Hort Innovation

Final Report

Carbohydrate monitoring to predict yield

Project leader:

Harley M. Smith

Delivery partner:

CSIRO Agriculture & Food

Project code:

AV19006

Project:

Carbohydrate monitoring to predict yield (AV19006)

Disclaimer:

Funding statement:

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

Publishing details:

ISBN 978-0-7341-4712-7

Published and distributed by: Hort Innovation

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Content

Summary

Avocado productivity is influenced by irregular bearing, which is a significant challenge to this horticulture industry. The three drivers of irregular bearing are poor fruit set, high fruit abscission and biennial bearing. Research from AV16005 has demonstrated that conditions which negatively impact stem starch reserves reduce flowering and fruit set and increase the rate of fruit abscission, suggesting that tree carbohydrate status is a major driver of irregular bearing. Avocado growers have conceptualized the requirement for a cost-effective system(s) to rapidly and non-destructively estimate tree carbohydrate status in order to evaluate the reproductive potential of the orchard.

The first component of this project was a desktop review of technologies that could potentially be used for non-destructive, in-field determination of tree carbohydrates in avocado, with the aim of identifying the technology most amenable for future development as a tool for commercial use. The first, introductory, chapter of the review provides background knowledge of non-structural carbohydrates, including the sources of these substances and how they are used for growth. This chapter also reviews current knowledge on storage carbohydrates and their impact on reproductive development. In the second chapter, non-destructive methods with potential application for measuring carbohydrates in plants were reviewed, considering the requirement for in-field measurement. Results from this literature review conclude that diffuse near-infrared (NIR) reflectance spectroscopy has the greatest potential to be developed into a cost-effective and rapid tool for assessment of carbohydrates in trees. The third chapter of the review considered an alternative approach to a sensing technology for predicting the tree carbohydrate status, through a modelling approach. This chapter introduces the concept of Photothermal Quotient (PTQ), a method which has been successfully used as a surrogate of carbohydrate levels for estimating yield in annual plants. In addition, this chapter discussed how PTQ can be used evaluate the impact of environmental stresses at different times during the reproductive cycle and how this influences yield.

The second component of this project was a proof-of-concept to demonstrate the potential of NIR reflectance spectroscopy for determining non-structural carbohydrate levels in avocado. With the time and resources available, it was determined that the most effective way to perform this proof of concept was a laboratory-based trial using field collected samples and hardware used for on-the-go field measurements. Avocado leaves and stems were scanned with an NIR hyperspectral imaging system, which produces a 2D image of the object (leaf or stem), where each pixel consists of an entire reflectance spectrum, which can be used for, at least partial, identification and quantification of the object's chemical constituents. This was achieved by building calibration models for non-structural carbohydrates, including starch and/or soluble sugars such as sucrose, glucose, fructose, perseitol and mannoheptulose. The models utilised machine learning tools to correlate features of the reflectance spectra with carbohydrates determined using standard wet chemistry methods. Results from this proof of concept confirm that NIR reflectance spectroscopy has the potential to be further developed to assess total non-structural carbohydrates.

The final component of this project trialed the use of PTQ to assess carbohydrate status avocado. Firstly, regional long-term climatic data were calculated and seasonal environmental conditions assessed for their potential impact on yield. While growing regions receive similar amounts of radiation used to drive photosynthesis, differences in maximum temperature and vapour-pressure deficit (VPD) influence yield potential. In addition, yield potential is also impacted by climate events including frost and heat stress. Secondly, with the commercial yield data made available to this study, poor associations between avocado yield and PTQ were observed. Finally, when multi-season starch measurements were extracted from previously published studies in Australia and the USA, a close association was observed between stem/branch starch levels and the monthly mean PTQ. This close association over a number of seasons, from two independent studies, indicates that PTQ has potential to be developed into an in-season assessment of carbohydrate status of avocado.

Keywords

Avocado, non-structural carbohydrates (NSC), starch, mannoheptulose, perseitol, near infra-red radiation (NIR), NIR reflectance spectroscopy (NIRS); hyperspectral imaging, photothermal quotient (PTQ), vapour pressure deficit (VPD).

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 barrier for maximizing yields, stabilizing supply and maintaining market development efforts.

Carbohydrates, or sugars, are essential substrates required for the growth and development of flowers, fruits, leaves, stems and roots (Eveland and Jackson, 2012; Barbier et al., 2015). Non-structural carbohydrates (NSC), which primarily includes soluble sugars and starch, are utilized to for the synthesis of key macromolecules which required for growth and development (Oliveira and Priestley, 1988; Hoch et al., 2003). In addition, soluble carbohydrates including glucose, sucrose and trehalose-6-phosphate act as signaling molecules that regulate key developmental processes including flowering, branching and shoot growth (Eveland and Jackson, 2012; Barbier et al., 2015).

All carbohydrates produced in a tree are derived from photosynthesis in the canopy. Once produced, carbohydrates are transported throughout the tree and incorporated into biomass during growth, utilized by respiration to produce the energy required for plant process, or stored as reserves for future use (Oliveira and Priestley, 1988; Hoch et al., 2003; DeJong, 2019). Although NSC can be stored in any live tissues, long-term storage is typically occurs in the xylem parenchyma cells of the woody tissues (stems, branches, trunks and roots). When growth and energy demands exceed the photosynthetic capacity of the tree, stored carbohydrates are mobilized to support growth. In avocado, fluctuations of stored starch in stems/branches correlates with reproductive and vegetative growth patterns in the tree (Scholefield et al., 1985; Whiley et al., 1996; Liu et al., 1999). Therefore, the timing and degree of starch accumulation, as well as mobilization, in woody tissues appears to be an indicator of tree carbohydrate status that plays a major role influencing reproductive growth and yield (Whiley and Wolstenholme, 1990; DeJong, 2019). This hypothesis is supported by research from AV16005, which provides evidence that avocado tree carbohydrate status is a major factor that influences flowering, fruit set and fruit abscission. (A. Haberman, M. Goetz and H. Smith, unpublished). Therefore, having capability to effectively manage carbohydrate accumulation in winter, control starch mobilization at flowering and direct sugars to flowers and fruits during the growing season is predicted to increase yields in avocado. In a step toward carbohydrate management and yield prediction, growers require a rapid, on-farm and non-destructive technology to estimate carbohydrate levels in trees.

The first objectives of this project were to perform a desktop analysis to review and identify a non-destructive approach(es) for estimating carbohydrate levels in avocado plant tissues that has the potential for further commercial development. Through this desktop review, two non-destructive technologies were identified to estimate carbohydrates in avocado leaves and trees, including near-infrared (NIR) reflectance spectroscopy and photothermal quotient (PTQ). The subsequent objective for this project was to undertake a proof of concept for these non-destructive approaches, where they were evaluated in order to assess the effectiveness of these technologies for estimating carbohydrates in avocado trees. Based on the outputs and outcomes of this project, recommendations and risks are discussed for future R&D investment.

Methodology

Project reach

Project activity was performed at the Waite Campus, including the Waite Campus Orchard, CSIRO High Value Laboratory Unit and a commercial orchard in the Riverland. For PTQ analysis, desk-top analysis was performed at the St Lucia campus of The University of Queensland. Weather data was downloaded from the Queensland Government's Silo database and from the Bureau of meteorology. Yield data from Commercial orchards was supplied by growers in Queensland, New South Wales and Western Australia.

Target Audience

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

Key research activities

Project activity was subdivided into two sections. The first section of the project involved the development of a desktop review. This review consisted of three chapters: (1) introduction to avocado carbohydrate dynamics, (2) potential methods for field measurements of plant carbohydrates and (3) introduction to photothermal quotient modelling as a potential surrogate for tree carbohydrate status. In summary, diffuse NIR reflectance spectroscopy was identified a potential non-destructive approach to estimate carbohydrates in avocado. The desktop analysis also suggests that PTQ may be adapted to horticultural trees as a surrogate for tree carbohydrate levels and used for estimating inter-seasonal variation in yield. The desktop review was provided with milestone 103 and is attached in Appendix B.

The second section of the project, proof of concept demonstrations, was subdivided into two activities: a trial of NIR reflectance spectroscopy and a sample application of PTQ to avocado using historical yield data.

Developing an appropriate NIR sensor, together with data analytics and trials in the field was well beyond the capacity of a project of this size. Therefore, the NIR proof of concept was undertaken by using fresh leaf and stem samples collected from the field, scanning them with a laboratory-based NIR-hyperspectral imaging system, using traditional wet-laboratory analytical measurements to determine the concentration of key non-structural carbohydrates and then developing a chemometric calibration between the NIR scans and the results of the wet-laboratory analysis. In order to perform this, a tissue preparation and analysis workflow was developed for the collection of leaves and stems in the field, transport to the laboratory, hyperspectral imaging and carbohydrate determination. The hyperspectral imaging allowed hundreds of NIR spectra per sample to be collected. Next, a computational workflow was developed to process the hyperspectral images, including identifying the tissue sample from the background, processing the spectra to account for lighting variation and generating a single representative spectra per sample to be used for calibration development. In order to develop an effective calibration, it was essential that the leaves and stems collected display as large a range of carbohydrate content as possible. Therefore, leaves and stems were collected from trees infested with *Phytophthora cinnamomic*, a major soil pathogen that impacts tree productivity, as well as healthy trees. In addition, leaves were collected from water stressed trees derived from a AV16005 trial, in which the irrigation was reduced by 25 and 50% at the time of flowering.

Concentrations of up to six individual carbohydrates were determined in the collected and scanned leaf samples, including starch, glucose, fructose and sucrose as well as the two C_7 sugars, mannoheptulose and perseitol, uniquely prevalent in avocado. Together, these carbohydrates were expected to represent the vast majority of NSC in avocado. Nine NSC data sets were established using various combinations of these six carbohydrates and used to develop the calibrations against hyperspectral imaging data using machine learning computational tools. Three of the data sets included the C_7 sugar measurements, as experimental studies suggest that these sugars have a storage function (Liu et al., 2002; Tesfay et al., 2012). Eight of the data sets were derived from leaves. The ninth data set was derived from xylem tissue in stem, in which starch only was measured.

The second proof of concept activity tested the use of PTQ analysis, as a surrogate for carbohydrate supply to explain yield variation in avocado. Firstly, long term Climate data was downloaded for seven regional locations from Atherton in Queensland, down to Loxton in South Australia and across to Yoongarillup in Western Australia to assess regional yield potential. Climate data are presented for maximum temperature (°C), minimum temperature (°C), radiation (MJ m^{-2} d^{-1}), VPD_{max} (hPa), PTQ (MJ m^{-2} d^{-1} $°C^{-1}$), daily evaporation (mm d^{-1}) and monthly rainfall (mm).

Secondly, growers were invited to supply their commercial yield data via brief story in Avocados Australia newsletter 'Guacamole' and by an email invitation from Simon Newitt, Avocado Horticulturalist at QDAF. Six growers provided yield data sets for PTQ analysis. Daily weather data was downloaded from SILO's gridded data sets for the point (location) closest to the orchard being examined. Due to a lack of observational data a 'flowering date' of September 30th was assumed for all sites to separate the pre-flowering and post-flowering (fruit growth) periods. Grower yields were plotted against pre- and post-flowering PTQ to investigate relationships between the two.

Finally, the literature was searched for multi-year data sets tracking year to year avocado tree carbohydrate status. Daily weather data was downloaded from SILO's gridded data sets for the point (location) closest to the experimental site. In-season carbohydrate status and PTQ was plotted on the same axis to examine possible correlations between these two variables.

Outputs

Detailed results for the outputs are provided in Appendix A. The desktop review and project management are located in Appendix B and C, respectively.

Output 1: Project Management:

- Program logic and monitoring and evaluation plan
- Project risk management
- Stakeholder engagement/communication plan

Output 2: Desktop Review:

- A comprehensive review on: (1) the role of tree carbohydrate status on productivity and (2) technologies for evaluating tree carbohydrate statues including non-destructive methods.
- Diffuse NIR reflectance spectroscopy was identified as the non-destructive technology for the proof of concept.
- The review of photothermal quotient modeling indicated that this approach may be feasible in tree crops, such as avocado, but would likely need the previous seasons yield to be included as a co-variate.

Output 3: Proof of concept – Hyperspectral imaging:

- Workflow developed for sampling, transport and hyperspectral imaging of avocado leaves and stems prior to starch and carbohydrate determination.
- Nine non-structural carbohydrate data sets produced.
- Workflow established for processing hyperspectral images and the use of machine learning tools to build calibrations against the non-structural carbohydrate data sets.
- Wavebands that associate with leaf starch and/or total NSC levels were identified.

Output 4: Proof of concept – Photothermal Quotient

- Yield and weather data compiled from commercial orchards and regional climatic data. Regional long-term climatic data was interpreted, as this likely impacts vegetative growth, flowering, fruit set, fruit abscission and yield in avocado.
- Association between PTQ and commercial yield assessed. Note: yield should correlate with radiation. However, in practice less predictable events, such as frost and heat stress, that impact yield and over-ride PTQ.
- Association between PTQ and seasonal starch measurements determined for published data sets. Analysis and comparison of published, multi-year seasonal variation in tree carbohydrate status was plotted against PTQ and results indicate agreement between the two variables.

Output 5: Assessment of NIR reflectance spectroscopy and photothermal quotient

- NIR reflectance spectroscopy: The laboratory-based hyperspectral imaging system used in this proof of concept indicates that NIR reflectance spectroscopy can be used to estimate starch and/or total NSC levels in avocado leaves. Additional work is required to expand this initial calibration into a robust commercially applicable calibration and, critically, to develop suitable hardware to reliably determine NIR reflectance spectra under field conditions. Acceptably accurate assessment of soluble sugars in whole tissues was not demonstrated, but would likely be possible with a larger data set. Additional work is also required to determine the most appropriate application of this technology for estimating carbohydrate levels in stems.
- PTQ analysis: While it is logical that yield should correlate with radiation, in practice weather extremes experience by orchards that impact yield over-ride PTQ analysis. However, analysis of published data sets which measured NSC in stem/branch tissues over a two-year period was plotted against PTQ and results indicate agreement between these two variables.
- An article that summarizes the results of the desktop study is under preparation for publication in “Talking Avocados” (AV18003), and will be submitted in early July 2021.
- A second, technical, article that summarizes the results of the NIR proof of concept is under preparation for publication in a Australian Tree Crop industry journal.

- A third article is in preparation for the Australian Tree Crop industry journal for the PTQ work performed in this project.
- Results from this project will be disseminated to industry at regional grower workshops and forums conducted by AV17005 (Avocado industry development and extension).
- A final report completed referencing the outputs from above (Milestone 190)

Industry publications:

- An article is in preparation that summarizes the results of the desktop study is under preparation for publication in "Talking Avocados" (AV18003)
- A second, technical article is in preparation for the NIR proof of concept for publication in the Australian Tree Crop industry journal. To maximize dissemination to the Avocado Industry, an issue where avocado is a featured crop will be targeted for publication.
- A fourth article is in preparation for the Australian Tree Crop Magazine on PTQ work performed in the project.

Conference and industry presentations

No conference or industry presentations as of yet. However, the project team has communicated with Simon Newett, the avocado industry development and extension specialist and project leader for AV17005, that we are willing to present the results of the project at Avocado Regional Forums in the future.

Outcomes

The intended outcomes for measure of success are discussed below.

Technologies for non-destructive evaluation of avocado tree carbohydrate status have been identified

A major outcome of this project was the identification of non-destructive approaches for the estimation of NSC in avocado with the potential for commercial in-field application. A range of non-destructive technologies for identification of chemical constituents of intact samples were reviewed. Further, these technologies were also considered for on-the-go measurements vs point-based measurement. The following technologies were reviewed:

- X-Ray Fluorescence (XRF) Spectroscopy
- Ramen Spectroscopy
- Attenuated total reflection (ATR) – Fourier Transform Infrared (FTIR) Spectroscopy
- Diffuse Reflectance Spectroscopy
- Terahertz (THz) Spectroscopy
- Nuclear Magnetic Resonance (NMR) Spectroscopy

After reviewing these technologies, diffuse reflectance spectroscopy, specifically at near infrared (NIR) wavelengths, was identified as the most promising technology for a proof-of-concept practical trial.

Additionally, a modelling approach, termed photothermal quotient (PTQ), was also considered. This approach utilizes light radiation, temperature and vapor pressure deficit, all of which impact photosynthetic CO_2 assimilation to surrogate carbohydrate supply available to the orchard per unit of development. This approach combines these climate factors together with historic yield data in order to estimate production.

Concept for non-destructive assessment of tree carbohydrate status has been demonstrated with an identified technology

The purpose of the proof of concept was to provide a practical demonstration that the technology identified by the desktop review did indeed have the potential for further development into a commercially viable tool. The project was not only successful in assessing NIR reflectance spectroscopy, producing a preliminary calibration from commercially available NIR imaging hardware with a high degree of accuracy for measurement of both leaf starch and of total NSC, but also began to address some of the issues around applicability of the technique, such as identification of key wavebands, choice of sample tissue, measurement of different carbohydrates, dealing with differences in sample illumination, etc. The outcome of this technology proof of concept is a demonstrated successful application of NIR reflectance spectroscopy to measure avocado leaf carbohydrates, as well as providing a solid basis to assess the viability of future work towards developing the hardware and software suitable for widespread industry adoption.

In irrigated well managed annual agricultural crops, light radiation correlates with yield due to extensive breeding and optimized management inputs. In contrast, semi-domesticated tree crops, including avocado, are optimized for survival and reproduction rather than maximal yield (Whiley and Schaffer, 1994). In addition, the competition for assimilate between vegetative and reproductive growth, as well as among reproductive units, during the growing season likely impacts carbohydrate availability and partitioning to flowers and fruits (Wolstenholme, 1990). In each of the avocado growing regions, the peak in PTQ occurs in the lead up to flowering in the winter months. In cooler regions there is likely to be competition for assimilate between maturing fruit on the tree and developing flowers, while in warmer regions these two phases are separated by several months, as fruits are harvested well before flowering. Commercial yields examined in this project were not related to either pre- or post-flowering PTQ. The conclusion of this project was that there are too many competing sinks in avocado for PTQ to correlate with yield. In addition, chance weather events such as frost, heat and high-VPD may adversely impact yield in any season. However, during the course of this study, carbohydrate data was extracted from two published, multi-year studies of avocado. When seasonal variation in tree carbohydrate status was plotted on the same axis as PTQ there was good association between stem/branch starch levels and PTQ.

Developmental pathway identified of a non-destructive carbohydrate assessment to provide commercially relevant yield prediction insights for avocado growers

NIR reflectance spectroscopy, implemented as hyperspectral imaging was demonstrated as a potential non-destructive approach to measure NSC in avocado. This assessment was based on a laboratory-based system with controlled lighting conditions. Developing this to a commercially viable tool for grower use would require several further steps:

1. generate considerable leaf and stem carbohydrate data sets, to not only increase accuracy of NSC measurements but also to establish whether key carbohydrates can be individually determined, such as C7 sugar levels, as these unique sugars are highly abundant, used for transport and may play a significant role in storage.
2. Develop field hardware. This step will need to consider cost-effectiveness of hardware vs accuracy of potential measurement and develop appropriate mechanisms to cope with mixed natural lighting or develop an appropriate active lighting system.
3. Develop large scale calibrations based on field collected data using samples across a broad section of the avocado industry to ensure applicability in all growing regions and management systems.
4. Assuming that avocado canopy remains the tissue of choice for NSC determination, ensure the hardware developed is suitable for on-the-go application and that accurate data can be collected from the chosen vehicle (whether ground or air).
5. Determine commercial partners and a commercialisation pathway for the field-proven system, including assuring cost-effectiveness and user-friendly software tools.

While it intuitively makes sense that yield would be related to PTQ, in practice, competition among sinks for assimilate and chance weather events make it unlikely to establish a correlative relationship between yield and PTQ. However, the observed correlation between in-season stem/branch starch levels derived from Scholefield et al., 1985 and Liu et al., 1999 and PTQ indicates that this method could be utilized to predict in-season tree carbohydrate storage. These findings would need to be tested and validated, as a component of future work examining rapid, cost effective methods of assessing the carbohydrate status of avocado orchards for predicting yield.

Avocado industry understands the potential risks and benefits of further investment in non-destructive carbohydrate assessment

Experimental studies indicate that stem/branch starch reserves are a major factor that influences reproductive development (DeJong, 2019; Scholefield et al., 1985; Whiley et al., 1996; Haberman, A., Goetz, M., and Smith, H, unpublished AV16005). To date, an understanding of how flowering, fruit set, fruit abscission and yield is impacted by total NSC content in the leaf canopy is not understood. Therefore, a potential risk for further investment is that carbohydrate content in the leaf canopy doesn't match tree carbohydrate status and therefore, has little impact on the reproductive potential of the tree. However, experimental studies indicate that in well managed orchards, increased yields were associated with the interception of light radiation (DeJong, 2019). Therefore, on the go non-destructive sensors may provide a research tool to further address the role of canopy leaf carbohydrate levels with key reproductive events in avocado. The second risk is centered on the idea that trees exhibit a level of branch or shoot autonomy (DeJong, 2019), which isolates reproductive organs, such fruits, from sources of carbohydrates in other parts of the tree. Therefore, overall tree status in the canopy may not match yield. A third risk for future investment is that a cost-effective hardware solution cannot be developed due to high costs associated with building a sensor and/or a light illumination system. The fourth risk, is that a calibration with enough accuracy and broach applicability (different regions, cultivars, management scenarios, etc) for useful tree carbohydrate management is not achievable. Lastly, there are no management practices adequate to make use of an accurate non-destructive approach to estimate tree carbohydrate for practical improvement in yield variability.

Yield data from growers and insufficient management may make it difficult to perform and utilize PTQ to surrogate carbohydrate and predict yield. In this study, PTQ analysis was focused on climate factors at flowering; however, yield is primarily determined by factors that impact fruit set and abscission. These two reproductive events are sensitive temperature extremes, humidity as well as the previous seasons crop and carbohydrate partitioning. In addition, yield is also impacted by vegetative flushes, which likely competes for carbohydrates with flowers and fruits during the growing season. The fact that PTQ was unable to predict yield in grower data set provided indicates that flowering is not the only reproductive event that contributes to yield. In fact, research outputs from AV16005 indicate that fruit set and fruit abscission are the major drivers of yield.

Monitoring and evaluation

Four "Key Evaluation Questions (KEQ)" were used to evaluate the performance of the project.

The first KEQ regards the effectiveness of the project.

To what extent has the project achieved its expected outcomes?

- The project completed the desktop study on carbohydrate monitoring in avocado, which included a review of carbohydrates in avocado, field measurement of plant carbohydrates and photothermal quotient (PTQ) as a surrogate for carbohydrate status in avocado.
- Through the desktop review a non-destructive method to measure carbohydrates in avocado was identified. The first method identified was diffuse reflectance spectroscopy, as this can provide a quantitative measure of NSC and is non-destructive (where deep tissue does not need to be illuminated). Moreover, this method is non-contact, meaning that it has to potential to be used on-the-go and has a wide variety of potential hardware implementations, which can be developed as a cost-effective approach for practical in-field evaluation with precision and repeatability (Magwaza and Opara et al., 2015). Diffuse NIR spectroscopy can detect C-H and other bonds found in carbohydrates and has been successfully used to calibrate grape berry composition (Edwards et al., 2020). As an alternative to NIR reflectance spectroscopy, PTQ was the second approach discussed in the desktop review and considered for assessment. In any broadacre or horticultural crop species, PTQ is the underlying mechanism that drives photosynthesis and the resulting growth and rate of development. Successful identification of ways that PTQ could be used to predict carbohydrate status and yield of avocado would benefit the avocado industry
- The project performed a proof of concept for NIR diffuse reflectance spectroscopy and this method was assessed for estimating non-structural carbohydrate levels in avocado leaves. The project achieved the expected outcomes as this method was shown to have the potential to estimate starch and possibly total carbohydrate (starch and soluble sugars).
- The project assessed the application of PTQ to surrogate tree carbohydrate status in avocado. While PTQ did not correlate well with yield derived from a number of orchards, this method may be valid only in well managed orchards. Interestingly, an unexpected outcome of this analysis indicates that PTQ is able to predict stem/branch starch levels in avocado derived from two independent data sets (Scholefield et al., 1985; Liu et al., 1999).

The second KEQ regards the relevance of the project.

How relevant was the project to the needs of intended beneficiaries? The beneficiaries or growers/farm managers are interested in managing tree carbohydrates in order to increase yield. A step in this direction involves the identification and development of non-destructive approaches to estimate carbohydrates in avocado trees.

- The project delivered a comprehensive review on carbohydrates in avocado and identified diffuse NIR reflectance and PTQ as possible non-destructive technologies to estimate carbohydrates in avocado.
- The project has performed a proof of concept for diffuse NIR reflectance using hyperspectral imaging. Based on the initial assessment using a laboratory-based hyperspectral imaging system with whole tissue samples collected from the field and the demonstrated ability to predict starch and/or total NSC in leaves, further development of this technology for in-field use is warranted and clearly has the potential for use by commercial growers given suitable hardware. While PTQ could be expected to correlate well with yield of avocado in well managed avocado orchards, in practice there many other management and environmental factors that can also affect the yield outcome thereby limiting the widespread use of PTQ to predict yield. However, the association between stem/branch starch levels derived from Scholefield et al., 1985 Liu et al., 1999 and PTQ calculated during a two-year growing period may provide a pathway for in-season monitoring of PTQ to predict carbohydrate status of avocado trees.

The third KEQ regards the process appropriateness for the project that includes the level of engagement.

How well have intended beneficiaries been engaged in the project? Grower engagement into the project was through the Project Reference Group (PRG).

- At the PRG meeting held on 12 January, growers engaged in discussions about the non-destructive carbohydrate measurement approaches identified in the desktop study. Discussions were raised about the applicability of PTQ as a surrogate for carbohydrate status and possible issues of calculating thermal time for avocado.
- Target audiences of the project (growers, farm managers and consultants) will be engaged through future "Talking Avocados" and Australian Tree Crop publications developed by the project. These articles will be submitted for publication in the next month. The project leader and key staff will participate and engage with industry at future Avocado Open Forums organized by AV17005 (Avocado industry development and extension) and Avocados Australia Limited.

Recommendations

-Non-structural carbohydrates derived from photosynthesis and stored starch in woody tissues mediates avocado growth and development. Preliminary data from the proof of concept indicates that LASSO modeling can be utilized to predict total NSC levels in leaves, as well as starch in stems, from NIR reflectance spectra. To validate these results and extend to broad industry applicability, much larger data sets will need to be generated in a future study.

-Collection of diffuse NIR reflectance spectra followed by LASSO mediated modelling demonstrate the potential use of this approach for predicting total NSC levels in leaves and stored starch in stems. However, the assessment was made using a laboratory-based hyperspectral imaging system under controlled illumination. The next step in evaluating the usage of NIR reflectance spectroscopy followed by LASSO modeling is to undertake in-field data collection. Additional experimental steps will be required before this objective can be achieved. Hardware capable of collecting NIR reflectance spectra can range from AU$10,000 to AU$250,000+, furthermore the project has also considered the possibility of taking a multispectral approach (reduced to eight wavebands). The next step is to assess the relative merits of different hardware approaches in-field and their suitability, not only for collecting data able to be used to build accurate calibrations, but also for commercial viability in regards to hardware cost. Secondly, the use of varying natural lighting and different artificial NIR sources need to be assessed for their impact on effective data collection. Thirdly, new calibrations will need to be built using the chosen hardware and at a much larger scale than used in the proof of concept described here, ideally encompassing the entire avocado industry plantings. Interaction with commercial growers needs to be maintained throughout this process to ensure that chosen solutions will be cost-effective (labour, hardware and software) and practical in their anticipated final form.

-Analysis of regional long-term climatic data identified differences between locations that are likely to have negative, and in some cases positive, impacts on the production system. Impacts of temperature on the crop cycle alters the timing of key developmental stages in relation to the environmental conditions. Investment into research to optimize the management of orchards in relation to the environment could result in improvements to the region-specific management of orchards. PTQ did not correlate well with yield of avocado in the data sets that we examined. While it is reasonably expected that there could be associations between PTQ and yield for well managed avocado orchards, in practice there are other management and environmental factors that can impact on yield thereby limiting the widespread use of PTQ. However, the agreement between measured carbohydrate extracted from the literature (Scholefield et al., 1985; Liu et al., 1999) and PTQ calculated over the same time period does provide the possibility of in-season monitoring of PTQ to predict carbohydrate status of avocado trees. This finding warrants further examination starch data sets and discussions with researchers to validate the method in commercial orchards.

Refereed scientific publications

No refereed scientific publications were published during the reporting period.

References

Barbier, F.F., Lunn, J.E., Beveridge, C.A. (2015) Ready, steady, go! A sugar hit starts the race to shoot branching. Current Opinion in Plant Biology, 25:39-45

DeJong, T. (2019) Advances in understanding fruit tree growth. In: Achieving sustainable cultivation of temperate zone tree fruits and berries. Volume 1: Physiology, genetics and cultivation. (eds) Lang, G.A. Cambridge, UK, Burleigh Dodds Science Publishing. pp. 73-92.

Edwards, E.J., Thomas, M.R., Gensemer, S., Lagerstron, R., Khokher, R., Liao, Q., Sun, Chamngming, C., Wang, D., Hargrave, C., Ralston, J. (2020) New non-destructive technologies for simultaneous yield, crop condition and quality estimation. Final Report to Wine Australia, Adelaide, South Australia.

Eveland, A.L., Jackson, D.P. (2011) Sugars, signalling and plant development. Journal of Experimental Botany 63:3367-3377.

Hoch, G., Richter, A., Körner, C. (2003) Non-structural carbon compounds in temperate forest trees. 26:1067-1081.

Liu, X., Robinson, P.W., Madore, M.A., Witney, G.W., Arpaia, M.L. (1999). 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology. Journal of American Society of Horticultural Science 124:671-675.

Liu, X., Sievert, J., Arpaia, M.L., Madore, M.A. (2002) Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. Journal of American Society of Horticultural Science 127:108-114.

Magwaza, L.S., Opara, U.L. (2015) Analytical methods for determination of sugars and sweetness of horticultural products – a review. Scientia Horticulturae 184:179-192.

