Final Report

Transforming subtropical/tropical tree crop productivity

Project leader:

Dr John Wilkie

Delivery partner:

Department of Agriculture and Fisheries, Queensland Alliance of Agriculture and Food Innovation, The University of Queensland, NSW Department of Primary Industries.

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Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Content

Summary

Orchard intensification of temperate tree crops such as apple, has led to significant increases in productivity. In this research program, we aimed to adapt the principles and management systems that have made these intensive systems so productive to the less developed, extensive orchard systems of subtropical and tropical tree crops. For the work we chose the commercially important avocado, macadamia and mango, because of their high potential for improved productivity.

The key components of modern apple orchard systems that allow them to be so productive are orchard light relations, vigour management, crop load, and tree and shoot architecture. Our approach has been to improve understanding of these key components through research, which we used to develop alternative management options and radically redesign orchard production systems. This research used applied field experiments, molecular genetics and plant modelling. A major focus of the program has been large scale Planting Systems Trials for each crop, where we investigated how the key components interacted with each other at different tree planting densities, scion varieties, rootstocks, and tree training systems.

In addition to applied field experiments, more fundamental research has also been undertaken to improve understanding of the orchard systems. We used Functional-Structural Plant Modelling to develop virtual branches, trees and orchards to model the distribution of light and movement of carbohydrates in different orchard designs. Through molecular genetics we developed genetic databases for genes expressed in the three crops and used them to identify candidate genes for key physiological processes and to map their expression over time. We investigated how flowering can limit crop load and through targeted molecular physiology experiments, we learnt how floral initiation is regulated.

Early in the project, we established fundamental relationships in mango, between total light interception and orchard yields and between inflorescence density and yield efficiency. By intensifying mango orchard systems we increased total light interception during early orchard life, which led to increased early yield per hectare, particularly for the precocious varieties. At the high-density of 1,250 trees per hectare, trellised ‘Keitt’ and ‘Calypso’ yielded 53 and 43 tonnes per hectare, respectively, five years after planting.

Selective pruning in a Bundaberg commercial macadamia orchard produced greater nut-in-shell yield than the industry standard mechanical hedging and topping. As with mango, intensive planting systems in macadamia had greater canopy volume per hectare and greater total light interception during early orchard life. This led to higher and earlier orchard yields in the precocious flowering variety ‘A203’, which at 5 years of age, when planted at high density (1,000 trees per hectare) produced 4.9 t per hectare nut in shell compared with 2.9 t per hectare for the low density (312 trees per hectare).

In ‘Hass’ avocado, we established fundamental relationships between total light interception and yield per hectare and between inflorescence density and yield efficiency. Early in the life of the intensive avocado orchard trial, the canopy volume per hectare and total light interception of the high and medium density systems were greater than the industry standard low-density systems. High-density systems had higher orchard yields two years after planting, however by year five, orchard yields of the low-density industry standard orchard system was significantly greater than when grown at high-density. The lower vigour ‘Ashdot’ rootstock has consistently produced greater yield per hectare than the higher vigour industry standard ‘Velvick’ rootstock in the Planting Systems Trial.

Keywords

Macadamia; mango; avocado; intensification; tree crops; Planting Systems Trials; tree training; pruning; canopy management; high density; crop load; vigour; architecture; tree structure; light interception; productivity; allocation; partitioning; rootstocks; dwarfing; vigour management; alternate bearing; functional-structural plant modelling; molecular physiology; precocity.

Introduction

In the latter part of the 20th century, some temperate tree crops, such as apple, underwent orchard system intensification. This intensification involved planting more trees per hectare using vigour managing rootstocks and using advanced tree training systems and crop load management systems. The intensification resulted in improved orchard light relations, improved vigour management and allocation of energy into fruit production, higher early yields and greater maximum yields of high-quality fruit.

Subtropical and tropical tree crop systems tend to be less intensive than many temperate tree crop production systems. Both fundamental understanding and the practical tools to manage vegetative vigour are limited. Therefore, these subtropical and tropical tree crops tend to be grown in large tree, low density systems to increase the length of time required before canopy management operations to prevent orchard crowding are required.

We identified components of modern intensive apple and stone fruit orchard systems that have led to the increased productivity compared with traditional systems. These key orchard systems components are orchard light relations, vigour management, tree architecture and the development of crop load.

Orchard light relations refers to the movement and interception of light within an orchard. It consists of total light interception and light distribution. Total light interception is an estimate of the proportion of photosynthetically available light that falls on an orchard that is intercepted by the canopies as opposed to falling on the ground between the trees or being reflected from the tree canopies. Across a range of tree crops, yield per hectare increases as total light interception increases from low levels, up to at least 60% to 80%, depending on the species. After this initial increase, yield per hectare is then often reported to plateau or decline as total light interception increases above 80% and 90%. Within canopy light distribution refers to the movement of light through orchard canopies. It is affected by row and within row tree spacing, canopy size, canopy density and tree architecture. For a range of tree crops, light distribution has been shown to affect crop load development processes such as floral initiation, fruit set and retention and fruit growth. It also affects the maintenance and growth of shoots and leaves in different portions of the canopy. Understanding the relationships between total light interception and distribution and fruit yield and quality were important steps in the productivity advances in temperate tree crops and led to the movement towards high-density, small tree orchard systems that tend to have more efficient orchard light relations. Understanding the light relations required for optimum orchard productivity in subtropical and tropical tree crops is an important step in developing more productive orchard systems.

A substantial part of the success of modern intensive orchard systems is due to the ability to manage vegetative vigour. Excessive vigour can lead to reduced partitioning of resources to the crop compared to vegetative growth; lead to high levels of within-canopy shading, with subsequent detrimental effects on crop load development; and require severe pruning which can in turn lead to excessive re-growth, exacerbating the issues above. Dwarfing rootstocks and low vigour scion varieties are a substantial part of the vigour management toolkit in temperate tree crops such as apple. However, orchard management techniques also play a role through vigour-moderating tree training and pruning techniques, optimized crop load, and chemical growth regulator applications. Subtropical and tropical tree crops tend to have high levels of vegetative vigour. Low vigour rootstock and scion varieties and vigour-moderating orchard management practices are also generally less available for subtropical and tropical tree crops. Therefore, understanding vegetative vigour, the factors affecting the allocation of resources between vegetative and reproductive development and development of management tools to manage vigour for subtropical and tropical tree crops are a key part of developing high productivity orchard systems.

Orchard productivity is not only affected by the ability of the canopy to intercept light, it is dependent on the canopy's ability to turn this intercepted energy into harvestable fruit and nuts. That is, the development of crop load. The development of crop load refers to all the processes required for inflorescence production, fruit set, fruit retention, fruit growth and return flowering in the following year. Crop load may be limited at any of these points along the crop development cycle. Inflorescence production has been demonstrated to limit yield in macadamia, mango and avocado. For macadamia, yield efficiency has been reported to increase linearly with raceme density from low levels up to a threshold level of raceme density, above which yield efficiency plateaus. Understanding these relationships for mango and avocado will be important for understanding limitations in the development of crop load and developing crop load management systems. Fruit set and retention may also limit yield.

Understanding the effect of tree architecture and responses to manipulation on the orchard light environment, development of crop load and subsequent shoot growth is essential to efforts to improve orchard systems productivity and should inform new planting systems design. It is important to understand architecture at a range of scales. Tree height, tree width, alley-way width relative to row spacing and canopy shape, described as orchard scale architecture, have very strong effects on total orchard light interception and distribution and orchard productivity. Whole-tree scale architecture, including branch structure and tree training system, have important effects on precocity, within canopy light environment, vegetative responses to manipulation, and the development of crop load. At the limb and shoot scale, architectural traits such as angle of growth can affect flowering and growth. For example, in apple, horizontal limbs are preferred due to moderated vegetative growth and increased flowering.

Intensive research in molecular biology has been carried out on apple as a model orchard species, contributing to the improvement of these crops. Contrastingly, mango, avocado and macadamia trees can be considered as orphans in term of molecular biology and molecular physiology. The genetic resources available for these crops is almost non-existent and the knowledge of their molecular physiology is quite limited. The aim of this project is to improve the genetic resources available for these species and to get insight into the molecular changes correlating with tree phenology, focusing on bud burst, flowering and juvenility. Two layers of experiments (time-course and molecular physiology experiments) have been designed to address this. Ultimately, the knowledge and resources obtained during this project will provide useful information for crop management and will accelerate future research in these crops.

The aim of this project has been to improve our understanding of the above orchard systems components in avocado, mango and macadamia and then attempt to adapt relevant principles from highly productive temperate tree crop orchard systems into our focus crops to optimize the performance of these orchard systems components. However, it is well understood that these components of the orchard system do not act in isolation but interact in a complex manner. An essential part of our work is to undertake research to understand how the orchard systems interact and to develop planting systems that optimize the components. One tool that has been used is large scale Planting Systems Trials, comparing factors such as plant density, tree architecture (tree training) and scion and rootstock variety.

A second tool that has been used is Functional-Structural Plant Models (FSPMs). These models explicitly describing the development over time of the architecture or structure of plants as governed by physiological processes that, in turn, are driven by the environment (Vos et al. 2010). The level at which architecture and physiology are modelled can vary widely depending on the objective of the research and the data available and can capture detail from the cellular and organ level to whole orchards. It provides a platform in which we can test competing ideas as to why a given phenomenon occurs and to what extent these are governed by structure and topology of the plant, as well as showing emergent properties of the models and generating new hypotheses. The way in which we create FSPMs has been to use L-Systems, which were first proposed by Lindenmayer (1968), and were later developed into the software packages LStudio (Windows) and VLAB (Linux/Mac) at the University of Calgary. An important component of the software system is its ability to place the FSPM within an environment, such as a representation of the sun and the sky at a given location and date for simulating the amount of light received by each leaf. The combination of a “plant architecture” in an environment and physiology within the plant makes FSPM a powerful tool to investigate how management practices such as planting density, training, pruning or limb bending can affect light interception, vegetative growth, flowering and fruit growth, and from there, productivity.

Once developed, intensive orchard systems have the potential to offer greater profitability for the macadamia, avocado and mango industries and make them more competitive internationally. It is widely recognized that subtropical and tropical tree crop systems are underperforming and greater productivity per hectare could be achieved. In temperate tree crop industries where intensive orchard systems have been adopted, orchard establishment costs and often annual maintenance costs are higher than in the traditional systems, but the intensive systems still provide greater profitability. It is likely that constraints on land and water resources will only continue to increase into the future and greater productivity per hectare with intensive orchard systems offers a path to use available resources more effectively.

Methodology

During the initial stages of planning of this Initiative, aimed at transforming the productivity of subtropical and tropical tree crops, we tried to identify the components of apple orchard systems that underpinned their high productivity and the components of tropical and subtropical tree crops that limited their productivity.

For apple, the key components emerged through an analysis of the evolution of apple orchard systems from the literature and commercially over the previous 40 years. Apple orchards had evolved from low density, large tree orchards planted on vigorous seedling rootstocks and pruned to a vase tree structure, to high density, small tree systems planted on dwarfing rootstocks and pruned to central leader tree structures. These changes led to underlying changes in orchard light environment, tree architecture, the development of crop load and tree vigour.

For the subtropical and tropical tree crops mango, macadamia and avocado, it was apparent that the orchard systems were in a similar level of development as apple orchard system during the mid-20th Century with many of the same orchard attributes and the underlying physiological limitations to productivity the same.

Having identified these orchard systems components (light, vigour, architecture and crop load) as those limiting productivity in our focus tree crops, we decided that our attempts to transform productivity in these tree crops needed to focus on understanding these components of the orchard systems and optimising their performance. Much is known about these orchard systems components in temperate tree crops and the strategies used to optimize their performance. However, having some understanding of the differing physiologies and suitability of the genetics between temperate tree crops and our focus subtropical crops, our philosophy was to attempt to adapt relevant concepts and technologies from the highly productive crops such as apple, while taking into consideration the specific physiologies and phenologies of our subtropical focus crops.

The intention of the work has been to begin a process of understanding the factors underlying productivity in subtropical and tropical tree crops and use that understanding to re-design more productive orchard systems. It is therefore implicit that to achieve this goal, this must merely be the first five-year phase of a much longer program of work due to the long-term nature of tree crops.

This program encompassed work across three tree crops in several growing regions across NSW and Queensland, using scientists and extension officers from a range of disciplines and three organisations, with postgraduate students forming an important part of the research effort. The group has worked toward understanding and improving the management systems and the genetics (particularly rootstocks) for our tree crops, as we believe both are limiting.

Below, we describe the experimental and extension activities undertaken within the research program. The descriptions of the experimental work are not intended to be a detailed ‘Materials and Methods’ of all of the experimental systems, as these are described in the attached Appendices. Rather, the descriptions below seek to provide an overview of the work undertaken. First, we describe research undertaken to understand and improve individual orchard systems components. Second, we describe the research undertaken to integrate this understanding, understand the interactions between orchard systems components and develop new systems. One of our major integrating activities are the Planting Systems Trials. Third, we describe the more fundamental research activities aimed at improving our understanding of the systems: Functional-Structural Plant Modelling and Molecular Biology. Finally, we describe our extension and communication activities.

Systems Trials

The systems trials undertaken in the project generally had two purposes. First, to understand interactions between the key orchard systems components and their effect on productivity. Second, to trial management systems with the potential for adoption by growers. The systems trials have either been undertaken in mature commercial orchards, using mature plantings on research stations, or establishing new trials on research stations. These large, long-term trials have consumed considerable amounts of resources both in their initial set up and their subsequent evaluation, particularly the three Planting Systems Trials established at the commencement of the project.

Due to the implicit long-term nature of tree crops, the Planting Systems Trial have so far only provided information on the early orchard phase.

Mango Planting Systems Trial

The purpose of the mango Planting Systems Trial is to understand the effect of scion variety, tree density and tree structure on orchard productivity and to develop improved understanding of the orchard systems components underlying productivity.

There were two major hypotheses tested in this trial. First, increased tree density will lead to greater early yield per hectare. Second, increased tree density will lead to greater maximum yield per hectare at maturity due to improve light use efficiency. Thirdly, that tree training will lead to reduced tree vigour resulting in an extended productive life for the orchard.

The Mango planting systems experiment was designed with six replications of the experimental treatments which consisted of three varieties (‘NMBP-1243’, Keitt, ‘Calypso’), three planting densities: conventional or low (8 m x 6 m; 208 trees/ha); medium density (6 m x 4 m; 416 trees/ha); and high density (4 m x 2 m; 1250 tress/ha) and three tree-training systems; conventional industry training and pruning; single leader; and espalier grown on trellis). Details of trial design are presented in Appendix 12. Measurement of tree growth and performance are discussed individually in the methods sections outlined below.

