after full bloom (DAFB). Results showed that on day 113 the trees had dropped an average 78% of their crop (Figure 9A). Moreover, an average 73% of the crop was dropped within the first 34-days of the trial. In figure 9B and C, we provided images of tree #4 and #12 at 65, 86, 99 and 113 DAFB. Based on the images for tree #4, there appeared to be a greater production of leaves with most of the canopy being produced between 86 and 113 DAFB. While tree #4 dropped 32% of the fruit between 65 and 86 DAFB, a greater percentage of the fruitlets (45%) abscised between 86 and 113 DAFB. In tree #12, there was a substantial increase in leaf production from 65-86 DAFB. After 86 DAFB, leaf production appeared to be continuous. Results showed that a greater percentage of the fruitlets (46%) abscised between 65 and 86 DAFB. By 99 and 113 DAFB, tree #12 dropped an additional 25 and 26% of the crop, respectively. Overall, the association of the vegetative summer flush with fruitlet abscission displayed in tree #4 and #12 was similar with the other 13 trees examined in this trial. In summary, results from this trial support the hypothesis that the peak fruitlet abscission coincides with the greatest period of leaf production. It should be pointed out that in contrast to the spring flush, the duration of the summer flush appears to extend for at least two-months in WA. Further, the outgrowth of the summer flush is not synchronized or uniform across the canopy such that vegetative shoot growth can be initiated at different times in different parts of the tree. Thus, management of summer flush may be more challenging than the spring flush.

Evaluating systems to test the efficacy of NAA, 1-MCP and DIECA on reducing fruitlet abscission

This activity was focused on evaluating two fruit inducible systems to determine if they could be used to better test the efficacy of NAA and 1-MCP on reducing the summer fruit drop. The aim was to identify a treatment that would induce a 30-50% fruit drop event. PGRs, such as NAA and/or 1-MCP, could be applied, either before or right after the abscission treatment and fruit drop could be evaluated to determine if these PGRs reduced abscission.

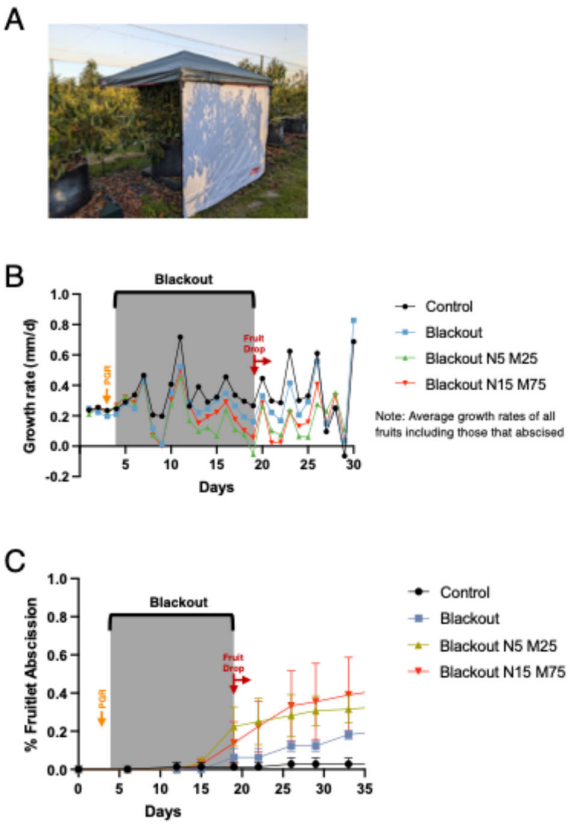

Figure 11. Evaluation of PGRs on fruitlet abscission in trees exposed to reduced light sunlight. (A) Tent-like structure used to block light termed "blackout treatment". (B) Growth rate of control and blackout treated trees with and without PGR treatments. (C) Percent fruitlet abscission in control and blackout treated trees with and without PGR treatments. N5 M25, NAA 5 mg/L + 1-MCP 25 mg/L; N15 M75, NAA 15 mg/L + 1-MCP 75 mg/L). Note: the blackout treatment was applied for 15-days starting on day 4 of the trial. The blackout treatment was removed when fruit drop was apparent on day 19.

The first fruit inducible system that was evaluated utilized Ethephon to determine if this ethylene releasing PGR could induce a wave of abscission. In AV16005, we showed that Ethephon, caused trees to drop approximately 50% of their crop (Smith and Goetz, 2023). As fruitlet abscission was induced rapidly when trees were treated with Ethephon, we needed to demonstrate exposure of trees to this PGR induced abscission in a similar manner as natural fruitlet abscission. The Ethephon trial was established in collaboration with Amnon Haberman at QDPI in 2024. To determine if Ethephon induced fruitlet abscission in a similar manner as natural abscission, we evaluated whether this PGR caused fruitlets to undergo growth arrest. In addition, we also evaluated whether seed coat senescence was associated with Ethephon induced abscission. Results showed Ethephon treated trees dropped approximately 30% of their crop, while only 10% of the fruitlets abscised in the control trees (Figure 10A). In figure 10B, we evaluated whether Ethephon treatment caused fruits to undergo growth arrest prior to abscission using Phytech fruit dendrometers. Results showed that when Ethephon was applied to trees on day 5 and 6 of the trial, as indicated by the orange arrow heads, the fruitlets underwent growth arrest and shrank in size before dropping from the tree (Figure 10B). Thus, the growth arrest and shrinkage patterns displayed in Ethephon mediated fruit abscission appeared to be highly similar to natural fruit drop. Interestingly, the population of fruitlets that didn't abscise in response to Ethephon also underwent growth arrest (Figure 10C). However, the growth rate for these fruitlets eventually recovered and followed a similar growth pattern as control fruitlets (Figure 10C). To evaluate whether Ethephon induced seed coat senescence during the abscission process, recently abscised fruitlets from control and Ethephon treated trees were collected and the seed coat was evaluated for senescence. Results showed that seed coat senescence was visibly apparent in recently abscised fruits derived from control trees (Figure 10D). In contrast, the seed coat examined from abscised fruitlets derived from Ethephon treated trees displayed no visible signs of senescence (Figure 10E). Thus, we concluded that Ethephon induced fruitlet abscission occurred via a different pathway to natural fruitlet abscission. We speculate that ethylene released by Ethephon may act directly on the abscission zone to induce abscission. Taken together, we concluded that Ethephon induced fruitlet abscission is not an appropriate system that can be used to trial the efficacy of NAA, 1-MCP and DIECA on immature fruit drop.