Oliveira, C.M., Priestley, C.A. (1988) Carbohydrate reserves in deciduous fruit trees. In: Horticultural Reviews. Volume 10: (ed) Janick, J., Portland, Oregon, USA, Timber Press. Pp. 403-430.

Rawson, H.M. (1988). Effects of high temperatures on the development and yield of wheat and practices to reduce deleterious effects. pp. 44-61. In: A.R. Klatt (ed.). Wheat production constraints in tropical environments. (CIMMYT: Mexico).

Scholefield, P.G., Sedgley M., Alexander D.M. (1985) Carbohydrate cycling in relation to shoot growth, floral initiation and development and yield in the avocado. Scientia Horticulturae 25:99-110.

Tesfay, S.Z., Bertling, I., Bower, J.P., Lovatt, C. (2012) The quest for the function of 'Hass' avocado carbohydrates: clues from fruit and seed development as well as seed germination. Australian Journal of Botany 60:79-86.

Whiley, A.W., Rasmussen, T.S., Saranah, J.B., Wolstenholme, B.N. (1996) Delayed harvest effects on yield, fruit size and starch cycling in avocado (*Persea americana* Mill.) in subtropical environments. II. The late-maturing cv. Hass. Scientia Horticulturae 66:35-49.

Whiley, A.W., and Schaffer, B. (1994) Avocado. In: Schaffer, B. and Andersen, P.C. (eds) Handbook of Environment Physiology of Fruit Crops, Volume 2, Subtropical and Tropical Crops. CRC Press, Boca Ranton, Florida, pp. 165-197.

Whiley, A.W., Wolstenholme B.N. (1990) Carbohydrate management in avocado trees for increased production. South African Avocado Growers' Association Yearbook 13:25-27.

Wolstenholme, B.N. (1990) Resource allocation and vegetative-reproductive competition: opportunities for manipulation in evergreen fruit trees. Acta Horticulturae 275, 451-460.

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report

Acknowledgements

The project team from CSIRO Waite Campus consisted of Harley Smith (Project Lead), Everard Edwards (Science Lead for NIR reflectance spectroscopy), Amnon Haberman (Post-doctoral Fellow) and Marc Goetz (Research Officer). Amnon Haberman collected and scanned leaf and stem samples, while Marc Goetz performed the starch and soluble carbohydrate measurements. Ryan Lagerstrom (Research Officer) at CSIRO Marsfield performed the calibration using machine learning tools. Anthony van Herwaarden (Science Lead for PTQ analysis) was a collaborator at the University of Queensland for modelling. The authors wish to acknowledge the investment contribution by Hort Innovation that enabled this innovative study to proceed. We would like to thank growers for contributing yield data sets that have allowed the analysis of environmental impacts on avocado yield. Finally, the authors would like to thank researchers, commercial consultants and growers for their valuable discussions of orchard practices, crop responses and validation of ideas.

Appendix A: Detailed Report

Contents

Detailed Methods

Materials

The *Persea americana* Mill. Cultivar used in this study was Hass. Leaf and stem material used in this study was collected from Hass trees at the Waite Campus and an orchard in the Riverland. To calibrate the lab-based NIR-hyperspectral imaging system it was essential to collect leaf and stem samples that display variation in carbohydrate content. To achieve this goal, leaves and stems were collected from healthy, abiotic- and biotic-stressed trees, as these stress conditions were likely to effect leaf and stem carbohydrates. Healthy leaf and stem samples were collected from three-year-old trees grown in pots at the CSIRO Waite Campus laboratory unit and from five-year-old trees at an orchard in the Riverland. For the biotic stress samples, leaves and stems were collected from 12-year-old *Phytopthora cinnamomi* infested trees at the Waite Campus Orchard. Linkage between AV19006 and AV16005 was established by utilizing drought stressed trees at an orchard in the Riverland. To induce a "drought" phenotype, irrigation was reduced by 25 and 50% in five-year-old trees. Both of these conditions resulted in significant reduction in stomatal conductance, which reduced starch levels in leaves and stems.

Sample collection and preparation

As a significant number of leaf and stems samples were obtained from an orchard in the Riverland, a four-hour drive from Adelaide, a trial was performed to determine if leaf and stem spectra were altered over a 24-hour period after harvesting the tissue. Results from this time course experiment showed that the spectra for leaves was not altered for up to 24 hours, as long as the leaves remain at 4°C (data not shown). As starch reserves accumulate in xylem parenchyma cells in woody tissues (DeJong, 2019), stems were cut longitudinally and the outer layer part of the stem consisting of epidermis, cortex and phloem was separated from the xylem and pith layers. Hyperspectral imaging of the xylem stem samples was used for the calibration. In contrast to leaf samples, the spectra for xylem-stem samples were altered when samples were kept at 4°C, over a 24 h period (data not shown). Whether the alteration in the xylem-stem spectra over time is significant remains to be determined. To reduce spectra variability, all samples were collected at mid-day and scanned at 24 hours after collection. This procedure ensured that the spectral integrity will be minimal and consistent between the samples.

Hyperspectral imaging

NIR reflectance spectroscopy was identified as the most practical non-destructive technology, with the most viable pathway to commercial use, for the estimation of carbohydrate levels in avocado (see desktop study, Appendix B). Non-contact NIR spectroscopy, which would be required for on-the-go field measurement also requires identification of target tissue (i.e. leaves). Hyperspectral imaging, where each pixel of a 2D image consists a reflectance spectrum, provides the most comprehensive NIR reflectance data source to simplify this requirement.

Consequently, NIR hyperspectral imaging was chosen for the proof-of-concept method to measure carbohydrates in avocado leaves and stems.

An NIR imaging system capable of being deployed in the laboratory or the field was used, but all data was collected in the laboratory to ensure that the potential of the method was assessed without the limited resources for the proof-of-concept being committed to solving problems with field deployment. A Headwall Micro eVNIR line-scan camera with Headwall halogen light source (Portable Analytical Solutions, NSW, Australia) was used in combination with a Zaber linear stage (Edmund Optics, Singapore) to move the sample past the camera. This camera is able to image 144 wavebands over a spectral range of 550-1650 nm at 300 pixels per line. The horizontal resolution was determined by the speed of the linear stage, as a line scan camera works by concatenating individual line scans (rows of 300 pixels) to form an image. Each pixel containing the full spectrum captured (144 wavebands). All imaging was carried out in a dark room/box where illumination was provided by an adjustable high intensity quartz tungsten halogen lamp. The camera position and linear stage speed were adjusted to provide approximately 20 pixels per cm in both image dimensions. A dark reference spectrum (covered lens, no illumination) was taken immediately prior to each sample scan and white reference spectra was taken during each sample scan. Samples were scanned on a custom built, 3D printed tray, which held white reference material provided with the Headwall Camera. All data manipulation and processing were carried out using R version 3.2 (R Core Team, 2015).

After imaging, the native camera data was corrected to reflectance using the following equation:

$$refl = (sample - dark)/(white - dark)$$

Where *refl* is the reflectance value, *sample* is the raw value from the camera, *dark* is the dark reference value and *white* is the white reference value. The reflectance value represented the intensity of return and ranged from 0 to 1. An example single band image is shown (Figure 1).

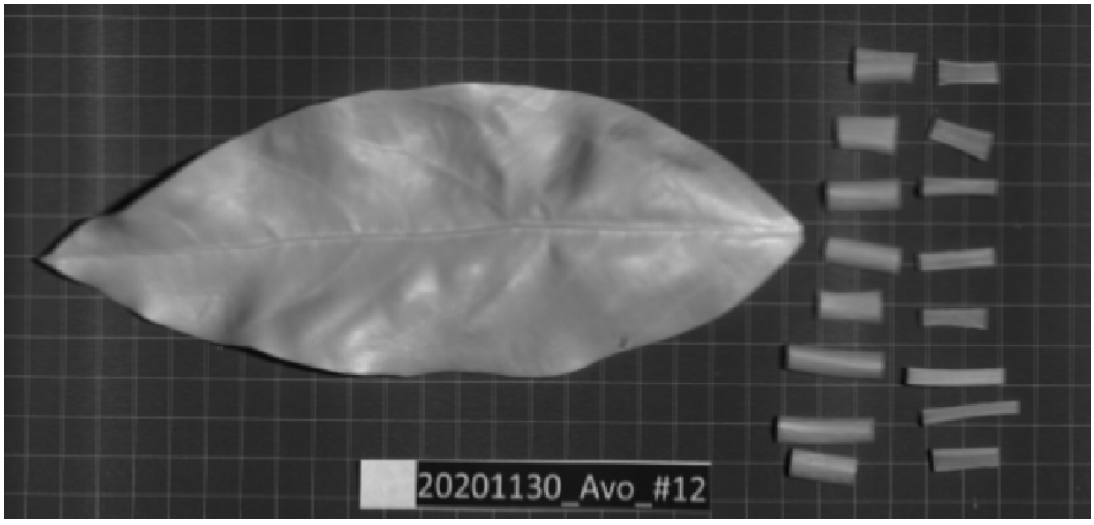


Figure 1: A typical sample scan, consisting of an avocado leaf scanned together with young stem sections taken from the same tree, illustrated using a single wavelength band.

As leaves are not perfectly flat (Figure 1), the illumination and the signal returned to the camera was uneven, which results in large amplitude variations in reflectance within homogenous samples. To account for the variations in reflectance, each spectrum is standardized by subtracting the spectrum mean reflectance and then dividing by the spectrum's standard deviation. The impact of standardising the variation of reflectance allowed the spectral shape to be leveraged in the quantification stage. The two plots in figure 2 show how the illumination variation was reduced by standardising variation in reflectance spectra.

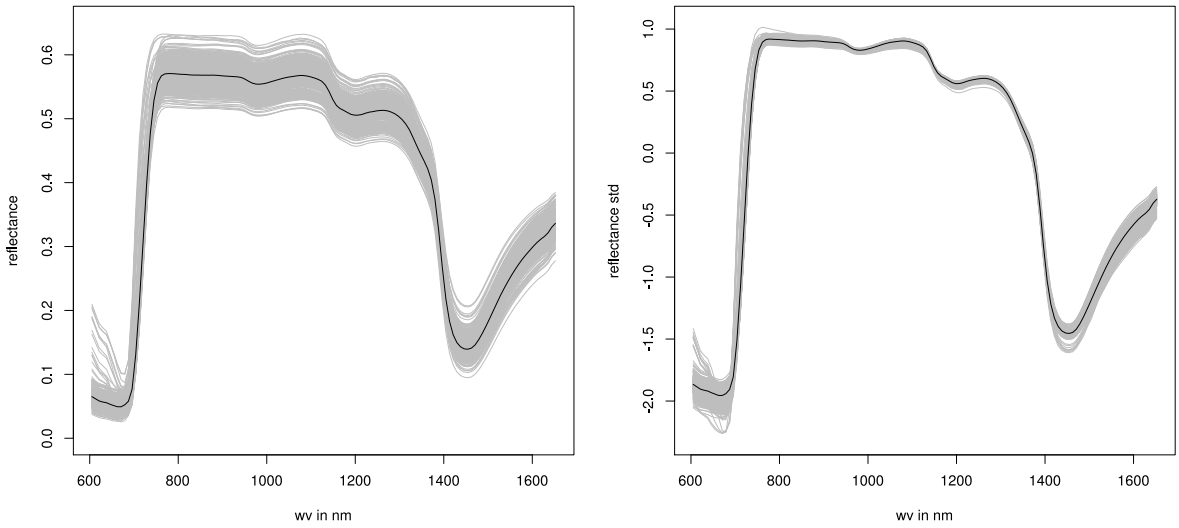


Figure 2: Leaf reflectance spectra from a single scan (left) and the same spectra after standardisation (right).

To develop carbohydrate calibrations, a representative spectrum was developed for each sample, as the entire sample was used for wet chemistry carbohydrate determination. For the leaf samples this process was initiated by isolating the pixels in the image which correspond to leaf tissue using the Spectral Angle Mapping (SAM) (Kruse et al., 1993). A library was built to apply the SAM approach by manually sampling leaf spectra over the entire imaging data set. In addition, pixels that were likely not exposed to enough light to generate a representative reflectance spectrum were excluded, based on the maximum reflectance over the entire wavelength range (spectral maxima less than 0.4). An example of this approach is displayed in Figure 3. A small dark area in the leaf where pixels were excluded to inadequate illumination is also identified in this figure.

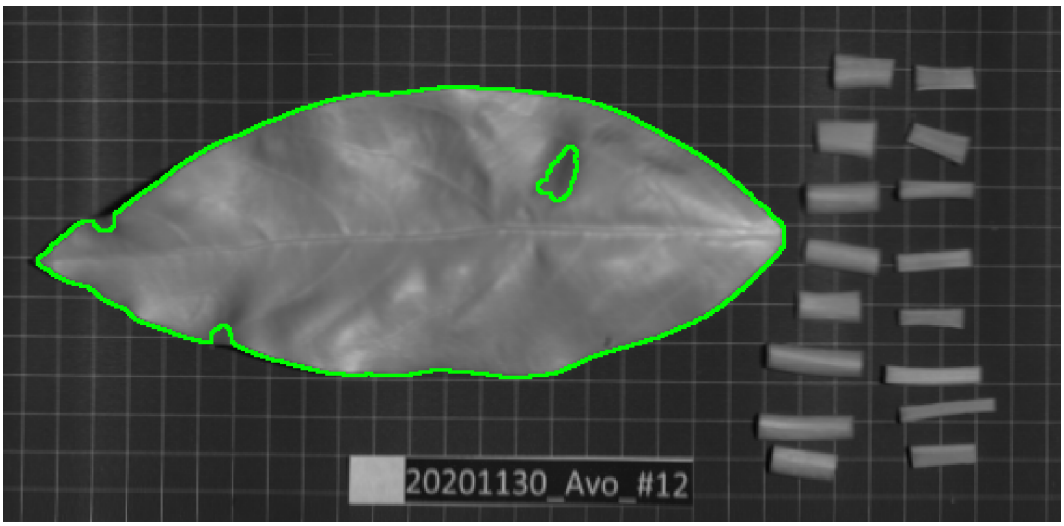


Figure 3: The same scan shown in figure 4 following automated leaf pixel selection. Pixels within the green curve are identified as leaf tissue.

Finally, to calculate a single spectrum for each carbohydrate measurement, the median spectrum was calculated over all the pixels identified as leaf tissue. An example median spectrum is shown in Figure 4.

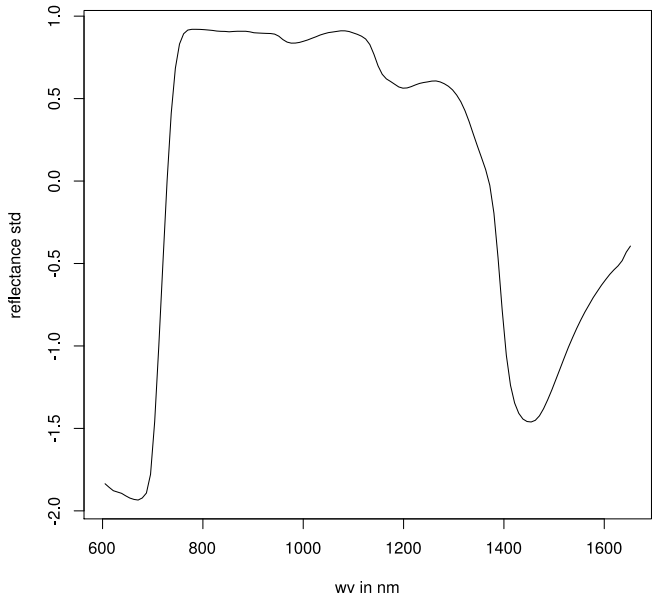


Figure 4: Example median leaf spectrum for one of a single leaf sample.

A similar process was utilized for obtaining representative spectra for the xylem-stem samples, other than a different library being built for the SAM step (using xylem spectra). Figure 5 (left) provides an image showing the pixels associated with xylem tissue and a plot (right) shows the median spectrum for this particular sample. Note the difference in the spectral patterns between the leaf (Figure 4) and xylem-stem (Figure 5) samples.

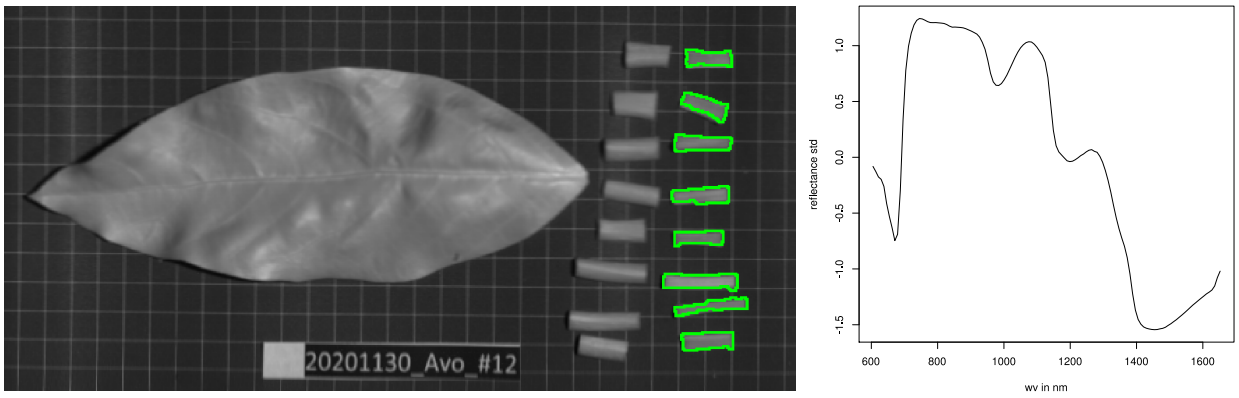


Figure 5: The same scan as figure 4 following automated xylem-stem pixel selection (left) and the median xylem-stem spectrum (right).

Tissue non-structural carbohydrate concentration

For extraction of non-structural carbohydrates, leaf and stem tissues were freeze-dried for 12 h and ground to a fine powder using the Retsch Cryomill grinder (Metrohm Australia Pty Ltd, AUS). 20 mg of leaf or stem freeze dried powder was transferred to a 10 mL screw cap tube and mixed with 3.0 mL of 80% ethanol. Next, the tube was incubated for 30 min at 80^{0}C and the insoluble material was centrifuged a 10,000 xg for 5 min. The supernatant was decanted into a clean 15 mL falcon tube and the sample was re-extracted two more times with 3.0 mL 80% ethanol. After each centrifugation step, the supernatant was decanted into the 15 mL falcon tube to combine all three soluble extracts. The final volume of the soluble extract was 9.0 mL, which was stored at -80^{0}C before analysis (note: this soluble extract contains the soluble carbohydrates, while the insoluble pellet contains the starch).

For starch analysis, the pellet was dried for 2.0 h at 55^{0}C. The Megazyme total starch kit (K-TSTA) was used to estimate starch levels in leaf and stem samples (Bio-Strategy Pty Ltd, AUS). To extract starch, dried pellets were resuspended in 300 µL of 2 M KOH and incubated for 20 min at 4^{0}C. Next, 1.2 mL of 1.2 M sodium acetate buffer (pH 3.8) was added to each tube and mixed thoroughly. After the total extract was transferred to a 2.0 mL Eppendorf tube, 20 µL of thermostable alpha-amylase and 20 µL of amyloglucosidase was mixed thoroughly with the insoluble extract and incubated for 30 min at 50^{0}C. After digesting the insoluble with alpha-amylase and amyloglucosidase, the samples were centrifuged at 12,000 xg for 5 min and the soluble extracted was transferred to a new Eppendorf tube for starch estimation. To measure glucose released during the starch extraction procedure, a standard curve was produced using glucose standards ranging from 0-50 µg/mL. Using a 96-well plate, 20 µL of glucose standard and soluble extracts were transferred to each well in duplicate and 280 µL of glucose oxidase/peroxidase reagent was added to each well and mixed. The 96-well plate was carefully covered in parafilm and alfoil to prevent evaporation and light exposure before the plate was incubated at 20 min at 50^{0}C. Using the FLUOstar Omega microplate reader (BMG LabTech, Australia) spectrophotometer, the absorbance of each sample was determined at 510 nm. The mean starch concentration (%/dry weight in grams) for each sample was quantified using the standard glucose curve. As 1% /g dry weight is equivalent to 10 mg/g dry weight, all starch measurements were converted to mg/g dry weight using the conversion 1%/g dry weight = 10 mg/g dry weight.

To quantify the soluble sugars, glucose, fructose, sucrose, mannoheptulose and perseitol, 2.0 mL of the soluble extract was placed in an Eppendorf tube and dried in a Gene miVac Quattro (SP Industries, USA) for 2.0 h at 55^{0}C. Each dried sample was resuspended in 20 µL sterile H_2O. Glucose, fructose, sucrose, mannoheptulose and perseitol were separated by High Performance Liquid Chromatography using the Sugar-Pak cation-exchange column (Waters, Rydalmere, AUS). The Aglient Technologies 1200 G1362A infinity refractive index detector (Aglient Technologies, USA) was used to identify and quantify separated sugars in each of the samples by comparison to the glucose, fructose, sucrose, mannoheptulose and perseitol standards.

Hyperspectral calibration models

The purpose of the calibration was to provide a predictive model for avocado carbohydrate content using the NIR reflectance spectra data. Calibration models were generated using the paired carbohydrate and median spectrum data (i.e. one pair per sample). Traditional multivariate linear regression techniques were not appropriate due to the autocorrelation that is present within spectral data. Edwards et al. (2020) utilised the same hardware/software systems as this current project and demonstrated two methods that were effective at producing predictive models for chemical composition of plant tissue. The first was Least Absolute Shrinkage and Selection Operator (LASSO) (Tibshirani, 1996), which extends multiple linear regression by performing regularisation of regression coefficients and variable selection. The second was Random Forest regression (RF) (Breiman, 2001), a commonly used method for prediction that is non-linear. RF models are an ensemble of regression trees formed on subsets of the predictor variables (spectra) and the independent variables (carbohydrates). RF can also be used for variable selection, providing the potential to identify key wavebands for future development.

Calibration models were built for the major non-structural carbohydrates in the leaves (starch, individual C_7 soluble sugars, total C_7 and standard C soluble sugars (sucrose, glucose and fructose) and total soluble sugars) and for various combinations of these. Only starch was used for a xylem tissue calibration model. The two approaches mentioned above (LASSO and RF) were compared as prediction methods. Generally, this was performed by first splitting the

data sets, with 70% of the observations, selected at random, used for training the methods, and 30% used for testing. This process was repeated nine additional times, with the training/test data selected at random each time. The performance of the models was assessed with the coefficient of determination (R^2) between the predicted and observed chemical values. Additionally, we evaluate the models with the Root Mean Squared Error (RMSE), which presents an error metric relative to the scale of the data. It is useful to consider both metrics as a high R^2 value can occur in the presence of a poor RMSE. The R^2 and RMSE were averaged over the ten repeats for reporting. Both models require some parameter selection, e.g. the mtry parameter in RF, but no attempt was made to optimise parameter values across the ten repeats. In all cases model parameters were optimised only for the range of values in each specific training set.

Both models report values that indicate which wavelengths in the spectra are driving the calibration models. For LASSO these are the regression coefficients (or weights) and for RF it is called variable importance. These values were assessed for some of modelling efforts.

Photothermal quotient Analysis

Climate characterization of avocado growing regions

Weather data was downloaded from the Silo database hosted by the Queensland Government, https://www.longpaddock.qld.gov.au/silo/ . SILO is a database of Australian climate data from 1889 to the present. It provides daily meteorological data sets for a range of climate variables in ready-to-use formats suitable for biophysical modelling, research and climate applications. Monthly mean weather data was downloaded for seven regional locations from Atherton in Queensland, down to Loxton in South Australia and across to Yoongarillup in Western Australia to assess regional climate characteristics that may affect yield potential. The Photothermal Quotient (PTQ) was calculated using radiation and temperature (Rawson, 1988; Equation 1) and Vapour Pressure Deficit (VPD) was calculated using the Vapour Pressure (VP) observed 9:00am at and daily maximum temperature to derive the VPD at the maximum temperature (VPD_{max}). Climate data are presented for maximum temperature (°C), minimum temperature (°C), radiation (MJ m^{-2} d^{-1}), VPD_{max} (hPa), PTQ (MJ m^{-2} d^{-1} $°C^{-1}$), daily evaporation (mm d^{-1}) and monthly rainfall (mm).

$$PTQ = \frac{Daily\ Radiation}{(Max°C+Min°C)/2-Base°C} \qquad \text{Eqn. 1}$$

Problems with base temperature

The 'base temperature' or 'threshold temperature' is particular to a species and is the temperature below which growth ceases. There are few published reports for the base temperature for avocado but Lahav and Trochoulias (1982) and Wang et al (2018) reported that the base temperature for avocado tree growth was 12°C while Wolstenholme (1987) and Zamet (1990) estimated the base temperature for growth and development was 10°C. Interestingly, when using the standard method for calculating thermal time (Equation 2) and a base temperature of 10 °C, the PTQ values calculated became non-sensical at some locations on the days following low minimum temperatures.

$$Thermal\ time = (Max°C + Min°C)/2 - Base°C \qquad \text{Eqn. 2}$$

Days in which the mean daily temperature approached 10°C, a daily increment of thermal time between -1 and +1 was observed. When divided into the observed daily radiation, a significant positive or negative value for PTQ can eventuate. In Figure 6, PTQ is calculated for each day in the year 2008 for Loxton in SA, Yoongarillup WA, Childers, QLD and Atherton QLD (Figures 6a, c, e, g). There were 8 values of PTQ greater than 50 or less than -50 for Loxton and Yoongarillup (data not shown). These data show the non-linearity created when the mean daily temperature approaches the base temperature of the species in question. At Childers and Atherton where mean daily temperatures rarely approach the base temperature, the standard method provides a reliable estimate of the daily increment of thermal time.

This issue was solved by calculating thermal time as day degrees (◦Cd), with the algorithms used by Whish et al. (2020) that divided each day into eight 3-h time periods, by fitting a circadian curve between the daily maximum and minimum temperatures to generate eight sub-daily temperatures. This approach excludes 3-h periods where the mean temperature is less than the base temperature to arrive at a more accurate estimate of the thermal time

spent above the base temperature in a 24-hour period. The method is described within the APSIM code (github.com/APSIMInitiative). In this study, the Thermal Time Calculator was accessed at Thermal Time Calculator (TTC) (shinyapps.io) to calculate thermal time from weather files downloaded in the APSIM format. From here on this is referred to as the TTC approach. PTQ was then calculated in the usual way by dividing the daily radiation by the daily thermal time increment.

PTQ calculated using the TTC method-for each of the 4 locations in 2008 (Figure 6b, d, f, h). For the colder locations at Loxton and Yoongarillup, mean annual temperature 16.5°C and 16.7°C respectively, it can be seen that the TTC method removes the non-linearity seen at low minimum temperatures at these sites when using Equation 2, while at the warmer locations of Atherton and Childers, mean annual temperature 20.5°C and 21.6°C, it can be seen that the estimate of PTQ remains largely unchanged between the two methods.

Avocado yield variation and PTQ

Growers across all the avocado growing regions were invited to provide yield data to the project via a brief story in Avocados Australia newsletter 'Guacamole', as well as by an email invitation from Simon Newitt, Avocado Horticulturalist with QDAF. Twelve growers responded offering to share data; however, four growers were unable to send their data, 2 sets of data were not suitable. Therefore, six growers provided data sets spanning 4-10 years that were suitable to evaluate. Weather data was downloaded from the Silo database hosted by the Queensland Government, https://www.longpaddock.qld.gov.au/silo/. Daily weather data was downloaded from SILO's gridded datasets for the point (location) closest to the orchard being examined. Gridded datasets are constructed by spatially interpolating the observational data from nearby weather stations. Data sets covered the period from 1st January 2008 to 31st December 2020. Due to a lack of observational data, a 'flowering date' was estimated as the peak of the flowering window presented in the regional 'Crop Cycle Calendars' which are available as part of the Best Practice Resource on the Avocados Australia website (Table 1). PTQ was calculated for base temperatures of 10°C using the TTC method. The average PTQ_{10deg} was calculated for a 120-day (Pre-flowering) period prior to the flowering date (Table 1) and for a 150-day (Post-flowering) period. Grower yields were plotted against pre- and post-flowering PTQ to investigate associations between PTQ and yield.

Is PTQ associated with within-season starch variation?

Finally, the scientific literature was searched for studies measuring multi-season avocado tree carbohydrate status. At the time of writing, two studies were found; one from New South Wales, Australia (Scholefield et al, 1985) and one from California, in the United States of America (Liu et al, 1999). The Australian study measured carbohydrate in branches every 2-3 months over 3 years and the California study measured carbohydrates in stems every month over 2 years. The carbohydrate data was extracted from graphs to test for associations with the month-to-month seasonal variation in PTQ. Australian weather data was downloaded (1976-1979) as above and the Californian weather data was downloaded (1994-1996) from the University of California Statewide Integrated Pest Management Program location at the University of California, Irvine campus (Pest and plant models, degree-days, and weather - UC Statewide IPM Program (ucanr.edu)). PTQ was calculated for base temperatures of 10 °C using the TTC method. Monthly mean PTQ_{10deg} was calculated for the duration of each experiment.