Avocado Planting Systems Trial

The purpose of the avocado Planting Systems Trial is to understand the effect of rootstock and tree density on orchard productivity and to develop improved understanding of the orchard systems components underlying productivity.

There were three major hypotheses being tested in this trial. First, increased tree density planting would lead to greater early yield per hectare. Second, increased tree density would lead to greater maximum yield per hectare at maturity due to improved orchard light relations. Third, ‘Ashdot’ rootstock would be better suited to high-density than ‘Velvick’ due to its smaller size and greater yield efficiency.

The trial was established in July 2014 at the Bundaberg Research Facility in a deep-red Ferrosol soil. The tree rows were mounded prior to planting to a height of approximately 40 cm to improve drainage.

The trial compared ‘Hass’ at three tree densities: conventional or low tree density (9 m x 5 m, 222 trees/ha); medium density (6 m x 3 m, 556 trees/ha); and high density (4.5 m x 2 m; 1111 trees/ha). At each of the tree densities, two rootstocks were compared: the industry standard and vigorous ‘Velvick’ rootstock; and the reportedly lower vigour ‘Ashdot’ rootstock. At each tree density the trees were trained and pruned as was considered appropriate for the density. The low-density trees received minimal pruning in the first years of the trial, with selective pruning to improve light levels within the canopy commencing at four years after planting. The medium-density trees were trained to a central leader for the first five years of the trial. The high-density trees were trained as a central leader to a vertical trellis for the first four years and thereafter less emphasis was placed on the central leader tree structure.

An industry advisory group of local avocado growers and consultants was created at the commencement of the trial. The group met every six to 12 months and provided advice on local industry best-practice for aspects of the trial management such as pruning (for conventional pruning treatments), tree nutrition and pest control. Given their exposure to the intensive avocado systems in the trial, it was our expectation that these growers would be well placed to adopt any successful systems.

Macadamia Planting Systems Trial

The purpose of the macadamia Planting Systems Trial is to understand the effect of planting density, tree training and scion variety on macadamia orchard productivity and to understand the key orchard systems components underlying productivity.

There were two major hypotheses being tested in this trial. First, increased tree density will lead to greater early yield per hectare. Second, increased tree density will lead to greater maximum yield per hectare at maturity due to improved light-use efficiency.

The overall trial consisted of a main trial and a smaller 'trellis plot' trial. The main trial consisted of three plant densities: the conventional tree density (8 m x 4 m; 312.5 trees/ha); a medium density (6 m x 3 m; 556.5 trees/ha) and a high density (5 m x 2 m; 1000 trees/ha). Tree training systems were also compared, with the Bundaberg conventional tree training system applied as a treatment in all densities and central leader treatments applied to the medium and high densities. These tree density and tree training treatments were applied to two commercially planted scion varieties: 'A203' and '741'. The 'trellis plot' was planted at 4.5 m x 1.5 m (1481 trees/ha) with both 'A203' and '741'. The trees were trained to a vertical trellis using a central leader tree structure. During the first three years of the trial the practice of bending and tying the lateral limbs arising from the central lead was compared with allowing those limbs to retain their natural angle.

An industry advisory group of local macadamia growers and consultants was created at the commencement of the trial. The group met every six to 12 months and provided advice on local macadamia industry best practice for aspects of the trial management such as pruning (for conventional pruning treatments), tree nutrition and pest control. Given their exposure to the intensive macadamia systems in the trial, it was our expectation that these growers would be well placed to adopt any successful systems.

Macadamia high density remediation trial

At the commencement of the project a large scale commercial high-density macadamia orchard was located in the Bundaberg region. We took the opportunity to undertake a canopy management trial within this orchard. The purpose of the work was to compare the standard practice of mechanical hedging and topping with manual selective pruning and test effect of a commercially available growth regulator uniconazole on canopy re-growth following pruning. The major hypothesis being tested in this trial was that severe mechanical hedging and topping would lead to more vigorous re-growth and competition with developing fruit than selective pruning. It was also hypothesized that reduced shoot growth due to uniconazole application may lead to reduced competition with fruit development.

The trial was conducted over three consecutive seasons with light interception and light distributions and a range of canopy and crop-load development variables measured.

NSW macadamia selective pruning and tree height control trial

Tree height control in commercial orchards is one of the most difficult aspects of macadamia canopy management. Mechanical topping has previously been reported to severely impact yield compared with no pruning while selective pruning for tree height control has only been reported for extreme reductions in tree size.

A tree height control trial was established in an experimental orchard at the Alstonville Centre for Tropical Horticulture. The orchard consisted of '816' and '246' planted at 7 m x 3.8 m and was eight years old at the commencement of the trial, with the trees approaching 6 m in height. There were two main purposes of this work. First, compare the effect of annual selective limb removal tree height control to 6 m in height, with a once-off reduction in tree height using selective pruning after four years of un-checked growth. Second, determine if the trees were large enough at a height of 6m to achieve the maximum orchard yield. Yield, canopy dimensions and light distribution were measured.

Tree vigour

One of the major factors limiting subtropical tree crops being planted in more intensive systems is the high vegetative vigour, which leads to orchard crowding. Therefore, understanding the factors affecting vegetative vigour and developing strategies to moderate vigour have been one of the project's major aims. Managing scion vigour with rootstocks, tree training and pruning systems, growth regulators, and crop load management are tools from temperate tree crop production systems that have been investigated in this program.

Mango vigour

Investigations on vigour management in mango have taken two approaches, both of which have proven to be useful in other species. The first is a canopy training approach where branches are trained to the horizontal to reduce their apical dominance concentrating it in a central leader, and the second approach is to use vigour-reducing rootstocks to manage the vigour of scion canopies.

Managing mango vigour through canopy training

The purpose of this research was to understand the effects of orchard intensification on mango tree vigour. The experiment tested the three major factors of orchard design (planting density, tree training and variety) to understand the interactions between them. This experiment was conducted within the mango Planting Systems Trial that is a major platform for this project, on which we were able to study many additional aspects of orchard intensification as outlined further in this report.

Mango vigour managing rootstock trial

Although many mangoes throughout the world are propagated by grafting, very few use proven vigour reducing rootstocks. A short review of rootstock research is presented in Appendix 9. In this project, we aimed to identify vigour-controlling rootstocks for mango by screening 97 candidate rootstocks for their vigour reducing performance under two scion varieties, 'NMBP-1243' and 'NMBP-4069' and to compare them to the standard Australian rootstock, 'Kensington Pride'. Candidate rootstocks were selected from polyembryonic accessions in the Australian National Mango Genepool and progeny from the Queensland Mango Breeding Program. Rootstock-Scion combinations were evaluated in a replicated field trial planted on Walkamin Research Station at 6 m x 3 m, with canopies pruned conventionally after the third year of growth. Candidate rootstock-scion combinations were planted over 3 years between 2014 and 2016 due to propagation logistics. Tree performance was measured biannually for tree growth characteristics and the canopy volume and canopy surface areas were calculated as a measure of tree size and vigour. Yield efficiency was assessed in rootstock-scion combinations as yield per canopy volume and yield per canopy surface area to ensure rootstock induced low-vigour trees have the potential to produce high yields when planted at high density in intensive orchard systems (Appendix 9). Selection and evaluation work such as this is a long-term endeavour in tree crops, expected to take longer than the five-year duration of this project. However, selection work initiated during this project will be continued in the following project, AS18000.

Avocado vigour managing rootstock assessments

The objective of the avocado rootstock work was to identify currently commercially available rootstocks that would manage scion vigour, increase partitioning of resources to reproductive growth and improve productivity in a high- density scenario. This work differed from the macadamia and mango rootstock components of the project in that there was no opportunity to draw on a local germplasm collection or breeding program to develop new rootstocks; thus, the work focused on commercially available material. While a substantial amount of avocado rootstock evaluation research has previously been undertaken in Australian conditions, none of this was undertaken with the specific purpose of identifying rootstocks that lead to improved productivity in an intensive orchard environment.

Domestically available rootstocks were selected following evaluation of the literature and discussions with industry experts. A review of the potential to import vigour managing rootstocks was undertaken as part of the project. The review concluded that only one rootstock, 'Steddom', would be useful to evaluate, however subsequent reports indicated that this rootstock had limited vigour control potential, so this was eventually abandoned. The review also concluded that several international groups were investigating alternate scion varieties to manage vigour in view of the lack of past success and limited future potential to use rootstocks for this purpose.

It was originally intended that a single large rootstock trial using 'Hass' as the scion would be planted. However, differences in delivery times between nurseries meant that the trial was planted in two phases. Both phases were planted at the Bundaberg Research Facility at 4.5 m row spacing and 2 m tree spacing with a vertical trellis. Phase 1 was planted in May 2016 with 'Hass' on nine different rootstocks. Phase 2 was planted in January 2018 with 'Hass' on six different rootstocks as well as the scion varieties 'Gem' and 'Maluma', each on one rootstock. A tree training factor was also incorporated into the Phase 2 trial, with a comparison between three-dimensional tree structures and two-dimensional tree structures, the hypotheses being that two-dimensional tree structures may allow improved productivity due to improved light distribution within the canopy, more ability to rejuvenate fruiting branches and/or improved canopy-root area ratio.

Macadamia vigour managing rootstocks and genetics

To identify potentially useful rootstocks for improving yield efficiency through the control of early fruiting, yield and vigour of the scion, we propagated 30 macadamia genotypes of seedlings and cuttings from diverse origins and sources in April-October 2014 (Appendix 5). In July 2016, a commonly grown scion 'HAES741' was grafted onto the rootstocks, and the trial was planted on 4th April 2017 following the procedure described in Appendix 5. Growth characteristics were measured for both scion and rootstock from November 2017- April 2019. Every year, grafted trees were phenotyped for flowering, fruiting and yield. Phenotyping will be continued until final measurements are taken at age 7 in 2024.

Additionally, we investigated macadamia vascular systems to identify vascular traits associated with reduced tree size. Details of this research were published in an article in Plant and Soil journal titled "Anatomical structures associated with vegetative growth variation in macadamia". The methods were as follows: stem sections from the most recent mature flush were collected from five replicates of three macadamia varieties: 'D4' (high vigour), 'B25' (intermediate vigour) and 'B63' (low vigour). Sections were imaged under a fluorescence microscope in order to measure the number and size of all xylem vessels in the stem. Data was analysed to determine relationship of vascular morphology with tree vigour.

Orchard light environment

The orchard light environment refers to both total light interception, the proportion of photosynthetically active radiation (PAR) intercepted by the canopy, and light distribution, the way this PAR moves within the canopy. Both total light interception and light distribution are significant drivers of tree crop productivity and quality. Overall, the aims of this work have been to understand the relationships between total light interception, canopy characteristics and productivity and to understand the effects of light levels within canopy on shoot growth, flowering, fruit set and quality. The outcomes in understanding from these aims have been applied in the design and management of more productive orchard systems.

Throughout the project, total light interception was measured using different equipment, using a range of methods and under both direct and diffuse light conditions, depending on the requirements of the experiment. In all of these situations, the methods have been based on the principles of Wunsche et al. (1995) for the measurement of total light interception in orchards. Initial measurements were undertaken using 80 cm long bar ceptometers with PAR sensors and data loggers. Ceptometers have the advantage that they are versatile and transportable and can be adapted to a range of types of light measurement. However, when collecting total light interception for large numbers of large plots, measurement can be slow. To increase the pace of light interception measurement, while maintaining accuracy, in our large-scale Planting Systems Trials, we built 'light trolleys'. These light trolleys consisted of a horizontal bar fitted approximately 4 m wide with PAR sensors at regular intervals, connected to a data logger and situated on top of a trolley which is pulled through the plots of the trials (Figure 1) recording data 10 times/second.

Figure 1. Purpose built light trolley measuring total light interception in the macadamia Planting Systems Trial.

Light distribution was also measured for all three crops, with aims and measurement techniques differing in each crop according to the experimental systems design and the different physiologies of each crop. For example, macadamia produces racemes from axillary buds of shoots of a range of ages, often situated well back from the periphery of the canopy, we therefore decided to measure light distribution using horizontal transects through the canopy.

Avocado

At the commencement of this project very little was known about avocado orchard light relations. Our first aim was to develop relationships between total light interception, canopy volume and yield per hectare in commercial orchards. It is well known that total light interception is a strong driver of yield per hectare in a range of tree crops, however the relationships vary. This information is very fundamental to understanding how much light needs to be intercepted for optimum productivity and the nature of the relationships, including if yield per hectare will decline at the very highest levels of light interception. The relationships developed are a baseline for avocado orchard light-use efficiency, against which experimental intensive orchard systems can be compared.

To develop these baseline avocado orchard light interception relationships, small plots of trees in blocks ranging from ‘recently planted’ up to ‘canopy crowding’ were selected in a commercial orchard. Over two years, canopy volume, total light interception and yield per hectare were measured.

Total light interception was measured annually in the avocado Planting Systems Trial and related to productivity and canopy development. The purpose here was to compare the light relations in the more intensive planting systems to that in the conventional systems to understand if the anticipated benefits of reduced row spacing in light-use efficiency were being realized.

Due to the likely impacts of localized light levels on flowering, fruiting and canopy development, canopy light distribution was studied in the Planting Systems Trial. These light distribution studies were undertaken over multiple years to understand effects of the plant densities on the distribution of light along the canopy periphery and within the canopy. Light availability in these portions of the canopy was correlated with localized flowering intensity, fruit set and leaf retention.

Mango

As with avocado, prior to this project there was little research work done on light relations in mango orchards, particularly relating to the benefits of increased light from increasing orchard planting density or alternative tree training systems. The main emphasis of this research in mango orchards focused on two areas: light interception and light distribution.

Three light interception experiments were conducted in mango. The first aimed to establish a baseline of current mango light interception in conventional 'Kensington Pride' orchards. This experiment surveyed light interception and its relationships with planting density, canopy volume and yields in several mango orchards of differing ages over two years. The second experiment used the Mango Planting Systems Trial on Walkamin Research Station to understand how light interception was influenced by variety, planting density, and tree training system over four years (Appendix 10). A third experiment aimed to improve the methodology for light interception measurement in tree crop orchard systems. This experiment compared the use of the PAR measuring light trolley with the newer LiDar technology by comparing results from both technologies on the Planting Systems Trial on Walkamin Research Station. This work was done in collaboration with Sydney University and co-funded by a DAF Innovation project.

Four experimental approaches were used to investigate light distribution: a baseline light distribution experiment to understand light distribution in conventional mango orchards, an experiment on the effect of pruning strategy within conventional orchards on light distribution, and two experiments investigating the effect of canopy architecture and planting density on light distribution.