In the second trial, we evaluated whether a temporary blocking of sunlight using a tent-like enclosure, termed “blackout treatment” (Figure 11A), could be used to induce a wave of abscission. As natural abscission appears to be induced by a reduction in carbohydrate supply to developing fruitlets, we reasoned that a “blackout treatment” would reduce carbohydrate availability throughout the tree causing a wave of fruitlet abscission to occur until the treatment is removed. The “blackout treatment” was applied to three sets of trees for 15-days four-days after fruitlets were tagged with the Phytech dendrometers. In one set of trees, NAA plus 1-MCP was applied at 5 mg/L plus 25 mg/L, respectively. In the second set of “blackout” trees, a mixture of NAA at 15 mg/L plus 1-MCP at 75 mg/L was applied. The wetting agent used in the PGR applications was applied to the third set of “blackout” trees and used as a control. The two PGR treatments and wetting agent were applied to the appropriate set of trees 1-day before the “blackout” treatment was applied (Figure 11B). In addition, a fourth set of control trees that were not enclosed in a tent-like structure was also used to compare fruit growth rates and abscission to the “blackout” sets of trees. Results showed that approximately 5-days after the “blackout treatment” was applied the growth rate of fruitlets declined relative the untreated control (Figure 11B). The reduction in fruitlet growth rate continued for an additional 10-days and after this time point the “blackout” treatment was removed when fruit abscission became apparent at day 19 of the trial (Figure 11B and C). After exposure to light, the growth rate of fruitlets in the “blackout” treated recovered and matched the growth rate of fruitlets in untreated control trees (Figure 11B). To our surprise, results showed that abscission was highest in the “blackout” treated trees where the PGR combinations were applied compared to the “blackout” wetting agent control. After reviewing the literature, it became apparent that a severe reduction in light can induce ethylene production in plants (Harkey et al., 2019). Therefore, we propose that the “blackout” treatment may have increased ethylene in these trees. Further, as a result of ethylene production, the “blackout” treated trees may have become sensitized to NAA at 5 and 15 mg/L, causing more ethylene to be produced and more fruit to drop compared to the “blackout” control.

The results from the Ethephon and “blackout” treatments indicate that while these systems can induce fruitlet abscission, they are no suitable to evaluate the efficacy of NAA and 1-MCP.While neither of these systems yielded a positive result, we were able to extract learnings from these trials. First, the ability of ethylene to induce abscission independent of seed coat senescence when externally applied indicates that there may be multiple pathways that regulate fruitlet abscission in avocado. Second, during periods of stress, applications of NAA may enhance ethylene production causing a greater population of fruitlets to abscise. As a result, we advise that NAA should not be applied during periods of stress, as this may further enhance the stress response causing trees to drop more fruitlets.

Additional Learning - Role of canopy in summer fruit abscission and yield

Maintaining a healthy canopy with sufficient leaf coverage is critical for yield, as leaves are the primary site of photosynthesis. In avocado, it has been speculated that carbohydrates used to support fruit development can be derived from: (1) leaves via photosynthesis and (2) storage reserves located in woody tissues, such as stems. It is generally accepted that storage reserves are primarily mobilized when growth exceeds the rate of photosynthesis (DeJong, 2019) In avocado, the proportion of carbohydrates derived from the canopy and woody reserves that are used to support fruit development during the summer is not understood. Further, if carbohydrate supply from leaves or woody reserves become limiting, we don’t know the degree in which summer fruit abscission will be impacted. Determining how much the canopy contributes to fruit development during the summer is critical for canopy management. Research from AV16005 demonstrated that defoliation induced a massive fruit abscission event that was initiated between 10- to 15-days after the treatment was applied. Given that it takes approximately 10-12 days for a fruitlet to abscise from the tree suggests that the storage reserves are limited in their ability to support the growth of fruitlets throughout the tree when photosynthesis absent. In AV23000, we had an opportunity to further examine the importance of the leaf coverage (ie: canopy photosynthesis) on summer the abscission. In the 2023-24 season, the trees selected in our trial were carrying a lower-than-expected crop load due to a low rate of fruit set. Leaf coverage in these trees appeared to be adequate, as there were minimal gaps in the canopy (Figure 12A). In addition, results showed that fruit abscission was minimal in the trees (Figure 12B). Thus, we speculated that due to adequate leaf coverage, the trees selected in 2024 were able to support the growth and

development of the fruitlets. In contrast, crop load was higher in 2025 due to a high rate of fruit set. In 2025, leaf coverage was sparse as there were multiple gaps in the tree (Figure 12C). We reasoned that due to a large crop and reduced canopy/leaf area, the size of the fruitlets produced in 2025 was substantially smaller than in 2024. Further, we speculated that the sparse canopy was a major determinant that contributed to fruitlet abscission, as the sparse leaf coverage was unable to simultaneously support the development of the fruitlets and vegetative summer flushes. As a result, summer fruit abscission was considerably higher in 2025 compared to 2024 (Figure 12C and D). Interestingly, yield (kg/tree) and the number of fruits per tree at harvest were significantly higher in 2024 compared to 2025 (Figure 12E and F). In addition, the average weight of the fruit was significantly higher in 2024 compared to 2025 (Figure 12G). ***Taken together, we propose that managing leaf area or canopy coverage is an important factor for reducing summer fruit abscission and increasing yield. It would be interesting to determine at what extent NAA (5 mg/L) and 1-MCP (150 mg/L) could reduce summer fruitlet abscission and increase yield with greater canopy coverage in a high cropping year.***