Table 1: Key periods in the Haas Avocado crop cycle for selection of Australian growing regions

Region	Peak flowering	Flowering date	Harvest period
Perth, WA	16th - 30th Oct	22 Oct	Aug – Nov
Tristate	19th Oct – 3rd Nov	27 Oct	Jul – Nov
Mid Nth Coast, NSW	1st – 15th Oct	7 Oct	Aug – Nov
Northern NSW	11th – 25th Sep	18 Sep	May - Jul
Central QLD	14th – 28th Sep	22 Sep	May - Jul
Nth QLD	26th Aug – 10th Sep	3 Sep	May - Jun

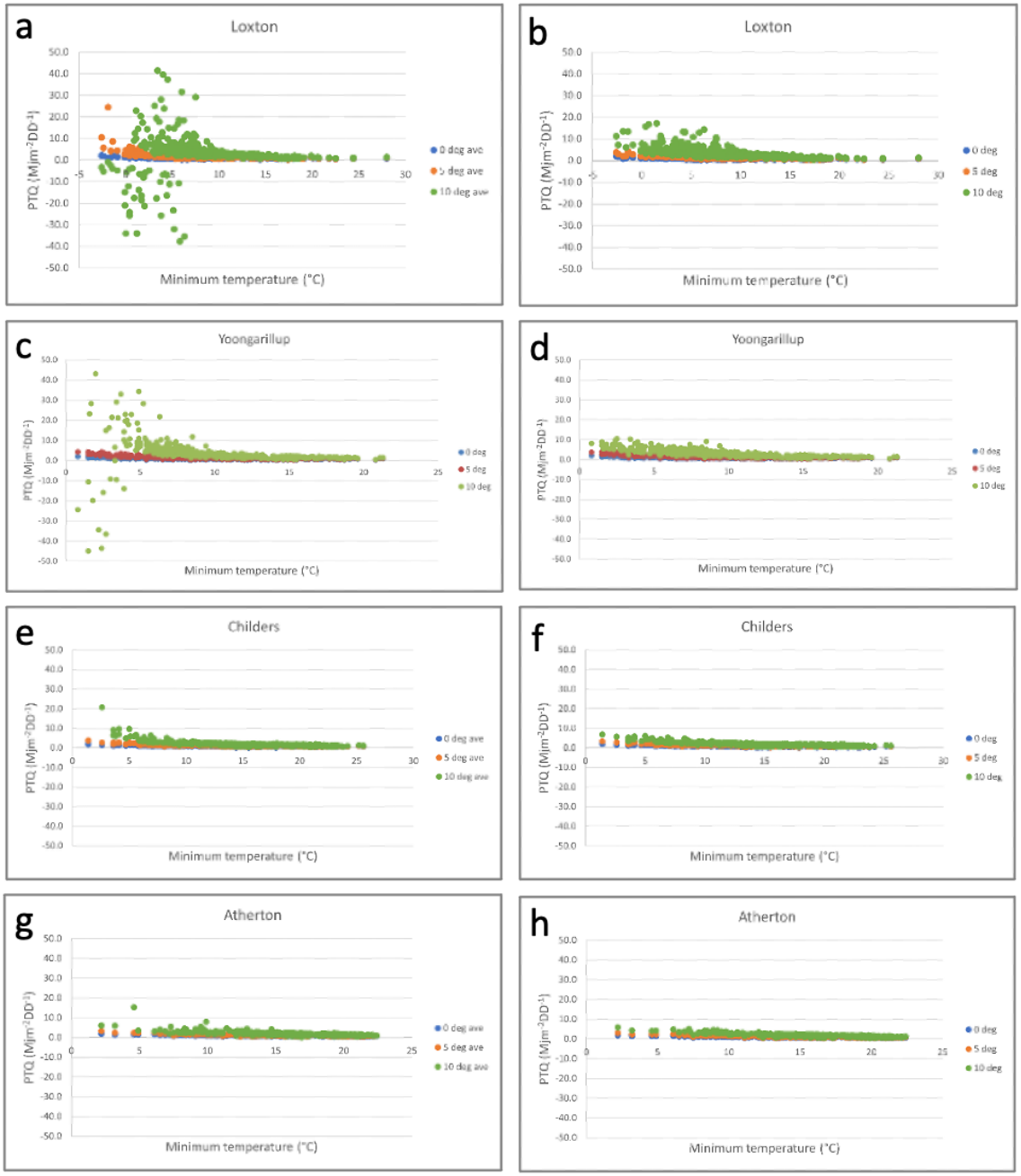


Figure 6: Photothermal quotient plotted against the overnight minimum temperature for the year 2008 at (a,b) Loxton, SA; (c,d) Yoongarillup, WA; (e,f) Childers, QLD; and (g,h) Atherton, QLD. PTQ was calculated with base temperatures of 0 °C, 5 °C, 10 °C using the (a,c,e,g) standard method and the (b,d,f,h) TTC method. (a,b) note: the range of PTQ was restricted to +/-50 in with 8 missing values from each of the two graphs.

Carbohydrate data sets

The accuracy and broad applicability of chemometrics based on multivariate calibration models are highly dependent on the data sets utilized, particularly the range and distribution of the data and the degree to which this represents the potential usage of the calibration. The concentration of six carbohydrates (starch, glucose, fructose, sucrose, mannoheptulose and perseitol), that compose the vast majority of the NSC pool in avocado tissues, was determined for each of the 300 leaf samples. Only starch content was determined for the xylem-stem samples due to time and resource constraints. It was anticipated that starch would be the largest component of NSC (>50%), but on average it was only 18%. However, starch concentration had greater variability than any of the other components of NSC measured and accumulated to a higher maximum concentration than any of the other carbohydrates analyzed (Table 2).

Table 2: Description of non-structural carbohydrate data sets used for calibration models

Leaf	No samples	Mean	Maximum	Minimum	SD	%RSD
1. Starch	300	50.8	307.1	2.1	61.6	121.3
2. Starch (>10 mg/g)	202	72.9	307.1	10.0	64.3	88.2
3. Starch + TSS	252	183.0	479.8	101.4	66.0	36.1
4. Starch + TC7	252	126.5	405.9	56.5	60.0	47.4
5. TSS	252	140.2	210.1	92.3	21.7	15.5
6.TC7	252	83.4	165.2	46.2	18.5	22.2
7. Mannoheptulose	252	50.8	102.2	24.1	15.6	30.7
8. Perseitol	252	32.7	63.0	15.0	8.6	26.3
Stem (xylem)						
9. Starch	108	67.2	393.8	1.4	98.6	146.7

Values for mean, maximum, minimum and standard deviation (SD) in mg/g dry weight

% RSD: Relative Standard Deviation

TSS (Total soluble carbohydrates): Glucose + Fructose + Sucrose + Mannoheptulose + Perseitol

TC7: Mannoheptulose + Perseitol

Calibration models were built using nine data sets derived from the wet-lab carbohydrate data (Table 2). The first data set consisted of all the starch measurements derived from 300 leaf samples, ranging from 2.1-307.1 mg/g dry weight (Table 2). Examining the distribution of this data showed that 98 samples had extremely low starch levels, less than 10 mg/g dry weight (Figure 7). A majority of these leaves analyzed with significantly low starch levels were collected from trees experiencing drought stress, which was introduced into this data set specifically to widen the range of starch observations. To avoid any potential bias in the calibration model as a result of the skewed distribution, a second starch data set was generated that consisted of the 202 samples with concentrations above 10.0 mg/g dry weight (Table 2, data set 2. Starch (>10 m/g)). The third data set represented total non-structural carbohydrates (starch + total soluble carbohydrates [TSS]), which consisted of 252 samples as soluble carbohydrate data was not available for 48 of the leaf samples. Total NSC concentrations ranged from 479.8 to 101.4 mg/ gram dry weight. Data sets for TSS, mannoheptulose + perseitol (TC7), mannoheptulose and perseitol derived from 252 leaves were also used for the calibration. The data sets for TC7 sugars were evaluated, as these carbohydrates are highly abundant and are predicted to act as storage carbohydrates (Liu et al., 2002; Tesfay et al., 2012). The last data set was derived from the 108 stem xylem samples for which starch concentration was available, with levels ranging from 393.8 to 1.4 mg/g dry weight.

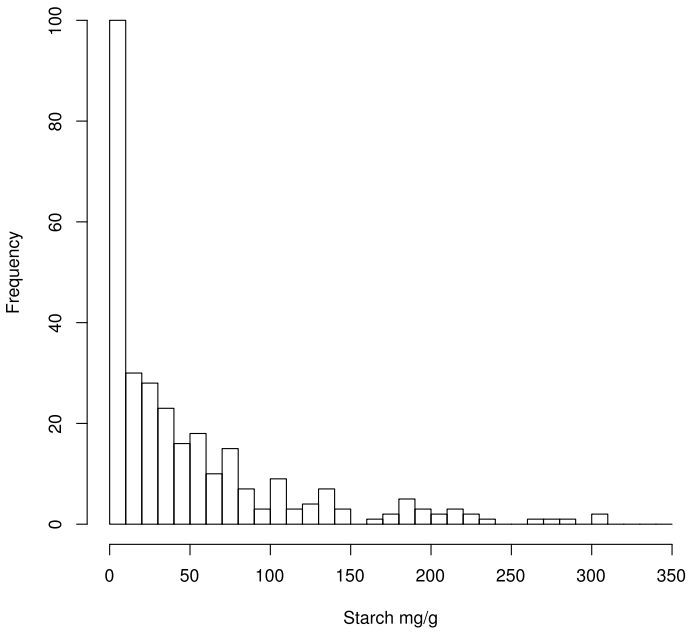


Figure 7: Histogram of leaf starch concentration (% DWt)

Estimating non-structural carbohydrate concentration using NIR-hyperspectral imaging

As described above, six carbohydrates were analysed using wet lab methods (enzymatic and HPLC) and two methods were used to generate calibration models for leaf and stem carbohydrates, LASSO and RF. Typically, leaf starch represents the major NSC pool in leaves, with soluble sugars being fairly tightly regulated to minimise osmotic impacts (Stitt and Zeeman, 2012). However, avocado also synthesises significant quantities of C7 sugars, which are thought to have a storage role (Liu et al., 2002; Tesfay et al., 2012). Consequently, of the possible combinations of carbohydrates, we concentrated on starch (key storage carbohydrate), total NSC (sum of all analysed carbohydrates) and the C7 sugars.

For each data set, calibration models were built using the two methods outlined above and are presented below. In addition, where the full set of hyperspectral wavebands were used for a model, LASSO weights and RF importance values were produced. These indicate the wavebands that are contributing to the calibration and are proportional to their impact on the model.

Leaf starch

Both the LASSO and RF models produced statistically significant fits between predicted and observed leaf starch content (R^2 = 0.89 and 0.73 for LASSO and RF, respectively). Figure 8 provides a visual representation of a single iteration of the ten iterations used to build the models. In addition, the RMSE values (21 and 33 mg/g for LASSO and RF respectively) indicate that the noise around the predictions is fairly low, validating the approach taken, particularly considering the relatively small number of samples available for this proof-of-concept study. The results also show that the LASSO model outperforms the RF model on both measures.

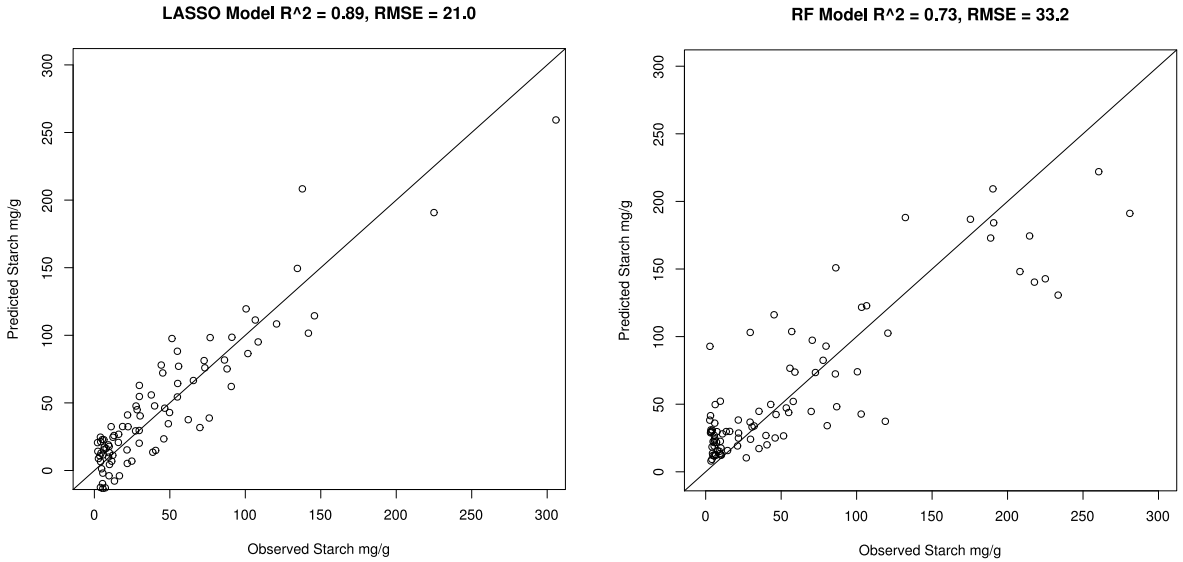


Figure 8: Observed vs predicted leaf starch content (mg/g) for one of the ten calibration models built with all 300 leaf samples using LASSO (left) or RF (right) methods.

The LASSO weights and RF variable importance plots for these models are shown in Figure 9. The LASSO weights (coefficients) suggest that the longer wavelengths in particular are major drivers of the calibration and this is supported by the RF importance values. The green curve shows an example spectrum for reference. Comparing the weights/importance values with the sample spectrum suggests that the RF model is quite reliant on the 'red-edge' at around 700 nm, but the LASSO model is not.

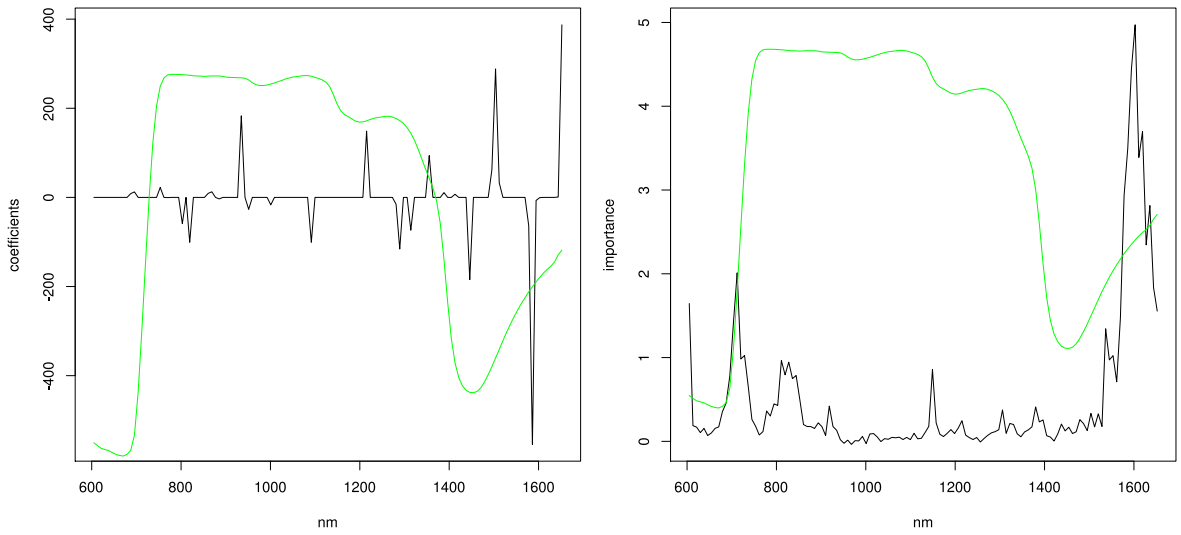


Figure 9: LASSO weights (left) and RF variable importance (right) for one of the ten calibration models of starch content using all 300 leaf samples generated per calibration method.

However, it was suspected that the RF model was biased towards attempting to perform well with the significant number of data points less than 10 mg/g of starch. This was perhaps supported by importance values being close to the main water absorption bands in our spectra (970, 1190 and 1470 nm) as well as the red edge, with the low starch values primarily occurring in drought stressed trees (Figure 9).

Consequently, a second model data set was generated by including only those samples where starch was > 10 mg/g). With this exclusion, there was a higher risk of overfitting the calibration model, as the number of starch measurements (202) was close to the number of wavebands, predictors (144). To compensate for this, we reduced the number of predictors from 144 to 8 by examining the weights (LASSO) or variable importance (RF) of models with all 144 predictors (Figure 10), by selecting the wavebands with the highest weight or importance across the ten iterations per model method. Wavebands adjacent to already selected wavebands were excluded to maximise the spread of the eight chosen predictors across the spectra. For LASSO, the wavebands were 695, 868, 992, 1149, 1297, 1495, 1585 and 1652 nm. For RF, the wavebands were 703, 811, 1149, 1239, 1314, 1388, 1495 and 1594 nm. The chosen wavebands were also similar to key wavebands in the calibration performed on the first starch data set.

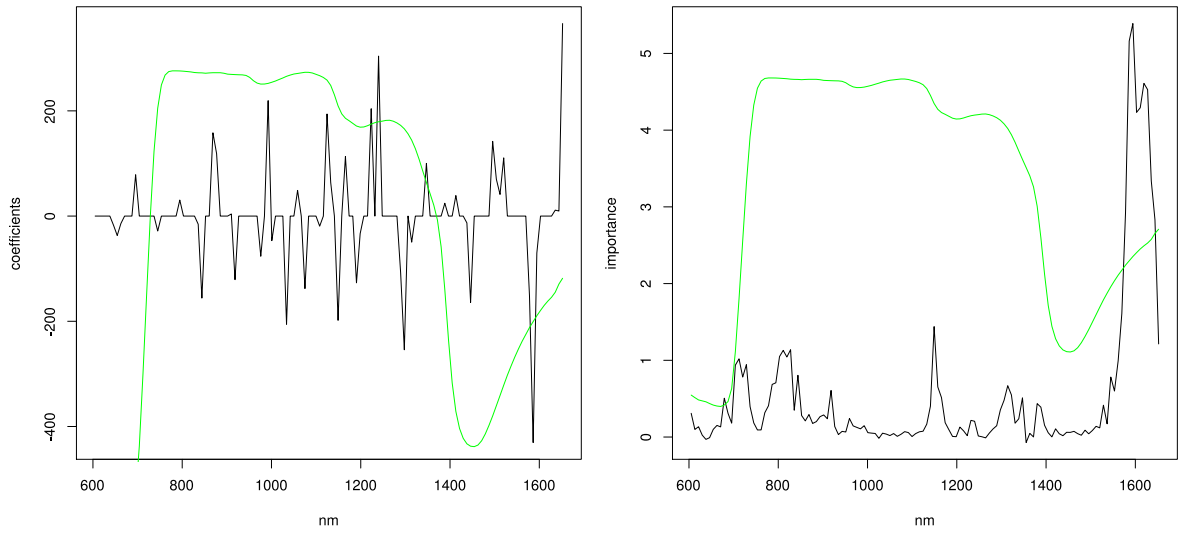


Figure 10: LASSO weights (left) and RF variable importance (right) for the models of starch content generated using all predictors with leaf samples where starch was > 10 mg/g

LASSO and RF models using the reduced starch data set (n=202) and reduced predictors (n=8) were then built as previously described. Perhaps not surprisingly, R^2 and RMSE of both models for observed vs predicted starch content (Figure 11) were reduced in comparison with the models generated using the full data set (Figure 8), but the fits were still statistically significant and the LASSO model again outperformed RF (Figure 11).

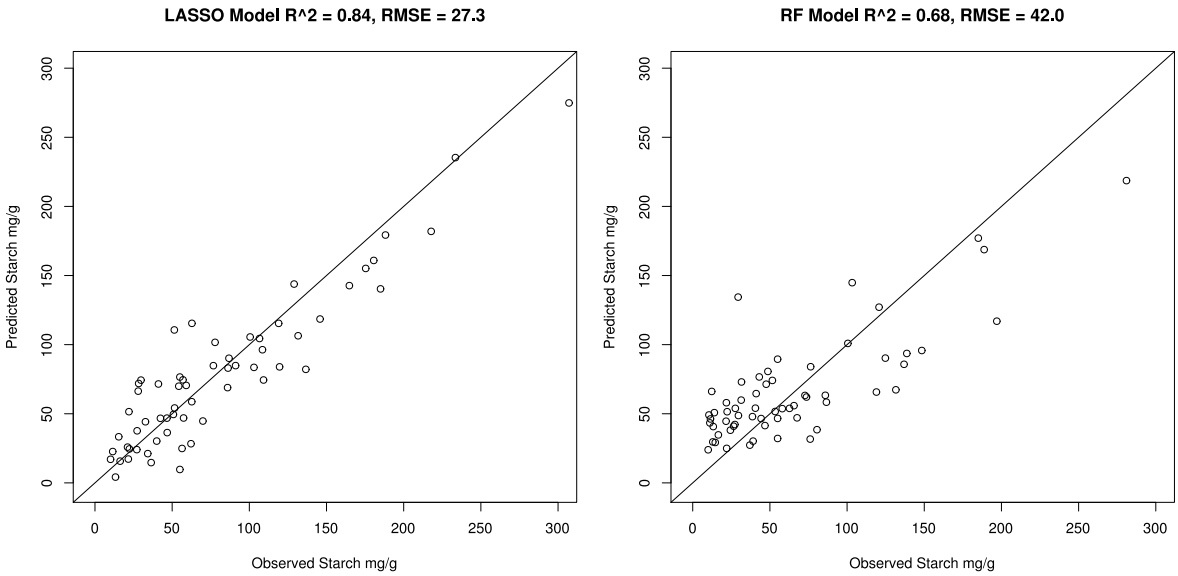


Figure 11: Observed vs predicted leaf starch content (mg/g) for one of the ten calibration models built with leaf samples where starch content was > 10 mg/g using LASSO (left) or RF (right) methods.

Using the same models to predict the entire data set (including leaf samples with starch < 10 mg/g) had limited impact on the fit (R^2), but increased the RMSE by around a third (Figure 12) and the low starch values were less well predicted than by the model built using the entire data set (Figure 8). Consequently, even if the models built with the full data set were biased by the skewed distribution of starch values, accounting for this did not provide a more robust prediction of observed starch values.

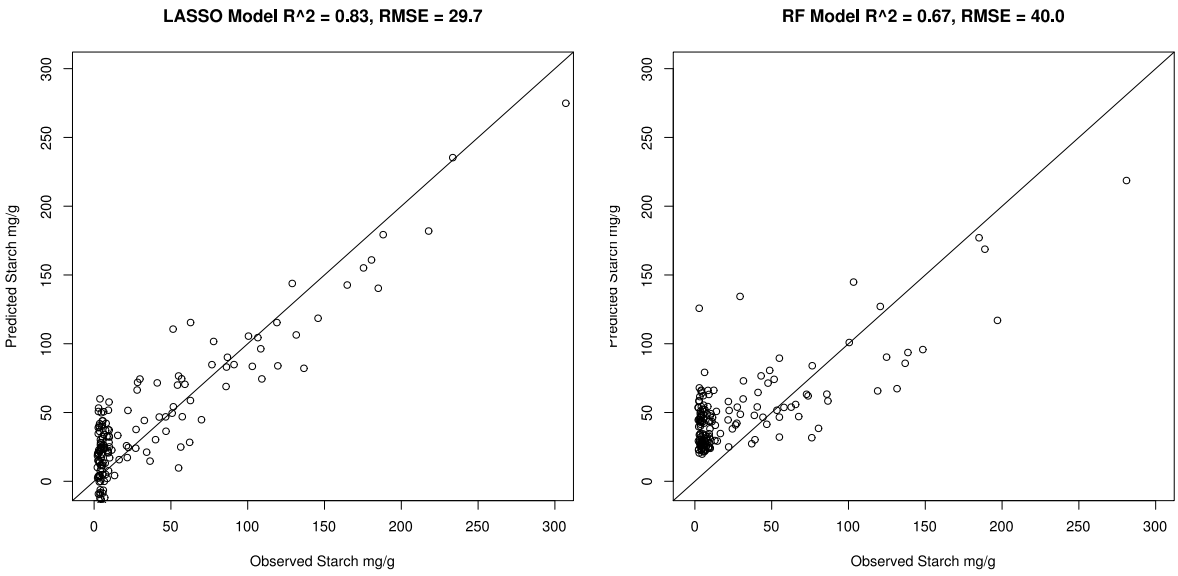


Figure 12: Observed vs predicted leaf starch content (mg/g) for the entire data set (n=300) using one of the ten calibration models built with leaf samples where starch content was > 10 mg/g using LASSO (left) or RF (right) methods.

Leaf total non-structural carbohydrates

Estimated total NS) using a non-destructive method was the primary aim of this proof-of-concept work. NSC was defined as the sum of starch and the five soluble sugars (C_6 and C_7) analysed, which were expected to contribute the vast majority of NSC in avocado tissue (Liu et al., 2002; Tesfay et al., 2012). Consequently, the models discussed in this section are built against the single total NSC value, despite the multiple chemical identities that this number represents. Summing the predicted concentrations for models of the individual components did not result in any meaningful difference to the single model for NSC (data not shown).

For this calibration, Figure 13 shows the weights for the LASSO model and variable importance values for the RF model. Both plots are relatively sparse, indicating they don't rely on small gradients in the spectral curves and are therefore unlikely to be over-fitted. The LASSO model achieves an R^2 value of 0.88 with an RMSE of 25, indicating good predictive power from the model, despite the multiple components it represents. This can also be seen visually in the left plot of Figure 14, where observed versus predicted values are plotted along with a line of perfect fit. The RF model does not fare as well with an R^2 value of 0.65.

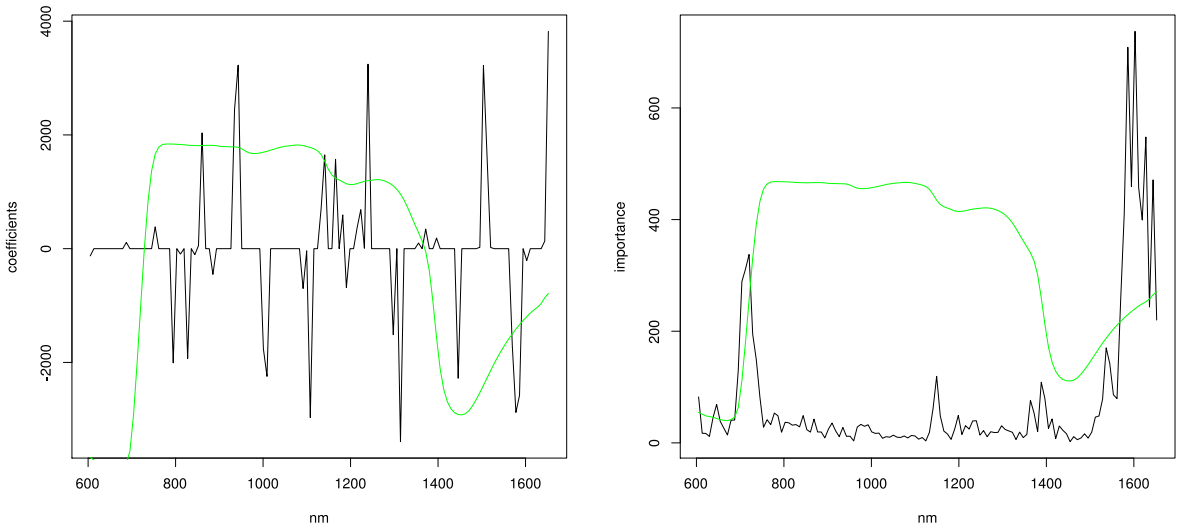


Figure 13: LASSO weights (left) and RF variable importance (right) for one of the ten calibration models of NSC using all available leaf samples (n=252) generated per calibration method.

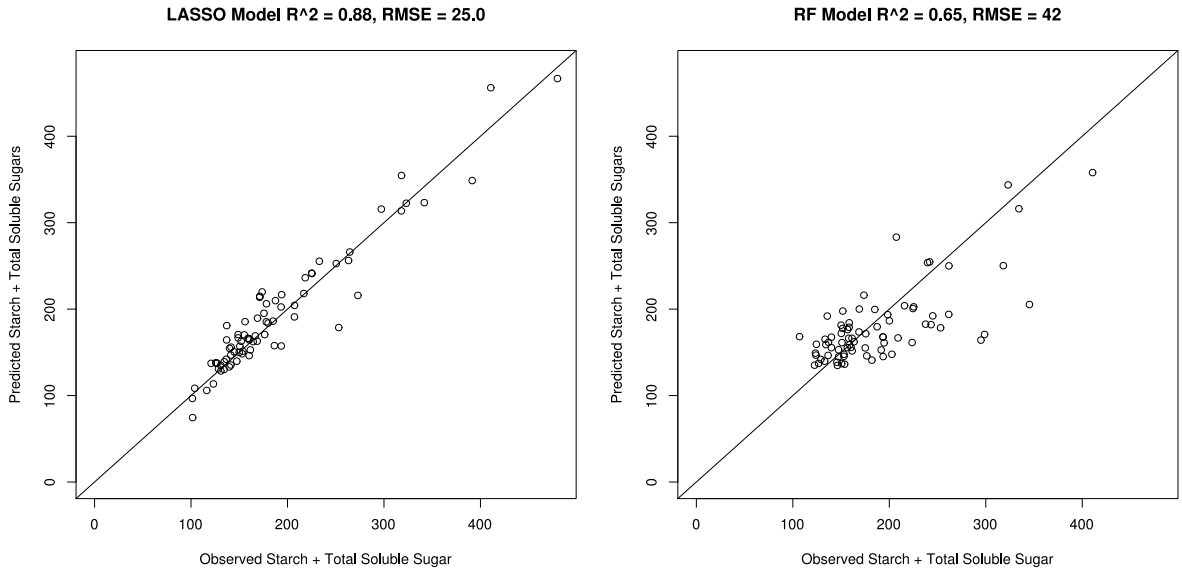


Figure 14: Observed vs predicted leaf NSC content (mg/g) using LASSO (left) or RF (right) methods

Leaf storage carbohydrates excluding sucrose, glucose and fructose

If the C7 sugars found in avocado are indeed considered as storage carbohydrates as suggested above, the combination of starch and the C7 sugars may represent the medium-term reserves available for future use, with sucrose, glucose and fructose representing the immediately available tree carbohydrate. Models were tested against this scenario, as well as those of starch alone and total NSC. The weights and variable importance measures for both models are shown in Figure 15. The LASSO weights indicate a fairly complex model compared to the calibrations in earlier sections. The adjacent positive and negative weights around 1000nm suggest there may be some over fitting going occurring. The overall accuracy of prediction wasn't quite as good as for total NSC, despite the model including fewer carbohydrates, the R^2 for the LASSO being 0.85, however, the difference was small and this still indicates a good relationship between observed and fitted values (Figure 16). The RF is again outperformed by LASSO.