The first light distribution investigation aimed to document the current range of light distribution in the canopies of 'Kensington Pride' trees between 1 and 30 years of age, in North Queensland. Trees were classified by tree height for this baseline investigation.

The second experiment aimed to develop a critical understanding of how four different pruning systems affect canopy light distribution in mature commercial 'Kensington Pride' orchards and relate light distribution within the canopy with yield and fruit quality. Data from this experiment was also used to parameterise and verify the light component of a mango light model to predict light distribution inside the canopy based on pruning methods (Mango modelling section of this report).

A third experiment looked at light distribution in conventional and single-leader trained mango canopies at low, medium and high planting densities. This experiment looked at the light distribution patterns within the canopies of low-density conventional, medium-density single-leader and high-density espalier mango trees and was conducted on the Mango Planting Systems Trial on Walkamin Research Station.

A fourth experiment further developed the understanding of light distribution, investigating how variety, density and training systems influenced light distribution at the branch level and relationships with leaf distribution, flowering and cropping over two years in the Mango Planting Systems field trial on Walkamin Research Station (Appendix 10).

Macadamia

Prior to this project, macadamia was probably one of the better studied subtropical tree crops in terms of orchard light relations. For example, the effects of total orchard light interception and canopy crowding on productivity had previously been reported. The areas of focus for macadamia have therefore been slightly different than for mango and avocado.

Work early in the project examined the effect of pruning techniques in a commercial high-density macadamia orchard on PAR, fruit and leaf distribution within the canopy. The macadamia Planting Systems Trial was then used to study the effect of planting density and early tree training on total light interception and interactions between canopy volume and total light interception during early orchard life. The effect of tree training and planting density on the distribution of light, leaf area, flowers and fruit within the canopy was also studied.

Crop load

Understanding of the development of crop load varied substantially between the three focus crops at the outset of this project. The crop load develops in a cycle that begins with floral initiation and encompasses floral development, fruit set and retention, and fruit growth. It is referred to as a cycle because the crop load in one season can affect the crop load in the following season.

Our early work focused on understanding the processes in the crop load development cycle that were most limiting yield. This understanding has then been used to inform further crop load experimentation and inform our planting systems designs and canopy management strategies.

There are important interactions between the development of crop load and shoot vigour and architecture and their combined effects on competition between reproductive development and vegetative growth. Understanding competition between tree organs and how it affects the allocation of resources within the tree is an important step in developing management systems with greater crop load carrying-capacity.

Avocado

A whole tree avocado crop load manipulation experiment was undertaken to understand the relationships between inflorescence density, fruit set, yield efficiency, vegetative growth and fruit quality. The purpose of the work was to develop basic understanding of the processes limiting the development of crop load in avocado and the plasticity in the responses when manipulated. This initial crop load work was undertaken on young, cropping 'Hass' avocado in a commercial orchard in the Bundaberg region. Inflorescence density was manipulated on the selected trees by removing varying proportions of the inflorescences (from 10% to 95% inflorescence removal) at anthesis. The remaining inflorescences were counted and subsequent fruit set, shoot and canopy growth and yield were monitored.

Follow-on crop load work aimed to develop management techniques to improve spring fruit set by reducing competition with spring shoot growth in the current year and through growth regulator application to increase branching and thus flowering positions. The work was undertaken across two seasons in a commercial orchard in the Bundaberg region. Treatments aimed at reducing shoot competition with fruit set included 'tipping' (removing) all or part of the growing shoot by pruning once or repeatedly throughout the spring. The treatments aimed at increasing canopy complexity included the growth regulator 'Cytolin' with or without a range of the shoot tipping treatments.

Detailed crop load work was then undertaken in a range of experiments in the avocado Planting Systems Trial and at other sites to influence crop load through use of a variety of plant growth regulators, fruit thinning and branch girdling, and to further understand the factors affecting the development of crop load in avocado.

Mango

The mango crop load research aimed to investigate flowering and crop manipulation as a tool for managing yield and fruit quality and, to understand the relationships between flowering, crop load, yield and vegetative growth in mango. The crop load research also aimed to determine the relative influence of flowering and resource allocation on productivity and how reproductive growth impacts on vegetative growth.

The investigations were conducted on mature 'Calypso' and 'Kensington Pride' trees in two separate experiments on farms in the Mareeba district of North Queensland. These experiments thinned flowers and or fruit at various intensities between 0 and 95% and observed the effects on yield, fruit size, fruit retention and fruit quality. The influence of flowering and crop load on the subsequent season's flowering, yield and vegetative growth was also investigated to determine the effect of flowering and crop load on biennial or irregular bearing.

A second approach used comparative analyses of data from the mango Planting Systems Trial with the aim of understanding the effect of flowering on yield efficiency.   Comparisons included floral density, yield efficiency and fruiting rate.

Macadamia

At the commencement of the project there was already a sound understanding of the relationships between flower and raceme density and yield efficiency, the potential for compensation in fruit set and fruit growth (nut size) processes, and thus the limitations to the development of crop load in macadamia. Therefore, crop load experimentation for macadamia aimed to improve understanding of competition between shoot growth and fruit set, develop management strategies to reduce competition and assess the crop load carrying capacity of the treatments in the macadamia Planting Systems Trial.

Canopy management strategies to minimise competition between vegetative growth and fruit set were investigated in the ‘macadamia high-density remediation trial’, described above. This work re-enforced the existing understanding that reducing competition during early fruit set was critical to high crop load in macadamia. The work, in part, led to a subsequent series of two experiments to further understand competition within and between organ types and the effect of the relative timing on growth. The first of these experiments looked at the relative timing of growth between shoots on their ability to compete for resources. The second experiment looked at the relative timing of fruit development and shoot growth following tip-pruning on the ability of fruit and shoots to compete for resources.

Detailed crop load experimentation was also undertaken in the Planting Systems Trial. The combination of tree density, tree training and variety treatments has enabled this work to separate out the effects of precocity (early flowering), PAR interception and canopy management on early orchard crop load development in macadamia.

Tree architecture

Architectural assessments at a range of scales have been undertaken across all three focus crops. Relatively little architectural work had been undertaken on these crops and so much of our initial focus was around improved understanding of aspects of the architecture of these crops. First, understand the patterns and processes controlling growth. Second, understand the vegetative and fruiting responses to our pruning and tree training treatments. Third, understand the relationships between shoot characteristics and flowering and fruiting. This improved understanding could then be used to help design planting systems with minimised vigour, optimised flowering and fruiting, and optimised light environment.

Undertaking architectural assessments at a range of scales aids in the interpretation of results, their practical importance and helps understand the mechanism responsible for observed responses. Architectural assessments were undertaken at the orchard, tree, branch unit (limb), shoot and growth unit scale. For example, on one extreme, tree canopy dimensions were used to calculate tree scale and orchard scale canopy volumes. On the other extreme, detailed assessments of vegetative, floral and fruiting characteristics and topology at the growth unit scale were also undertaken.

Avocado

Architecture experimentation for avocado was undertaken in commercial orchards and in the avocado Planting Systems Trial. The primary focus of the detailed avocado architecture assessments has been to identify factors affecting fruit set and retention.

Early in the project an architecture study was undertaken in a commercial avocado orchard in Childers using ‘Hass’ with the aim of better understanding summer fruit drop. Detailed architectural assessments of limbs in mature trees recorded a range of fruiting and vegetative characteristics. The work commenced in late spring and measured characteristics including the timing of growth of current growth units, presence or absence of fruit and the presence of leaves. Subsequent measurements later in the cropping season measured characteristics and location of any subsequent vegetative growth and the retention of previously measured fruit.

The detailed architectural assessments in the avocado Planting Systems Trial were undertaken over three cropping seasons with three rounds of measurements per year. These detailed measurements recorded the timing, location and patterns of vegetative growth, the location and type of flowering and the extent and location of fruit set and retention. The work was aimed at identifying underlying factors affecting fruit set and retention, the patterns of vegetative growth and the underlying factors for observed differences between the Planting Systems Trial treatments.

Mango

Mango tree architecture research aimed to establish and understand patterns of vegetative and reproductive development and their responses to manipulation in different varieties, to determine the advantages and disadvantages of managing vegetative and reproductive growth on productivity. This knowledge will assist the appropriate selection of varieties suited to different planting densities and training systems. The approach taken in the architectural investigations was to measure structural, functional, temporal and topological relationships, at the tree, limb and growth-unit levels using trees in the mango Planting Systems Trial at Walkamin Research Station between 2014 and 2018. Analyses of this data will allow ongoing investigation of how fundamental growth relationships respond to management and environmental factors associated with mango orchard intensification. This research collaborated with staff and students from the French research institute, CIRAD, and will form the basis for ongoing collaborations between DAF and CIRAD into the future.

Macadamia

A significant portion of the macadamia architecture work aimed to understand factors affecting early orchard (precocious) flowering, due to the recalcitrant nature of macadamias and to the large impact early flowering has on early orchard yield. Much of this was undertaken in the macadamia Planting Systems Trial using detailed architectural assessments to compare the shoot growth and flowering responses of the tree training treatments and. One of the Maroochy Research Facility based Macadamia Industry Breeding Program trials was used to identify architectural factors affecting early flowering and yield efficiency across macadamia genotypes.

Shoot and limb angle and limb bending were given attention, due to their well-documented effects on flowering and vigour in apple orchard systems and the anecdotal and macadamia industry perceptions of the effects of limb angle on nut set. A range of experiments contributed to this work, including treatments in the macadamia Planting Systems Trial, mature tree limb bending experiments and the previously mentioned Macadamia Industry Breeding Program Trial.

Another focus of the architecture work in macadamia has been to compare the two commercial varieties used in the macadamia Planting Systems Trial, to provide background understanding for observed treatment effects.

Molecular/genetic regulation of floral initiation and juvenility

Creation of genetic resources for orphan crops and gene discovery

At the start of the project, no genetic resources were available to identify target genes and design the primers to monitor their expression. We therefore had to create such resources. To do so, we extracted the RNA from different tissue types (leaf, bud, stem, fruit, flower and root), pooled the resulting RNA, converted it to cDNA and normalized the cDNA in order to make a library for each species, representative of the different genes (Figure 2; Chabikwa et al., 2020). Before making these resources publicly available at the end of the project, we decided to annotate these libraries using the other genetic resources published in the meantime. This provides the scientific community with resources in a more useful format than raw assemblies (Figure 2; Chabikwa et al., 2020). Target genes from the created cDNA libraries were identified by sequence homology to selected genes from other species based on the literature.

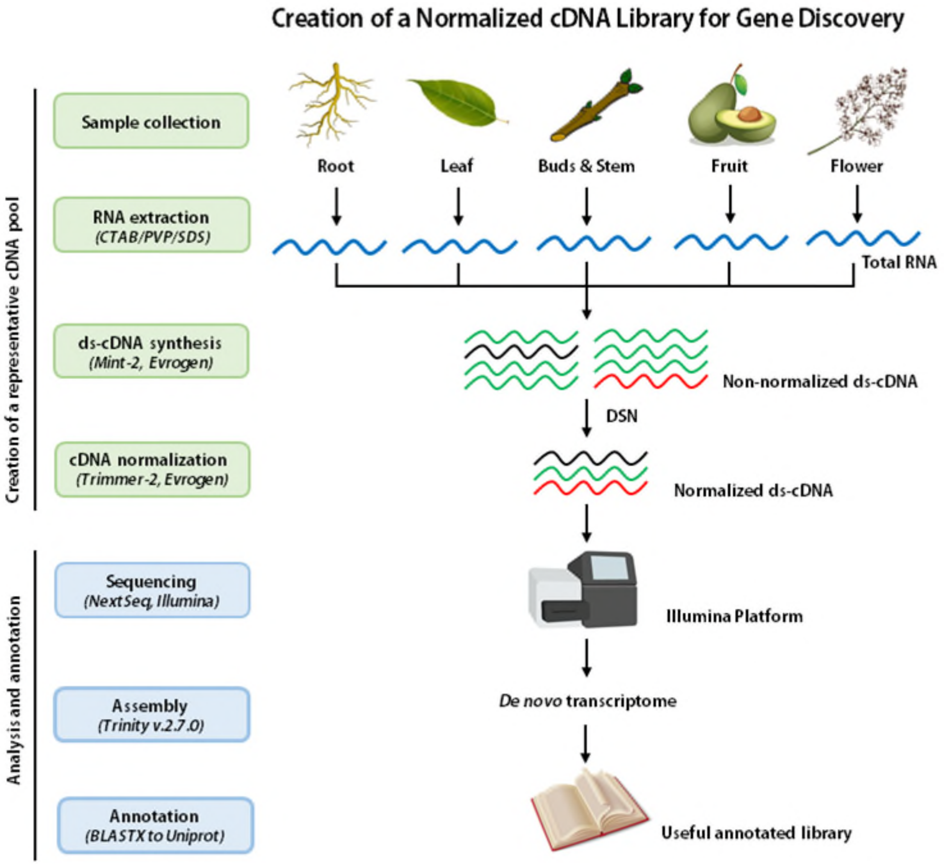

Figure 2. Experimental procedure to create the cDNA libraries. (Simplified from Chabikwa et al., 2020)

RNA extraction and gene expression

In order to efficiently extract RNA from the different sample types, a new RNA extraction method had to be created for this project. Given that the project has generated a significant number of samples, we developed a semi-high-throughput method that was safer than existing methods, in order to decrease the risk for the staff members in charge of this step (Barbier et al., 2019) and the extracted RNA was then used for gene expression.

Time-course experiment

The time-course experiment is an assay that was performed over four years on the three crops of the project. The aim of the experiment was to assess the relevant changes in the physiology of the trees and compare them to the tree phenology, focusing on bud burst, flowering and juvenility. Over the four-year time-course, leaves (inside and outside of the canopy) and buds (axillary and terminal) have been collected every 3-5 weeks and sent to the University of Queensland for further processing as described above.

Molecular experiments

The results obtained with the time-course experiments allowed design of a finer series of experiments aimed at testing the response of physiological genes in response to different conditions/treatments. The aim of these experiments was to investigate the physiological processes underlying the impact of different factors on tree growth and development.

Functional-structural plant modelling

The modelling component of the project was designed to help integrate the architectural, developmental, physiological and molecular aspects of the research, in order to provide insights into the key research components of avocado, macadamia and mango orchard productivity. The underlying framework for this work is called functional-structural plant modelling (FSPM, Vos et al. 2010), which produces virtual plants, whole or in part, (Room et al., 1996) via simulations of plant development and growth with both mechanistic and empirical inputs, that can be used to run virtual experiments.