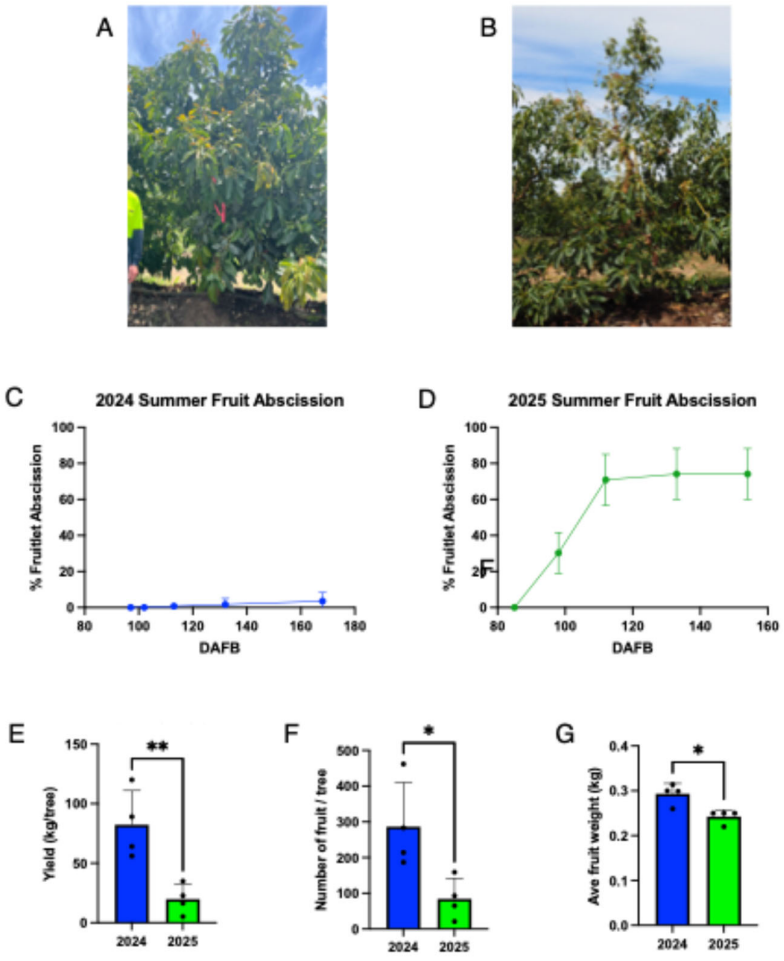

Figure 12. Representative image of an untreated control tree in (A) 2024 and (B) 2025. Percent summer fruit abscission in (C) 2024 and (D) 2025. Comparison of (E) yield, (F) number of fruit per tree and (G) average weight of fruit per tree in 2024 and 2025. *$p < 0.05$, **$p < 0.01$.

Summary

- In this project, we gained an understanding of PGRs that could be used to manage summer fruit abscission. Results from this project indicate that treatment of tress with NAA at 5 mg/L or 1-MCP at 150 mg/L may reduce summer fruit drop. Note: this assessment was only determined for one season, as abscission was minimal in the first year of the project due to an unexpected low crop load.

- The impact of NAA, 1-MCP and DIECA on the physiology of the seed coat was evaluated by quantifying IAA, IAA-Asp, ACC and soluble sugars in the seed coat derived from PGR treated and control trees. Overall, our results suggest that PGR treatments assessed in our trials did not collectively change the levels of sugar and hormone metabolites that would negatively impact seed coat function.

- It has been speculated that the outgrowth of the vegetative summer flush is a causal factor that induces summer fruit abscission. Here, our results suggest that the period in which the greatest number of shoots in the canopy undergo extension appeared to coincide with the greatest level of fruit drop. Therefore, management of summer fruit drop may also involve the usage of plant growth retardants that reduce the growth potential of vegetative shoots.

- Results from the project suggests that managing leaf area or canopy coverage is an important factor for reducing summer fruit abscission and increasing yield.

Objective 2: Clarifying the opportunity to limit irregular bearing using PGRs

To address the objective aimed at clarifying the opportunity to manage irregular bearing, an article was published in Talking Avocados titled “Opportunities to manage irregular bearing in avocado” in the 2024 spring issue (see appendix). In addition, speaking invitations at three avocado forums on irregular bearing (fruit abscission, fruit set and using PGRs to manage vegetative growth) were used to clarify the opportunity to manage irregular bearing with PGRs. The presentations not only covered the usage and risks of using PGRs to manage irregular bearing but also other technologies including spray induced gene silencing and genome engineering.

Presentations at Avocado forums (see Appendix)

- 2024 Western Australia Forum (Pemberton) – Opportunities to address irregular bearing in avocado
- 2024 Tristate Avocado Forum (Renmark) – “Using plant growth regulators to manage vegetative growth”
- 2025 NSW Avocado Forum (Stuarts Point) – “Avocado fruit set – Limiting factors and knowledge gaps”

In addition to presenting at these forums, Harley Smith also participated in the Irregular Bearing Workshop and served on the AV23014 PRG committee. At the workshop Harley Smith presented a talk titled “An understanding of avocado fruit abscission and opportunities to management”.