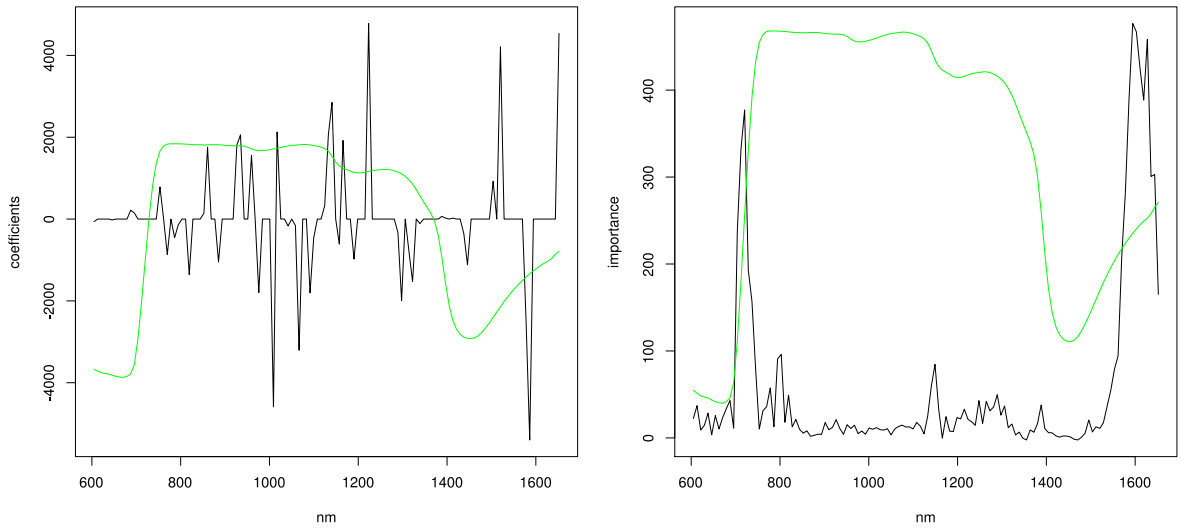


Figure 15: LASSO weights and RF variable importance for starch + C7 sugar models.

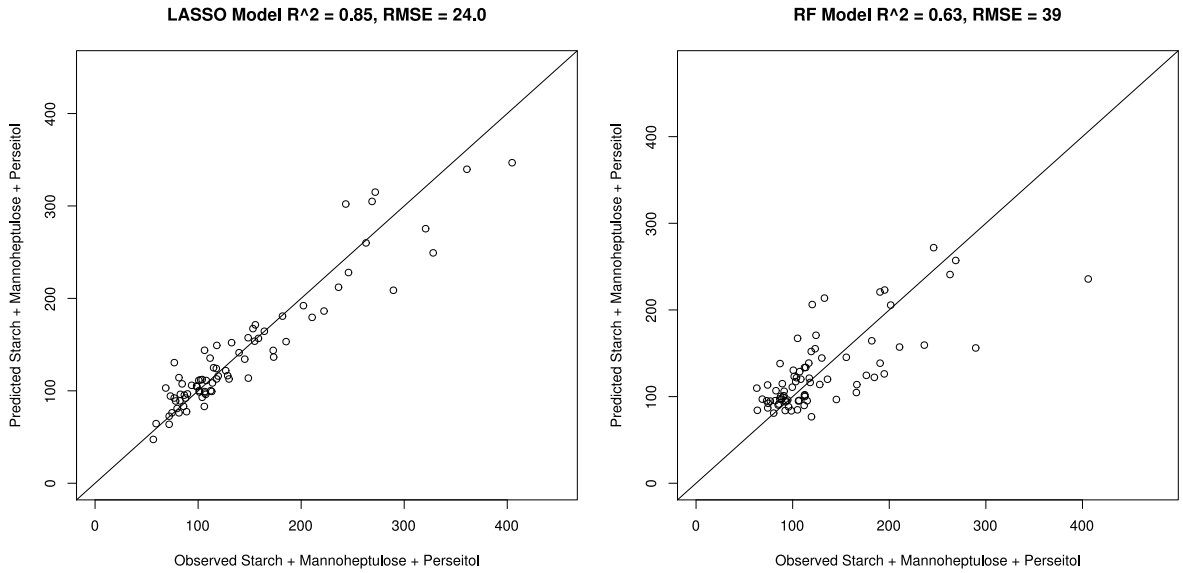


Figure 16: Observed vs predicted starch + C7 sugars for leaves based on median spectra

Leaf soluble carbohydrates

A number of additional models were built against soluble carbohydrates. In some tissues, soluble sugars have been quite amenable to NIR reflectance spectroscopy, with accurate calibrations produced (Decruyenaere et al., 2011; Ramirez et al., 2015; Hu et al., 2017). Soluble sugars often represent the carbohydrates available at any given point in time and thus a calibration against total soluble carbohydrates may have value independently of total non-structural carbohydrates or reserves. Further, models were built against the two C_7 sugars, as these unusual sugars are major end products of photosynthesis that accumulate to high levels in avocado tissues (Liu et al., 2002; Tesfay et al., 2021).

The LASSO models for combinations of soluble carbohydrates were both fairly poor, compared the those that included starch (Figure 17), R^2 values between 0.40 and 0.45. Potentially this reflected the data sets used, which had much lower variation than the starch data sets (Table 2). When individual calibrations were built for the two C_7 sugars (Figure 18), the predictive power was improved somewhat ($R^2 > 0.5$ in both cases), but still greatly inferior to the calibrations that included starch. However, all of these calibrations would likely be improved with a larger data set that had a greater variation.

In all four cases the RF models were very poor, with the best (mannoheptulose) having an R^2 much lower than the worst (total soluble sugars) of the LASSO models. Whilst, the RF models including starch didn't match the equivalent LASSO model in any case, they did still have reasonable accuracy, but the poor performance with the soluble sugar data sets suggests that the LASSO method should be preferred.

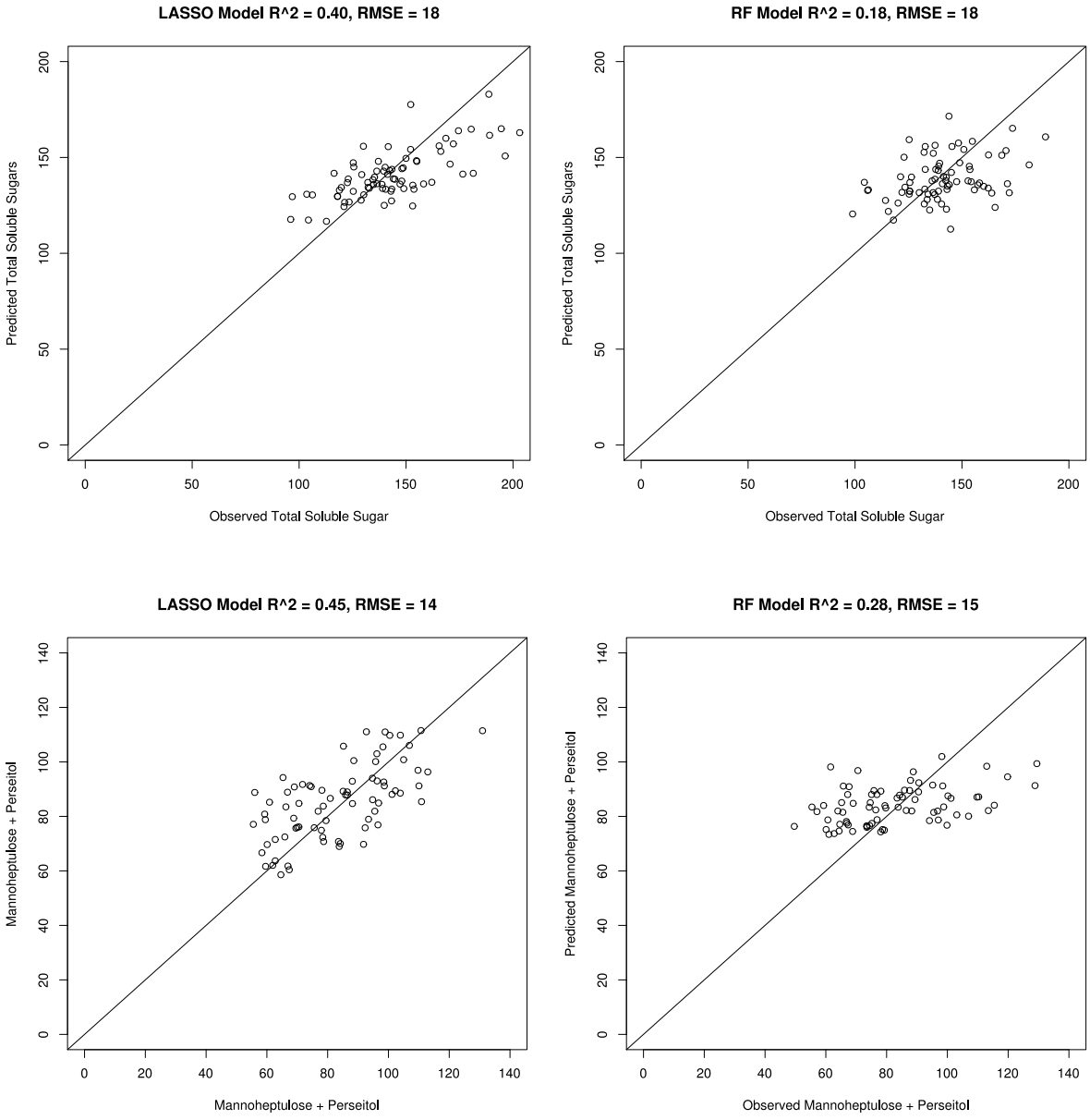


Figure 17: Observed vs predicted leaf total soluble carbohydrate content (top panels) and total C_7 sugar content (bottom panels) using one of the ten calibration models built using LASSO (left panels) or RF (right panels) methods (n=252 in each case).

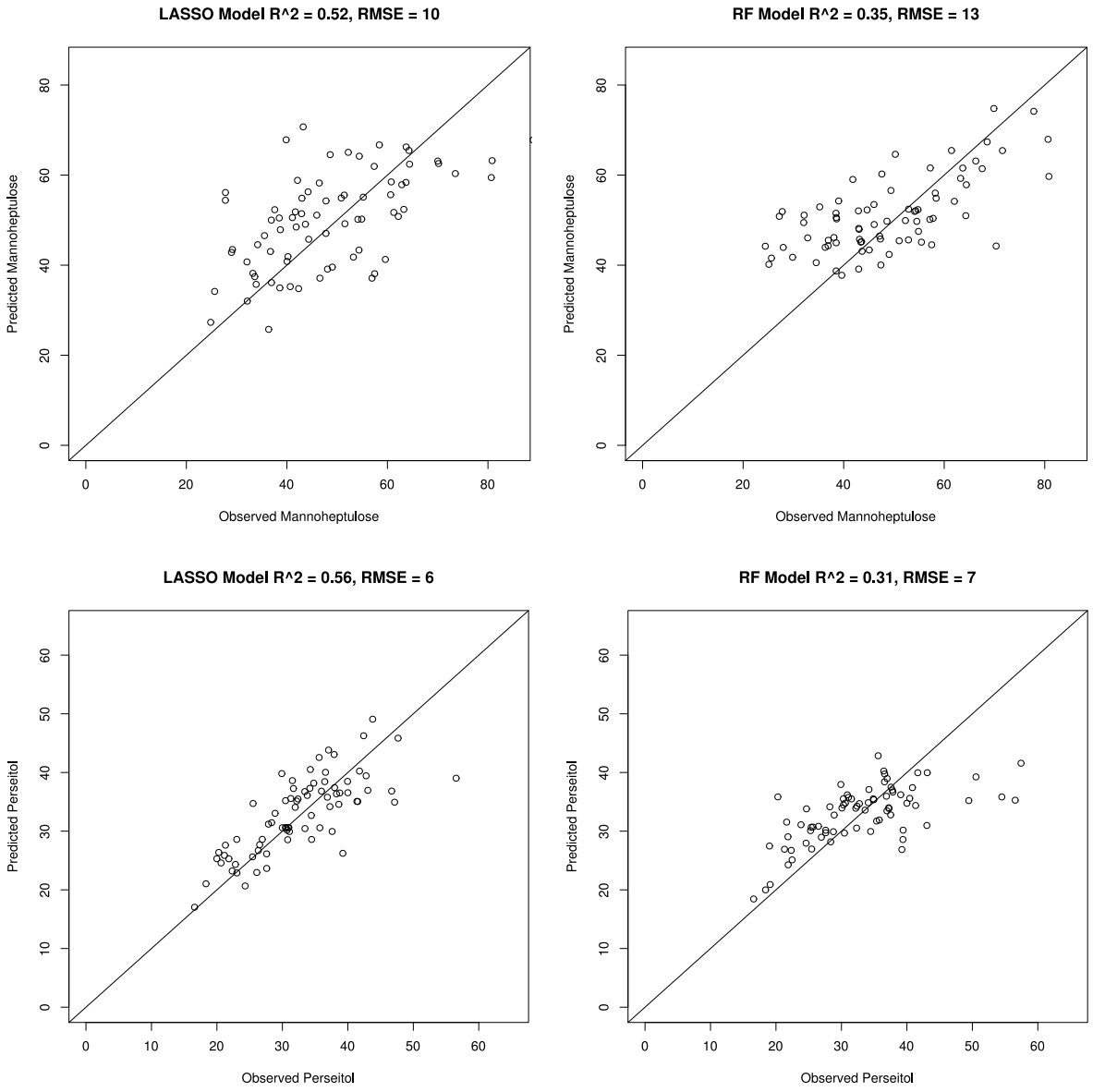


Figure 18: Observed vs predicted leaf mannoheptulose content (top panels) and perseitol content (bottom panels) using one of the ten calibration models built using LASSO (left panels) or RF (right panels) methods (n=252 in each case).

Stem xylem tissue starch

The final data set used was for a different tissue type, young stems. Leaves were the preferred tissue, as they are easily visible in the field and, therefore, amenable to on-the-go NIR spectroscopy from a vehicle. Stems are likely to require point measurements. Furthermore, as noted above, the xylem parenchyma is the site of carbohydrate storage and will mostly occur below the penetration depth of NIR (typically <5 mm; Lammertyn et al, 2000), requiring the stem to be cut and therefore a destructive sampling methodology.

The data set size was limited to the resources available and preference given to the leaf samples and only 108 measurements of xylem-stem sample starch were available. To decrease the potential risk of overfitting the calibration model, the number of wavebands were reduced from 144 to 8 predictors using the same process as for the second leaf starch data set (see above) and the LASSO and RF models built as previously described. Figure 19 shows the observed versus predicted values for both LASSO and RF models for one iteration of the calibration modelling. While the overall R^2 value for the LASSO model, 0.82, indicates a reasonable linear relationship between observed and predicted, the plot highlights the short comings of such a small sample, with the vast majority of observed values at the lower end of the absolute range. The RF model's performance was less convincing and likely suffers due to the small sample size. Given the limited data set and wavebands used there is clear potential for a successful model to be built.

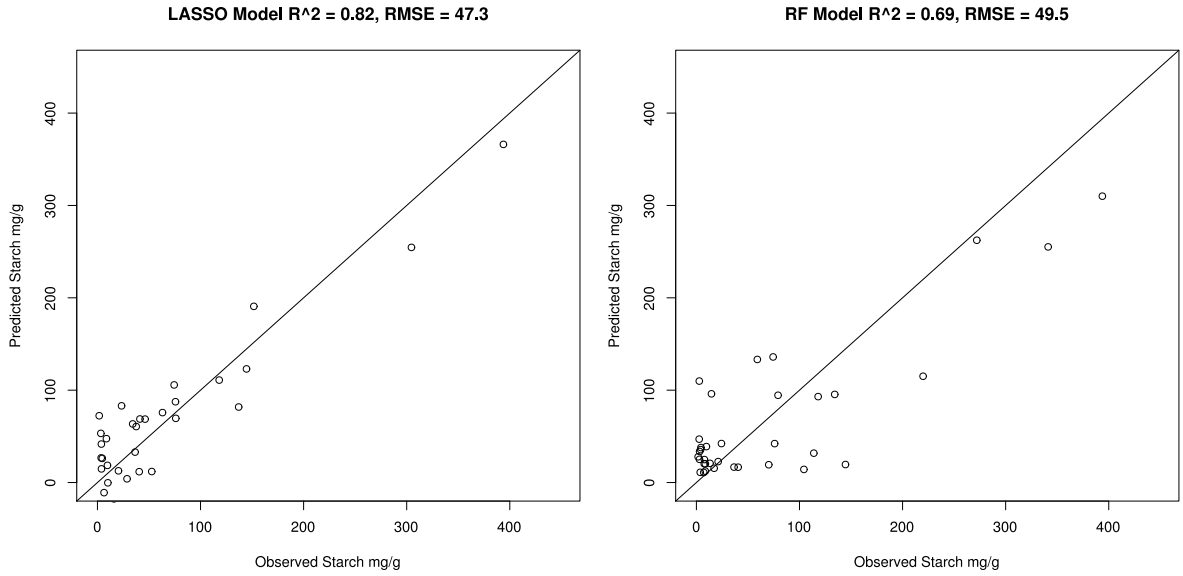


Figure 19: Observed vs predicted xylem-stem starch content (mg/g) for one of the ten calibration models built using LASSO (left) or RF (right) methods (n=108).

Summary of NIR reflectance spectroscopy

In table 3, a summary of model performance is shown over all the calibrations carried out. The table reports only on the LASSO models as they universally outperform the RF equivalents. Generally, those calibrations which include starch achieve good relationships between observed and predicted values, even though starch was usually less than half of the total NSC. Modelling involving soluble carbohydrates on their own tend towards poor relationships, but this was possibly due to limitations of the data sets that were possible for this proof-of-concept demonstration.

Table 3: Summary of R^2 and RMSE for LASSO models		
Leaf datasets	**R^2**	**RMSE**
1. Starch	0.89	21
2. Starch (>10 mg/g)	0.84	27
3. Starch + TSS	0.88	25
4. Starch + TC7	0.85	24
5. TSS	0.4	18
6. TC7	0.45	14
7. Mannoheptulose	0.52	10
8. Perseitol	0.56	6
Xylem dataset		
9. Starch	0.82	50

Values for mean, maximum, minimum and SD in mg/g dry weight
TSS (Total soluble carbohydrates): Glucose + Fructose + Sucrose + Mannoheptulose + Perseitol
TC7 (Total C7 sugars): Mannoheptulose + Perseitol

The use of photothermal quotient to understand yield variation in avocado

Seasonal fluctuations in stem and branch starch levels that correlate with reproductive and vegetative growth is thought to be an indicator of tree carbohydrate status (Whiley and Wolstenholme, 1990). Photothermal Quotient (PTQ; Nix, 1976) is defined as the ratio of daily solar radiation to the daily increment of Thermal Time (TT), where TT is defined as the mean daily temperature ((Max + Min)/2), which is subtracted by the base temperature (Nix, 1975). The base temperature for a crop is the temperature at which growth ceases. Thus, PTQ is a surrogate for the assimilate/carbohydrate supply available to the crop per unit of development. PTQ can be calculated for different periods during the season to examine the sensitivity of yield to conditions during a particular period. Averaged over time, PTQ summarizes a large volume of weather data into a single measure. A higher PTQ indicates that conditions are favorable for growth and yield because there is a high assimilate supply available to the crop per unit of growth and development. If a crop is able to efficiently use of the sunlight it receives, then in the absence of other limiting factors, it will yield more under cooler temperatures than under higher temperatures.

When using PTQ it is assumed that crops are well managed, not under pest and pathogen pressures, appropriately irrigated and will yield close to their potential. In other crops, it has opened research opportunities for researchers to explore critical growth periods for yield formation and physiological factors limiting yield across regions. In addition, PTQ has allowed farm consultants and growers to develop management practices to optimise yield according to seasonal conditions. Once PTQ is calibrated to a particular crop, the data can also be used to identify years in which crops failed to meet their yield potential and explore the reasons why (Rawson 1988). Perennial species are likely to be more complex for applying PTQ due to competition for available assimilate among reproductive sinks and between reproductive and vegetative growing units. In addition, these competition events will be influenced by the previous seasons' crop load and degree of vegetative growth cycles.

This section evaluates the use of PTQ to explain seasonal and regional variation of yield in avocado. It draws on the commercial yield data from avocado growers, weather data available online for research and climate applications and previously published data to create knowledge aimed at identifying management and breeding opportunities to improve the productivity and profitability of Australian avocado industry.

Climate characterisation of avocado growing regions

The long-term monthly means were determined for maximum temperature (°C), minimum temperature (°C), radiation (MJ m^{-2} d^{-1}), VPD_{max} (hPa), PTQ x5 (MJ m^{-2} d^{-1} $°C^{-1}$), daily evaporation (mm d^{-1}) and monthly rainfall (mm) for seven locations spanning the avocado growing regions in Australia (Figures 20-26). Maximum temperatures were highest at Kabra and lowest at Yoongarillup. Minimum temperatures were warmest at Bundaberg and lowest at Loxton falling to below 5 °C in July. The greatest range between maximum and minimum temperatures was at

Loxton followed by Kabra, while the least range was at Alstonville followed by Bundaberg. Yoongarillup had the highest levels of radiation in Summer months but the lowest radiation in winter months. This range is likely due to the Mediterranean climate as shown by the strongly winter-dominant rainfall. At the other extreme, Atherton with its tropical, summer-dominant rainfall, had the lowest summer radiation but the highest late-autumn/early winter radiation. PTQ was the lowest and varied the least at Atherton due mainly to lower radiation and warmer temperatures. PTQ varied the most at Loxton due primarily to high radiation and low minimum temperatures. Interestingly, all sites displayed a peak in PTQ between June and October though there was an almost 3-fold difference in the peak PTQ between Atherton (2.6 MJ m^{-2} d^{-1} $°C^{-1}$) and Loxton (6.2 MJ m^{-2} d^{-1} $°C^{-1}$). VPD_{max} varied considerably across sites with the highest at Loxton where the mean monthly VPD_{max} was above 30 hPa in the summer months from December to February. The lowest levels of VPD_{max} occurred at Loxton and Yoongarillup over the winter months of June to August and the least range in VPD_{max} was at Alstonville and Macksville presumably due to the close proximity of the ocean and the influence of sea breezes.

Multiple linear regression was used to model avocado yield in Israel (Lomas and Zamet, 1994). Statistically significant climatic variables explained up to 83% of the interannual variability in avocado yield. Factors included 5-day mean minimum temperatures in winter, soil temperature during frost events, heat stress events and VPD. While it is difficult to protect against the occurrence of these weather events it could be possible to mitigate against heat and VPD by ensuring that orchards are well irrigated prior to such events. The deleterious effects of cold stress to roots are well documented (Calleja-Cabrera et al., 2020) and a method to mitigate against cold stress is through mulching each winter to insulate the roots against the cold.

Studies in avocado indicate that floral induction occurs in late autumn/early winter in temperate regions (Ziv et al., 2014). Furthermore, floral induction is triggered in part by a period of cool day/night temperatures (Buttrose and Alexander, 1978). Peak flowering varies across the Australian avocado growing regions, which is likely due to variation in the duration of cold winter temperatures (www.avocado.org.au). For example, in a warmer winter climate, such as Atherton, peak flowering occurs in early September; whereas, peak flowering occurs in late October in the cool winter climate regions of South Western Australia and the growing region along the Murray River. The other important factor that impacts PTQ is the effect of climate on fruit maturation. In warmer climates, fruit maturation is accelerated and as a result, fruits are harvested much earlier in the season. However, in cooler climates, fruit maturation and harvest is delayed.

Results from this proof of concept indicates that long-term mean weather data highlights favorable PTQ in the months leading up to flowering, which likely facilitates starch accumulation in woody tissues (stems, branches and trunks) to drive vegetative and reproductive growth during spring and summer months (Scholefield et al., 1985; Whiley et al., 1996; Liu et al., 1999). The other striking feature of the long-term mean data is that relative to spring PTQ, the post-flowering PTQ is low during late summer and into autumn, which coincides with the period of assimilate demand used to drive fruit growth and development, as well as the summer and autumn vegetative flushes. Seasonal variation in PTQ favors a large flush of inflorescence and vegetative growth followed by a period of high premature fruit drop. The low PTQ during fruit growth and development reinforces the importance of stored reserves for fruit development and highlights the vulnerability of young fruit to periods of low assimilate supply that may be precipitated by heat and VPD shock, periods of cloudy weather and water deficit; phenomena that likely contribute to summer fruit drop.

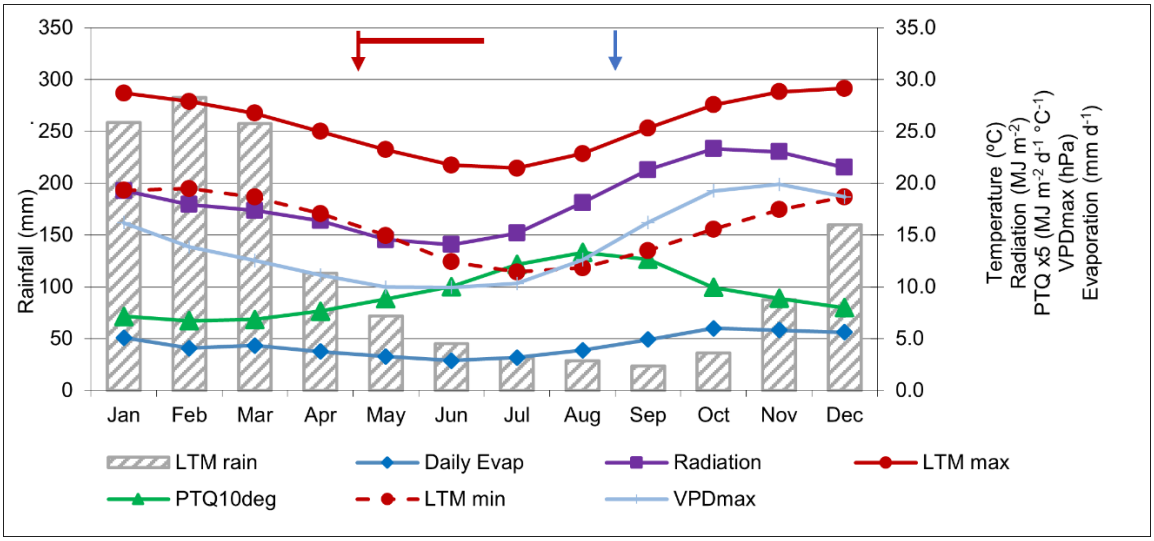


Figure 20: Long-term mean monthly weather data for Atherton, QLD

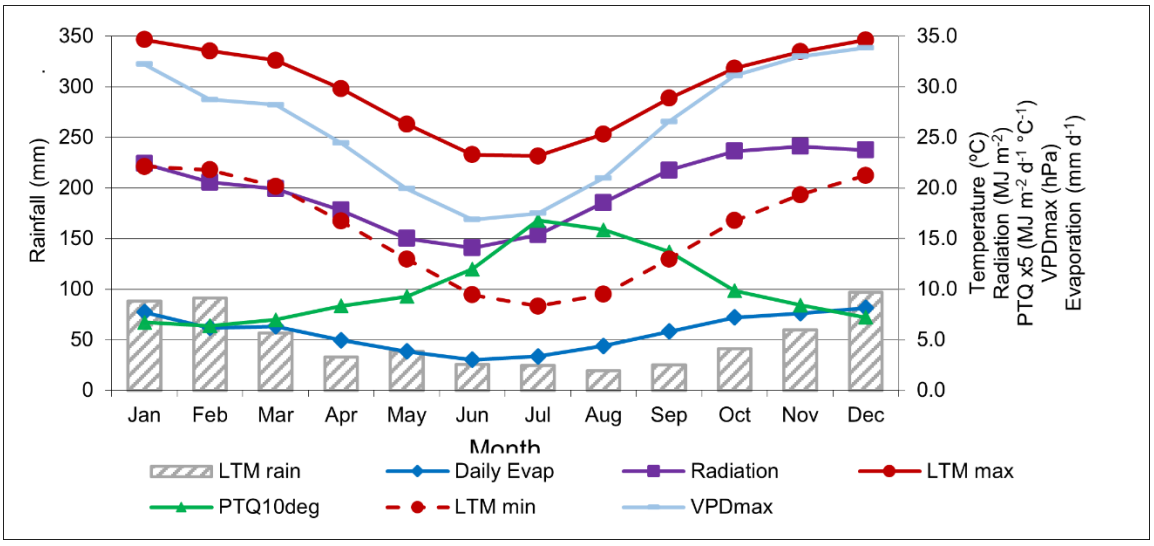


Figure 21: Long-term mean monthly weather data for Emerald, QLD

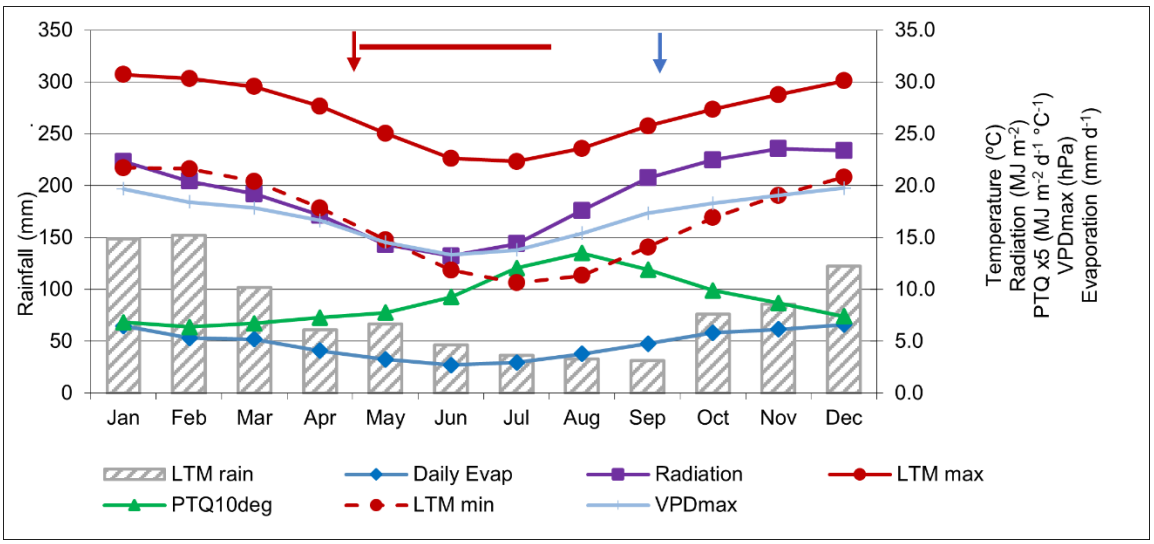


Figure 22: Long-term mean monthly weather data for Bundaberg, QLD.