Physiological crop models are an important part of research for farming systems. These models provide a way to conduct experiments that would be difficult or costly to run and allow novel management practices and plant growth responses to be studied in a meaningful way. However, underlying assumptions of uniform canopy structure used in most such models do not apply well to orchard canopies. Functional-Structural Plant Models (FSPM) can provide a more complete and explicitly modelled 3D world and so tackle a class of problem where plant physiology interacts with plant structure (including biomechanics) and spatial distribution of resources (including the light environment). Novel cultural practices, especially in long-lived fruit and nut trees, can be manipulated and tested against current practices in a timely manner, even allowing for the time required for model development. FSPM was developed in response to the need for better understanding of the role of plant architecture (structure) and physiology (function) and how these interact to influence plant growth.

The central computer tools in this work are the software platforms from the University of Calgary called Vlab (Prusinkiewicz et al., 2000) and L-studio (Karwowski and Prusinkiewicz, 2004), which implement the plant-modelling languages cpfg and lpfg, based on the formalism of L-systems (Lindenmayer 1968, Prusinkiewicz et al., 1988) have allowed the exploration of a wide range of functional processes including self-organisation, carbohydrate partitioning, hormone flux, plant development at the cellular level, and responses to management.

L-systems are parallel rewriting grammars that are used to model biological systems where actions occur simultaneously across the developing structure. An L-system is defined by an alphabet of symbols representing plant components, a starting string or axiom, and set of rules that define the production of new elements such as internodes, leaves and flowers. The symbols are arranged in strings, with the sequence of symbols defining their position and connectivity in the plant structure. Each of the symbols can carry information (such as the dry weight of the fruit, area of the leaf) and interact with a simulated environment. Events such as bud break can be mediated through these interactions and can be applied in a deterministic or stochastic manner depending on our understanding of the process and the needs of the modelling.

This technology is applied through a collaborative spiral, mixing virtual and real-world experiments, reinforcing and extending the physiological hypotheses developed by field researchers through application of the models to more situations than it is economically feasible to implement in the field. In addition, models can be used to “experiment” with simulated data that cannot be easily measured in the field, for example, incident light at individual leaves, i.e. canopy light distribution. In the context of this project, we focus on whole plant responses in conditions of adequate water and minerals and no pest pressure, but with changing local temperature and light conditions.

The modelling team has been comprised of Dr Jim Hanan (QAAFI), Dr Neil White (DAF), and Dr Inigo Auzmendi and Dr Liqi Han (QAAFI project research fellows). In addition, two of the project PhD students, Dr Anahita Mizani and Dr Ming Wang, incorporated modelling components in their research work. The chief collaborators on the field work side were Dr John Wilkie and Helen Hofman (DAF, macadamia and avocado), and Dr Ian Bally and Dr Paula Ibell (DAF, mango), along with several DAF technical staff, UQ summer and winter research students, as well as grower input. In addition, international collaborators have included Prof Prusinkiewicz and Dr Cieslak (U Calgary), Dr Alla Seleznyova and Dr Grant Thorp (New Zealand Plant and Food Research), Prof Dejong (UCDavis), and Dr Costes and Dr Pallas (INRA, France).

The modelling effort was initially focused on the KRCs of the project (architecture, light, vigour, and crop load) with architectural development modelled for all three crops, and the other aspects targeted in separate crops, the idea being to integrate the different aspects over the course of the project by re-parameterizing according to species.

Collection of underlying hypotheses and assumptions for the different KRCs (Appendix 13) made it apparent that carbon allocation should underlie models in all areas. The focus of research was shifted to development of carbon allocation models at different scales appropriate to different questions. Three levels of abstraction/scale were indicated:

1. Self-organising model at canopy scale; likely to be best for rapid assessment of planting density and orchard-scale management effects, particularly related to light.

2. Local carbon pool driving flushing shoot and autonomous units carbon allocation model (AUCAM); useful for local scale processes, such as crop load and responses to management such as fruit thinning. It has been used for aggregation to tree canopy and then orchard scale, simulating growth and yield with virtual trees.

3. Carbon transport-resistance allocation model (CTRAM) at internode/leaf scale; likely to be the best for understanding interactions of carbon allocation, and growth processes controlled by hormones or genetic regulatory networks, particularly in response to management and light environment.

In the self-organising approach (White and Hanan, 2016), the activity of terminal buds is modelled using L-Systems based on the approach of Palubicki et al. (2019). This uses a simplified shade model to determine the availability of light on an individual leaf basis, accumulates it through the branching structure and then distributes the resulting “resource” based on demand and structural parameters. If enough resource reaches a bud it grows out into the nearest open space. Underlying parameters and processes can be tuned against more detailed models or against aggregate data for a canopy, such as light interception measurement.

An orchard scale macadamia model was created to explore aspects of canopy management and light interception. This approach relies on a relatively small set of parameters to determine specific structural aspects, e.g. leaf size and shape; number of leaves in a whorl; branching angles, but other aspects such as bud fate and growth of new metamers are determined by the environment, principally the availability of space and light. The latter are controlled by a set of parameters whose values are validated against field data. This is a much larger set of parameters that relate to the accumulation of vigour and light, but others control the way that light is reduced within the canopy, the sphere of influence that one plant part has on another and the way in which decisions are made in terms of the fate of buds.

In the simulation of growth and yield with virtual trees (Appendix 14), photosynthate is accumulated on a per leaf area and light availability basis and distributed based on potential growth-demand of individual organs to drive the development and growth of a flushing shoot. Initially we designed a simple, generic whole-shoot carbon pool model of local shoot and fruit growth considering measured solar radiation and temperature, as a means of integrating our key research components of vigour, architecture, light and crop load (Auzmendi et al., 2017).

We added the complexity of a young tree canopy structure and included stem growth to simulate size at harvest of individual fruits in a whole tree assuming carbon autonomy between shoots. We used this virtual tree to simulate the effects of different training systems, crop loads, light availability and warmer temperature on fruit size distribution, and discuss them from a theoretical point of view (Auzmendi and Hanan, 2018).

We designed and implemented programs to read canopy structure data measured in the field and represent them in our modelling software (Auzmendi and Hanan, 2019).

We proposed a new modelling method for simulating carbon allocation within the plant considering autonomy at branches of different ages in a tree (autonomous units carbon allocation model, AUCAM) and used it to design a simple functional-structural macadamia model to simulate tree and fruit daily growth during one growing season. These simulations were employed to explain some of the results of previous experiments, as well as to investigate the autonomic scale of a young macadamia tree measured in the field (Auzmendi and Hanan, In press).

Considering 2-year-old branches autonomous with regard to carbon, we simulated yield and light interception in young macadamia trees growing in an orchard environment with a range of different planting densities, tree size and shapes. These results were discussed and compared with previous experiments (Auzmendi and Hanan, 2020).

In the CTRAM approach (Appendix 15, Seleznyova and Hanan 2017, 2018) photosynthate production of individual leaves is simulated, typically using a ray-tracing program such as *QuasiMC* (Cieslak et al. 2008) to determine PAR availability. The photosynthate is distributed from these sources to the eventual sinks (growing leaves, shoots and fruit), considering the connectivity of the structure, the resistance to flow in each internode, and relative sink strength. Among our three approaches, this has the most physiologically detailed parameters, some of which need to be estimated computationally, in particular the transport resistance parameters within internodes. This is also the most computationally expensive approach. High-performance computing techniques (Appendix 17) allow application at scales from tree canopy to orchard.

The question of why/how flushing stops and reasons for biennial bearing are target processes that could be investigated with these systems. We can hypothesize connections with root growth, carbon reserve levels, and/or hormonal and genetic-regulatory processes affecting the interplay between fruiting and flushing, and model these in abstract ways to help determine appropriate real-world experiments that may shed light on these questions.

Modelling of light distribution at a canopy scale is very computing intensive so a rapid canopy shape prototyping system referred to as CanopyShapes was developed (Appendix 16). This does not utilise L-Systems developmental aspects but does set up the tree and orchard structures to interact with the light environment model QuasiMC. This model shared much of field and laboratory data used to create the self-organising model: leaf spectral properties; leaf arrangement; light extinction within a canopy and rate of photosynthesis. The CanopyShapes model allows rapid prototyping of different shapes for light distribution testing, without the time-consuming aspects of a model incorporating tree growth and management.

The original QuasiMC ray tracer was successfully parallelised by Liqi Han and Jian Cao to its high-performance version – HP-QuasiMC (Appendix 17), which has been deployed to UQ’s computing clusters and Australia’s Raijin Supercomputer (through collaboration with the Research Computing Centre). Given 1000 cores, the running of a virtual orchard light experiment would take the original serial ray-tracer 6 days while our high-performance version took no more than 26 minutes.

Following the significantly increased computing efficiency (with no sacrifice of accuracy) enabled by HP-QuasiMC, a mangoL tree/orchard simulator was then developed by Liqi Han using a mango tree dataset from Anahita Mizani (Mizani, 2020) as its first instance. In a number of preliminary virtual experiments, the mangoL model has demonstrated its configurability for pre-evaluation of mango orchard designs (for example, with different planting densities, row orientations, and unconventional orchard layouts) and to help optimise pruning and training practices towards the maximisation of light absorption and carbon productivity (Appendix 17).

The process of building and refining the models will be instrumental for developing new hypotheses and ideas that can challenge our current understanding of the production systems. Progress for individual species is detailed in Appendix 21, which describes work by our graduated PhD students, Dr Anahita Mizani, Dr Ming Wang and Dr Ben Toft. Dr Wang’s work also incorporates ideas from the ecologically based pattern-oriented modelling approach to refine methods for parameter estimation, and model verification and validation based on data collected at different scales (Wang et al. 2016, 2018).

In the area of international collaborations, several important links have been developed, in addition to the link with Dr Alla Seleznyova for CTRAM modelling described above. A pilot project for improvement of computational techniques for modelling light interception at an orchard scale using High Performance Computing was undertaken in collaboration with Dr Liqi Han, Weifang University, China and Dr Evelyne Costes at INRA, France (Han et al., 2017) based on apple data. This led to Dr Han joining our group and refining the techniques with visiting scholar Jian Cao (Appendix 17).

Dr Ian Bally and Dr Paula Ibell initiated a future modelling collaboration with the CIRAD mango group run by Dr Frederic Normand in Reunion. This was followed up by a meeting at the ISHS Modelling Symposium in Montpellier, France in June 2015 between Dr Jim Hanan and associated French researchers lead by Dr Frederick Boudon. Our more detailed carbon models are a good complement to their multi-scale modelling architectural models using Markov chains.

Collaboration with Evelyne Costes’ group at Supagro in Montpellier was further developed through visiting scientist Benoit Pallas, who developed a model to investigate biennial bearing (Pallas et al. 2017) using apple data in the first instance.

Extension and Communication

Extension and communication activities encompassed communication between researchers within the project and extension of results and activities to industry. Early in the project we identified that for this large project, which was being undertaken across three crops by researchers from diverse scientific disciplines and dispersed geographically, communication within and external to the project would be critical for success. DAF extension officers, first Peter Rigden and later Bridie Carr, were allocated to coordinate project communication activities.

Within project communication

One of the potential benefits of this large project across three crops, was that the researchers from the different crops undertaking related activities with similar aims would be able to learn from each other's experiences. The main aim of the within-project communication, therefore, was to keep the research team updated on the aims and progress of the research in each crop and in the modelling and molecular components. The major activities were undertaken to achieve this:

- Annual team meetings were held where the researchers presented their recent work and intended follow-on work. These meetings were held at locations where major project activities were being undertaken so that the entire team could view these key field and laboratory activities as part of the meeting.
- Team webinars were held several times per year. These webinars were another opportunity to update the researchers on work being undertaken in the project and on any upcoming events.
- A project management group was established that consisted of the leaders of the different scientific disciplines within the project, management representatives and the DAF project communications coordinator. The purpose of the management group was to coordinate major project activities and to ensure all groups were up to date with new developments.

Industry extension

The primary intention of the project was to undertake long-term strategic research. However, a high priority was placed on extension throughout the entire project. One reason for this was that we realised the importance of bringing industry along on our intensive orchard systems journey from the beginning so that they could see the benefits and challenges of intensive systems throughout the life cycle of the orchard systems. Additionally, a range of short-term project outputs and outcomes were relevant for the conventional orchard systems of the focus crops. Extension activities consisted of:

- Annual field days held in the major Planting Systems and Rootstock Trials
- Conference and field day presentations of project results
- Industry bulletin articles
- Macadamia and avocado trial advisory group meetings.

Figure 3. The avocado Planting Systems Trial Advisory Group - John Wilkie, Chris Searle, Simon Grabbe, Simon Newett, Laurie McCloskey, Lachlan Donovan, Helen Hofman and Jarrad Griffin.

Outputs

The major project results are summarised below. The detailed reports are contained in the attached Appendices or in the previously published works listed in Appendix 1.

Systems Trials

Avocado Planting Systems Trial

Tree height in the high-density systems increased more rapidly than in the medium and low-density systems, presumably due to the support of the trellis. From one year after planting there were significant differences in canopy volume/tree, with the low and medium density canopy volume/tree significantly greater than the high-density canopy volume/tree. The high-density and to a lesser extent, medium-density systems, filled their allotted space rapidly. The canopy volume/hectare of the high and medium-density treatments has not increased much since 2016/17, whereas the low-density treatments have continued to increase in canopy volume/hectare over the entire period of the trial. The canopy volume for 'Ashdot' trees has generally been smaller than for 'Velvick' trees, although in 2018/19 the difference was not statistically significant (Appendix 2).

Yield/tree decreased with increasing tree density after the first crop, as would be expected given the effect of tree density on canopy volume/tree. In the first cropping season, 2015/16, the increased planting density in the high-density treatment led to greater yield/hectare compared with the medium and low-density systems. From the 2016/17 season on, however, the increased planting density in the high and medium density treatments did not compensate enough for smaller yields per tree to produce greater yield/hectare than the low-density systems. In fact, by the fourth cropping season, 2018/19, the yield/hectare of the high-density systems was significantly lower than that of the medium and high-density systems. From the second cropping season onward, the yield/tree of 'Ashdot' rootstocks has been significantly greater than that of 'Velvick' rootstock.

Mean fruit weight, an important commercial fruit quality characteristic for avocados, has generally been large in the trial so far. There has been no effect to date of tree density on mean fruit weight, however, 'Ashdot' rootstock has consistently produced fruit with a significantly greater mean weight than 'Velvick'.

Figure 4. High density central leader trees supported by trellis in the Avocado Planting Systems Trial a little over two years after planting (upper) and almost five years after planting (lower).