Outputs

Table 1. Output summary

Output	Description	Detail
New knowledge on the impact of PGRs that target hormones involved in summer fruit abscission	Evaluation of the plant growth regulators, NAA, 1-MCP and DIECA, for their ability to reduce summer fruit abscission	The project identified two PGRs with the potential to reduce summer fruit abscission. • NAA at 5 mg/L has the potential to reduce summer fruit abscission. Results from this project indicate that NAA at 5 mg/L doesn't appear to cause major changes in the levels of key hormone and sugar metabolites in the seed coat that would impair fruit development. • 1-MCP at 150 mg/L also has the potential to reduce summer fruit abscission. Results from this project indicate that 1-MCP at 150 mg/L doesn't appear to cause major changes in the levels of key hormone and sugar metabolites in the seed coat that would impair fruit development.
A better understanding of the impact of the summer vegetative flush on fruitlet abscission	Evaluation of the timing and duration of the summer flush and its association with fruitlet abscission	Project results support the hypothesis that the outgrowth of the summer flush is associated with summer fruit abscission. • The period in which the greatest number of shoots in the canopy undergo extension appears to coincide with the greatest level of fruit drop. • Outgrowth of the summer flush is not synchronized or uniform across the canopy such that vegetative shoot growth can be initiated at different times in different parts of the tree during the summer months. • Due to the variability in the temporal and spatial dynamics of vegetative growth between different trees and within the canopy, management of the summer flush may be more challenging than reducing the growth potential of the spring flush.
Clarifying the opportunity to manage summer fruitlet abscission using PGRs	Publication in industry magazine and presentations at regional avocado forums	• An article was published in Talking Avocados titled "Opportunities to manage irregular bearing in avocado" in the 2024 spring issue (see appendix). • Presentations were given at three avocado forums on irregular bearing that covered the impact of fruit abscission and fruit set on production and using PGRs to manage vegetative growth. Here, the presentations illustrated the opportunity to manage irregular bearing drivers with PGRs. • The presentations covered other technologies, including spray induced gene silencing and genome

		engineering, that may also have the potential to reduce summer fruitlet abscission.

Photos/images/other audio-visual material

< Refer to *Attachment A3: Final report guide* or the guidance note at the start of this template >

Outcome

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Availability of new knowledge aimed at improving orchard management practices to increase production and yield consistency based on improved knowledge of tree physiology.	The outcome from this project is consistent with the Avocado Strategic Investment Plan, outcome 2, which aims to improve supply, productivity and sustainability. KPI: Pathway created for the development of a plant growth regulator application.	Improved knowledge on the potential usage of plant growth regulators that target candidate hormones implicated in avocado fruitlet abscission	Knowledge developed for the PGRs, NAA and 1-MCP, on fruit development and abscission. Opportunity to reduce irregular bearing using NAA and 1-MCP. Recommendation of future R&D on the usage of NAA and 1-MCP manage summer fruit abscission.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project achieved its expected outcomes?	The project delivered new knowledge on the potential usage of PGRs for managing fruitlet abscission. The project team provided evidence that the peak summer flush is associated with the greatest amount of fruitlet abscission. Exogenous application of the ethylene releasing PGR, Ethephon induced fruitlet abscission in a pathway distinct from natural fruit abscission.	Further R&D on the usage of NAA and 1-MCP to mitigate summer fruit abscission. R&D on the usage of PGRs to manage the summer vegetative flush alone and in combination with NAA and/or 1-MCP.
How relevant was the project to the needs of intended beneficiaries?	The project developed a knowledge base for using NAA and 1-MCP to mitigate summer fruitlet abscission.	Publication on the potential usage of NAA and 1-MCP in reducing summer fruit abscission.
How well have intended beneficiaries been engaged in the project and were the engagements appropriate to the target audiences of the project?	The project team engaged with Australian Avocado Industry through: (1) publication in Talking Avocados and (2) presentations at three avocado regional forums, in regions impacted by irregular bearing impacts production.	Accept future opportunities to present at avocado forums on the potential usage of NAA and 1-MCP in mitigating summer fruit abscission.

Recommendations

Practical application of the project findings

- The project provided evidence that PGRs, NAA and 1-MCP, have the potential to be used to mitigate summer fruit abscission.

Possible future RD&E that directly flow from the work undertaken and its results

Future RD&E to develop the usage of NAA and/or 1-MCP in combination with plant growth retardants to delivery practical application to growers to manage summer fruit abscission.

- Perform small-scale trials with NAA and 1-MCP to determine if these PGRs have the potential to be used as PGRs to manage summer fruit abscission. In this trial, evaluate whether higher concentrations of 1-MCP can be used to better reduce summer fruit abscission. Also, evaluate the usage of plant growth retardants (ie: paclobutrazol or uniconazole) on the summer flush and fruit abscission.
- If NAA and/or 1-MCP show potential to be used as PGRs to manage summer fruit abscission, perform medium scale trials with (1) NAA, (2) 1-MCP and (3) NAA + 1-MCP. Evaluate the usage of plant growth retardants on summer flush and fruit abscission
- If NAA and/or 1-MCP show potential to be used as PGRs to manage summer fruit abscission, perform medium scale tria ls with (1) NAA, (2) 1-MCP or (3) NAA + 1-MCP with and without plant growth retardants. This activity will need to be repeated for 3-years to obtain necessary information to register NAA and 1-MCP for avocado (8 evaluation trials in at least two regions). In addition, residue trials would also need to be performed with NAA, which can be performed in conjugation with the trials outlined above.

Development and adoption activities that would ensure full value from the project's findings for industry. >

- In a possible future RD&E project, develop a commercialization pathway for product registration, NAA and/or 1-MCP, in avocado. Future RD&E will need to ensure the proposed trials above are aligned with Australian Pesticides and Veterinary Medicines Authority requirements to register NAA and 1-MCP in avocado. In addition, eight independent trials will also need to be performed alongside the trials above to evaluate residue levels for NAA.