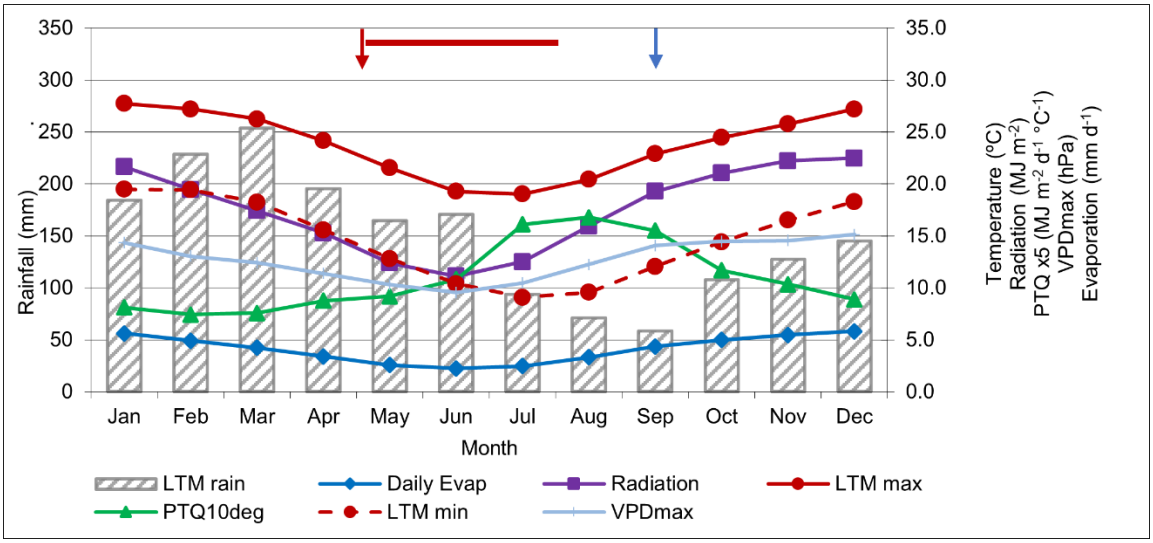


Figure 23: Long-term mean monthly weather data for Alstonville, NSW

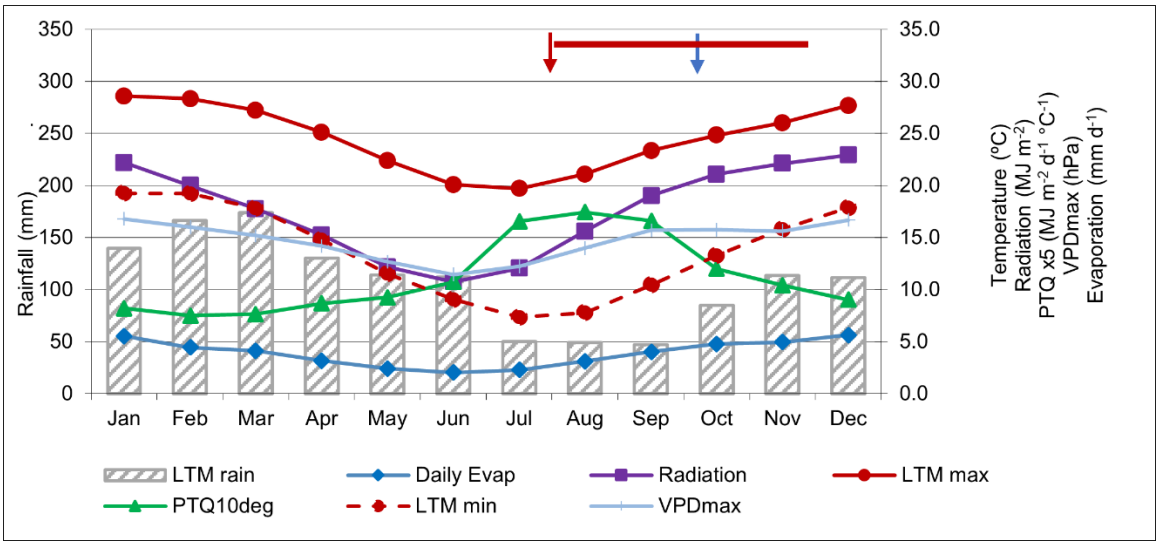


Figure 24: Long-term mean monthly weather data for Macksville, NSW.

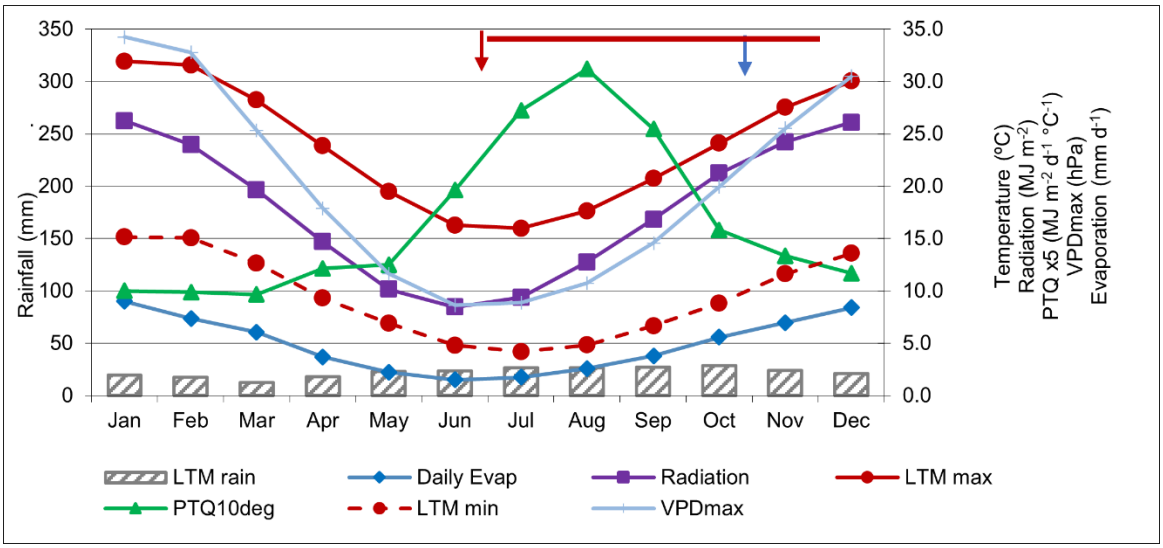


Figure 25: Long-term mean monthly weather data for Loxton, SA..

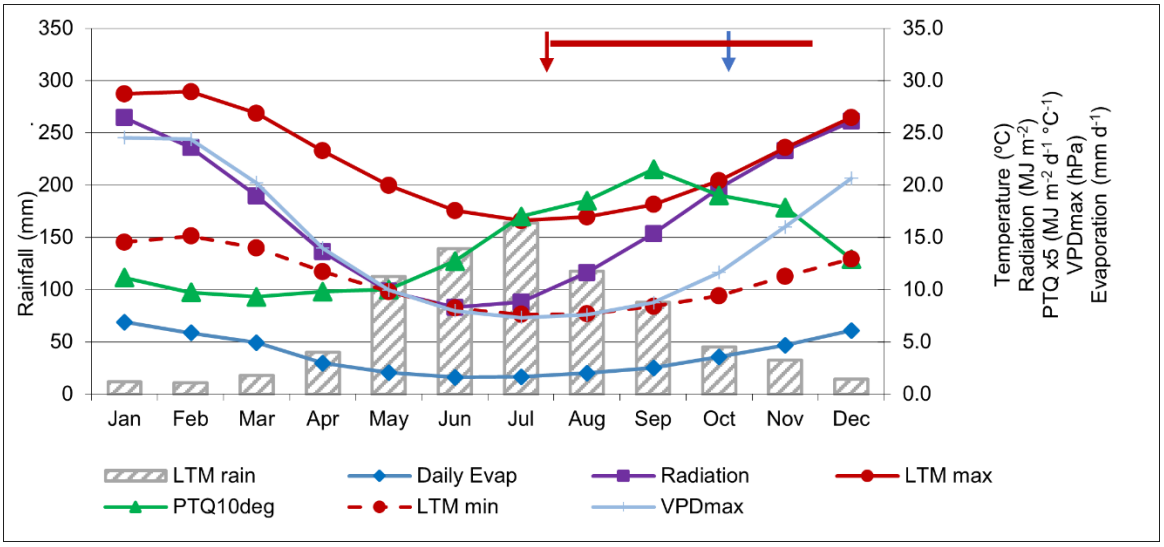


Figure 26: Long-term mean monthly weather data for Yoongarillup, Western Australia.

Note: For each figure, red arrow denotes the start of harvest and the red bar denotes the length of the harvest period. The blue arrow denotes the 'peak-flowering' date. Information derived from Crop Cycle Calendars prepared by Avocados Australia.

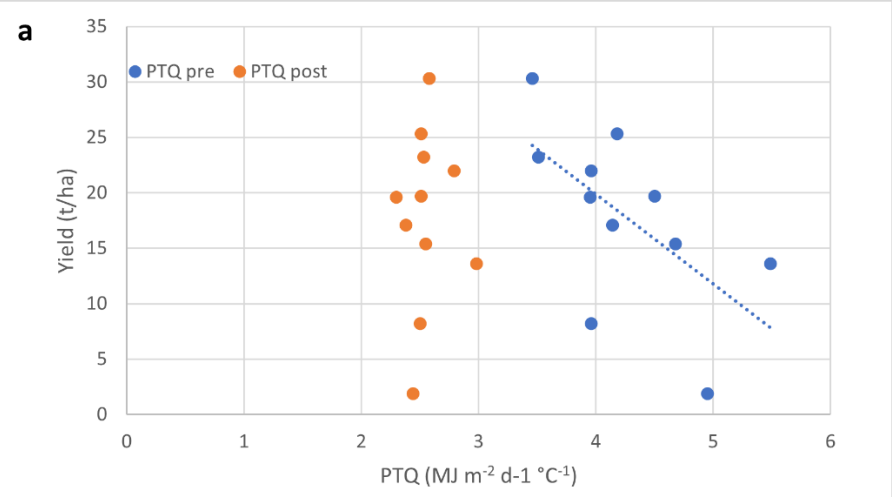
Avocado yield variation and PTQ

In annual species such as wheat, canola and sorghum, the use of PTQ to explain seasonal and environmental impacts on tillering and yield has allowed breeders to better select traits relevant to the target environments. Work by Rawson (1988), Fischer (1985), Peak and Angus (2009) demonstrated the power of using PTQ as a surrogate for assimilation per unit of development to predict kernels per ear and yield for wheat crops. More recently Faraji, (2012a, 2012b) has used PTQ to explain year to year variation in oil concentration of canola crops. These are examples of the strong relationships that are possible in annual crops between yield and PTQ. This has allowed breeders to better select traits relevant to the target environments. It has opened opportunities for researchers to explore critical growth periods for yield formation, physiological factors limiting yield across regions and it has allowed farm consultants and growers to develop management practices to optimize yield according to seasonal conditions.

Early work by Wolstenholme (1987) suggested pathways to yield improvement for avocado breeding and Stephenson and Chapman (2012) demonstrated that well managed avocado orchards, at its upper yield levels of 38 t/ha had equivalent performance to crop benchmarks, such as citrus and apple, when expressed on an energy-equivalent basis. The challenge is to maintain maximum yield potential annually.

Historic avocado yield (t/ha) derived from an orchard in south west WA, north QLD, central QLD and south QLD, were plotted against pre- and post-flowering PTQ (Figure 27). Results showed that the relationship between PTQ, pre- or post-flowering, and the commercial yield of avocados was insufficient. Further, no consistent relationship between pre-flowering PTQ and yield was observed; sometimes loosely positive and sometimes loosely negative. One could speculate that a negative relationship between PTQ and yield may be driven by high temperatures and VPD_{max} which could associate with high radiation in some environments or years. Indeed, Lomas and Zamet, 1994 found negative yield trends with radiation in autumn and positive yield trends in winter. The hypothesis that heat-stress and high VPD events negatively impact yield deserves further investigation.

Year to year variation in yield was substantial in the commercial yield data studied. There was no evidence of biennial bearing in any of the yield data (data not shown) which suggests that infrequent weather extremes, such as frost (Lomas and Zamet, 1994), heat-stress (Wolstenholme, 2013), high VPD (Lomas and Zamet, 1994) and biotic stress, such a disease, influenced the interannual variation of yield in most regions. There was also a weak relationship between yield and post flowering PTQ across these orchards. This is somewhat surprising considering that yield loss due to the early immature fruit abscission event follows the post-flowering period. The post-flowering PTQ displayed less interannual variation than the pre-flowering PTQ and was the main reason for the absence of any relationship with yield. This suggests that pre-flowering accumulation of carbohydrate reserves is more important to determining yield that post-flowering assimilation. It is possible that the level of stored carbohydrate reserves influences the level of fruit set and abscission.



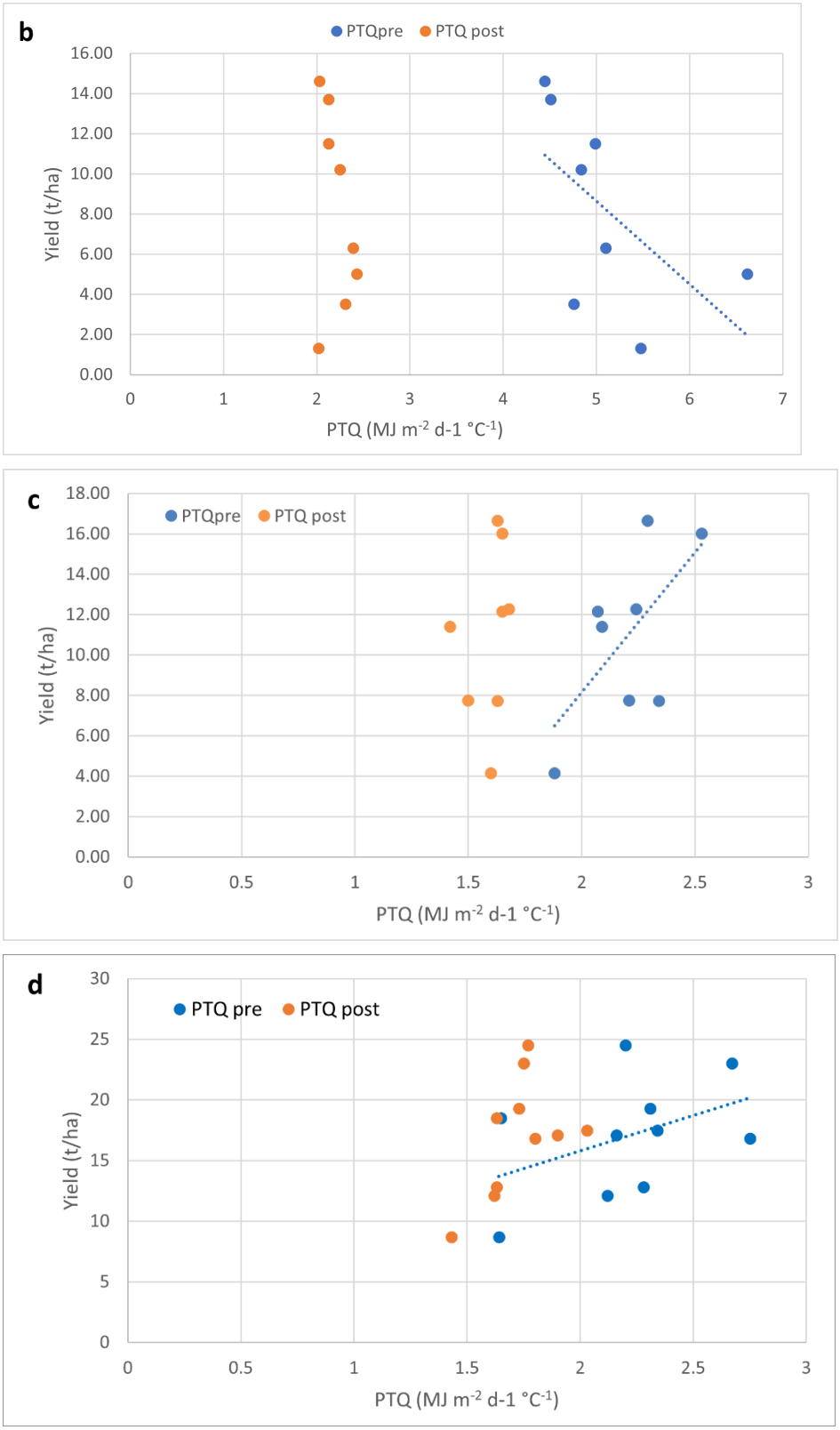


Figure 27: Typical plots of Yield (t/ha) of avocado fruit plotted against PTQ 120 days pre-flowering (blue circles) and 150 days post flowering (orange circles) for an orchard in (a) south west, WA (2010-2020), (b) south, QLD (2013-2020), (c) central, QLD (8 years between 2001-2020) and (d) north, QLD (11 years between 2004-2020).

Figure 28 presents the VPD_{max} and observed fruit drop for a 2-month period between November 2019 and January

2020 for an orchard in south west, WA (H. Smith unpublished data). As mentioned earlier in this report, fruit abscission may be associated with high VPD 'stress' events. While not conclusive, the figure does show fruit drop events in the days following brief periods of high VPD. Data mining of existing observations of fruit abscission and investigation of associations with weather extremes warrants further investigation.

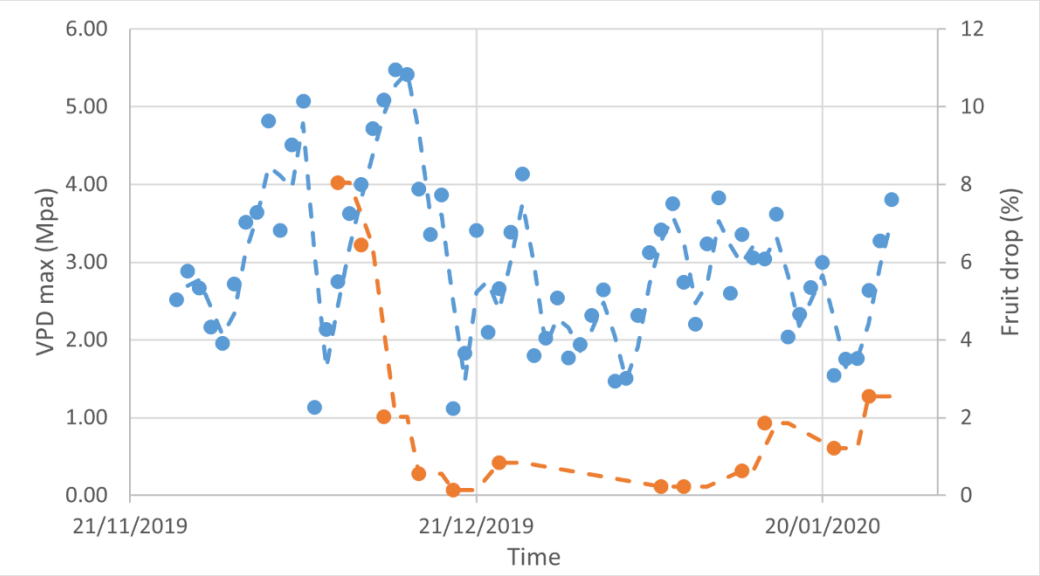


Figure 28: VPD_{max} and observations of fruit drop plotted against a 2month period November 2019 and January 2020 for an orchard in south west, WA . (H. Smith unpublished data)

In-season Carbohydrate monitoring

During literature searches and investigations into the possible associations between avocado yield and PTQ, two key publications were identified that contained in-season measurements of starch reserves in branches and stems (Scholefield et al., 1985, Liu et al., 1999). The branch starch data of Scholefield et al., 1985 was derived from an orchard in New South Wales, Australia, while the stem starch data of Liu et al., 1999 was derived from an experimental site in Southern California, USA. Scholefield et al., 1985 measured carbohydrate in branches every 2-3 months over 3 years and Liu et al., 1999 measured carbohydrates in stems every month over 2 years. The carbohydrate data were extracted from graphs to test for associations with the month-to-month seasonal variation in PTQ. Australian weather data was downloaded (1976-1979) from SILO (https://www.longpaddock.qld.gov.au/silo/) and the Californian weather data was downloaded (1994 1996) from the University of California Statewide Integrated Pest Management Program (UC-IPMP) for the weather station at the University of California, Irvine campus (Pest and plant models, degree-days, and weather - UC Statewide IPM Program (ucanr.edu)). PTQ was calculated for base temperatures of 10°C using the TTC method and monthly mean PTQ_{10deg} was calculated for the duration of each experiment.

Figure 29a plotted the branch starch levels (orange circles, from Scholefield et al., 1985) and monthly mean PTQ (blue circles, from SILO) for the duration of the trial. Figure 29b plotted stem starch levels (orange circles, from Liu et al., 1999) and monthly mean PTQ (blue circles, UC-IPMP) against time. The close association between starch concentration and PTQ over a number of seasons and the close agreement between two independent studies, indicate that PTQ is influencing the in-season storage of starch in branches and stems close to the photosynthetic source. The stem starch levels in Figure 29b essentially tracks the PTQ, while branch starch levels in Figure 29a appears to show a lag of approximately one month. This retrospective analysis of two, independent, previously published studies and comparison to new weather analysis suggests that seasonal variation in PTQ is influencing carbohydrate storage in avocado trees. Monitoring of PTQ shows potential to be developed into an in-season assessment of carbohydrate status of avocado trees. Its pilot testing and validation could be a component of a larger project to develop rapid and cost-effective carbohydrate monitoring for avocado trees.

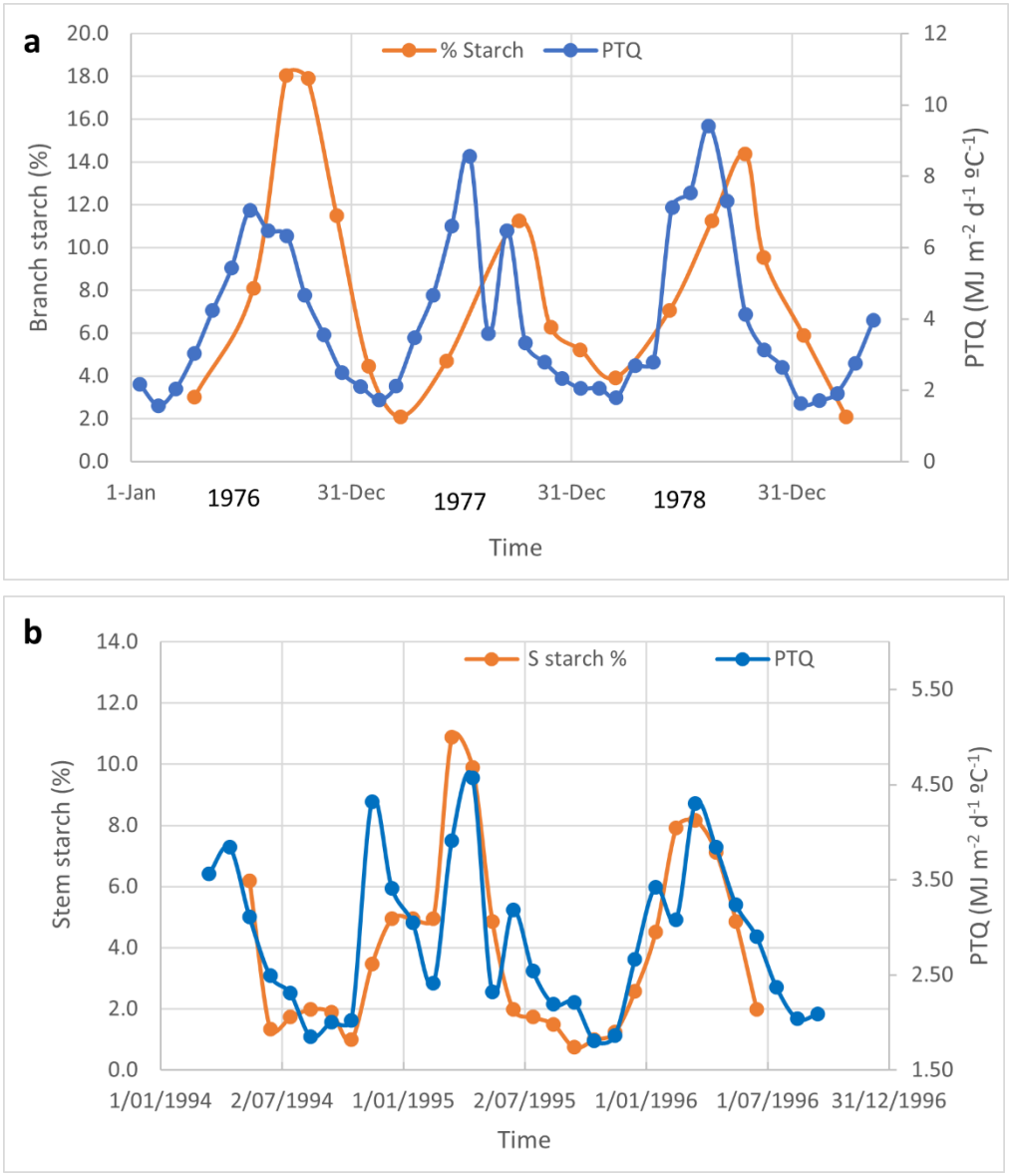


Figure 29: (a) branch starch (orange circles, from Scholefield et al., 1985) and monthly mean PTQ (blue circles, from SILO) plotted against time and b.) stem starch (orange circles, from Liu et al., 1999) and monthly mean PTQ (blue circles, UC-IPMP) plotted against time.

Summary of PTQ investigation in avocado

The long-term mean weather data highlights the favorable PTQ in the months leading up to flowering in avocado for all growing regions. An increase in PTQ prior to flowering would facilitate the growth and development of inflorescences, flowers and vegetative spring flushes. NSC reserves may be mobilized during the fruit development to increase the pool of carbohydrates available for growth. Low PTQ during summer and into autumn coincides with the period of demand for assimilates used to drive fruit growth, as well as the summer and fall vegetative flushes and root growth. Seasonal variation in PTQ appears to favor a large flush of inflorescences and vegetative shoots followed by a period of immature fruit drop to adjust yield potential to a more rapid rate of fruit growth and development, as temperatures warm but constrained by little change in assimilate supply. For the commercial yield data examined, there was a low relationship between pre- or post-flowering PTQ and yield. Post-flowering PTQ displayed less interannual variation than the pre-flowering PTQ. This further suggests that pre-flowering accumulation of carbohydrate reserves are more important to determining yield than post-flowering assimilation. We speculate that the level of stored carbohydrate reserves is a critical driver of fruit set and is equally as important as photosynthesis during fruit development. The close association between starch levels in avocado stems and branches with PTQ and the close agreement between the two independent studies, indicates that PTQ is influencing

the in-season starch reserves close to the photosynthetic source. This finding shows potential to be developed into an in-season assessment of carbohydrate status of avocado orchards.

References

Avocados Australia: https://avocado.org.au

Breiman, L. (2001) Random forests. Machine Learning 45:5-32.

Buttrose, MS, and Alexander, DM. (1978). Promotion of floral initiation in 'Fuerte' avocado by low temperature and short daylength. Scientia Horticulturae 8:213–219.

Calleja-Cabrera, J., Boter, M., Oñate-Sánchez, L., Pernas, M. (2020) Root growth adaptation to climate change in crops. Frontiers in Plant Science 11:544

Decruyenaere, V., Clément, C., Agneessens, R., Losseau, C., Stilmant, D. (2011) Development of near-infrared spectroscopy calibrations to quantify starch and soluble sugar content in the roots of *Rumex obtusifolius*. Weed Research 52:1-5.

DeJong, T. (2019) Advances in understanding fruit tree growth. In: Achieving sustainable cultivation of temperate zone tree fruits and berries. Volume 1: Physiology, genetics and cultivation. (eds) Lang, G.A. Cambridge, UK, Burleigh Dodds Science Publishing. pp. 73-92.

Edwards, E.J., Thomas, M.R., Gensemer, S., Lagerstron, R., Khokher, R., Liao, Q., Sun, Chamngming, C., Wang, D., Hargrave, C., Ralston, J. (2020) New non-destructive technologies for simultaneous yield, crop condition and quality estimation. Final Report to Wine Australia, Adelaide, South Australia.

Faraji, A. (2012a) Flower formation and pod/flower ratio in canola (*Brassica napus* L.) affected by assimilates supply around flowering. International Journal of Plant Production 4:271–280.

Faraji, A. (2012b) Oil concentration in canola (Brassica napus L.) as a function of environmental conditions during seed filling period. Int J Plant Prod 6:267–278

Fischer, R.A. (1985). Number of kernels in wheat crops and the influence of solar radiation and temperature. Journal of Agricultural Science 105:447–461.

Hu, J., Ma, X., Liu, L., Wu, Y., Ouyang, J. (2017) Rapid evaluation of the quality of chestnuts using near-infrared reflectance spectroscopy. Food Chemistry 231:141-147.

Kruse, F.A., Lefkoff, A.B., Boardman, J.W., Heidebrecht, K.B., Shapiro, A.T., Barloon, P.J., Goetz, A.F.H. (1993) The spectral image processing system (SIPS)-interactive visualization and analysis of imaging spectrometer data. Remote Sensing of Environment 44:145-163.