Mango Planting Systems Trial

We have demonstrated that intensification of mango orchards is possible with positive outcomes in the first six years of an orchard's life. Increasing plant density in association with alternate tree training and precocious varieties has lifted yields early in the orchards' development compared to conventionally trained trees planted at the lower densities common in the Australian mango industry. In the sixth year after planting, annual yields varied significantly between the trial orchard systems. At the high planting density (1,250 trees per ha), when 'Keitt' was espalier trained, it was the most productive variety with a yield of 49.15 t/ha, which was 334% higher than the low density conventionally trained trees (208 trees per ha) with 14.68 t/ha. Further details of productivity information in the mango planting systems field trial including a review of fruit quality and pack outs at 5 and 6 years after planting are presented in Appendix 12. Other outcomes from the mango Planting Systems Trial are reported under the headings below.

Figure 5. Mango Planting Systems Trial high density espalier Calypso (upper) and Keitt (lower) supported by vertical trellis leading up to harvest.

Macadamia Planting Systems Trial

Canopy volume increased over the five years of the trial on both a per tree and per hectare basis (Appendix 4). There was no difference in canopy volume/tree between tree density treatments until four years after planting when the low and medium density treatment tree canopy volumes became larger than the high-density treatment trees, with the differences becoming larger five years after planting. Across the five years of the trial, canopy volume/hectare increased with tree density and at five years after planting the canopy volume per hectare of the high-density treatments was greater than the medium density treatments which was in turn greater than the low-density treatments. Although not comparable statistically, the canopy volume per hectare of the high-density trellis trees appeared to be greater than that of the high-density trees in the main trial.

Overall, there were three main treatment effects on yield during this early orchard phase of the trial. First, nut in shell (NIS) yield/hectare increased over time as the orchard developed from planting. Variety ‘A203’ and ‘741’ produced their first, albeit small, crops two and three years after planting, respectively. The first commercially relevant yield for ‘A203 was three years after planting (2017) with 2.3 t NIS/ha for the high-density treatment, increasing to 4.9 t NIS/hectare five years after planting.

Second, there have been strong significant effects of planting density on NIS yield/hectare. The higher-density treatments for both varieties have tended to have greater yield/hectare than the lower density treatments. For example, in 2019, the high-density ‘741’ treatment produced approximately 2 t NIS/hectare compared with 1.1 t NIS/hectare for the low-density treatment, and the high-density ‘A203’ treatment produced 4.9 t NIS/hectare compared with 2.9 t NIS/hectare for the low-density. The high-density trellised plot, although not directly comparable statistically to the main trial, showed both earlier yields and higher yields/hectare than the high-density treatments in the main trial.

Third, the yield of ‘A203’ has generally been greater than for ‘741’. For example, in 2018, the yield/hectare of ‘A203’ ranged from 1.6 to 2.9 t NIS/hectare in the main trial and for ‘741’ between approximately 0.5 and 1.5 t NIS/hectare. The difference in yield/hectare for each density to date has been more dramatic for ‘A203’ than for ‘741’.

Figure 6. High density central leader ‘A203’ in the macadamia Planting Systems Trial approximately 18 months after planting in 2015 (left) and five years after planting in 2019 (right).

Macadamia high-density remediation trial

Selective pruning significantly increased NIS yield/hectare compared with the industry standard mechanical hedging and topping, in this trial within a commercial high-density macadamia orchard (Appendix 5). The hedged and topped treatment produced a mean of 3.89 t NIS/hectare across the three years of the trial and the selective pruning treatment produced 5.42 t NIS/hectare. There were indications that the application of the growth regulator uniconazole was able to reduce the negative impact of mechanical hedging and topping on yield with the NIS yield/hectare of the hedged and topped trees sprayed with uniconazole significantly greater than the hedged and topped trees, across the three years of the trial. However, the kernel yield/hectare of the hedged and topped treatment with uniconazole was non-significantly greater than the hedged and topped trees.

NSW macadamia selective pruning and tree height control trial

Annual selective pruning was able to maintain tree height at approximately six metres across the five years of the trial (Appendix 8). After four years of annual selective pruning tree height control, the un-pruned trees were 1.1 m taller than those that had been controlled in height. There were no significant differences in yield between the annual selectively pruned and un-pruned trees until the season following the pruning of the previously un-pruned trees down to six metres, when the yield of the previously un-pruned ‘816’ trees was significantly lower than the annually pruned trees. There was no significant difference in yield/tree between the annually pruned and previously un-pruned ‘246’ trees in the final year although a severe drought had caused a yield drop to approximately 2 kg NIS/tree from approximately 12 kg NIS/tree in the previous year, which likely affected the results.

Tree vigour

Managing mango vigour and canopy size through tree training and planting density

Orchard canopy volumes measured in 26 to 30-year-old commercial ‘Kensington Pride’ orchards in North Queensland were between 5,588 m^3/ha and 18,519 m^3/ha and in 7-8-year-old trees were between 1,895 m^3/ha and 3,157 m^3/ha. In the mango Planting Systems Trial, orchard canopy volumes in 5-year-old trees planted at high density, were between 3,250 m^3/ha and 5,500 m^3/ha depending on variety, a 1.7 to 2.9 times increase in age equivalent orchard canopy volume (Appendix 10). By six years of age, tree canopy volumes in the mango Planting Systems Trial were generally lower in the espalier-trained trees compared to single leader or conventional trained trees, however the relationship was not always statistically significant and was dependent on variety.

For example, tree canopy volumes in ‘Calypso’ were similar in all training and planting density systems. However, when an orchard wide approach was taken, orchard canopy volumes (m^3/ ha) increased significantly at the high planting density to be, on average, 4.2 times higher than the low-density conventionally trained plots. Orchard volumes were influenced by each combination of variety, planting density and training system. The trade-off between lower tree canopy volumes at higher planting density is overcome at the orchard level due to the increased number of trees. Increasing the orchard canopy volumes through high-density planting was a major driver for other parameters such as light interception and yield. The combination of these factors led to large increases in yield in the high-density orchard system much earlier in the orchard’s life (Appendix 10).

Trunk cross-sectional areas of trees have been used as an indicator of tree size, volume and biomass; however, these relationships can be less reliable when trees are pruned and as they age. Our investigations of trunk cross-sectional areas in six-year-old trees in the mango Planting Systems Trial found a similar relationship to tree volume, with conventional low and medium density trees having the largest trunk cross-sectional area followed by single leader trees at medium density and espalier trees at high density with the smallest trunk cross-sectional areas. The single leader and espalier tree training system successfully reduced individual stem growth resulting in smaller volumes, a critical factor for high-density intensive orchard systems.

Mango vigour management through rootstocks.

A replicated field trial on Walkamin Research Station was established between 2014 and 2016 to evaluate 97 candidate vigour-reducing rootstocks under two scions. At the end of this project, trees in the trial are between four and six years old. The candidate rootstocks have induced a range of tree sizes in the two scions, with some rootstocks showing scion specific effects and others affecting both scion varieties similarly. Early indications show that the best vigour-reducing rootstocks for each scion had statistically similar or better yield per canopy volume (m^3) than when ‘Kensington Pride’ was used as the rootstock. We believe that selected rootstocks will be a useful technology for managing scion vigour in intensive mango orchard systems. A short list of suitable rootstocks is currently being developed from tree size and productivity data to progress the selection of the most suitable rootstocks for mango. In the next project, the shortlisted rootstocks will be evaluated for their performance at high planting density and with additional scions. Appendix 9 provides details on the trial design and performance of rootstocks to this point. We expect to have confirmed a shortlist of rootstocks for further testing by 2021 and to plant additional field trials by 2022.

Figure 7. Grafting of mango rootstocks for the vigour managing rootstock trial (upper) and an example of differing tree size in the trial due to rootstock (lower).

Avocado vigour management

The avocado Planting Systems Trial compared two rootstocks, the industry standard ‘Velvick’ and the less popular ‘Ashdot’. Over the five years of the trial, ‘Ashdot’ consistently had smaller canopy volumes and trunk cross-sectional areas than ‘Velvick’ (Appendix 3).

In the Phase 1 rootstock trial there were significant effects of rootstock on rootstock and scion (trunk) cross-sectional area, and the number and cross-sectional area of pruning cuts in both 2017/18 and 2018/19. In the 2017/18 season there were no significant effects on canopy volume and in the 2018/19 season all of the trees were large enough to be pruned to their maximum allowable dimensions and so canopy volume was no longer measured. There was no significant effect of rootstock on yield in 2017/18, but there was in 2018/19 with ‘Ashdot’ rootstock having a significantly greater yield than the other rootstocks (Appendix 3).

In both the phase 1 rootstock trial and the Planting Systems Trial, even the rootstocks on the lower end of the vigour scale, such as ‘Ashdot’ were ultimately too vigorous for the high-density system with intensive pruning required, which in turn induces vigorous regrowth.

Figure 8. Two-dimensional tree training treatment in the Phase 2 Avocado Rootstock Trial in January 2020.

Figure 9. The Phase 1 Avocado Rootstock Trial during flowering in spring 2018

Macadamia vigour management

A range of work has focused on developing understanding and tools for macadamia orchard vigour management.

The macadamia rootstock component generated phenotypic data on the effect of rootstocks on scion productivity, precocity and vigour. Twenty-seven rootstocks were genotyped using DArT markers, and the genetic diversity is presented in Appendix 6. Rootstocks will be propagated after final selection in 2024.

Based on a study of vascular systems, we concluded that the size of xylem vessel can explain the variability in plant size. The three varieties showed distinct differences in height ($P < 0.001$). Change in tree height was correlated with the xylem vessel characteristics of mean vessel area ($R^2 = 0.57$, $P < 0.001$), pith size ($R^2 = 0.70$, $P < 0.001$) and total additive vessel area ($R^2 = 0.41$, $P < 0.01$). Further investigation on large number of genotypes and the mechanism of xylem-vessel mediated vigour control is required (see Toft et al., 2019 for details).

In the macadamia high-density remediation trial, shoot growth following pruning was greater in mechanically hedged and topped trees compared with the selectively pruned trees. It is believed that the greater shoot growth from the decapitated stems in the hedged and topped trees led to greater competition with fruit set than in the selectively pruned trees. In two out of the three years of the trial, the growth regulator uniconazole significantly reduced shoot growth following mechanical hedging and topping and there were indications that this reduced shoot growth led to reduced competition with fruit set (Appendix 5).

Orchard light environment

Avocado

In the conventional avocado orchard light-relations study, we described relationships between canopy volume/hectare and total light interception, total light interception and yield/hectare and canopy volume/hectare and yield/hectare (Wilkie et al., 2019). As canopy volume/hectare increases from low levels, total light interception increases almost linearly up to approximately 30,000 m^3 canopy/hectare, above which the rate of increase in total light interception slows and eventually begins to plateau, presumably due to selfshading within the canopy. Yield/hectare increases strongly as total light interception and canopy volume/hectare increase from low levels up to approximately 80% light interception and 30,000 m^3 canopy/hectare, respectively. Yield/hectare then begins to plateau as total light interception and canopy volume/hectare increase further. At the highest levels of total light interception and canopy volume/hectare, there was some evidence that yield/hectare declined, although the statistical confidence for this was too low to state this with certainty.

In the avocado Planting Systems Trial, total light interception increased between planting and the final measurement in 2019. The total light interception of the high-density treatments was greater than the medium-density, which were in turn greater than the low-density treatments. In 2016, two years after planting, yield/hectare was significantly related to total light interception. By 2017, however, the relationship was no longer significant, indicating that factors other than total light interception were strong drivers of yield/hectare amongst the density and rootstock treatments (Appendix 2).

Light distribution experimentation was also carried out in the avocado Planting Systems Trial (Appendix 2). One set of measurements measured light and tree responses along the periphery of the canopy. There were no effects of tree density on peripheral canopy PAR. This may be because in the two years this work was undertaken, the low-density trees were far from filling their canopy space and so were still well irradiated. PAR and tree responses were also measured in horizontal transects through the canopy from one inter-row to the other through the trunk. There were significant effects of tree density on PAR levels within these transects, with high and medium-density treatments having greater levels of PAR within the canopy than low-density treatments. However, the work does not suggest that there is a direct relationship between PAR availability and fruit set. This is of course contingent on there being sufficient PAR to retain flowering terminals, because where there are no flowering shoots there can be no fruit.

Mango

Light interception

A baseline study of light interception in current ‘Kensington Pride’ orchards in Queensland found that light interception generally increased with tree age and as canopy volume increases. Light intercepted reached its maximum at around 67% of full sunlight, in 26-31-year-old trees with average orchard canopy volumes of between 6,186 m^3 and 7,524 m^3/ ha. Light interception did not increase beyond this regardless of increased canopy volume per hectare. Comparing light interception with yield found they were significantly correlated, indicating the light captured by orchard canopies is a significant driver of orchard productivity. Further details of the baseline light interception study are presented in Appendix 10.

Orchard light interception increased much more rapidly over time as planting density increased and was commensurate with the rapid increases in canopy volumes in the higher density systems. At five years of age the light interception of high-density orchards, had reached 40% of full sunlight with a canopy volume of 3,000-7,000 m^3/ha and achieved between 20 and 40 t/ha. This is significantly higher than the 15% light interception in conventional ‘Kensington Pride’ orchards of the same age, in the baseline study. We have shown that the rapid increases in canopy volumes of high-density systems enables them to capture and use more sunlight energy earlier than low density orchard systems which is a major factor in the large increases in yields early in the life of high-density orchard systems.

Mango Light distribution

The interaction of tree canopies with sunlight is not just a matter of the total light intercepted, but also the way that sunlight is distributed throughout the canopy. In commercially grown ‘Kensington Pride’ trees up to 30 years old we found highly variable light distribution between canopies with different pruning systems. The percentage of light transmitted into canopies increased as trees aged and grew higher, despite the different pruning styles. Young immature trees up to 1.5 m high had the lowest light penetration into their canopies at <47% of full sunlight. The highest light transmission into the canopy was measured in mango trees between 3.8 and 4.3 m high. We also found large variation in light levels in within individual canopies. Light transmission was at its highest in the top 75% of the canopy dropping to its lowest between the ground and 25% of canopy height. There was an overall the tendency for light transmission to increase with increasing distance from the trunk to the edge of the canopy. Light levels were highest in the outer northern and southern aspects of the canopy but tended to be higher in the eastern rather than the western side of any given position of the canopy.