Industry publications

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Intellectual property

No project IP or commercialisation to report

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Appendix A: Industry article and Regional Forum Announcements

Opportunities to manage irregular bearing in avocado

By Harley Smith, CSIRO Agriculture & Food

Avocado irregular bearing is a considerable challenge for stabilizing annual production for domestic and international market development. Based on a survey and analysis of yield data derived from avocado orchards across Australia it was concluded that irregular fluctuations in annual yield is due primarily to poor fruit set and high fruit abscission, which are influenced by environmental factors[1]. While biennial bearing can negatively impact annual yield, it appears to be more prominent in orchards that experience hot and dry summers. As avocado production continues to increase and oversupply is impacting profitability in years of high production, the ability to mitigate the drivers of irregular bearing is becoming increasingly important for the sustainability of the industry.

Fruit set and immature fruit abscission are major drivers of irregular bearing

Fruit set or the initiation of fruit growth is dependent upon successful pollination and fertilization to activate cell proliferation in the ovary of the pistil, the female reproductive structure in the flower[2]. As avocado fruit set is highly sensitive to environmental conditions[3,4], this phase of fruit development can be a considerable challenge for growers who maintain orchards in regions that experience temperatures well above or below 25°C day and 20°C night regime during anthesis. However, growers have indicated that in certain years adequate levels of fruit set occurred under cool to cold day and night temperatures, which suggests that additional environmental factors impact fruit set. In support of this observation, relative humidity levels below 75% reduce pistil viability resulting in poor fruit set in 'Hass' (Iñaki Hormaza, personal communication). Therefore, understanding the impact of temperature x humidity on fruit set in growing regions, such as Manjimup/Pemberton, Toowoomba and Tablelands, will better equip growers with tools to predict weather conditions that will impact fruit set. Furthermore, this understanding will also be critical for creating a framework to manage fruit set when orchards experience unfavourable cool to cold weather conditions during anthesis.

During avocado fruitlet development, a high percentage of immature fruits will exit the growing phase and undergo abscission[5] (Figure 1A). A high rate of immature fruit abscission (IFA) occurs during the initial stages of fruit development, when the average size of the fruitlets is < 15 mm in diameter[6]. The absence of fertilization is a major contributing factor during the first two weeks of this initial fruit drop event[3,7]. During the summer, a significant percentage of fertilized fruitlets abscise when immature fruits acquire up to 40% dry matter[6,7,8]. IFA is a major challenge for growers in Central QLD (Simon Newett, personal communication) and coastal regions of southwest WA[6], as these regions typically have favourable conditions that support fruit set during anthesis. It has been postulated that flushes of vegetative growth during the spring and summer (Figure 1B and C) are a primary driver that induces IFA. As gibberellin promotes the expansion of shoots[9], plant growth regulators (PGRs) that inhibit the biosynthesis of this hormone, including paclobutrazol and uniconazole, are used to reduce the growth potential of the vegetative flushes. However, the success of these applications in increasing yield is often variable indicating that other hormones and/or metabolites could be targeted to reduce IFA. In addition to the flushes of vegetative growth, hot-dry conditions also stimulate IFA, which can be managed by the usage of overhead sprinklers to reduce canopy temperatures.

What is known about the physiology of fruit set and fruitlet abscission?

Experimental studies show that the accumulation of starch in the pistil of avocado flowers is highly variable indicating that carbohydrates are not evenly partitioned to each flower[10]. Further, the level of starch in the pistil correlates with fruit set and retention. Therefore, stored starch in the pistil appears to be important factor for fruit set, as this carbohydrate reserve likely functions to support pollen tube growth required for fertilization, as well as cell proliferation initiated at the onset of fruitlet growth.

Results from AV16005 show that fruitlets undergo growth arrest prior to abscission[6]. Further, results suggest that fruitlet growth cessation is triggered by a decrease in carbohydrate supply during the summer fruit drop event. The reduction in carbohydrate supply is likely due to the outgrowth of the summer flush, which may divert carbohydrates away from a subset of developing fruitlets. Moreover, reduced carbohydrate availability may increase competition among developing fruitlets causing additional fruit drop. Thus, carbohydrate availability appears to be a common theme that determines whether a flower can set fruit and if a fruitlet can maintain a critical growth potential that prevents abscission.

What opportunities are available for reducing irregular bearing?

Yield is a function of genetics x environment x management (Figure

2A). As irregular bearing impacts annual yield, effective management and/or breeding strategies have the potential to improve fruit set in a cool to cold climate, reduce fruit drop and minimize biennial bearing. Research in model plants show that the integration of sugar and hormone signaling regulates growth[11]. Therefore, identifying candidate hormones that regulate the drivers of irregular bearing can be leveraged to develop PGR based applications to limit irregular bearing. Hormone profiling studies show that fruitlet growth arrest and abscission is associated with changes in the levels of auxin, ethylene, jasmonic acid and abscisic acid in the fruit[6]. Therefore, manipulating the levels of at least one of these hormones using plant growth regulators (PGRs) may offer a pathway to temporarily maintain the growth potential of fruitlets to limit abscission during the summer drop event. In AV23000, at least three considerations are being addressed to develop a PGR application(s) to suppress summer fruit abscission (Figure 2B). First, research efforts are focused on identifying a working concentration for a PGR(s) that targets auxin, ethylene, jasmonic acid and/or abscisic acid. For example, results from AV16005 show that a decline in auxin activity in the arresting fruitlet is associated with summer fruit abscission[6]. Therefore, it can be hypothesized that maintaining auxin activity in the fruitlet will reduce abscission. 1-naphthaleneacetic acid (NAA) is a highly stable synthetic auxin that is effective at delaying ripening in domesticated grapes when applied at 50 mg/L[12]. When NAA was applied at 25 mg/L to avocado trees during the summer, this synthetic auxin elicited an ethylene response that caused the wilting of shoots and leaves (Figure 3). Thus, the potential usage of NAA to limit summer fruitlet abscission requires research to identify a concentration that doesn't negatively impact tree health. The second consideration in the development of a PGR application is to determine the time of application. For example, applying a PGR too early or late will have little impact on the summer fruit drop. Therefore, identifying the window of time where the PGR can impact summer fruit drop will be essential for the effectiveness of the application. In addition, as a PGR application(s) is an added cost for growers, it is important to develop a method that minimizes the number of sprays applied during the summer months. Thus, determining the timing of application becomes a critical factor for reducing the production cost for a PGR based application to limit summer fruit drop. Lastly, the final consideration to examine, if possible, is whether a PGR is translocated to developing fruit to maintain a normal growth potential during the summer fruit drop period (Figure 2B).