Lahav, E., Trochoulias, T. (1982) The effect of temperature on growth and dry matter production of avocado plants. Australian Journal of Agriculture Research 33:549-558.

Lammertyn, J., Peirs, A., De Baerdemaeker, J., & Nicolaï, B. (2000). Light penetration properties of NIR radiation in fruit with respect to non-destructive quality assessment. Postharvest Biology and Technology 18:121–132. https://doi.org/10.1016/S0925-5214(99)00071-X

Liu, X., Robinson, P.W., Madore, M.A., Witney, G.W., Arpaia, M.L. (1999). 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology. Journal of American Society of Horticultural Science 124:671-675.

Liu, X., Sievert, J., Arpaia, M.L., Madore, M.A. (2002) Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. Journal of American Society of Horticultural Science 127:108-114.

Lomas, J., Zamet, D. (1994) Long-term analysis and modelling of agroclimatic effecs on national avocado yields in Israel. Agriculture and Forest Meterology. 71:315-336.

Nix, H.A. (1975). Australian climate and its effects on grain yield and quality. In: A. Lazenby and E.M. Matheson (Editors), Australian Field Crops, Vol. I: Wheat and Other Temperate Cereals. Angus and Robertson, Sydney, pp. 183-226.

Nix, H.A., (1976). Climate and crop productivity in Australia. In: Climate and Rice. International Rice Research

Institute, Los Bafios, Philippines, pp. 495-508.

Peake, A.S., Angus, J.F. (2009) Increasing yield of irrigated wheat in Queensland and Northern NSW. GRDC Northern Region Grains Research Updates, Goondiwindi, 3-4 March.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramirez, J.A., Posada, J.M., Handa, I.T., Hoch, G., Vohland, M., Messier, C., Reu, B. (2015) Near-infrared spectroscopy (NIRS) predicts non-structural carbohydrate concentrations in different tissue types of a broad range of tree species. Methods in Ecology and Evolution 6:1018-1025.

Rawson, H.M. (1988). Effects of high temperatures on the development and yield of wheat and practices to reduce deleterious effects. pp. 44-61. In: A.R. Klatt (ed.). Wheat production constraints in tropical environments. (CIMMYT: Mexico).

Scholefield, P.G., Sedgley M., Alexander D.M. (1985) Carbohydrate cycling in relation to shoot growth, floral initiation and development and yield in the avocado. Scientia Horticulturae 25:99-110.

Stephenson, R.A. and Chapman, J. (2012) Striving for the yield potential of Macadamia. Acta Horticuturae 45:113-118.

Stitt, M., Zeeman, S.C. (2012) Starch turnover: Pathways, regulation and role in growth. Current Opinion in Plant Biology 15:282-292.

Tesfay, S.Z., Bertling, I., Bower, J.P., Lovatt, C. (2012) The quest for the function of 'Hass' avocado carbohydrates: clues from fruit and seed development as well as seed germination. Australian Journal of Botany 60:79-86.

Tibshirani, R. (1996) Regression shrinkage and selection via the Lasso. Journal of the Royal Statistical Society. Series B, 58:267-288.

Wang, M., White, N., Grimm, V., Hofman, H., Doley, D., Thorp, G., Cribb, B., Wherritt, E., Han, L., Wilkie, J., Hanan, J. (2018) Pattern-oriented modelling as a novel way to verify and validate functional-structural plant models: a demonstration with the annual growth module of avocado. Annuals of Botany. 121:941-959.

Whish, J.P.M., Lilley, J.M., Morrison, M.J., Cocks, B., Bullock, M., 2020. Vernalisation in Australian spring canola explains variable flowering responses. Field Crops Research 258:107968. doi:10.1016/j.fcr.2020.107968.

Whiley, A.W., Wolstenholme B.N. (1990) Carbohydrate management in avocado trees for increased production. South African Avocado Growers' Association Yearbook 13:25-27.

Whiley, A.W., Rasmussen, T.S., Saranah, J.B., Wolstenholme, B.N. (1996) Delayed harvest effects on yield, fruit size and starch cycling in avocado (*Persea americana* Mill.) in subtropical environments. II. The late-maturing cv. Hass. Scientia Horticulturae 66:35-49.

Wolstenholme, B.N. (1987). Theoretical and applied aspects of avocado yield as affected by energy budgets and carbon partitioning. Proceedings of the World Avocado Congress, Pretoria, South African Avocado Growers Association Year book 10: 58-61.

Wolstenholme, B.N. (2013). Ecology: climate and soils. In: Schaffer B, Wolstenholme BN, Whiley AW, eds. The avocado: botany, production and uses, 2nd ed. Wallingford, UK: CAB International, 86–117.

Zamet, D.N. (1990) The effect of minimum temperature on avocado yields. California Avocado Society Yearbook 74:247-256.

Ziv, D., Zviran, T., Zezak, O., Samach, A., Irihimovitch, V. (2014). Expression Profiling of FLOWERING LOCUS T-Like Gene in Alternate Bearing 'Hass' Avocado Trees Suggests a Role for PaFT in Avocado Flower Induction. Plos One 9:e110613.

Appendix B: Desktop Study

AV19006 Desktop Review

Identification of a rapid carbohydrate monitoring system(s) in avocado

Everard Edwards[1], Anthony van Herwaarden[2] and Harley M. Smith[1]

[1]CSIRO Agriculture and Food, Waite Campus, Urrbrae, SA

[2]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Brisbane, QLD

Annual avocado yields are well below the theoretical production potential of 32 t/ha indicating that significant gains in yield can be achieved through innovative management and breeding (Wolstenholme, 1986, 1987). The major developmental processes that reduce avocado yield and contribute to irregular bearing are low fruit set and high fruit abscission, as well as biennial bearing (Newett, 2013). Abiotic and biotic stresses impact these processes, which further increase the severity of irregular bearing, adding to the difficulty of assessing and predicting the production capacity. To maximize production, growers require rapid and cost-effective system(s) to evaluate the production potential of trees to effectively apply management inputs at key time points during the season.

Mechanisms that drive plant growth, development and yield are dependent upon carbohydrates or sugars (Eveland and Jackson, 2011). A hypothesis was developed indicating that increased productivity can be achieved via tree carbohydrate management (Wolstenholme and Whiley, 1989; Whiley and Wolstenholme, 1990). The ability to manage carbohydrate storage, mobilization and partitioning requires tools to rapidly estimate or measure these substances without the use of laborious laboratory methods. The goal of this review is to identify a non-destructive approach(es) to measure carbohydrate levels in avocado tissues. This desktop review is divided into three chapters. The first chapter is an introductory chapter subdivided into three sections. The first section covers the seasonal developmental patterns of avocado and how growing units can impact yield. In section two, the two primary sources of carbohydrates are highlighted, as well as the types of sugars partitioned to growing units and storage tissues. In addition, sink-source relations are covered in this section. The last section highlights studies that integrated avocado growth and development with seasonal fluctuations in carbohydrates. Together, these sections pinpoint tissues used for the proof-of-concept, a preliminary assessment of a non-destructive technology for measuring carbohydrates. The second chapter discusses non-destructive technologies for measuring carbohydrates levels in plants. Devices that utilize near infrared (NIR) and other parts of the electromagnetic spectrum will be highlighted as they have potential to estimate carbohydrate levels in avocado in a non-destructive manner. A non-destructive technology will be identified for a proof-of-concept, which will be performed to evaluate the use of this tool in avocado. In the last chapter, photothermal quotient (PTQ), which is defined at the ration of daily solar radiation to mean daily temperature, will be discussed. This chapter will highlight how, PTQ is used to surrogate carbohydrate supply for reproduction and yield in annual crops. Moreover, a pathway for PTQ analysis in avocado will be presented and a proof-of-concept will be performed based on the information in this chapter.

Chapter 1: Introduction to avocado

Phenology of avocado

In temperate growing regions, avocado trees undergo seasonal patterns of reproductive, vegetative and root growth (Wolstenholme and Whiley, 1989; Whiley and Wolstenholme, 1990; Whiley et al., 2013). Reproductive bud development is initiated in response to low temperatures in the fall and early winter months (Nevin and Lovatt, 1989; Salazar-Garcia et al., 1999; Salazar-Garcia et al., 2006). The floral inductive signal switches the fate of a subset of buds from vegetative to reproductive resulting in the formation and development of an inflorescence (Ziv et al., 2014). As inflorescence development is marked by the production of flowers, the floral transition is a key step in the reproductive process (Wilkie et al., 2008; Bangerth, 2009; Samach and Smith, 2013). At flowering in the spring, pollination and the pollen-pistil interactions involved in fertilization are key factors that influence fruit set (Davenport 1986; Alcaraz et al., 2013). Pollen-pistil interactions, such as pollen germination, pollen tube growth and ovule penetration, as well as fertilization, are highly sensitive to temperature and humidity (Sedgley, 1977; Sedgley and Annells, 1981; Alcaraz and Hormaza, 2009). Therefore, growing regions that experience periods of low or high temperatures, as well as low humidity conditions during flowering, often display a low level of fruit set. Further, as a majority of flowers abscise, even under favorable conditions (Sedgley, 1977; Sedgley and Annells, 1981; Alcaraz and Hormaza, 2009; Garner and Lovatt, 2016), low fruit set is one of the major factors that limit production in avocado (Newett, 2013). After fertilization, fruit growth and development is initiated and maintained throughout the growing season (Schroeder, 1953; Bower and Cutting, 1988; Alcaraz et al., 2013). However, a significant number of fertilized fruits abscise in a continuous manner and waves of fruit drop during the growing season (Davenport, 1986; Garner and Lovatt, 2008; Alcaraz and Hormaza, 2009). Thus, fruit abscission is another major factor that limits production (Whiley and Schaffer, 1994; Salazar-Garcia and Lovatt, 2013; Newett, 2013).

Up to three vegetative flushes of growth occur in temperate growing regions, including the spring, summer and fall flushes (Salazar-Garcia and Lovatt, 2013). Growth and development of the summer and fall flushes are key for the initiation of reproductive patterns of growth the following season, as the majority of inflorescences and flowers are produced by these two vegetative flushes (Davenport et al., 1982; Salazar-Garcia et al., 1998; Salazar-Garcia et al., 2006). For example, the inhibition of summer and fall vegetative growth in trees with a heavy crop results in low flowering the following season (Salazar-Garcia et al., 1998; Salazar-Garcia et al., 2006; Ziv et al., 2014). Two flushes of root growth occur in a single season (Whiley et al., 2013). The first flush of root growth occurs between the arrest of the spring flush and the initiation of the summer flush. The second cycle of root growth peaks in autumn after summer flush arrests. In high cropping trees, root growth is significantly reduced (Whiley et al., 2013). While the seasonal patterns of avocado vegetative, reproductive and root growth have been characterized, little is known about the timing and duration of secondary growth in trunks, limbs and other woody tissues.

Understanding the seasonal patterns of vegetative and reproductive growth is important for identifying key management intervention points where competition and/or dominance between growing units may limit productivity (Wolstenholme and Whiley, 1989; Whiley and Wolstenholme, 1990). For example, the predisposition of avocado trees to produce multiple vegetative flushes in a growing season is predicted to compete for resources required for fruit set and development (Wolstenholme and Whiley, 1989; Salazar-Garcia et al., 2013). To decrease the growth potential of vegetative flushes, growers apply plant growth regulators that inhibit gibberellin biosynthesis, such as paclobutrazol and uniconazole (Salazar-

Garcia et al., 2013; Whiley et al., 2013; Menzel and Le Lagadec, 2014). These growth retardants function to reduce stem elongation, which is a major carbon sink in developing vegetative shoots. It is hypothesized that reducing the growth potential of stems increases carbohydrate availability for fruit set and fruit retention. However, despite the widespread usage of these growth retardants, low fruit set and high fruit abscission are nevertheless major factors that limit production (Newett, 2013).

Photosynthesis and carbohydrate partitioning

Photosynthesis is a complex process primarily carried out in leaves (Buchanan et al., 2015). During the process, CO_2 and H_2O together with light energy are used to synthesize glucose ($C_6H_{12}O_6$) and O_2 is released. Glucose can be converted to fructose, another monosaccharide, and together these sugars serve as building blocks for sucrose, a non-reducing disaccharide that is translocated from leaves to growing organs via the phloem (Stein and Granot, 2019). Glucose also serves as a building block for polysaccharides, such as starch and cellulose (Buchanan et al., 2015). In addition to glucose, fructose and sucrose, mannoheptulose and perseitol are major non-structural 7-carbon sugars (C7) produced in mature avocado leaves (Liu et al., 2002). Together, mannoheptulose and perseitol can constitute over 10% of the total dry matter in avocado tissues, including leaves, stems, trunks and roots, as well as flowers and fruits (Lie et al., 1999a, 1999b, Tesfay et al., 2012a, 2012b). In addition to sucrose, these C7 sugars are translocated from leaves to growing organs (Liu et al., 2002). Therefore, sucrose, perseitol and mannoheptulose are key translocated carbohydrates allocated to shoots, fruits, roots and other growing tissues to mediate growth.

Starch is the major carbohydrate reserve in plants (MacNeill et al., 2017). Starch reserves provide carbohydrates for growth and respiration in non-photosynthetic tissues or at times when photosynthesis is limited, such as night and during the burst of vegetative and reproductive growth in the spring. Thus, the translocated sugars, sucrose, perseitol and mannoheptulose, together with starch reserves are key factors of carbohydrate partitioning (Liu et al., 2002; Tesfay et al., 2012). It should also be pointed out that oligosaccharides, amino acids and lipids also display a storage function that can be utilized for carbohydrate production in trees (Hoch et al., 2003).

Environmental factors such as light intensity, temperature and CO_2 levels have a major impact on the rate of photosynthesis (Stein and Granot, 2019). Photosynthesis is also influenced by growth demands in the tree (Paul and Foyer, 2001; Fernie et al., 2020). For example, an increase in carbohydrate demands by growing vegetative and/or reproductive units feedback to increase the rate of photosynthesis in leaves (Iglesias et al., 2002; Urban et al., 2004 Haouari et al., 2013). Due to recurrent flushing, the photosynthetic capacity of the canopy likely changes over the season. First, during each vegetative flush, carbohydrate production via photosynthesis occurs when leaves reach 80% the final size, which takes approximately 17-24 days after leaf initiation (Whiley 1990; Liu et al., 2002). Further, leaves attain maximum photosynthetic capacity after 40-50 days of development (Liu et al., 2002). As up to three flushes of shoot growth occur every season, photosynthetically active leaves become shaded by newly formed leaves, which may impact carbohydrate availability for fruit production. However, the photosynthetic potential may be more stable in heavy cropping trees, as shading from summer and fall flushes would be limited. In environments where summer and fall flushes are initiated in relatively high cropping trees, the overall photosynthetic potential may change with new flushes of vegetative growth, which may influence fruit abscission.

Relationship between carbohydrate levels and seasonal growth cycles in avocado

In tree crops, bud burst is a major developmental event in which a subset of dormant buds transition to a metabolically active state (Cooke et al., 2012; Barbier et al., 2019). During this period of growth stored carbohydrates and nutrients are actively mobilized for shoot growth (reproductive and vegetative) and respiration. In forest trees, experimental studies show that starch stored in the current and previous year are used for reproductive and vegetative growth (Dietze et al., 2014).

In avocado, the relationship between vegetative and reproductive patterns of growth and carbohydrate levels were evaluated. In temperate environments, starch levels fluctuated over a two-year period in stems (Scholefield et al., 1985; Liu et al., 1999a). In both studies, starch accumulates in stem tissues from winter to early spring. Subsequently, starch reserves decline from mid-spring to its lowest levels by autumn. This decrease in starch reserves correlates with the initiation and development of reproductive shoots and organs (inflorescences, flowers and fruits) and vegetative shoots (spring, summer and fall flushes). Therefore, it has been hypothesized that starch reserves are mobilized in stem tissues and partitioned to provide carbohydrates necessary for reproductive and vegetative growth during the spring and summer months (Scholefield et al., 1985; Liu et al., 1999a). In winter, the increase in starch levels in stems corresponds to a time in which vegetative growth terminated and fruit growth is significantly reduced. The accumulation of starch reserves in stem tissues is likely derived by photosynthesis occurring in the leaves produced from spring, summer and/or fall flushes (Scholefield et al., 1985; Liu et al., 1999a). Interestingly, lower levels of stem starch reserves accumulate in trees with a high crop load, indicating winter photosynthates are directed away from storage tissues in stems to support fruit development in high yielding trees (Scholefield et al., 1985). As high crop load inhibits growth of the summer/fall flushes, winter accumulation of starch would be derived from leaves of the spring flush. As the photosynthetic capacity of leaves decreases after 50-days, leaves derived from the spring flush are predicted to produce less carbohydrates than leaves from the summer and fall flushes. It has been speculated that winter starch accumulation in stems plays a critical role in inflorescence development in the spring (Liu et al., 1999a), as inflorescences produced following an “on-crop” are smaller and produce fewer flowers (AV16005: Haberman and Smith unpublished). Defoliation of avocado trees in the summer results in a rapid decline in starch in stems, which correlates with massive fruit drop event (AV16005: Haberman and Smith, unpublished). Lastly, stem starch levels at peak flowering also correlate with fruit set (AV16005 Haberman and Smith unpublished). Taken together, these studies indicate that stem starch levels are a critical factor for determining the reproductive potential of the tree.

In addition to stems, starch also accumulates in trunks and lignified roots (Liu et al., 1999a). In trunk samples, starch levels were relatively stable throughout the two seasons. The differences between seasonal starch levels in stems and trunks raises two interesting points. Firstly, starch that accumulates in the inner layers of the trunk produced in previous years may be inaccessible for mobilization and partitioning for growth. Secondly, experimental evidence suggests that the storage potential in woody parenchyma tissues of trunks and branches may act as an active sink for carbohydrates (Cannell and Dewar, 1994; DeJong, 2019). As a result, woody parenchyma tissue would actively compete for carbohydrates with vegetative and reproductive growing units during the spring and summer months. Together, these two points may explain why Liu et al., 1999a did not observe seasonal starch fluctuations in trunk tissues.

While the seasonal patterns of vegetative, reproductive and root growth have been determined in avocado, little is known about the timing and duration of secondary growth in large limbs and trunks. In

trunks and limbs, secondary growth is driven by the activity of the vascular cambium, which is a large sink for structural and storage carbohydrates (Ye and Zhong, 2015). Further, results from poplar and forest trees indicate that the vascular cambium undergoes seasonal periods of growth and dormancy (Huang et al., 2020). Understanding the timing and duration of secondary growth and how this impacts carbohydrate availability for reproductive growth will further enhance our understanding for facilitating the improvement of avocado management.

Managing tree carbohydrates for reproductive growth

Tree carbohydrate status as indicated by carbohydrate availability is a key driver of yield. In tree crops, carbohydrate availability is dependent upon the rate of photosynthesis and the size of the storage reserves, as well as the mechanisms that mobilize and transport sugars to growing units, including flowers and fruits. Methods to measure tree carbohydrate levels throughout the growing season is the first step for estimating sugar availability. Once carbohydrate monitoring systems are adapted to avocado, management tool development can be aimed at increasing the carbohydrate storage capacity in woody tissues and partitioning sugars to reproductive growing units at times when competition and/or dominance impacts yield. To achieve this management capability, an understanding of how trees store, mobilize and partition carbohydrates for growth and reproduction is key to developing tools to manipulate the carbohydrate storage capacity and partitioning to flowers and fruits for increasing yield. Lastly, a carbohydrat monitoring system can also be used to identify the best canopy system to maximize photosynthesis and carbohydrate production.

References:

Alcaraz, M.L., Hormaza, J.I. (2009) Avocado pollination and fruit set – A perspective from Spain. California Avocado Society Yearbook 92:113-135.

Alcaraz, M.L., Hormaza, J.I., Rodrigo, J. (2013). Pistil Starch Reserves at Anthesis Correlate with Final Flower Fate in Avocado (*Persea americana*). Plos One *8*.

Bangerth, F. (1989). Dominance among fruits sinks and the search for a correlative signal. Physiologia Plantarum 76:608-614.

Barbier, F.F., Dun, E.A., Kerr, S.C., Chabikwa, T.G., Beveridge, C.A. (2019) An update on the signals controlling shoot branching. Trends in Plant Science 24:220-236.

Bower, J.P., and Cutting, J.G. (1988). Avocado fruit development and ripening physiology. Horticulture Reviews 10:229-271.

Buchanan, B.B., Gruissem, W., Jones, R.L. (2015) Photosynthesis. In: Biochemistry and molecular biology of plants, 2nd edition, Wiley-Blackwell, Hoboken, NJ: pp. 508-565.

Cannell, M.G.R., Dewar, R.C. (1994). Carbon allocation in trees: A review of concepts for modeling. In: Advances in Ecological Research, volume 25. M. Begon and A. H. Fitter, eds. Academic Press, London, pp. 59–104.

Cooke, J.E.K., Eriksson, M.E., Junttila, O. (2012) The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell and Environment 35:1707-1728.

Davenport, T.L. (1982) Avocado growth and development. Proceedings of the Florida State Horticultural Society 95:92–96.

Davenport, T.L. (1986). Avocado flowering. Horticulture Reviews 8:247-289.

DeJong, T. (2019) Advances in understanding fruit tree growth. In: Achieving sustainable cultivation of temperate zone tree fruits and berries. Volume 1: Physiology, genetics and cultivation. (eds) Lang, G.A. Cambridge, UK, Burleigh Dodds Science Publishing. pp. 73-92.

Dietze, M.C., Sala, A., Carbone, M.S., Czimczik, C.I., Mantooth, J.A., Richardson, A.D., Vargas, R. (2014) Nonstructural carbon in woody plants. Annual Review of Plant Biology 65:667-687.

Eveland, A.L., Jackson, D.P. (2011) Sugars, signalling and plant development. Journal of Experimental Botany 63:3367-3377.

Fernie, A.R., et al., (2020) Synchronization of developmental, molecular and metabolic aspects of source-sink interactions. Nature Plants 6:55-66.

Garner, L.C., Lovatt, C.J. (2008). The relationship between flower and fruit abscission and alternate bearing of 'Hass' avocado. Journal of American Society of Horticultural Science 133:3-10.

Garner, L.C., Lovatt, C.J. (2016). Physiological factors affecting flower and fruit abscission of 'Hass' avocado. Scientia Horticulturae 199:32-40.

Haouari, A., Van Labeke, M.C., Steppe, K., Mariem, F.B., Braham, M., Chaieb, M. (2013) Fruit thinning affects photosynthesis activity, carbohydrate levels, and shoot and fruit development of olive trees grown under semiarid conditions. Functional Plant Biology 40:1179-1186.

Hoch, G., Richter, A., Körner, Ch. (2003) Non-structural carbon compounds in temperate forest trees. 26:1067-1081.

Huang et al., (2020) Photoperiod and temperature as dominant environmental drivers triggering secondary growth resumption in Northern Hemisphere conifers. Proceedings of the National Academy of Sciences of the USA 117:20645-20652.

Iglesias, D.J., Lliso, I., Tadeo, F.R., Talon, M. (2002) Regulation of photosynthesis through source: sink imbalance in citrus is mediated by carbohydrate content in leaves. Physiologia Plantarum 116:563-572.

Liu, X., Robinson, P.W., Madore, M.A., Witney, G.W., Arpaia, M.L. (1999a). 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology. Journal of American Society of Horticultural Science 124:671-675.

Liu, X., Robinson, P.W., Madore, M.A., Witney, G.W., and Arpaia, M.L. (1999b). 'Hass' avocado carbohydrate fluctuations. II. Fruit growth and ripening. Journal of American Society of Horticultural Science 124:676-681.

Liu, X., Sievert, J., Arpaia, M.L., Madore, M.A. (2002). Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado Journal of American Society of Horticultural Science 127:108-114.

MacNeill, G.J., Mehrpouyan, S., Minow, M.A.A., Patterson, J.A., Tetlow, I.J., Emes, M.J. (2017) Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. Journal of Experimental Botany 68:4433-4453.

Menzel, C.M., Le Lagadec, M.D. (2014) Increasing the productivity of avocado orchards using high-density plantings: A review. Scientia Horticulturae 177:21-36.

Nevin, J.M., Lovatt, C.J. (1989) Changes in starch and ammonia metabolism during low temperature stress-induced flowering in Hass avocado – A preliminary report. South African Avocado Growers' Association Yearbook 12:21–25.

Newett, S.D.E. (2013) Scoping study for avocado alternate bearing research. Hort Innovation AV12028 Final Report p1-20.

Paul, M.J., Foyer, C.H. (2001) Sink regulation of photosynthesis. Journal of Experimental Botany 52:1383-1400.

Salazar-García, S., Lord, E.M., Lovatt, C.J. (1998) Inflorescence and flower development of the 'Hass' avocado (*Persea americana* Mill.) during 'on' and 'off' crop years Journal of American Society of Horticultural Science 123:537–544.

Salazar-García, S., Lord, E.M., Lovatt, C.J. (1999) Inflorescence development of the 'Hass' avocado: commitment to flowering. Journal of American Society of Horticultural Science 124:478–482.

Salazar-Garcia S, Cossio-Vargas LE, Lovatt CJ (2006) Crop load affects vegetative growth flushes and shoot age influences irreversible commitment to flowering of 'Hass' avocado. Hortscience 41:1541–1546.

Salazar-Garcia, S., Garner, L.C., Lovatt, C.J. (2013). Reproductive Biology. In: Schaffer, B., Wolstenhome, N.B. and Whiley, A.W. (eds) The Avocado: Botany, Production and Uses. CABI, New York, NY. pp. 118-167.

Scholefield, P.G., Sedgley M., Alexander D.M. (1985) Carbohydrate cycling in relation to shoot growth, floral initiation and development and yield in the avocado. Scientia Horticulturae 25:99-110.

Schroeder, C.A. (1953) Growth and development of Fuerte avocado fruit. American Society for Horticultural Science 61:103-109.

Sedgley, M. (1977) The effect of temperature on floral behaviour, pollen tube growth and fruit set in avocado. Journal of Horticulture Science 52:135-141.

Sedgley, M., Annells, C.M. (1981). Flowering and Fruit-Set Response to Temperature in the Avocado Cultivar Hass. Scientia Horticulturae 14:27-33.

Smith, A.M., Stitt, M. (2007) Coordination of carbon supply and plant growth. Plant Cell and Environment 30:1126-1149.

Smith HM, Samach A (2013) Constraints to obtaining consistent annual yields in perennial tree crops. I: Heavy fruit load dominates over vegetative growth. Plant Science 207:158–167

Smith, A.M., Zeeman, S. (2020) Starch: A flexible, adaptable carbon store coupled to plant growth. Annual Review of Plant Biology. 71:217-245.

Stein, O., Granot, D. (2019) An overview of sucrose synthases in plants. Frontiers in Plant Science 10:95.

Tesfay, S.Z., Berting, I., Bower, J.P. (2012a) D-mannoheptulose and perseitol in 'Hass' avocado: Metabolism in seed and mesocarp tissue. South African Journal of Botany. 79:159-165.

Tesfay, S.Z., Bertling, I., Bower, J.P., Lovatt, C. (2012b) The quest for the function of 'Hass' avocado carbohydrates: clues from fruit and seed development as well as seed germination. Australian Journal of Botany 60:79-86.

Urban, L., Léchaudel, M., Lu, P. (2004) Effect of fruit load on girdling on leaf photosynthesis in Mangifera indica L. Journal of Experimental Botany 55:2075-2085.

Whiley, A.W., Wolstenholme B.N. (1990) Carbohydrate management in avocado trees for increased production. South African Avocado Growers' Association Yearbook 13:25-27.

Whiley, A.W. (1990) CO_2 assimilation of developing fruiting shoots of vc Hass avocado (*Persea americana* Mill). 13:28-30.

Whiley, A.W., Schaffer, B. (1994) Avocado. In: Schaffer, B. and Anderson, P.C. (eds) Handbook of Environmental Physiology of Fruit Crops: Volume II. Sub-tropical and Tropical Crops. CRC Press, Boca Raton, FL, pp. 3–35

Whiley, A.W., Schaffer, B., Wolstenholme, B.N. (2013) Crop management. In: Schaffer, B., Wolstenhome, N.B. and Whiley, A.W. (eds) The Avocado: Botany, Production and Uses. CABI, New York, NY. pp. 118-167.

Wilkie, J.D., Sedgley, T., Olesen, T. (2008). Regulation of flower initiation in horticulture trees. Journal of Experimental Botany 59:3215-3228.

Wolstenholme, B. N. (1986) Energy costs of fruiting as a yield-limiting factor with special reference to avocado. Acta Horticulturae 175:121-126

Wolstenholme, B. N. (1987) Theoretical and applied aspects of avocado yield as affected by energy budgets and carbon partitioning. South African Avocado Growers' Association Yearbook 10:58-61.

Wolstenholme B.N., Whiley, A.W. (1989) Carbohydrate and phenological cycling as management tools for avocado orchards. South African Avocado Growers' Association Yearbook 12:33-37.

Ye, Z.H., Zhong, R. (2015) Molecular control of wood formation in trees. Journal of Experimental Botany. 66:4119-4131.

Ziv, D., Zviran, T., Zezak, O., Samach, A., Irihimovitch, V. (2014). Expression Profiling of FLOWERING LOCUS T-Like Gene in Alternate Bearing 'Hass' Avocado Trees Suggests a Role for PaFT in Avocado Flower Induction. Plos One 9:e110613

Chapter 2: Field measurement of plant carbohydrates

Introduction to carbohydrate analysis

Carbohydrates are an essential part of all living organisms, including the structure of plant cells. For this study, it is the freely available storage carbohydrates that are of interest, generally termed non-structural carbohydrate (NSC). NSC is typically composed of two pools: i) soluble carbohydrates, such as glucose, fructose, sucrose, and, in the case of avocado, mannoheptulose and perseitol, and ii) simple, easily decomposed single chain polysaccharides, such as starch and fructan. Given the ubiquity of carbohydrates in plant tissue, laboratory analysis has relied on some form of extraction, such as aqueous ethanol or water, that separates the soluble carbohydrates from insoluble carbohydrates. The extract is then either used directly to determine an estimate of total soluble carbohydrate, or the individual carbohydrates in the extract are separated and quantified individually. A further extraction and analysis process is subsequently required for the storage polysaccharides that make up the remainder of NSC. Generally, this involves hydrolysing the polysaccharides to their constituent monomers and measuring those in the same way as the soluble carbohydrates. The hydrolysis step is often performed enzymatically (Karkalas, 1985; McCleary et al., 1994) as further purification of the polysaccharide is not required for enzyme hydrolysis to be effective, but in purified samples hydrolysis can be achieved using other methods, such as acid (Pirt and Whelan, 1951) or ultrasound treatment (Mecozzi et al., 1999).