The pruning methods used in commercial ‘Kensington Pride’ trees also significantly affected the distribution of light within canopies, with light transmission generally increasing as the heaviness of pruning increased. In non-pruned and window-pruned trees, light transmission ranged between 8% and 87%. Commercially pruned also displayed a wide variation if light distribution, between 21% and 90% demonstrating the difficulty in getting light into mangoes thick vigorous evergreen canopies. In the heavier pruned trees where light transmission was greatest, yields were significantly lower, suggesting that there are net drawbacks from heavy pruning that are independent of light levels. Fruit quality was not affected by the pruning methods.

In the intensive mango orchard systems of the Mango Planting Systems field trial, we found light distribution was less variable with more light reaching the inner areas of canopies in the single leader and espalier trained trees. The most even light distribution was seen in the espalier trained trees planted at high-density with light distribution in the mid canopy height between 38.6% and 68.5%, compared to conventionally trained trees with light distribution between 23.2 % and 50.6%. Further details on light distribution within mango canopies and associated effects are presented in Appendix 10.

Macadamia

Similar to mango and avocado, the total light interception work in the macadamia Planting Systems Trial showed that total light interception was greater for high-density systems than medium-density systems, which in turn had greater total light interception than the low-density systems, during this early orchard phase. In 2019, five years after planting, the high-density systems were intercepting approximately 69% of PAR compared with 29% for the low-density systems. Although not comparable statistically, the high-density trellis system intercepted 81% PAR in 2019. Tree density, most likely due to row width, also affected the relationship between canopy volume/hectare and total light interception, with high-density systems intercepting more PAR for the same canopy volume/ha than medium-density systems, which in turn intercepted more PAR than the low-density systems (Appendix 4).

In 2017, 2018 and 2019, there were significant relationships between total light interception and NIS yield/hectare. In 2017 and 2019, NIS yield/hectare increased linearly with total light interception, whereas in 2018, there appeared to be a plateau in yield at the higher levels of light interception. In all three years there were differences in the relationship between varieties, with ‘A203’ having a greater NIS yield/ha for any given level of light interception than ‘741’. This indicates a greater efficiency of conversion of PAR to NIS yield in ‘A203’ compared with ‘741’. The reasons for this are unclear.

Light distribution was measured in horizontal transects through selected treatments in the Planting Systems Trial. In winter 2017, ‘A203’ high-density central leader trees had greater levels of PAR within the canopy than the high-density conventional trees, which were being mechanically hedged. There was no difference in PAR level within the canopy between the high-density conventionally pruned ‘A203’ and the low-density conventionally pruned ‘A203’, even though the high-density treatment had a significantly greater total light interception than the low-density. This suggests within canopy light distribution should be greater for high-density compared with low-density systems at canopy maturity.

Crop load

Avocado

The initial avocado crop load work aimed to develop basic whole-tree crop load understanding (Hofman et al., 2018). We found that yield efficiency increased significantly with inflorescence density. There was a significant relationship between inflorescence density and yield efficiency. Yield efficiency increased linearly at the lower and moderate levels of inflorescence density, however, it was unclear it the relationship remained linear at the very highest levels of inflorescence density or if yield efficiency began to plateau. There were a range of other effects of crop, with reductions in fruit size, dry matter and vegetative growth as yield efficiency increased.

The follow-on work aimed to improve spring fruit set by reducing competition with vegetative growth and by increasing the number of fruiting positions (Hofman et al., 2018). There were no treatment effects in the first year. In the second year, partial tip-pruning of the spring flush and partial removal of the spring flush plus ‘Cytolin’ increased spring fruit set relative to the control. The effect was presumably due to reduced competition between fruit and shoot growth. However, the gains in fruit set at end of spring were largely lost over summer due to increased fruit drop over the summer fruit drop period.

Crop load carrying capacity experimentation was undertaken over several years in the avocado Planting Systems Trial and has provided a substantial contribution to understanding the yield responses of the different treatments (Appendix 2). In 2018/19 there was no effect of density treatment on the number of flowering terminals/cm^2 branch cross-sectional area (BCSA), however high-density trees had fewer flowering terminals/tree than medium and low-density trees due to a smaller tree size. However, the high and medium-density systems had more flowering terminals/hectare than the low-density systems. In several of the cropping seasons, the medium and high-density treatments had significantly lower rates of fruit set and retention than the low-density systems, ultimately leading to poorer yield efficiency in the high and medium-density systems compared with the low-density systems from four years after planting. Competition between fruit and shoots for resources may be a significant factor in the poor fruit set during spring in the high-density systems, potentially due to re-growth from pruning.

Mango flowering and crop load management

The aim of the crop load research was firstly, to investigate flowering and crop manipulation as a tool for managing yield and fruit quality and secondly, to improve our understanding of the factors that influence crop load and how mango tree responds to different crop loads.

When we investigated flower thinning as a tool to manage yield and fruit quality in ‘Calypso’ and ‘Kensington Pride’, flower thinning intensity (% of flowers removed), had little or no effect on tree yields over a wide range of thinning intensities. We concluded that flowering is not always the key factor affecting yield in mango. ‘Kensington Pride’ trees compensated for fruit thinning in a similar way to flower thinning. Only multiple or late thinning of fruit showed an increase in the number of fruits per panicle, average fruit weight and percentage of dry matter. Fruit blush, colour and TSS were not affected by crop thinning. Early fruit thinning was not effective, as any benefits were lost due to mangoes high fruit drop at early and mid-fruit development.

Resource allocation and limitations within the tree play a major role in shaping final crop load or yield of a tree. Trees compensated for higher thinning intensity by retaining more fruit per inflorescence and increasing fruit size to maintain yield. In ‘Calypso’, yields were only significantly reduced when the thinning intensity was above 80% (Appendix 11). Fruit TSS (Brix^o) and fruit blush colour were not significantly affected by flower intensity in the first year but fruit TSS was higher with increased flower thinning in the second year for (Appendix 11).

Compensation for flower or crop load thinning is driven by the reallocation of photosynthetic carbon resources to the remaining fruit on other terminals. Very high thinning intensity exceeds the trees capacity to reallocate resources to remaining fruit as carbon is reallocated to vegetative growth which can compete with fruit for further carbon resources, limiting any increases in tree yield. In ‘Calypso’ this occurred when the flower thinning intensity was above 60%. In general, a floral density of 2 inflorescences per cm^2 of trunk cross sectional area was the approximate point where yield efficiency was maximised. This varied between varieties where the average yield efficiency (YE) of Keitt (0.8 kg fruit/TCSA) was greater than ‘Calypso’ (0.4 YE) which was greater than ‘NMBP-1243’ (0.2 YE) (Appendix 11).

The effects of flowering and fruit thinning on subsequent season's reproductive and vegetative growth were minimal. We did not find evidence of low inter-seasonal storage of carbon caused biennial or irregular bearing between years. The effect of weather and orchard management strategies on irregular bearing in mangoes may be greater than the effect of crop load of the previous year.

We found that there might be a potential to use flower and crop load thinning to manage yield and fruit quality, however we need to better understand the influences of seasonal variations in environment and management practices.

Macadamia

Macadamia crop load work in the Planting Systems Trial was aimed at assessing the crop load carrying capacity of the different systems and to identify the main factors limiting the development of crop load during early orchard life (Appendix 4). There were relatively few and small effects of tree density and tree training treatment on raceme density (flowering intensity; racemes/m^3 canopy volume) and yield efficiency (canopy fruiting density; kg NIS/m^3 canopy volume). One of our main tree training hypotheses for the trial was that limb bending would improve precocious flowering. Although the central leader trees (with and without bending) had significantly greater raceme densities than the conventionally pruned treatments at their respective densities in the trials first flowering in 2015, the amount of flowering and subsequent yield were not commercially relevant. There were some minor effects of tree density on crop load, but they were minor and inconsistent and overall there was little effect of tree density on raceme density and yield efficiency, even though there were large differences between tree densities in total light interception, indicating the high-density treatments are maintaining their conversion efficiency of PAR to fruit load even as they near their maximum level of total light interception. The most noticeable impact of the treatments was that of variety on early flowering, with 'A203' having significantly greater raceme densities throughout the trial compared with '741', leading to greater and earlier yield efficiency.

The primary limitation to the development of crop load in the Planting Systems Trial was poor early flowering. In 2016/17, 2017/18 and 2018/19 there were significant linear relationship between raceme density and yield efficiency. There was no sign of a plateau in these relationships in any of the three cropping seasons, indicating that raceme density was limiting the canopies ability to achieve their maximum yield efficiency. There was a significant effect of variety when added as a covariate to these relationships, with 'A203' achieving a greater yield efficiency than '741' for any given levels of raceme density. This indicates that there are factors in addition to the precocious flowering that led to the greater yield of 'A203'.

Macadamia crop load work also looked at the effect of competition with vegetative growth on fruit set and yield. In the high-density remediation trial, the vigorous vegetative re-growth induced by the mechanical hedging and topping led to lower yield than the selectively pruned trees, where the re-growth following pruning was far more moderate. It is likely that competition for resources between the re-growing shoots and developing fruit led to the lower NIS yield in the hedged and topped treatment (Appendix 5). We also investigated the relative timing of vegetative growth and fruit growth on the ability of individual organs to compete for resources by tip-pruning trees before and after anthesis to produce canopies with shoots and fruit growing at different stages relative to each other. We found that fast growing organs, whether they be fruit or shoots, were able to outcompete slower growing organs. The relative timing of organ growth affected the ability of the organ to compete for resources, because the growth rate of both fruit and shoots varies through the period of active growth. When shoots began growing before or around the time of anthesis, they had high growth rates and outcompeted setting fruit, leading to high rates of fruit abscission. When shoots began growing several weeks after anthesis, the rapidly growing fruit suppressed shoot growth rate and were able to effectively compete for resources, leading to lower rates of fruit abscission (Appendix 7).

Architecture

Avocado

Avocado provides significant challenges to high density systems in the complexities and variability of its branch architecture, including its terminal flowering habit, its indeterminate inflorescences, strong vertical growth tendency, a variable mix of sylleptic and proleptic growth, high levels of branch death and decline and the lack of branch renewal. Architectural studies over the course of the project have developed some understanding of the interactions of these factors and their implications for high density planting and identified some of the characteristics of shoots and inflorescences most likely to set and retain fruit. This includes the effect of determinant inflorescences on fruit set, the requirement for mature leaves for fruit retention and the inhibitory effect of the presence of fruit on localised bud burst and growth.

Mango

One of the aims of the mango architecture studies was to understand the differences in tree growth between varieties, training systems and planting densities and to determine the ability to moderate vegetative vigour and to optimise horticultural productivity.

Tree vegetative and floral growth varied significantly between varieties. 'Calypso' had more lateral growth units and less apical growth units than 'Keitt' or 'NMBP-1243'. There are also strong temporal variations in growth habit suggesting that, in addition to genetics, the environment and management can influence mango tree growth. Different architectural and growth responses between the three training systems can be partly explained by the ratios of growth unit orientation (horizontal, vertical or intermediate). Espalier trained trees had more horizontal than intermediate or vertical growth units. Single leader trained trees had similar numbers of horizontal and vertical growth units and conventional trained trees had predominantly intermediate or vertical growth units. These architectural differences between training systems contribute significantly to the tree responses discussed below. The technique of bending branches to the horizontal in the single leader and espalier training systems impacts tree growth by increasing the number and length of growth units following bending. However, trees with more lateral branching had their leaf area distributed more densely within the canopy and filled their allocated growing space in the orchard more effectively.

The rate of flowering (percentage of terminals flowering) was influenced by several factors. The number of growth units was not a significant factor on flowering, but growth unit orientation was. Horizontal growth units had a greater percentage of flowering terminals than vertical growth units. Varietal responses to flowering differed between seasons, with previous pruning, training system and crop load potentially affecting these trees. The combination of growth and management (training system and density) in the single leader training system resulted in the highest percentage of flowering per canopy volume as well as reduced tree size (trunk diameter and canopy dimensions). Despite smaller canopy volumes per tree in single leader trees grown at medium density, they had the largest percentage of their canopies flowering, higher number of vegetative terminal growth units and higher flowering per ha. Such outcomes demonstrate the interdependence and synergies of factors inherent in orchard systems that are not always apparent when studying specific factors alone.

Macadamia

Tree structure and architecture of variety ‘741’ and ‘A203’ were documented at a range of scales in the Planting Systems Trial. At the whole tree scale, trunk cross-sectional area and canopy volume (in some experiments non-significantly) of ‘A203’ was greater than for ‘741’. At the limb scale, ‘741’ had significantly more limbs/tree than ‘A203’ but the ‘A203’ limbs were larger, containing more growth units than those of ‘741’. At the growth unit scale, ‘A203’ growth units were longer, and the mean internode length was greater than those of ‘741’, however there was no difference in mean node number/growth unit. The mean growth unit angle from vertical was lower (closer to horizontal) in ‘A203’ than ‘741’ (Toft, 2019).

There was also a significant focus on identifying factors that affect precocious flowering in macadamia in the Planting Systems Trial detailed architecture assessments and analysis. We found that the likelihood of a growth unit flowering, significantly increased as growth unit length increased in variety ‘A203’, but there was no effect of growth unit length on flowering in ‘741’. There was also an effect of growth unit angle, with the likelihood of a growth unit producing a raceme increasing as the angle moved from vertical with the greatest likelihood of flowering at 150^{o}. The relationship was true for both varieties, however the maximum likelihood of flowering for ‘A203’ was much greater than for ‘741’.

Limb bending in mature macadamia trees significantly increased flowering when undertaken in late May, around the time of floral initiation, but not when undertaken in mid-February. Limb bending in young trees in the macadamia Planting Systems Trial that had not flowered or were still flowering inconsistently, had no effect on flowering. It may be that limb bending has no effect on early (precocious) flowering in macadamia or it may be that we observed no effect because we did not time the bending around the time of floral initiation (Toft, 2019). In both of these trials, limb bending resulted in decreased growth from the apex of the branch and increased lateral growth. When architectural relationships were developed from observations across genotypes, limb angle and raceme production was correlated, with greater raceme production as limb angle moved away from vertical. In the same work limb length, canopy volume and trunk-cross sectional area were all correlated with limb angle and were smaller as limb angle moved away from vertical (Toft et al., 2019). It therefore appears that shoot and limb angle both have a large impact on macadamia tree function, although manipulation of limb angle through bending had inconsistent effects.

Molecular/genetic regulation of floral initiation and juvenility

During the project, important discoveries have been made about the regulation of the tree phenology at the molecular level. Notably the experiments carried out revealed that the role of miR156 in vegetative identity was relatively conserved compared with other model plants. Contrastingly, the role of miR172 in promoting the transition to reproductive stage may be different between the small trees and other model plants (Ahsan et al., 2019a). It was also shown that the levels of phase change regulators in the scion is largely controlled by the scion, but importantly can be influenced by the rootstock leaves through the modulation of the miR156/miR172 pathway (Ahsan et al., 2019b). It was also determined that the AP1 and SOC1 genes could be used as markers of the reproductive phase (Ahsan et al., 2019a).