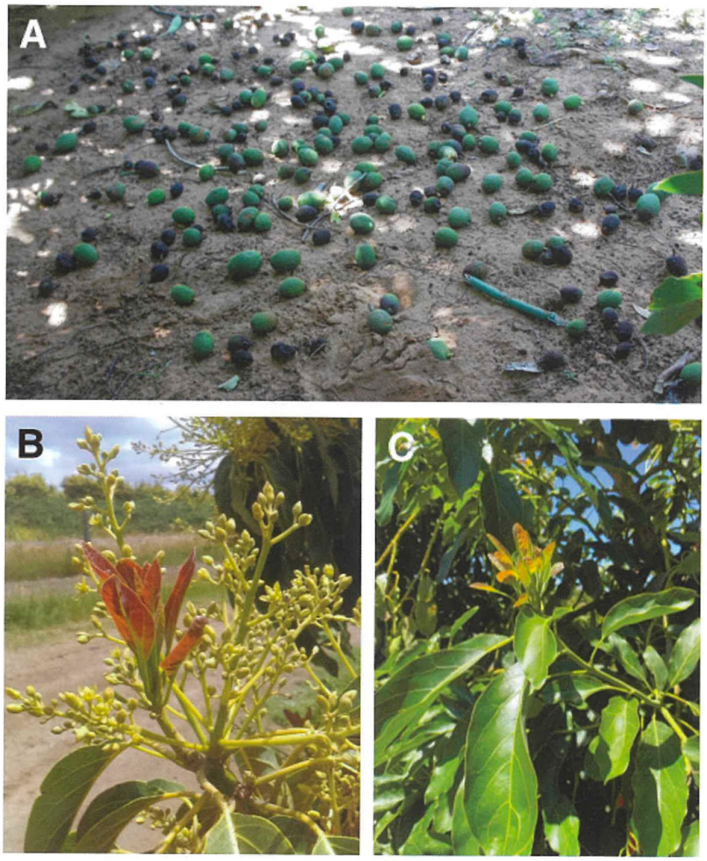

Figure 1: Fruitlet abscission and the outgrowth of vegetative flushes. (A) Abscission of immature fruits. Growth of the vegetative (B) spring and (C) summer flush.

Experimental studies indicate that spray-induced gene silencing (SIGS) has the potential to protect crops from pathogens and pests[13]. In this approach, a ribonucleic acid sequence is applied to a crop of interest to temporarily suppresses the function of a specific gene that allows a pathogen to infect or a pest to parasitize a plant. Given that candidate genes implicated in summer fruitlet abscission were recently identified[6], it would be intriguing to determine if this technology could be leveraged to suppress the function of growth arrest/abscission genes in avocado (Figure 2C). One of the challenges with using PGRs is the variable response elicited at different concentrations, as described above for NAA. In addition, PGRs can

target multiple hormone pathways. For example, paclobutrazol not only inhibits gibberellin biosynthesis but this chemical also inhibits an enzyme that breaks down abscisic acid[14]. Thus, applying paclobutrazol during a stress event may increase abscisic acid levels in the tree, which may affect the growth potential and abscission rate of developing fruitlets. In contrast, utilizing a SIGS approach has the potential to specifically target a gene(s) implicated in fruitlet growth arrest and abscission. To harness this technology, the efficacy and success of this approach must first be determined in avocado.

Breeding and development of new avocado varieties adapted to specific growing climates with improved fruit set, reduced fruitlet abscission and/or alternate bearing has the potential to stabilize annual production in climates where 'Hass' underperforms and/or displays irregular cropping patterns. While many avocado varieties have been developed, information regarding their performance in different environments and irregular bearing potential is limited[15]. With the recent release of the 'Luna UCR' by the University of California Riverside (*https://news.ucr.edu/articles/2023/10/24/luna-ucr-avocado-one-times-2023-best-inventions*), which is stated to have a reduced pruning requirement (i.e., lower level of vegetative growth), it would be interesting to evaluate the irregular bearing potential of this new variety in the diverse avocado growing environments in Australia.

One of the major drawbacks of the traditional avocado breeding strategy is the time it takes to develop new varieties. For example, Luna UCR™ took decades to develop. Therefore, alternative breeding strategies that have the potential to speed up variety development could be explored. For example, CRISPR-Cas gene-editing is a relatively new technology that can be used to create mutations to "knock-out" or manipulate gene function in existing varieties[16]. One of the major requirements for performing gene-editing is having the capability to regenerate plants from individual cells containing the edited gene(s) (Figure 2D). Recently, the biotechnology company GreenVenus LLC has edited a polyphenol oxidase (PPO) gene in 'Hass' (*https://www.greenvenus.com/wp-content/uploads/2023/06/GreenVenus-LLC-Achieves-Breakthrough-in-Avocado-Gene-Editing.pdf*), to reduce browning/oxidation of the fleshy part of the avocado fruit. The success in developing an avocado gene-editing system by GreenVenus LLC, opens the door to edit genes that impact production. However, to produce new gene-edited clones of 'Hass' with a reduced irregular bearing potential, it is essential to have identified candidate genes implicated in regulating fruit set and abscission, as well as biennial bearing. As candidate growth arrest/abscission genes have been identified in avocado[6], establishing this technology has potential to develop clones of avocado varieties with reduced summer fruit drop. However, additional molecular based research is essential for using this breeding technology to develop clones with improved fruit set and reduced biennial bearing (Figure 2D). Taken together, implementation of CRISPR-Cas gene-editing presents a new opportunity to develop new clones of avocado cultivars.