Total soluble carbohydrate content has traditionally been measured using the anthrone solution (Dreywood, 1946; Yemm and Willis, 1954; Edwards et al., 2011) or a phenol-sulphuric acid assay (DuBois et al., 1956; Masuko et al., 2005). Field measurements of total soluble solids in fruit with a high sugar content, such as grapes, have long been undertaken using handheld refractometers.

Individual soluble carbohydrates have been separated using many methods, including thin layer chromatography (e.g. Ghebregzabher et al., 1976), high-performance liquid chromatography (e.g. Hurst et al., 1979), gas chromatography (e.g. Davison and Young, 1969) and capillary electrophoresis (e.g. O'Shea et al., 1993), etc. Detection has been via various colourimetric/spectrophotometric methods (see above), refractive index (e.g. Raessler, 2011), mass spectrometry (e.g. Dell, 1987), etc. In addition, enzymatic based assays have allowed precise determination of individual carbohydrates from mixed samples (e.g. Giampietro et al., 1982).

Requirements for in-field measurement of tree carbohydrates

The overall objective of this project is to recommend a pathway for the development of a method to rapidly assess avocado carbohydrate status at scale in the field. The *ideal* method would assess every tree in the orchard, accurately assess whole tree carbohydrate status, be non-destructive, non-contact, and collect data with minimum user interaction either from an aerial vehicle or from a ground vehicle driving at normal speed without stopping. The *minimum viable* method would a rapid spot measure, use a single tissue sample with no further processing, be taken in a few seconds with handheld or vehicle mounted equipment and require minimal staff training. Irrespective of where a method sits within these extremes, it should provide a geolocated map of the spatial variation in the orchard in addition to summary data for each block.

From the summary above, it is clear that traditional laboratory methods are impractical in meeting these needs; at best being adaptable to a test kit that might meet the minimum viable method. However, over the last two decades many spectroscopic techniques that have previously only been possible in the laboratory are now practical, or potentially practical, in the field. Furthermore, advances in computing capacity have resulted in chemometrics, whether based on traditional statistical or newer machine learning algorithms, that previously required mainframe computing, now being able to be run on a local machine or instrument.

Most of these spectroscopic technologies can, at least theoretically, be adapted to a sensor that can be held against living avocado tissue to collect data, but an on-the-go sensor needs to work with reflected sunlight (*passive* sensor and the only realistic option for aerial observation), reflected illumination (*active* sensor) or emitted energy (may be passive or active sensor depending on source of emission activation). An on-the-go sensor is also likely to see a larger proportion of the avocado tree and is, therefore, also likely to have a more representative view of whole tree NSC. At the time of writing, the most appropriate tissue (canopy/stem/inflorescence) for assessment is also uncertain, but whether a point measure or an on-the-go measure, the chosen technology will need to be able to view or access the appropriate tissue.

Technologies to measure tissue composition *in-situ* and suitability for quantification of carbohydrates

Whilst a simple water extraction and chemical test is possible for field measurements of some compounds (e.g. there are many commercial kits for spot measurement of soil macronutrients and water quality) this approach would not be practical for large numbers of samples or extensive regular sampling. Furthermore, they would necessarily be destructive in nature.

A method that can identify and quantify NSC in intact avocado tissue is required. A range of technologies already exist that can quantify individual chemicals in a complex solid sample. Each of these exposes the sample to some form of electromagnetic energy and uses a detector to measure the absorbance, reflectance or emission of energy from the sample. The sample composition, or chemical of interest within the sample, is determined using spectroscopy; that is, the detected energy is split into its component wavebands, or frequencies, with variation in intensity between the wavebands dependant on the mix of chemicals within the sample, providing a 'fingerprint' that can be used to identify and quantify those chemicals.

Many of these techniques use infra-red (IR) radiation for the energy source, as the IR wavelengths correspond to the vibrational energies of bonds in molecules commonly of interest, including carbohydrates. Examples include near infra-red (NIR) spectroscopy, mid infra-red (MIR) spectroscopy, often implemented as attenuated total reflectance-Fourier transform infra-red (ATR-FTIR) spectroscopy, and terahertz (THz) spectroscopy. These examples all use reflectance or absorbance of electromagnetic energy by the sample, but other techniques use energy to excite electrons (x-ray fluorescence (XRF) spectroscopy), or atomic nuclei (nuclear magnetic resonance (NMR) spectroscopy), which then emit electromagnetic energy at specific wavelengths that can be detected and quantified.

With the exception of ATR-FTIR, none of these techniques has an absolute requirement for direct contact between sample and sensor and portable versions of instruments are increasingly becoming available.

XRF Spectroscopy allows the elemental composition of a sample to be determined. A beam of x-rays are directed at the sample, causing low-energy electrons to be knocked out of atoms within the sample. This, in turn, results in unstable atomic configurations and higher energy electrons dropping into the low-energy orbitals; the excess energy being emitted as a photon (fluorescence), which can then be detected. Different elements fluoresce at different wavelengths and the total energy emitted at a given wavelength is proportional to the amount of that element in the sample.

Penetration of the sample is dependent on composition, due to potential absorption of the fluoresced photons as they travel out of the sample, and the elements of interest, with the heavier the element the higher the energy of the emitted photons and the greater the penetration through to sample to the detector (Towett et al., 2016). Whilst, x-ray radiation has the potential to damage living plant tissues, visual damage was only observed in live Soybean leaves after 60 minutes of exposure (Montanha et al., 2020), so would be unlikely to cause problems in short-term field use.

Portable handheld XRF instruments suitable for field use are available from a range of manufacturers and attempts have been made to use these to assess the mineral composition of living plants under laboratory (Tsutsumimoto and Tsuji, 2007) and field conditions (Mason et al., 2015). Unfortunately these devices are only able to detect elements with the atomic number of 11 (sodium) or heavier and the elements that form carbohydrates (C, H, O) are lighter than this and thus only able to be measured using laboratory instruments (noting that H can't be measured using XRF). Even if a field portable XRF instrument capable of measuring C and O should be developed in the future, C, H and O are the three most common elements in plant tissue and it is unlikely that differences in NSC could be determined from changes in elemental composition of plant tissue alone. Consequently, there is no realistic prospect of using XRF techniques for non-destructive, in-field, assessment of NSC in avocados.

Ramen Spectroscopy is a form of vibrational spectroscopy discovered by C.V. Ramen in the 1920's (Singh, 2002) and uses monochromatic light (typically a laser) to illuminate a sample resulting in the emission of a spectrum of light (polychromatic) from the sample. Rather than being due to fluorescence from electrons changing energy states, Ramen spectroscopy is based on the scattering of light due to its interaction with the vibrational state of the chemical bonds within the sample. The illuminating light changes the vibrational state of those bonds, which in turn changes the energy (and therefore wavelength) of the illuminating light, producing a spectrum that can be measured. The various bonds in the sample and its chemical structure both affect the resulting spectrum, meaning that it can be used to identify complex chemicals in an intact sample, such as plant tissue.

Sample penetration is dependant on the wavelength of the laser used for the excitation and the sample; an IR laser will penetrate further into a plant sample than a green laser for instance. Compared with other IR based techniques, Ramen spectroscopy has an advantage when it comes to penetration of live tissue as, although water absorbs IR radiation strongly, it produces only weak Ramen scattering. With the use of techniques such as spatially offset Ramen spectroscopy, tissue penetration may reach several mm (Matousek, 2007). Ramen scattering represents an extremely small fraction of the illuminating energy (i.e. $<10^{-8}$) thus is easily affected by any fluorescence induced in the sample by the illumination or by extraneous non-monochromatic light.

Specific spectral signals can be associated with specific chemical bonds and their configuration in the wider structure of a molecule, thereby allowing positive chemical identification, and published Ramen spectra have included a range of carbohydrates (Wiercigroch et al., 2017). However, in mixed/intact

samples, chemometrics, such as multiple-regression models, are more commonly used and are able to quantify starch in intact algae (Chiu et al., 2017) or detect qualitative changes in storage carbohydrates in peanuts (Farber et al., 2020), for example.

As with XRF, a number of handheld field-portable instruments are available on the market, but many of these are designed to identify pharmaceuticals or explosives. The use of portable devices with intact plant tissue has been limited to date. Ramen spectrometers attached to microscopes have been used with live plant tissues to examine changes in leaf tissue during development (Butler et al., 2015) and effects of plant stress (Altangerel et al., 2017). The latter study also built a prototype 'remote' Ramen spectrometer, that was used at a distance of 10 cm from the leaf.

With appropriate development of hardware and analytical calibrations, Ramen spectroscopy has the potential to be used for spot measurements of carbohydrates in intact avocado tissue in the orchard. However, the development required would be significant, it is unlikely to ever be an on-the-go instrument and reliability of quantification is more difficult than related techniques due to the extremely low intensity of Ramen scattering (e.g. Baranska et al., 2006).

ATR-FTIR Spectroscopy is another form of vibrational spectroscopy and perhaps the most commonly used implementation of MIR spectroscopy. FTIR uses a polychromatic light source with an interferometer and a broadband detector. The interferometer modulates the polychromatic light, by combining a fixed and changing pathlength, producing an interferogram at the detector over the time taken for the changing pathlength to move from its minimum to maximum extent. Using a Fourier transform, the interferogram is converted to an IR spectrum. Originally, FTIR was performed with the sample between the modulated light beam and the detector, but ATR-FTIR adds an optically dense crystal with a high refractive index that creates a light path which travels through only a few µm of sample adjacent to the crystal surface, thereby generating an IR absorption spectrum of a sample simply by placing it against an instrument's optics.

ATR-FTIR instruments typically work in the MIR range (2,500-25,000 nm) and are available in handheld field-portable formats. As with Ramen spectroscopy, chemometrics is usually used to generate a calibration against the compound of interest in a specific background matrices (e.g. avocado tissue). Whilst there is no current published calibration for carbohydrates in avocado, calibrations for carbohydrates in other matrices have been generated, including fruit juice (Leopold et al., 2011), hardwood (Zhou et al., 2015) and grapevine tissue (Schmidtke et al., 2012). It is likely that developing a calibration for avocado would be relatively straightforward, although variations in water content between trees and orchards would need to be accounted for effectively.

ATR-FTIR techniques for NSC assessment in avocado orchards would necessarily be point measurements, with non-contact on-the-go measurement not possible.

Diffuse Reflectance spectroscopy is a form of absorbance spectroscopy, but rather than examining the absorption spectrum by measuring the wavebands that are transmitted through an object (e.g. a solution of carbohydrates in a spectrophotometer), the spectrum of light that is reflected back from an object surface (e.g. an avocado leaf) is measured and split into its constituent wavebands to determine which bands have been absorbed by the object. To achieve this, specular reflection (the direct reflection of the illumination source) must be excluded as this has not been significantly influenced by the object of interest.

Ultra-violet (UV) and visible (VIS) wavelengths correspond with the energies required to move electrons from one orbital to another in an atom or molecule. Consequently, absorption spectra in the UV-VIS range are limiting when the aim is to determine the composition of a complex mixture, such as biological tissue, because there are relatively few absorption bands. IR wavelengths correspond with vibrational energies of molecular bonds and provide much more detail in absorption spectra and, therefore, more potential for identification and quantification of compounds in mixed samples.

Previously, UV-VIS-NIR spectrometers generally used monochromators to scan through their waveband range and MIR spectrometers generally used interferometers (see FTIR above). However, the capabilities and specifications of photodiode array (PDA) detectors have steadily improved and offer the advantage of simultaneous recording of all wavebands at once, not possible with monochromators or interferometers.

A major advantage of reflectance spectroscopy compared with the technologies above is that the sample can be at distance from the sensor, with the spatial resolution required and the strength of illumination determining the practical distance. This provides the potential for non-destructive, non-contact spot measurements and, when a PDA-based sensor is used, for non-contact on-the-go measurements in the orchard.

Although MIR theoretically offers the most potential information for development of a calibration between diffuse reflection spectra and avocado NSC, NIR also contains information on a large number of binds, albeit dominated by bonds including hydrogen (Türker-Kaya and Huck, 2017). There have been extensive reviews comparing the use of VIS-NIR-MIR for soil composition, including carbon and carbon dominated fractions, which have confirmed the value of both NIR and MIR diffuse reflectance spectroscopy (e.g. Reeves, 2010; Soriano-Disla et al., 2014). There are fewer publications on NSC or other carbohydrate fractions in plant tissue than soil, but effective calibrations have been developed using NIR for NSC in grapevine (De Bei et al., 2017) total carbohydrates in foxtail millet (Chen et al., 2013), water soluble carbohydrates in clover (Inostroza et al., 2017) and seasonal changes in NSC in grasses (Shetty et al., 2012), albeit all using dried ground material.

THz Spectroscopy uses the range between infra-red and millimetre wave. Historically, it has been extremely difficult to generate THz waves, but in recent years more affordable hardware based on fast lasers has become available, driving development of THz spectroscopy. Terahertz waves have been used in medical and security imaging due to their ability to penetrate into solid material without having harmful biological effects (Yang et al., 2016), but as with shorter wavelengths, can also be used for spectroscopy. Their use in food applications was recently reviewed by Afsah-Hejri et al. (2019), including the detection of carbohydrates and the elucidation of carbohydrate structure. In general, these studies examined purified carbohydrates, but one study compared THz spectra from Mung bean seedlings with purified starch in order to estimate starch content of the seedlings and successfully developed a calibration model for starch in Mung bean seedlings (Nakajima et al., 2019).

At present, the hardware available is not practical for field use, even as a spot measurement and the chemometric experience with THz spectroscopy is quite limited. Furthermore, although THz waves will penetrate further into plant tissue than VIS-NIR-MIR waves, potentially improving the volume of tissue assessed and accuracy of quantification at scale, the high water content of plant tissues will still likely limit

this to 1 mm or less. Interestingly, this aspect of THz has been used to monitor plant water stress by measuring very small changes in water content (Born et al., 2014). Despite these shortcomings, THz spectroscopy may be a useful tool for assessing carbohydrates in the orchard at some point in the future.

NMR Spectroscopy uses a strong magnetic field to align the spin of charged atomic nuclei in the sample (usually protons or ^{13}C). By applying a radio wave pulse of the correct frequency the spin of some of these nuclei will be reversed and they will subsequently emit radio waves when they 'relax' back to their initial spin, which can be detected and will provide information on the composition and structure of the sample. Until recently NMR required the sample to be embedded in the magnetic field and used large expensive fixed equipment. Over the last 20 years, however, single-sided (open) benchtop NMR machines have been developed and more recently still, a small field portable device (WaveGuide Formµla™, Waveguide Corp, Cambridge, MA, USA). At the same time, the application of NMR has broadened from detailed analysis of molecular structure to composition of complex matrices (such as food or plant tissue). For example, the content of fat, water or even oxygen (reviewed in Capitani et al., 2017).

Sample penetration by NMR is dependent on the magnetic field of the equipment used, thus could be tuned to whatever volume of sample was required in the orchard. NSC quantification would be achieved by positive identification of the constituent carbohydrates and not rely on a chemometric calibration, therefore would be unaffected by other differences in tissue composition between regions or orchards. However, the open benchtop NMR machines are still unsuitable for field use, would require a destructive sample in practice and still only provide a spot measurement. The new portable device requires a liquid sample and thus would need destructive sampling and crude extraction. As with THz spectroscopy, NMR spectroscopy, may become a practical tool for orchard use in the future, but still requires significant development.

Conclusions and Recommendation

The various technologies described here each have pros and cons in regards to accuracy of quantification, ease of development for use in avocado orchards and technology readiness of available hardware. With the aim of an on-the-go method, minimising sensor cost and maximising the use of available scientific reports, NIR diffuse reflectance is the recommended approach. This has the potential to be:

- used as a passive sensor (not requiring its own light source), noting that an active sensor would be easier to develop;
- developed as a hand-held spot measurement device or as an on-the-go vehicle mounted device;
- developed as a point (large single pixel) or imaging sensor, the latter providing the potential for specific objects of interest, such as inflorescences, to be identified and assessed;
- built with a lower cost and more accessible detector (e.g. PDAs) than other diffuse reflectance wavebands, whilst still accessing bond vibrational states (thereby having a greater likelihood of accurate chemical identification).

References

Afsah-Hejri L, Hajeb P, Ara P, Ehsani RJ (2019) A Comprehensive Review on Food Applications of Terahertz Spectroscopy and Imaging. Compr Rev Food Sci Food Saf 18: 1563–1621.

Altangerel N, Ariunbold GO, Gorman C, Alkahtani MH, Borrego EJ, Bohlmeyer D, Hemmer P, Kolomiets M V., Yuan JS, Scully MO (2017) In vivo diagnostics of early abiotic plant stress response via Raman spectroscopy. Proc Natl Acad Sci 114: 3393–3396.

Baranska M, Schütze W, Schulz H (2006) Determination of Lycopene and β-Carotene Content in Tomato Fruits and Related Products: Comparison of FT-Raman, ATR-IR, and NIR Spectroscopy. Anal Chem 78: 8456–8461.

De Bei R, Fuentes S, Sullivan W, Edwards EJ, Tyerman S, Cozzolino D (2017) Rapid measurement of total non-structural carbohydrate concentration in grapevine trunk and leaf tissues using near infrared spectroscopy. Comput Electron Agric 136: 176–183.

Born N, Behringer D, Liepelt S, Beyer S, Schwerdtfeger M, Ziegenhagen B, Koch M (2014) Monitoring plant drought stress response using terahertz time-domain spectroscopy. Plant Physiol 164: 1571–1577.

Butler HJ, McAinsh MR, Adams S, Martin FL (2015) Application of vibrational spectroscopy techniques to non-destructively monitor plant health and development. Anal Methods 7: 4059–4070.

Capitani D, Sobolev AP, Di Tullio V, Mannina L, Proietti N (2017) Portable NMR in food analysis. Chem Biol Technol Agric 4: 1–14.

Chen J, Ren X, Zhang Q, Diao X, Shen Q (2013) Determination of protein, total carbohydrates and crude fat contents of foxtail millet using effective wavelengths in NIR spectroscopy. J Cereal Sci 58: 241–247.

Chiu L Da, Ho SH, Shimada R, Ren NQ, Ozawa T (2017) Rapid in vivo lipid/carbohydrate quantification of single microalgal cell by Raman spectral imaging to reveal salinity-induced starch-to-lipid shift. Biotechnol Biofuels 10: 1–9.

Davison PK, Young R (1969) Gas chromatography of carbohydrates the quantitative determination of the free sugars of plants as their trimethylsilyl ethers. J Chromatogr A 41: 12–21.

Dell A (1987) F.A.B.-Mass Spectrometry of Carbohydrates. *In* RS Tipson, D Horton, eds,Academic Press, pp 19–72.

Dreywood R (1946) Qualitative Test for Carbohydrate Material. Ind Eng Chem Anal Ed 18: 499.

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric Method for Determination of Sugars and Related Substances. Anal Chem 28: 350–356.

Edwards EJ, Downie AF, Clingeleffer PR (2011) A Simple Microplate Assay to Quantify Nonstructural Carbohydrates of Grapevine Tissues. Am J Enol Vitic 62: 133–137.

Farber C, Sanchez L, Rizevsky S, Ermolenkov A, McCutchen B, Cason J, Simpson C, Burow M, Kurouski D (2020) Raman Spectroscopy Enables Non-Invasive Identification of Peanut Genotypes and Value-Added Traits. Sci Rep 10: 1–10.

Ghebregzabher M, Rufini S, Monaldi B, Lato M (1976) Thin-layer chromatography of carbohydrates. J Chromatogr A 127: 133–162.

Giampietro O, Pilo A, Buzzigoli G, Boni C, Navalesi R (1982) Four methods for glucose assay compared for various glucose concentrations and under different clinical conditions. Clin Chem 28: 2405–2407.

HURST WJ, MARTIN RA, ZOUMAS BL (1979) APPLICATION OF HPLC TO CHARACTERIZATION OF INDIVIDUAL CARBOHYDRATES IN FOODS. J Food Sci 44: 892–895.

Inostroza L, Lobos I, Acuña H, Vásquez C, Tapia G, Monzón G (2017) Nir-prediction of water-soluble carbohydrate in white clover and its genetic relationship with cold tolerance. Chil J Agric Res 77: 218–225.

Karkalas J (1985) An improved enzymic method for the determination of native and modified starch. J Sci Food Agric 36: 1019–1027.

Leopold LF, Leopold N, Diehl HA, Socaciu C (2011) Quantification of carbohydrates in fruit juices using FTIR spectroscopy and multivariate analysis. Spectroscopy 26: 93–104.

Mason S, McNeill A, Mclaren T, Doolette A (2015) UA0313: XRF as a tool for measuring crop nutrient contents in the laboratory and the field FAST. Adelaide.

Masuko T, Minami A, Iwasaki N, Majima T, Nishimura S-I, Lee YC (2005) Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Anal Biochem 339: 69–72.

Matousek P (2007) Deep non-invasive Raman spectroscopy of living tissue and powders. Chem Soc Rev 36: 1292.

McCleary BV, Solah V, Gibson TS (1994) Quantitative Measurement of Total Starch in Cereal Flours and Products. J Cereal Sci 20: 51–58.

Mecozzi M, Amici M, Pietrantonio E, Acquistucci R (1999) Ultrasound-assisted analysis of total carbohydrates in environmental and food samples. Ultrason Sonochem 6: 133–139.

Montanha GS, Rodrigues ES, Marques JPR, de Almeida E, dos Reis AR, Pereira de Carvalho HW (2020) X-ray fluorescence spectroscopy (XRF) applied to plant science: challenges towards in vivo analysis of plants. Metallomics 12: 183–192.

Nakajima S, Shiraga K, Suzuki T, Kondo N, Ogawa Y (2019) Quantification of starch content in germinating mung bean seedlings by terahertz spectroscopy. Food Chem 294: 203–208.

O'Shea TJ, Lunte SM, LaCourse WR (1993) Detection of carbohydrates by capillary electrophoresis with pulsed amperometric detection. Anal Chem 65: 948–951.

Pirt SJ, Whelan WJ (1951) The determination of starch by acid hydrolysis. J Sci Food Agric 2: 224–228.

Raessler M (2011) Sample preparation and current applications of liquid chromatography for the determination of non-structural carbohydrates in plants. TrAC Trends Anal Chem 30: 1833–1843.

Reeves JB (2010) Near- versus mid-infrared diffuse reflectance spectroscopy for soil analysis emphasizing carbon and laboratory versus on-site analysis: Where are we and what needs to be done? Geoderma 158: 3–14.

Schmidtke LM, Smith JP, Müller MC, Holzapfel BP (2012) Rapid monitoring of grapevine reserves using ATR-FT-IR and chemometrics. Anal Chim Acta 732: 16–25.

Shetty N, Gislum R, Jensen AMD, Boelt B (2012) Development of NIR calibration models to assess year-to-year variation in total non-structural carbohydrates in grasses using PLSR. Chemom Intell Lab Syst 111: 34–38.

Singh R (2002) C. V. Raman and the Discovery of the Raman Effect. Phys Perspect 4: 399–420.

Soriano-Disla JM, Janik LJ, Viscarra Rossel RA, MacDonald LM, McLaughlin MJ (2014) The performance of visible, near-, and mid-infrared reflectance spectroscopy for prediction of soil physical, chemical, and biological properties. Appl Spectrosc Rev 49: 139–186.

Towett EK, Shepherd KD, Lee Drake B (2016) Plant elemental composition and portable X-ray fluorescence (pXRF) spectroscopy: quantification under different analytical parameters. X-Ray Spectrom 45: 117–124.

Tsutsumimoto K, Tsuji K (2007) Time-resolved x-ray fluorescence for monitoring the intake of mineral nutrients in living plants. X-Ray Spectrom 36: 324–327.

Türker-Kaya S, Huck CW (2017) A review of mid-infrared and near-infrared imaging: Principles, concepts and applications in plant tissue analysis. Molecules. doi: 10.3390/molecules22010168.

Wiercigroch E, Szafraniec E, Czamara K, Pacia MZ, Majzner K, Kochan K, Kaczor A, Baranska M, Malek K (2017) Raman and infrared spectroscopy of carbohydrates: A review. Spectrochim Acta Part A Mol Biomol Spectrosc 185: 317–335.

Yang X, Zhao X, Yang K, Liu Y, Liu Y, Fu W, Luo Y (2016) Biomedical Applications of Terahertz Spectroscopy and Imaging. Trends Biotechnol 34: 810–824.

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57: 508–514.

Zhou C, Jiang W, Via BK, Fasina O, Han G (2015) Prediction of mixed hardwood lignin and carbohydrate content using ATR-FTIR and FT-NIR. Carbohydr Polym 121: 336–341

Chapter 3: Photothermal quotient (PTQ) as a surrogate for carbohydrate status in avocado

Introduction to thermal time and photothermal quotient (PTQ)

The concept of thermal time was developed by Nix (1975), and expresses the sum of 'effective' growth temperature over time to explain the rate of plant growth and development. The effective growth temperature on a daily time step is usually calculated as the mean daily temperature (sum of daily maximum temperature and the daily minimum temperature divided by two) and then subtract the base temperature (Equation 1)

Effective growth temp (°C) = ((Daily max temp + Daily min temp)/2)-base temp Eq1

The 'base temperature' or 'threshold temperature' is particular to a species and is the temperature below which growth stops. Lahav and Trochoulias (1982) and Wang et al (2018) reported that the base temperature for Avocado tree growth was 12°C while Wolstenholme (1983) estimated the base temperature growth and development was 10°C.

There is a cold requirement for floral induction which requires mean daily temperature to fall to below 20°C (Lahav and Trochoulias, 1982; Olesen, 2005). In warmer environments, floral initiation is delayed. However, once initiated the rate of growth and development of flowers and fruits will increase in orchards with a warmer climate compared to cooler southern, or higher altitude, orchards.

Expressed as a sum over a period of time this 'thermal time' can be used to measure the heat units required for different stages of development;

Growing Degree Days = $\sum_{1\text{-}n}$ Effective growth temperature Eq2

where the effective growth temperature is summed over days 1 to 'n' for a particular period of crop development.

For example, in macadamia, Moncur et al (1985) found differences, across a number of locations varying in growth temperatures, in the number of days taken to get from floral initiation to the start of raceme elongation and from the start of raceme elongation to anthesis. By expressing the Growing Degree Days for these developmental periods at each site they were able to develop a model that predicted final raceme length across all locations.

Nix (1976) further explored plant growth and development using Photothermal Quotient (PTQ), defined as the ratio of solar radiation to the mean daily temperature minus the crop base temperature.

$$PTQ = \frac{Daily\ Radiation}{\left(Max°C + Min°C\right)/2 - Base°C}$$

Eq3

Thus, when averaged over time PTQ can summarize a large volume of weather data into a single measure. PTQ can be calculated for different growth periods during the season and is a surrogate for the assimilate supply available to the crop per unit of development. So a higher PTQ means that conditions are favourable for growth because there is a high assimilate supply available.

Use of PTQ in other crops

Fischer (1985) and Rawson (1988) demonstrated the strong correlation between kernel number and yield in wheat with PTQ. Faraji (2012a) demonstrated that flower formation and pod/flower ratio was

positively related to PTQ in canola *(Brassica napus*) and Faraji (2012b) demonstrated that here was a positive logarithmic relationship between PTQ during seed filling and the oil content of the seed. PTQ has also been applied to other broadacre, annual crops to explain environmental variation in yield and yield components. While PTQ has been used widely in the agronomy literature, and turf industry (Danneberger and Cushnahan, 2014) there are few articles where it has been applied to horticultural tree crops possibly due to the interaction between seasons and the complication of stored carbohydrates being carried across seasons. Given the success in applying PTQ in other species, it would appear that a comprehensive analysis of avocado yields across seasons and locations searching for associations with temperature, radiation and PTQ is warranted to explore season to season variation in yield. A simple model to predict avocado yields across regions, with some level of certainty, ahead of the harvest period would be of great value to the industry.

Variation in PTQ may also affect the rate of development of fruits within a tree or an orchard row. Intuitively one could speculate that fruits developing in sunshine, close to leaves receiving high radiation could be expected to develop faster and mature sooner that fruits held in cooler, more shady locations in the canopy. Is it possible that position in the canopy can at least partially explain the variation in time to fruit maturity. There has been little work on the variation in time to fruit maturity within trees. There have been some observations (C. McConchie pers. Comm.) that macadamia nuts held at the top of the tree and those on the northern side of the tree fall first, followed by nuts on the west and east fall next while shaded lower branches and southern side of tree (southern hemisphere) will drop last.

Avocado physiology and PTQ

There is data in tree species like macadamia (McFadyen et al 2012) and avocado (Alcaraz et al 2013) to indicate that factors which limit current assimilate supply to the developing fruit result in abscission of fruit prematurely and loss of yield potential. Since Photothermal Quotient (PTQ) (Nix, 1976) is defined as the ratio of daily solar radiation to the daily Growing Degree days, where GDD is defined as the mean daily temperature ((Max + Min)/2) and subtract the base temperature for the crop (Nix, 1975), PTQ can be used as a surrogate for the assimilate supply available to the crop per unit of development. PTQ can be calculated for different periods during the season to examine the sensitivity of yield to conditions during a particular period. A higher PTQ means that conditions are expected to be favourable for growth and yield formation because there should be a high assimilate supply available to the crop. Therefore, if the leaves of a crop are able to make best use of the radiation it receives then it should yield more under cooler temperatures than under higher temperatures.

When using PTQ we assume that crops are well managed, are not suffering from insect attack, are well watered and yield close to their potential. In field crops the use of PTQ has opened opportunities for researchers to explore critical growth periods for yield formation, physiological factors limiting yield across regions and it has allowed farm consultants and growers to develop management practices to optimise yield according to seasonal conditions. Once calibrated to a particular crop, the data can also be used to identify years in which production is below expectations and explore reasons for poor performance (Rawson 1988). Perennial species are likely to be more complex to deal with due to the influence of previous season on current year's growth and yield.