Gene expression data revealed that the molecular mechanisms involved in flowering such as FT, BRC1 or EBB1 are conserved in the small trees (Umair Ashan, thesis manuscript; Ye Gong, masters report). However, their regulation may differ, notably for macadamia for which the expression of FT in the axillary buds may be more important than its expression in leaves contrary to what has been published in other model plants (Appendix 21; Barbier et al., 2020). Molecular experiments also highlighted that alternate bearing in mango and avocado may, at least partly, involve carbohydrate partitioning (milestone report May 2018).

Functional-structural plant modelling

Macadamia

Self-organising models

An orchard-scale macadamia model was created to explore aspects of canopy management and light interception (Appendix 19) allowing pruning practices such as hedging, topping and removal of the central leader. The virtual orchard was planted with 7 m between rows and 4 m between trees and hedging was undertaken during the spring flush to create a 2 m alleyway. Light interception was 94% for unhedged canopies and 77.4% for hedged canopies. The model has been validated against fully digitised trees up to 2m tall. These results were similar to the values given in MacFadyen, et al. (2004).

CanopyShapes

The CanopyShapes modelling approach (Appendix 16) was developed with macadamia as the initial example, allowing efficient development and comparison of management canopy shapes and densities, targeting optimum light distribution. The high-density plantings, regardless of shape, showed a higher accumulation of carbon at ~18 g/ha/day for each 1 m^2 of effective leaf area compared to low density orchards which recorded ~14 g/ha/day. The approach can be used for other fruit and nut trees with relatively few changes.

Virtual orchards including light interception, tree growth and yield

An initial simple and generic whole-shoot carbon pool model of growth considering measured solar radiation and temperature was developed, focussed on principles underlying shoot and fruit growth and their interactions (Auzmendi et al., 2017).

The generic model was extended to incorporate young tree canopy structure with carbon autonomous shoots making it possible to simulate multi-factor experiments with different training system, crop load, light and temperature (Auzmendi and Hanan, 2018).

A program was developed to read canopy structure data measured in the field and represent them in our modelling software and was used to verify measured field data, carry out preliminary analysis, and use these visualizations for demonstrative purposes (Auzmendi and Hanan, 2019). They were employed as well to start simulations of plant growth with modified canopies for representing different management branch arrangements, i.e. standard and planar shapes (Auzmendi and Hanan, 2020).

A new, simple method was implemented to simulate carbon allocation based on assumptions usually made in field experiments. Simulations using this method in a virtual macadamia tree showed emergent properties that reproduced some of the effects that the within-tree scale of carbon autonomy has on the growth of fruit and canopy in the tree, e.g. organ variability within the tree, yield, leaf area and fruit load. Our simulations were employed to investigate and explain these effects, as well as to understand better the impacts that the scale of autonomy has on field experiments, using concepts like carbon demand and supply, as well as their ratio, which cannot be estimated in the field in a straightforward manner. These simulations indicate that assuming carbon autonomy at branch scale, i.e. two-year-old wood, showed the most realistic results for the macadamia tree that was measured (Auzmendi and Hanan, In press).

Growth simulations of a young macadamia tree assuming autonomy with regard to carbon for 2-year-old branches were able to reproduce the effects of tree size, shape and planting density on light interception and yield, mimicking the planting densities of our field experiments and extending the range to planting densities that have not yet been implemented in the field. Trends observed in the simulations with macadamia trees were emergent properties of the model that matched the conclusions of field observations and improved our understanding of concepts of planting density, and its effects on light interception and yield (Auzmendi and Hanan, 2020).

Mango

A temperature-driven model of mango flushing with varietal parametrisation was developed (Mizani, 2020). With measured canopy architecture as a base, this has been used to explore effects of different temperatures across a mango canopy, and within canopy light distribution.

A mango tree and orchard simulator, currently driven by a set of specific mango tree data (Mizani, 2020) but capable of using other datasets from different varieties and trial systems, has been developed to help investigate the impacts of orchard configurations (planting density, row orientation, unconventional layout and so on) and management practices (training and pruning systems) on light interception and distribution (Appendix 17). A highly parallel and scalable light simulator has been developed for fast-tracking the use of virtual experiments to help improve orchard designs and operations in a timely manner (Appendix 17). These tools can use other mango tree/orchard datasets and can be extended to simulate other fruit and nut trees by incorporating the appropriate structural or developmental model.

Avocado

A local pool model of the annual architectural development of avocado branch was developed and used to validate the use of Pattern-oriented Modelling approaches in FSPM (Wang et al. 2016, 2018, Appendix 18).

A new version of the CTRAM model was developed (Appendix 15), which converted underlying model parameters from the original electric-circuit inspired approach with photosynthetic source strength represented by electromotive force to a representation incorporating more realistic carbon concentrations (Seleznyova and Hanan 2017) and phloem/xylem water relations (Seleznyova and Hanan 2018). Initial parameterization of a CTRAM model for avocado flushing has been undertaken, still requiring details of leaf light reflectance before transport resistance parameters can be estimated.

Outcomes

Avocado

Apart from an increase in yield/hectare from the first crop of the high-density systems two years after planting, the high and medium-density planting systems have to date failed to deliver increases in yield/hectare compared with conventional low-density systems. In fact, five years after planting the high-density systems had significantly lower yield/hectare than the low-density conventional systems.

The medium and high-density systems filled their allotted space and had greater total light interception earlier in the life of the orchard, but apart from the very early benefit two years after planting, this greater yield potential was not converted into greater yield/hectare in the intensive systems. This occurred because the high and medium-density systems had lower rates of fruit set than the conventional low-density systems.

The reasons for the poorer fruit set in the medium and high-density systems are unclear. However, it is possible that greater competition between shoot growth and setting fruit is at least partly responsible. One hypothesis is that the intensive pruning required to maintain the desired canopy size of high-density systems may have led to excessive re-regrowth that competed for resources with spring fruit set. For the medium-density systems, the structural pruning to maintain a central leader tree structure may have had a similar impact.

Results from both the Planting Systems Trial and rootstock trial demonstrate that rootstock significantly affects productivity in high-density systems. In the Planting Systems Trial ‘Ashdot’ has consistently outperformed ‘Velvick’ and in the rootstock trial ‘Ashdot’ significantly outperformed all other rootstocks tested in the second year of cropping. Part of this is probably due to the lower vegetative vigour of ‘Ashdot’, but another important factor appears to be high levels of determinate inflorescences.

Despite the disappointing performance of the intensive systems that were trialled, the work has significantly improved our understanding of the factors underlying productivity in avocado. For example, orchard light environment work has identified optimum levels of total light interception and canopy volume for maximum yield/hectare. Light distribution studies have also provided us with an understanding of the effects of tree density on movement of light through intensive and conventional orchards. These light distribution studies have also provided understanding of the effect of PAR on growth and fruiting, with the somewhat surprising result that within canopy levels of PAR are of relatively minor importance to fruiting so long as they are sufficient to maintain leaf area and fruiting positions.

Crop load studies have reinforced the importance of sufficient flowering intensity to achieve the maximum crop load. They have also identified early fruit set as a critical point at which yield, in the central Queensland environment, is limited, with strong effects of competition with vegetative growth. Poor summer fruit retention has also been identified as a point where yield is limited, however, the factors affecting summer fruit retention are less well understood.

Architectural studies have identified the characteristics of shoots and inflorescences most likely to set and retain fruit. This includes the effect of determinant inflorescences in fruit set, the requirement for mature leaves for fruit retention and the inhibitory effect of the presence of fruit on localised bud burst and growth.

The work has clearly identified the importance of developing vigour management systems for intensive avocado systems. Excessive growth leads to direct competition with setting fruit, excessive shading and intensive pruning with likely subsequent indirect effects on fruit set. The currently available low vigour rootstock ‘Ashdot’ provided improved productivity for ‘Hass’ than the more vigorous industry standard rootstock ‘Velvick’, however this was still not sufficient to overcome the vigour of the ‘Hass’ scion. Our overall assessment is that we need to improve the combined vigour management systems (rootstocks, PGRs, pruning techniques) for intensive avocado systems to succeed.

This improved understanding has provided direction for a second phase of intensive systems trials.

Mango

Proof of the concept and early trials of Intensification of mango production have been successful and represent a truly novel approach to growing mangoes. Intensive orchard systems have the potential to sustainably increase the mango industry's productivity and profitability under the environment of increasing competition from global markets, higher labour, land and input costs.

Genetics or variety is a consistent factor in how mangoes respond to planting density, tree training, tree growth and crop load. Some varieties will be better suited to orchard intensification than others, and these are likely to require less maintenance, be more resilient to seasonal variations and to give more consistent high yields. Although most of the relationships between the major factors studied with growth and productivity were consistent across mango varieties, the differences in the scale and timing of these relationships will need to be reflected in management of specific varieties in intensive orchards.

Variety differences are mainly expressed in their architectures and by the morphology and topology of their growth units, variation in their growth cycles and their propensity to flower and fruit.   Some tree architectures are more suited to intensive production than others. Preferred architectures for intensification are inherently characteristic in some varieties, they can be induced by tree training or a combination of both. Trees with less apical and more lateral growth units have greater branch complexity, less annual vegetative growth and as such, are less vigorous, requiring lighter annual pruning. These trees also have a higher leaf area index, better light interception and distribution and use the area allocated for each tree in the orchard more effectively. This architecture essentially reduces energy spent on vegetative growth and directs more of it to flowering and fruiting. The single leader and espalier training systems induced the preferred architectural traits while reducing overall tree vigour.

Managing tree vigour is a key component of high productivity and longevity of intensive orchards. The mango industry currently uses annual pruning and the growth regulator paclobutrazol to manage tree vigour. The project has identified tree training, using branch bending, and rootstocks as two additional tools to manage vigour. Lower vigour varieties with optimised architecture, early flowering (precocity) and retention of multiple fruit per inflorescence may also contribute to early and consistent high yields.

Our crop load work has shown us that mango yields are strongly influenced by resource allocation and they can compensate for poor flowering or lower fruit set by increasing the number of fruit retained per inflorescence or fruit size. As with other traits yield efficiency varies between varieties and seasons, with an upper limit of fruit retention in mango of between 2 and 3 flowers per trunk cross sectional area.

In mango, this project has investigated multiple factors and how they contribute to growth and productivity, but much of the success has stemmed from studying both the individual factors and in combination in a systems approach that integrates the complex relationships between factors. Using this approach, we have identified how the fundamental physiology of mango varies from avocado, macadamia and temperate crops such as apple. This crop specific understanding is helping us customise successful management strategies for each tree crop in ways that are responsive to varietal and seasonal variations.

In the next project, our studies will continue to examine tree growth in mature cropping trees that have filled their allotted space in the orchard. This will provide a better understand the seasonal responses in growth and productivity of older trees and identify management practices that will maximise the useful life of intensive mango orchard systems.

Macadamia

We have demonstrated that high density macadamia planting systems are capable of producing substantially greater yield/hectare in early orchard life than conventional low-density planting systems. The effect is greatest for precocious varieties. We found little effect of tree training treatments on early flowering and yield.

The Planting Systems Trial has allowed us to clearly identify the factors limiting early yield in macadamia and the manipulations to the system that lead to early orchard yield. First, greater planting density allows the development of greater canopy volume/hectare earlier in the life of the orchard. This greater canopy volume/hectare intercepts a greater proportion of the available PAR, which provides increased yield potential for the system due to the relationship between total light interception and yield/ha. Total light interception, however, is not the only consideration, with raceme production on young trees providing a significant limitation to NIS yield/tree early in orchard life. Somewhat surprisingly, raceme density was still a limitation to yield in the precocious variety 'A203' in the crop five years after planting.

The results to-date are for the performance of the Planting Systems Trial up to five years after planting, which we still consider to be early in the life of the orchard and so it is possible that the intensive pruning required to prevent crowding of the intensive systems may lead to excessive competition and yield decline in subsequent years. However, in the high-density remediation trial over a period of three consecutive cropping seasons we demonstrated that selective pruning could maintain canopy size and high levels of yield in mature, high density macadamia orchards.

The work has reinforced the importance of competition between vegetative growth and fruit set in macadamia crop load development. We found that depending on the relative timing of their growth, and therefore their growth rate relative to each other, either fruit or shoots have the ability to outcompete the other for resources. The relative timing of growth, and therefore the allocation of resources can be manipulated through the timing of pruning.

The work has improved the understanding of macadamia orchard light relations. In addition to the relationships described above, we confirmed that tree density affected the way in which light is intercepted and distributed within the orchard. High density systems intercepted more PAR for the same level of canopy volume/hectare compared with low density conventional systems, presumably due to the narrower row width and less within canopy shading. Low and high-density conventionally pruned systems had similar levels of within canopy PAR despite the greater levels of total light interception in the high-density systems, indicating that high density systems should have improved within canopy PAR distribution when all systems have filled their allotted space.

Detailed architectural assessments provided important insights into the regulation of flowering early in macadamia orchard life. Shoot characteristics including growth unit length and growth unit angle from the vertical affected the likelihood of flowering, however the manipulations such as limb bending had no effect on early flowering.

The macadamia rootstock evaluation component has provided indications that rootstock significantly affects scion vigour and early flowering. However, it should be noted that this trial is in its early stages and several more years of evaluation are required before this can be confirmed. For future vigour managing rootstock evaluations, xylem vessel size can be used as a tool for early selection of reduced tree vigour. This finding provides a method to reduce cost of evaluation of large number of progeny for dwarfing characteristics.

Vigour management through rootstocks was not the only method we investigated. The high-density remediation trial clearly demonstrated that selective pruning led to less vegetative re-growth than the conventional mechanical hedging and topping. The less vigorous re-growth following the selective pruning was likely a significant contributor to the increased yield compared with the mechanical hedging and topping.

Molecular/genetic regulation of floral initiation and juvenility

Two major types of outcome have been achieved concerning the molecular part of the project. The first one is the creation of tools (RNA extraction method and genetic libraries) to enable and accelerate the research in the crops. These tools are described in Chabikwa et al., (2020) and Barbier et al., (2019) and have been successfully used to discover genes relevant to tree physiology (Chabikwa et al., 2020) and to monitor gene expression in different samples, leading to the second outcome.