In conclusion, developing management and breeding solutions to reduce the impact of irregular bearing is a strategic area of research, which requires a basic understanding of the physiological and molecular processes that mediate poor fruit set and high fruitlet abscission, as well as biennial bearing. However, management and breeding related research aimed at limiting irregular

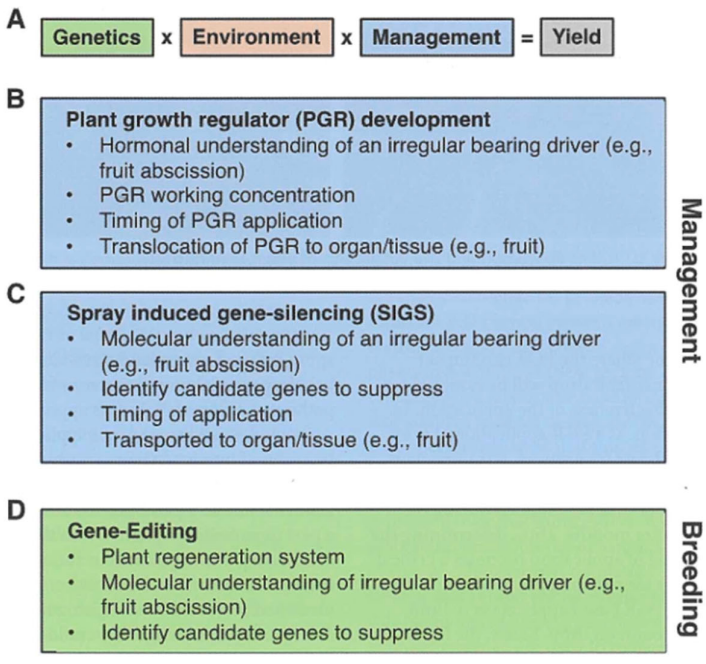

Figure 2: Opportunities to manage irregular bearing in avocado. (A) Yield is a function of genetics x environment x management. Opportunities to manage irregular bearing include the development of (B) plant growth regulator (PGR) applications and (C) spray induced gene-silencing. (D) Gene-editing is a breeding technology with potential to reduce irregular bearing and improve the genetics of existing varieties.

Figure 3: The impact of 1-naphthaleneacetic acid (NAA) at 25 mg/L on avocado tree health. Images of a vegetative shoot treated with (A) control spray and (B) NAA at 25 mg/L.

bearing comes with inherent risks that must be evaluated and managed on a regular basis to ensure that a practical(s) outcome is achievable.

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TRISTATE

Technical theme: Plant growth regulators (PGRs)

Australian avocado industry

Regional Forum

Wednesday 15 May 2024

Forum - Renmark Hotel, Murray Avenue

Orchard walk - Costa, Murtho Road

TIME	ITEM	PRESENTER
8:15 am	**Registration**	
9:00 am	Tristate regional lead welcome	Steven Falivene (NSW DPI) and Kym Theil (AAL)
9:10 am	Introduction to project	Bridie Carr (DAF)
9:15 am	Industry update	Mary Burton (AAL)
9:40 am	Industry benchmarking	Clinton Muller (RMCG)
9:55 am	Review of PGRs (current understanding)	Harley Smith (CSIRO)
10:15 am	**Morning tea**	
10:45 am	Review of PGRs (other experience and Chilean update)	Harley Smith (CSIRO)
11:10 am	Use of Plant Growth Regulators in avocado	Sebastian Recabarren (Imtrade)
11:40 am	Uniconazole - uses and experience	Frank Galluccio (Sumitomo)
11:55 am	Uses of Triazoles PGRs in avocado	Amnon Haberman (DAF)
12:25 pm	Other state grower use of PGRs	TBC
12:40 pm	**Lunch**	
1:20 pm	Q&A session	All
1:40 pm	Activity - regional opportunities and knowledge gaps	All
2:40 pm	Wrap up	Steven Falivene (NSW DPI)
3:00 pm	Depart for orchard	
3:40 pm	Orchard walk hosted by Costa	Mertho Road - *Instructions provided at forum*
5:00 pm	**End**	

WESTERN AUSTRALIA

Technical theme: Irregular bearing

Australian avocado industry

Regional Forum

INDUSTRY DEVELOPMENT & EXTENSION PROJECT

Thursday 27 June 2024

Forum - Pemberton Mill Hall, 15 Brockman Street

Orchard walk - TBC

TIME	ITEM	PRESENTER
8:15 am	**Registration**	
9:00 am	WA regional lead welcome	Rachelle Johnstone (WA DPIRD)
9:10 am	Introduction to project	Bridie Carr (DAF)
9:20 am	Industry update	John Tyas (AAL)
9:50 am	Industry benchmarking	Gurtej Sidhu (RMCG)
10:10 am	Crop load study	Declan McCauley (DPIRD)
10:30 am	**Morning tea**	
11:00 am	Irregular bearing	Harley Smith (CSIRO)
11:25 am	Round robin group session	Growers and speakers
12:30 pm	Wrap up	Rachelle Johnstone (WA DPIRD)
12:45 pm	**Lunch**	
1:30 pm	Departure for orchard walk	
2:00 pm	Orchard walk hosted by	
3:30 pm	**End**	