This component of project AV19006 will use PTQ to investigate seasonal and regional variation of yield in avocado. It draws on commercial yield data held by growers, weather data downloaded from the Silo database hosted by the Queensland Government, https://www.longpaddock.qld.gov.au/silo/ and expertise in crop physiology at QAAFI to create knowledge that can be applied to identify management and breeding opportunities to improve the productivity and profitability of Australian avocado orchards. SILO is a database of Australian climate data from 1889 to the present. It provides daily meteorological

data sets for a range of climate variables in ready-to-use formats suitable for biophysical modelling, research and climate applications.

Access to several growers yield data from each region to get a spread of yield data across a range of locations varying in radiation and temperature will maximise the range in conditions. Irrigated orchards and the best orchard managers are targeted to try and access yield data that is close to the regional potential. We need a number of years of yield data from each location to be able to look for relationships between yield and PTQ. With a broad range in data we can examine possible relationships within orchards including testing for alternate bearing by using the previous year's yield as a covariate in the analysis. In addition to site specific data we can pool regional data and also test for differences in yield potential between regions.

Climatic differences between avocado growing regions

Figures 1-3 show the long term monthly means of weather factors for three of the locations to represent a range of avocado growing regions. Maximum temperatures were highest at Emerald and lowest at Alstonville. Minimum temperatures were highest at Bundaberg and lowest at Alstonville, falling to almost 8 °C in July. The greatest range between maximum and minimum temperatures was at Emerald while Bundaberg had the least range. Radiation did not vary much between sites with the main differences occurring in winter and spring with sites at lower latitudes receiving less radiation. PTQ was remarkably similar across all sites in summer and autumn. In late winter and spring PTQ was highest at lower latitudes. Rainfall and VPD showed the greatest range across sites. Rainfall was lowest and VPD the highest at Emerald with the mean monthly VPDmax was almost 34 hPa in December. The lowest levels of VPDmax were at Alstonville.

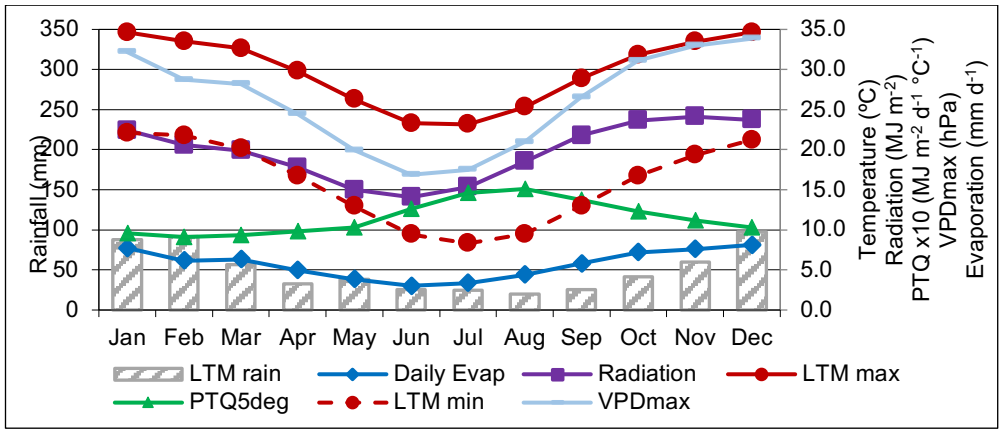


Figure 1: Long-term mean monthly weather data for Emerald

The long term mean weather data highlights the favourable PTQ in the months leading up to flowering which should facilitate floral bud differentiation and storage of non-structural carbohydrates that could be mobilised for flowering, fruit set and development. The other striking feature of the long term mean data is that, relative to spring, PTQ is low during late summer and into autumn which coincides with the period of greatest demand of assimilate for fruit growth. Seasonal variation in PTQ therefore favours a large flush of flowers and vegetative growth followed by episodes of premature fruit abscission. The low PTQ during fruit growth reinforces the importance of stored reserves for fruit development and the vulnerability of young fruits to periods of low assimilate supply due to events such as low radiation (cloud), heat and VPD shock, and water deficit; phenomena which all result in periods of low assimilate supply. Interestingly monthly mean maximum temperatures and VPD varied the most between the avocado growing regions while incident radiation and PTQ varied the least.

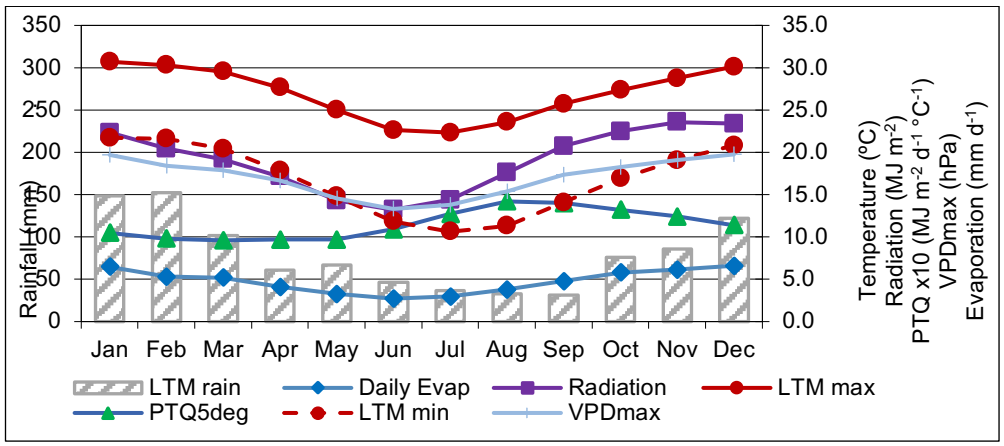


Figure 2: Long-term mean monthly weather data for Bundaberg

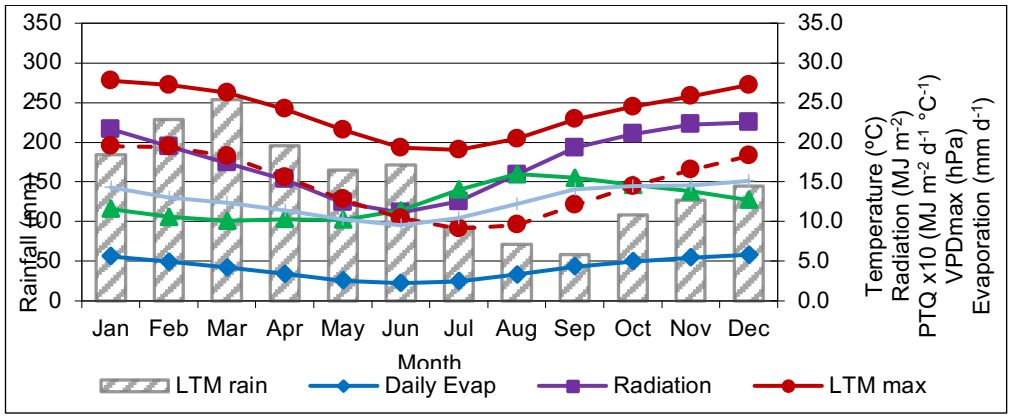


Figure 3: Long-term mean monthly weather data for Alstonville Research Station.

Model for the regulation of retention/abscission of plant organs

Ever since tree crops have been cultivated in orchards, those who have tended the trees have sought to better understand fruit ripening and fruit drop and the factors which accelerate or slow its timing. Orchardists have sought to manage abscission to allow them to better plan and schedule harvest periods and to avoid or accommodate weather events that may impact severely on yield and quality. Historically the loss of commercial yield as a result of pre-harvest drop was a serious issue in apple (*Pyrus malus*), pear (*Pyrus commums*), peach (*Prunus persica*), orange (*Citrus sinensis*) and grapefruit (*Citrus paradise*) orchards (Cooper et al., 1968).

In the past 20 years there has been a rapid expansion in our understanding of the process of abscission. It has become clear that shedding of plant organs such as leaves and fruit is a highly co-ordinated event involving multiple changes in cell structure, metabolism and gene expression (Taylor and Whitelaw, 2001). This means that if we were able to gain a better understanding of the process, we could commercially retain, promote or delay fruit and nut abscission by targeting action at a specific point in the process. We have learnt how to control abscission in some species but our knowledge of the sequence of events and signalling involved is limited. Because our knowledge of the process is incomplete we are unable to explain why the same chemical is effective in one tree crop but not as effective in other species or even cultivars of the same species.

Abscission usually occurs in highly predictable positions at morphological sites that are distinct (Taylor and Whitelaw 2001). Abscission is described as the culmination of altered gene expression, leading to changes in the balance of plant hormones. These hormonal changes lead to the loosening of adjacent cell walls within the abscission zone and subsequent cell separation. This final step in the loss of adhesion between cell layers in the abscission zone is brought about by the induction of cell wall-degrading enzymes that lead to cell-wall separation. The cells that comprise the abscission zone have been termed the 'abscission zone target cells' (AZTC). These cells appear to lay dormant or are not initiated while Auxin levels are high. A reduction in auxin levels are followed by a rise in ethylene and AZTC respond by enlarging, maturing and separating Roberts et al (2002) (Fig 4). Osborne and Sargent (1976) were able to demonstrate that until these cells were present, abscission could not be induced even by prolonged exposure to high concentrations of applied ethylene. The identification of transcription factors that regulate the differentiation of the abscission zone target cells and the process of cell separation is promising for horticultural industries seeking to retain fruit or to better manage the timing of abscission (Taylor and Whitelaw 2001).

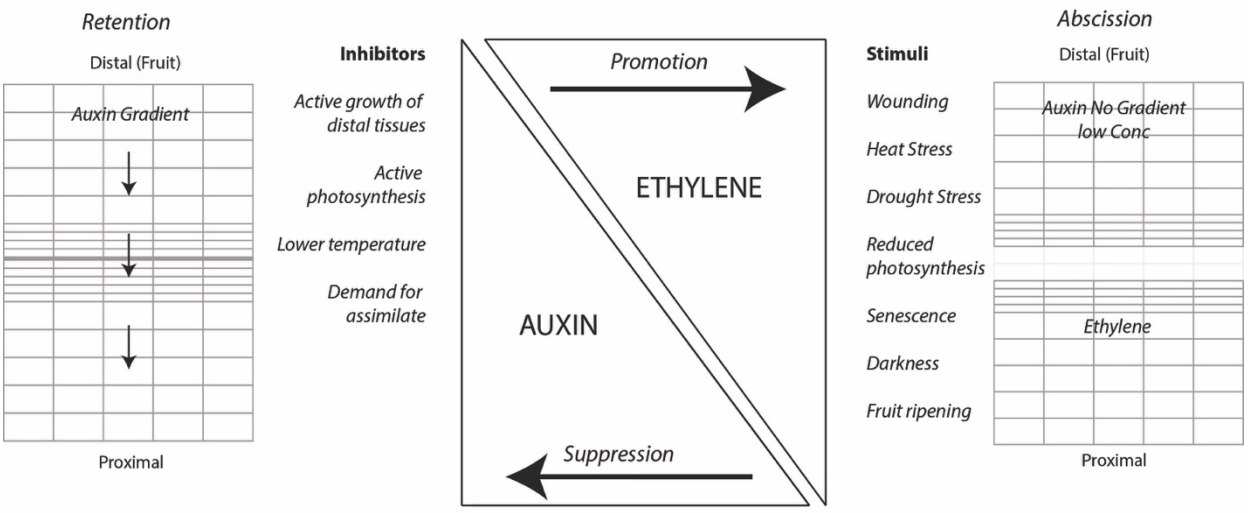


Figure 4: Schematic representation of the physiology of retention/abscission. When the fruit is metabolically active the auxin levels/gradient results in the AZTC being dormant and non-responsive to ethylene. It is proposed that the lack of carbon supply due to stress or maturation of the fruit, leads to a fall in auxin levels making the AZTC responsive to ethylene and triggering the cascade of gene expression leading separation of the abscission zone and fruit drop. (modified from Gonzalez-Carranza et al 1998).

A decline in assimilate supply to fruitlets is likely to reduce synthesis and transport of the abscission-inhibiting hormones auxin. The decline in auxin is followed by an elevation in ethylene and ABA, the abscission accelerating hormones that are also associated with growth retardation and senescence (Taylor and Whitelaw 2001). Evidence suggests that provided the flux of auxin to the abscission zone is maintained then the cascade of events leading to cell separation is inhibited (Addicott 1968, Fig 4). Metabolically active (growing) organs such as leaves and fruits produce auxin (Gonzalez-Caranza 2004) and a gradient of auxin from the subtended organ towards the plant axis maintains the abscission zone in a non-sensitive state (Addicott 1982). Reduction or reversal of the auxin gradient causes the abscission zone to become sensitive to ethylene. It seems reasonable to assume that as a fruit or nut nears maturity and metabolic activity slows that the production of auxin would slow. It is hypothesised that, as for leaves, deceasing auxin levels being produced by fruit thereby provide the stimulus for initiation of the abscission cascade. Taylor and Whitelaw (2001) concluded from physiological evidence that the balance between auxin and ethylene is crucial to the initiation of the abscission process and provided evidence of a molecular basis for auxin-ethylene interaction.

Conclusions

A greater knowledge of the photosynthesis, growth and water use of a crop in relation to radiation and temperature enables different segments in the supply chain from growers to processers to make predictions about yield, plan for harvest, schedule business operations, better manage their inputs and provides management and breeding targets for researchers.

The long-term mean weather data highlighted the favourable PTQ in the months leading up to flowering which should facilitate growth of inflorescences and storage of non-structural carbohydrates that could be mobilised during the fruit filling period to augment current assimilate. The other striking feature of the long-term mean data is that, relative to spring, PTQ is low during late summer and into autumn which coincides with the period of greatest demand of assimilate for fruit growth. Seasonal variation in PTQ therefore favours a large flush of flowers followed by episodes of premature fruit abscission. The low PTQ during fruit growth reinforces the importance of stored reserves for fruit development and the vulnerability of young fruits to periods of low assimilate supply.

Work by Wolstenholme (1987) and Stephenson and Chapman (2012) demonstrated that well managed avocado yields are similar to the tree-crop benchmarks of citrus and apple while another rainforest species macadamia, is commonly only yielding about 30% of that level, when expressed on an energy-equivalent basis. The strong inference from these analyses is that well managed avocado is capable of taking full advantage of the radiation it receives while macadamia cannot. Indeed, plots of diurnal wateruse of trees on full-sun days show truncated water use between 10am and 3pm (Daniel Manson, Pers comm.). Recent published work by South African researchers indicate that this truncation in wateruse is the result of stomatal closure due to VPD above 20-25hPa, to restrictions in water flow within the tree or possibly both. (Smit et al, 2020). It is clear that the stomatal closure at high VPD is limiting gas exchange thereby reducing photosynthesis in macadamia trees and limiting yield. While avocado does not appear to be as sensitive to higher VPD levels, it is possible that premature fruit drop could be associated with extreme VPD events.

During the course of this literature review it became apparent that while the industry has annual crop calendars to assist growers with orchard management decisions, the industry does not have a phenology model for avocado. While we think we have reasonable qualitative understanding of the drivers of seasonal development, the industry does not have a model for predicting/explaining developmental stage. By using existing phenology observations from across regions and coordinating observations of phenology across locations the investment required to develop a model could be minimized. Observations of avocado growth stages across several seasons from sites spread across the regions should allow an avocado seasonal development model (ASDM) to be calibrated and validated. Such a model would assist researchers and help growers schedule orchard operations by predicting growth stage based on weather conditions to date.

The concept of PTQ appears to have been largely ignored by horticultural tree crop physiologists. Nix (1976) described it as the simplest possible measure of the balance between crop growth and developmental rhythms. It is a gross measure of the light energy available for photosynthesis per unit of developmental time. While PTQ has been used widely in the agronomy literature, there are few articles where it has been applied to horticultural tree crops possibly due to the interaction between seasons and the complication of stored carbohydrates being carried across seasons. Given the success in applying PTQ in other species, it would appear that a comprehensive analysis of avocado yields across seasons and locations searching for associations with temperature, radiation and PTQ is warranted to explore season to season variation in yield.

A greater understanding of the factors which result in flower and fruitlet abscission is likely to assist the development of orchard-based strategies to increase profitability of orchards by retaining more fruit through to harvest.

References

Addicott, F.T. (1982). Abscission. Berkeley, CA, USA: California University Press.

Addicott, F.T. (1968). Environmental factors in physiology of abscission. (1968). Plant Physiol. 43: 1471-1479.

Alcaraz, M.L., Hormaza, J.I., Rodrigo, J., (2010). Ovary starch reserves and pistil development in avocado (Persea americana). Physiol. Plant 140, 395–404.

Avovados Australia website https://avocado.org.au

Buttrose, MS, and Alexander, DM. (1978). Promotion of floral initiation in 'Fuerte' avocado by low temperature and short daylength. Scientia Horticulturae 8, 213–219.

Cooper, W.C., Rasmussen, G.K., Rogers, B.J., Reece, P.C., Henry, W.H. (1968) Control of abscission in agricultural crops and its physiological basis. Plant Physiol 43: 1560-1576

Danneberger, T.K. and Cushnahan, M. (2014). Growing Degree Days. New Zealand Sports Turf Journal. 12-13.

Faraji, A. (2012a) Flower formation and pod/flower ratio in canola (Brassica napus L.) affected by assimilates supply around flowering. Int. J. Plant Prod. 4, 271–280 (2010).

Faraji, A. (2012b) Oil concentration in canola (Brassica napus L.) as a function of environmental conditions during seed filling period. Int J Plant Prod 6:267–278

Fischer, R.A. (1985). Number of kernels in wheat crops and the influence of solar radiation and temperature. Journal of Agricultural Science 105: 447–461.

Gardner F.E., Marth P.C. and Batjer L.P. (1939). Spraying with plant growth substances to prevent apple fruit dropping. Science 90: 208–209.

Gonzalez-Carranza, Z.H., Lozoya-Gloria, E., Roberts J.A. (1998).Recent developments in abscission: shedding light on the shedding process. Trends in Plant Science. 3: 10–14.

Lahav, E. and Trochoulias, T. (1982). The effect of temperature on growth and dry matter production of avocado plant Aust. J. Agric. Res., 33 (1982), pp. 549-558.

McFadyen, L., Robertson, D., Sedgley, M., Kristiansen, P. and Olesen, T. (2012). Time of pruning affects fruit abscission, stem carbohydrates and yield of macadamia. Functional Plant Biology. 39: 481–492.

Moncur, M.W., Stephenson, R.A. and Trochoulias, T. (1985). Floral development of Macadamia integrifolia Maiden and Betche under Australian conditions. Scientia Horticulturae. 27:87–96.

Nix, H.A. (1975). Australian climate and its effects on grain yield and quality. In: A. Lazenby and E.M. Matheson (Editors), Australian Field Crops, Vol. I: Wheat and Other Temperate Cereals. Angus and Robertson, Sydney, pp. 183-226.

Nix, H.A., (1976). Climate and crop productivity in Australia. In: Climate and Rice. International Rice Research Institute, Los Bafios, Philippines, pp. 495--508.

Olesen T. (2005). The timing of flush development affects the flowering of avocado (Persea americana) and macadamia (Macadamia integrifolia3tetraphylla). Australian Journal of Agricultural Research 56, 723–729.

Osborne DJ, Sargent JA. (1976). The positional differentiation of ethylene responsive cells in rachis abscission zones in leaves of Sambucus nigra and their growth and ultrastructural changes at senescence and separation. Planta 130: 203–210.

Rawson, H.M. (1988). Effects of high temperatures on the development and yield of wheat and practices to reduce deleterious effects. pp. 44-61. In: A.R. Klatt (ed.). Wheat production constraints in tropical environments. (CIMMYT: Mexico).

Roberts, J. A., Elliot, K. A. & Gonzalez-Carranza, Z. H. (2002). Abscission, dehiscence, and other cell separation processes. Ann. Rev. Plant Biol. 53, 131–158.

Taylor J.E., Whitelaw C.A. (2001). Signals in abscission. New Phytologist. 151: 323–39.

Wang, M, White, N., Grimm, V., Hofman, H., Doley, D., Thorp, G., Cribb, B., Wherritt, E., Han, L., Wilkie, J. and Hanan, J. (2018). Pattern-oriented modelling as a novel way to verify and validate functional–structural plant models: a demonstration with the annual growth module of avocado. Annals of Botany 121: 941–959.

Wolstenholme, B.N. (1987). Theoretical and applied aspects of avocado yield as affected by energy budgets and carbon partitioning. Proceedings of the World Avocado Congress, Pretoria, S. Afr. Avocado Growers, Assoc. Yearb., 10: 58-61.

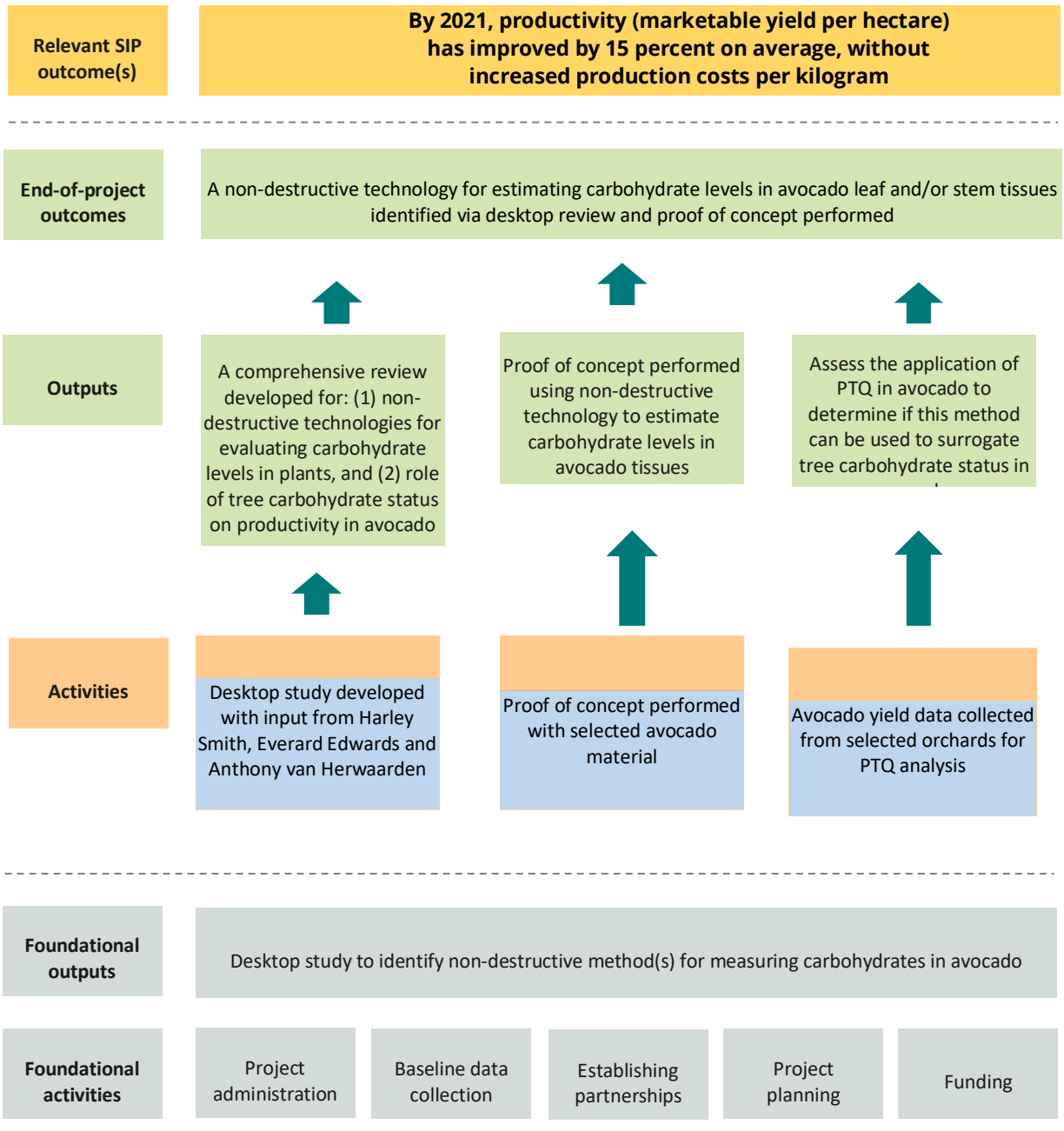
Wolstenholme, B.N. (2013). Ecology: climate and soils. In: Schaffer B, Wolstenholme BN, Whiley AW, eds. The avocado: botany, production and uses, 2nd edn. Wallingford, UK: CAB International, 86–117.

Appendix C: Project Management

Program logic

AV19006: Carbohydrate monitoring to predict yield and understanding fruit set

Figure 1: Logic model for AV16005



2 Project M&E scope

2.1 Audience

Table 1: M&E audience and their information needs

Audience	Information need
Primary	
Project team Harley Smith (Project Leader) Everard Edwards (Team Leader) Amnon Haberman (PDF) Ryan Lagerstrom (Statistics) Anthony van Herwaarden (PTQ)	Literature review on non-destructive methods and photothermal quotient (PTQ) for estimating carbohydrates in plants. Proof of concept of performed: Data collection from non-destructive technology and carbohydrate analysis Computing resources for statistics and modelling for calibration of data Yield data from select avocado orchards for PTQ analysis
Hort Innovation	Desktop study, proof of concept and PTQ analysis completed

2.2 Key evaluation questions

Table 2: Project key evaluation questions

Key evaluation questions	Relevant?	Project-specific questions
Effectiveness		
1. To what extent has the project achieved its expected outcomes?	yes	Has the project completed the desktop study on carbohydrate monitoring in avocado? Has the project identified a non-destructive method(s) to measure carbohydrates in avocado? Has the project performed a proof of concept to assess the non-destructive method(s) in avocado? Has the project assessed the application of PTQ to surrogate tree carbohydrate status in avocado?
Relevance		
2. How relevant was the project to the needs of intended beneficiaries?	yes	Has the project delivered a comprehensive review on non-destructive technologies and PTQ in plants? Has the project identified a non-destructive method(s) and PTQ, including a proof of concept, that could be further developed for predicting yield in avocado?
Process appropriateness		
3. How well have intended beneficiaries been engaged in the project?	yes	To what extent were the target engagement levels of industry levy payers achieved? Have regular project updates been provided through linkage with the industry communication project?
4. To what extent were engagement processes appropriate to the target audience/s of the project?	yes	Did the project engage with industry levy payers through their preferred learning style? How accessible were extension events to industry levy payers?
Efficiency		
5. What efforts did the project	no	

Key evaluation questions	Relevant?	Project-specific questions
make to improve efficiency?		
Other (if any)		

2.3 M&E budget

The project provides resources to cover researchers time to: (1) prepare desktop review, (2) perform a proof-of concept and (3) complete PTQ analysis. Harley Smith, Everard Edwards and Anthony van Herwaarden will write the desktop review. For the proof of concept, Amnon Haberman will collect and scan avocado samples with non-destructive technology, as well as measure carbohydrates in these tissues. Ryan Lagerstrom will perform the statistics and modelling to calibrate data for the proof of concept, as well as manage image data. Anthony van Herwaarden will perform the PTQ analysis. The project also provides resources to the project leader to prepare, review and submit reports. Lastly, project resources also cover laboratory costs associated with measuring carbohydrates and travel to orchards to collect avocado tissues. The key personnel involved in the project are Harley Smith (project leader), Everard Edwards (CSIRO: Team Leader), Amnon Haberman (CSIRO: Post-doctoral fellow), Ryan Lagerstrom (CSIRO: Research projects officer), Anthony van Herwaarden (University of Queensland: Scientist) and Shane Wynn (CSIRO: finance).

3 Performance expectations, data collection and analysis

Table 3: Project monitoring plan

Logic level	What to monitor	Performance expectation (KPIs) and/or monitoring questions	Data collection – method (e.g. survey) and source (e.g. growers)	Timing of, and responsibility for, data collection
Foundational activities	Provision and analysis of data	Monthly provision of data	Laboratory and computational record keeping	Monthly by project team members
Activities and outputs	Literature Review Proof of concept evaluation Carbohydrate analyses Statistics and modelling Publication in Talking Avocados	July to Oct Sept to May Sept to Feb Jan to May May	N.A. Record keeping Data collection Data collection & analysis	Project Leader Team members Project Leader

End-of-project outcomes	Non-destructive technology for evaluation of carbohydrate levels in avocados identified	Preliminary assessment performed	Assessment provided to avocado industry	End of project (Project Leader)

4 Evaluation

Table 4: Additional evaluation data requirements

KEQ	Data collection requirement	Source and method
1. To what extent has the project achieved its expected outcomes?	'NA'	Desktop study identified non-destructive technology for carbohydrate determination in avocado
2. How relevant was the project to the needs of intended beneficiaries?	'NA'	Grower interest in estimating tree carbohydrate status for yield assessment
3. How well have intended beneficiaries been engaged in the project?	'NA'	Project reference group meeting provided key industry members input into the project
4. To what extent were engagement processes appropriate to the target audience/s of the project?	'NA'	As above

Table 5: Independent evaluation studies (as required by Hort Innovation)

Type of evaluation	When (start and finish)
Mid-term evaluation	*Insert timing*
Final evaluation	*Insert timing*

5 Reporting and continuous improvement

Table 6: Project progress reporting

Report type	To whom	Timing
Milestone Reports	Hort Innovation	Six-month
Final Report	Hort Innovation	At end of project, May 2021
Articles	Industry magazine	At end of project, May 2021
Written and verbal update	Project Reference Group	Six-month

Table 7: Project continuous improvement activities

Continuous improvement process	Details	Timing
Reflection meeting with Hort Innovation R&D Manager	Meeting between R&D Manager and Research Team	At end of project
Team meetings	Meetings between project team members to discuss project trials and timelines	Monthly
Project Reference Group (PRG) meeting	A PRG meeting between project team members, Hort Innovation and industry representatives to gain feedback on project activities and refine methodology	Six-month