The avocado industry development and extension project (AV23010) has been funded by Hort Innovation, using the avocado research and development levy, contributions from the Australian Government and co-investment from the Queensland Government Department of Agriculture and Fisheries, NSW Department of Primary Industries, and the WA Department of Primary Industries and Regional Development. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

CENTRAL NSW

Australian avocado industry

Regional Forum

Technical theme: Yield variability & industry support

Date - Thursday 3 July 2025 | 8:30am - 3:30pm

Venue - Stuarts Point Community Hall - Ocean Ave, Stuarts Point NSW 2441

Orchard walk - Malcolm and Narelle Heather's orchard

TIME	ITEM	PRESENTER
8:30 am	**Registration - Stuarts Point Community Hall - Ocean Ave, Stuarts Point, NSW 2441**	
9:00 am	Central NSW regional lead welcome	Isabella Ellmers (AHR) & Ian Tolson (AAL)
9:15 am	National and Regional extension activities update	Renata Grunennvaldt (DPI) & Isabella Ellmers (AHR)
9:30 am	Industry update	John Tyas (AAL)
10:00 am	Roadmap for Enhancing Yield Consistency in the Australian Avocado Industry	Dr John Wilkie (Wilkie Horticulture)
10:30 am	**Morning tea - poster session**	
11:00 am	Avocado fruit set – Limiting factors and knowledge gaps in avocado	Dr Harley Smith (CSIRO)
11:30 am	Developing a non-destructive method to estimate carbohydrate levels in avocado	Dr Everard Edwards (CSIRO)
12:00 pm	Light interception and avocado orchard productivity	Dr John Wilkie (Wilkie Horticulture)
12:30 pm	Wrap up and grower evaluation	
12:40 pm	**Lunch**	
1:20 pm	Departure for orchard walk	
1:35 pm	Orchard Walk	Malcolm & Narelle Heather
2:15pm	Orchard walk round robin station sessions: • Carbohydrates, flowering and fruit set - Harley Smith • Yield consistency, canopy management and light interception - John Wilkie • Industry conversations - Ian Tolson, John Tyas, NSW DPI recovery response representative • R&D - Mary Burton & Gemma Burger	
3:30pm	**End**	

Hort Innovation AVOCADO FUND

Avocado irregular bearing workshop

Date - Wednesday 7 August - Friday 9 August 2024
Venue - State Library of Queensland, Cultural Precinct, Stanley Pl, South Brisbane

WEDNESDAY 7 AUGUST - BOARDROOM (LEVEL 5)

TIME	ITEM	PRESENTER
12.00 pm	**Registration & Lunch**	
12: 45 pm	Welcome, introduction and workshop aims	John Wilkie (Project lead)
1: 30 pm	Avocados Australia update	John Tyas (CEO of AAL)
2: 20 pm	Revisiting the Tony Whiley project and comprehensive survey	Simon Newett (Consultant)
2:50 pm	**Afternoon tea**	
3: 15 pm	Domestic survey of practices and regional perspectives discussion	Renata Grunennvaldt (DAF)
Theme 1 - Flowering, pollination & fruitset		
4: 30 pm	Flowering and fertilisation - International guest speaker	Inaki Hormaza (CSIC Spain)
5:30 pm	**FINISH**	
6:30 pm	**Optional group dinner (venue TBC)**	

THURSDAY 8 AUGUST - BOARDROOM (LEVEL 5)

TIME	ITEM	PRESENTER
8.30 am	**Welcome & recap**	
9: 00 am	Highlights and synthesis of literature review and Tony Whiley's report	John Wilkie (Project lead)
9: 30 am	Group discussion	
Theme 1 - Flowering, pollination & fruitset		
10: 00 am	Pollination	Stephen Trueman (Griffith Uni)
10:30 am	**Morning tea**	

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

Hort Innovation AVOCADO FUND Wilkie Horticulture Queensland Government

THURSDAY 8 AUGUST - BOARDROOM (LEVEL 5)

TIME	ITEM	PRESENTER
Theme 2 - Carbohydrates, competition and abscission		
10: 50 am	Abscission model	Harley Smith (CSIRO)
11: 20 am	Carbohydrate monitoring	Craig Boyce (Costa)
11: 40 am	Carbohydrate allocation and storage	Gerhard Rossouw (DAF)
12: 00 pm	Group discussion	
12:30 pm	**Lunch**	
Theme 3 - Crop load management		
1: 00 pm	Western Australian crop load trial	Declan McCauley (DPIRD)
1: 30 pm	New Zealand flower pruning trials - International guest speaker	Phillip West (NZ Avocado)
2: 00 pm	Group discussion on avocado crop load management	
2: 15 pm	Apple crop load management strategies	Sally Bound (Uni of Tasmania)
2: 45 pm	Group discussion on other crop load management strategies	
3: 00 pm	**Afternoon tea**	
3: 30 pm	Framing irregular and alternate bearing	Amnon Haberman (DAF)
4: 00 pm	Alternate bearing - International guest speaker	Vered Irihimovitch (ARO Israel)
4: 30 pm	Group discussion	
5: 00 pm	Recap key discussion points and outcomes	
5: 30 pm	**FINISH**	

FRIDAY 9 AUGUST - HERITAGE COLLECTIONS LEARNING ROOM (LEVEL 4)

TIME	ITEM	PRESENTER
8: 30 am	**Welcome, target outcomes, and plan for day**	
9: 00 am	Group discussions and workshop activity for road map development	
11: 45 am	Closing remarks	John Wilkie (Wilkie Horticulture)
12:00 pm	**END & LUNCH**	