The second type of outcome obtained is the improvement of the knowledge on the physiology of these trees. The results obtained during the project have allowed the drawing of some conclusions about the physiological regulation of the three crops. We have highlighted that some aspects of the tree physiology were similar to plant models, whereas other aspects are clearly distinct, probably reflecting some specific evolutionary adaptations to their environment. Some examples of these regulatory discoveries are presented in Ashan et al., 2019a,b; Ashan, 2019; Appendix 20, and Barbier et al., 2020.

Functional-structural plant modelling

There now exists across the three species, macadamia, mango and avocado, a suite of models that tackle different aspects of productivity within orchards. These exist at different structural scales, from a single branch to whole orchard level, and cover tree architecture and management practices, fruit growth and carbohydrate production.

The generic whole-shoot carbon pool model and its incorporation in a young tree canopy structure allowed us to discuss from a theoretical point of view the effects of different training systems, crop loads, light availability and warmer temperature on fruit size distribution. This facilitated debate with other project members about the results of our simulations in comparison with field observations in our Planting Systems Trials. The debate led to new hypotheses, new experiments and improved our understanding of tree physiology.

The simulations with the virtual macadamia tree at different scales of carbon autonomy were employed to explain some of the results of previous experiments, as well as to investigate the autonomy scale of a field measured young macadamia tree, improving our understanding. The new method developed represents a contribution as well to the international plant modelling community.

Our virtual macadamia trees considering branch autonomy and an orchard environment helped to have an idea of the effects that increase the understanding of and support hypotheses about the effects of planting distances and tree size on orchard yields, which could be useful for designing future field experiments and orchards.

The high-performance ray tracer HP-QuasiMC as well as the mangoL tree/orchard simulator developed in this project have enabled virtual experiments to adopt massive explicit rays and complex configurations at multiple scales (from a metamer to an entire orchard) while being shortened from days and even weeks (if run with the conventional serial programs) to minutes. These tools have broken a dilemma between computing efficiency and simulation accuracy that has long annoyed the horticultural modelling community, and will continue to support the pre-evaluation and optimisation of orchard designs and operations.

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Monitoring and evaluation

AI13004 pre-dates the requirements for formal M&E plans and, as such, a formal plan was not developed. However, the program was externally reviewed twice, and the focus of these reviews could be used as a reasonable framework for evaluation. Success of AI13004 as defined in the two external project reviews undertaken since the beginning of the project can broadly be grouped into:

1. Improved scientific understanding in areas required to improve subtropical and tropical tree crop productivity through the development of intensive planting systems.
2. Initial success in the form of improved early orchard productivity of ‘best bet’ intensive macadamia, mango and avocado orchard systems.
3. Effective communication and extension of program outcomes to the industries.
4. Program research being undertaken using robust scientific techniques to provide confidence in industry recommendations.
5. The research program and project team effectively managed and coordinated.
6. AI13004 contracted milestones met and program Outputs delivered.

Recommendations

Our primary recommendation is that the overall program of research should continue. The body of research, with the intent of designing more productive, intensive planting systems for these important subtropical and tropical tree crops was dubbed from the outset to be a long-term endeavour.

The end of this first six-year phase of the work is a useful time to reflect on the successes and failures of the range of systems and strategies that have been trialled, with the intention of learning from the experience and developing a plan for future research with the best possible chance of success.

Avocado

Recommendations for industry

- The conventional avocado orchard total light interception study found that for 'Hass' avocado, growers should aim for their orchards to be intercepting approximately 80% of available PAR. Canopies that were not intercepting less than approximately 80% PAR were unlikely to be achieving their maximum yield and as orchards approach 90% PAR interception, growers run the risk of yield declining.
- From our work to date it is not possible at this stage to recommend a density or tree training system that will reliably produce higher yields per hectare. It is clear, however, that the central leader shaping has not proved effective, at least without improved technologies for vigour control.
- The project has demonstrated the potential for improved yield through choice of rootstock. Growers are recommended to consider yield and canopy size attributes along with consideration of disease tolerance, soil suitability and vigour in their choice of rootstock.

Research recommendations

- The current avocado Planting Systems Trial is nearing the end of its usefulness. We recommend the final crop should be harvested in 2021. The trial has allowed us to develop a better understanding of, the physiological factors affecting avocado productivity; the underlying physiological challenges of intensive avocado systems; and the practical challenges in managing intensive avocado systems.
- A follow-on avocado intensive systems trial should be designed and planted using the lessons learnt from the first trial. This trial should include research to develop improved growth regulator treatments to manage vigour. The trial should also compare additional canopy structures that, through our previous work and international visits, have been identified as having improved characteristics for intensive systems. The work could also include treatments investigating the effects of rootstock and scion variety on vigour and some comparisons of tree density. Other varieties than 'Hass' should receive research attention.
- Experimentation into vigour management via genetics should continue to be investigated. First, the phase 1 rootstock trial should be continued for an additional two to three cropping seasons. Second, the phase 2 rootstock trial, which also includes scion varieties in addition to 'Hass' and is comparing two tree training systems, should be continued.
- Additional research is required to develop greater understanding of factors affecting the allocation of resources between vegetative and reproductive growth. This work should also investigate methods to manipulate allocation of resources in favour of fruit production.
- One hypothesis developed through the current project, is that the characteristics of the root systems in intensive systems are affecting their ability to set and retain fruit. We recommend comparisons of root system density and distribution between intensive and conventional avocado orchard systems be undertaken. The possibility of links to determinate flowering patterns should also be explored.

Mango

Recommendations for industry

- Past and future research findings be communicated with industry to facilitate the adoption in orchard intensification by industry.
- An intensive mango orchard production manual be written with strong attention to the performance of different mango varieties and training systems management.

Research recommendations

- That the mango Planting System Trial on Walkamin research station be extended for another 5 years to evaluate the effects of planting density, tree training and variety in a systems approach in mature trees that have reached their allocated orchard space. In combination with the past 6 years, another five years will provide information on the useful life of intensive mango orchards and management throughout the orchard's life.
- The mango rootstock selection trial should be continued for up to two years to confirm a shortlist of suitable vigour-reducing rootstocks. At that point, continued evaluation of the shortlisted accessions should be undertaken in an intensive mango orchard system at high-density, with espalier tree training and under more scion varieties.
- Establishment of a mother block of the shortlisted rootstock trees to supply seed for future role-out of the successful rootstocks to industry.
- Protection of the Intellectual Property of successful rootstocks in the short list through Plant Breeders Rights registration. This may involve a new comparative field trial to obtain data to support the PBR application.
- Research on light interception be repeated when the high-density treatments of the mango Planting Systems Trial mature and fill their allocated space.
- Light distribution research continues to define optimal light conditions for flowering, yield and fruit quality in intensive mango orchard systems.
- Continued collaboration with CIRAD, Reunion on understanding mango architectural relationships with tree growth, productivity and management.
- Continue the research collaborations established in this project with groups such as CIRAD, USQ, CRCNA, QAAFI, and develop new collaborations.

Macadamia

Recommendations for industry

- Selective pruning produced greater NIS yield than mechanical hedging and topping in the high-density remediation trial. Given these productivity improvements selective pruning should be considered as an option by grower, however, there are many factors considered by growers when deciding on a canopy management strategy.
- Limb bending was trialled as an early orchard tree training technique. There was no improvement in precocity and minor effects on vegetative growth. We do not recommend limb bending as a tree training technique for macadamia growers.

Research recommendations

- The current macadamia Planting Systems Trial should be continued for another five years, to understand the performance of the trialled systems in the mature orchard phase. This will also allow us to develop understanding the physiology underlying productivity of mature orchards. Relationships such as canopy volume/hectare and total light interception, total light interception and yield/hectare and the effects of variety and tree density can be extended.

- Set up a phase 2 intensive macadamia Planting Systems Trial, using the lessons learnt so far from the first trial. This second trial should incorporate additional scion varieties; investigate further tree training techniques in terms of their effects on early canopy development and flowering and orchard light relations; and vigour control strategies.
- Additional techniques for macadamia vigour management should be investigated. This should include further development of PGR treatments to reduce growth and improve allocation of resources to fruit production. One goal of this work should be the development of a commercial growth regulator treatment.
- Allocation of resources between vegetative and reproductive growth has been identified as a key factor affecting macadamia productivity. Factors such as the time of pruning have been shown to affect this allocation and the ability of different organs (fruit or shoots) to compete for resources. Further work on methods to alter allocation of resources should be undertaken investigating factors such as PGRs (manipulating shoot growth and fruit retention), nitrogen nutrition, scion variety and environmental factors such as temperature.
- Further work needs to be undertaken on the distribution of light through macadamia canopies. First, the effect of tree density and tree training system on within canopy light levels. Second, further work to understand the relationships between within canopy PAR levels, leaf retention, raceme production and fruit set. Third, develop light extinction coefficients to understand the rate of removal of PAR with leaf area.
- The rootstock trial is three years old now and will crop increasingly heavily over the coming years. We recommend continuing the existing rootstock screening trial for another five years to select elite performing rootstocks which have performed well in a young orchard. These elite stocks should be propagated for wider regional testing and the current trial at Nambour should be continued to determine performance in a mature orchard. Discovery of molecular marker (DNA, RNA or proteins) associated with rootstock-scion interactions could be useful for future selection of rootstocks for reduced growth and increased productivity. Investigating the vascular system over a large number of accessions would be helpful for rapid screening for reduced tree size.
- Detailed architecture measurements were a significant feature of the macadamia Planting Systems Trial experimentation. This data set is a significant resource and should be analysed further.

Molecular/genetic regulation of floral initiation and juvenility

The results obtained in the molecular project highlighted that the role of the molecular components involved in flowering in tropical trees, especially macadamia may differ from their role in other model crops. As an example, the role of FT in macadamia axillary buds and leaves may strongly differ from other crops, and it appeared that the axillary buds may also participate in the induction of FT. In terms of crop management, this may lead to the development of new pruning techniques, for example.

The project has also shown that the expression of some specific genes is well correlated with floral induction and bud break. This may be used in orchards as a tool to predict flowering time and intensity, allowing farmers to fine-tune crop management to the specific needs of the trees at a given time.

Some key components of flowering regulation have also been highlighted during this project. This basis of knowledge about the regulation of flowering in tropical trees may be used in crop engineering in order to genetically improve crop though genome editing. This approach was, for example, successfully used to improve yields and crop management in kiwi fruit.

The examples described above highlight the potential applications emerging from this first phase of the project. In order to see this theoretical knowledge being applied in orchards, further confirmation needs to be done to confirm the role of these molecular components in the regulation of tree phenology. In combination with the project AS17000, which will unravel more potential targets for crop improvement, the project AS18000 will carry out sets of experiments on trees to test the relevance of the identified targets. The experiments performed in the project AS18000 will be design in a way close to what could be potentially done in orchards to manage crops. Such experimental design would ultimately have the dual objective of testing the role of molecular components in tree phenology and to offer potential new ways of managing crops.

Functional-structural plant modelling

Research recommendations

- Future modelling work should be more integrated with field work and applied to more clearly defined problems.
- Creation of a parallel-computing version of lpfg should be supported to enable efficient simulation of orchards.
- The different scales of carbon allocation models should be adapted and applied to the three species as needed in supporting the process of further virtual experimentation.
- Due to the time cost of acquiring enough data to reproduce whole tree architectures, alternative methods should be explored.
- From the plant physiology point of view, the models are very simple now and adding some additional physiological processes would make them more applicable to a wider range of situations.
- Light distribution within the canopy rather than simply light interception at the canopy floor needs to be further investigated in terms of the findings from the CanopyShapes modelling.
- The modelling and computing technology should be further developed to investigate various combinations of training systems, pruning and hedging, density and orientations, forming a decision support tool to save time and reduce cost for development of future orchard systems.

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Intellectual property, commercialisation and confidentiality

Project activities may have generated additional IP to pre-project, background IP associated with rootstock germplasm. Project IP will be dealt with in accordance with HIA policy when germplasm, deemed as being suitable to protect and commercialise, is identified. DAF will manage the commercialisation process on behalf of contributing partners and IP equity will be split according to contributions of the specific component the IP was developed under. DAF will develop a commercialization and exploitation plan in consultation with Hort Innovation and other equity partners.

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Appendices

1. Project outputs
2. Avocado Planting Systems Trial
3. Avocado rootstocks for high density systems
4. Understanding the bases of early orchard productivity in macadamia: the effect of tree density, tree training and variety on orchard development and yield
5. Effects of selective limb removal, mechanical hedging and topping and plant growth regulators on yield and canopy development in high density macadamia
6. Macadamia rootstock screening for high performance: genetic diversity, striking rate, early field growth and precocity
7. The effect of the relative timing of growth on resource allocation in macadamia
8. A comparison of the responses of two macadamia cultivars to the use of selective limb removal to control tree height, with respect to yields, nut quality and insect damage
9. Mango Rootstock research
10. Mango Light relations summary
11. Mango crop load (part 1) - Reproductive growth
12. Mango fruit quality and pack out research in orchard intensification
13. Hypotheses and assumptions for Functional Structural Plant Modelling
14. Simulating growth and yield with virtual trees
15. CTRAM: a model for carbohydrate allocation
16. Modelling Light in Macadamia Canopies
17. Supercomputing and Complex Systems Modelling: The High-performance Ray Tracer and the mangoL Tree/Orchard Simulator
18. Model Development for Avocado, Mango and Macadamia
19. A model of macadamia with application to pruning in orchards
20. Insight into Flowering Regulation in Macadamia

Appendix 1

AI13004 project outputs

Articles in industry publications

Bally, I., Ibell, P., 2014. Can smaller trees in more intensive mango orchards lead to higher productivity? *Mango Matters*. 14(1), 14-15.

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Wilkie, J.D., Hofman, H., Toegel, H., Parfitt, C., Griffin, J., Toft, B. “The effect of plant density and cultivar on early orchard flowering, yield and light relations”. International Macadamia Symposium, Lincang, China, October 2018.

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Journal articles

Ahsan, M. U., Hayward, A., Irihimovitch, V., Fletcher, S., Tanurdzic, M., Pocock, A., Beveridge, C. A., & Mitter, N., 2019. Juvenility and Vegetative Phase Transition in Tropical/Subtropical Tree Crops. *Frontiers in plant science*, 10, 729. https://doi.org/10.3389/fpls.2019.00729

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Barbier FF, Chabikwa TG, Ahsan MU, Cook SE, Powell R, Tanurdzic M, Beveridge CA, 2019. A phenol/chloroform-free method to extract nucleic acids from recalcitrant, woody tropical species for gene expression and sequencing. *Plant Methods* 15: 62

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Toft, B.D., Alam, M.M., Wilkie, J.D., Topp, B.L., 2019. Phenotypic association of multi-scale traits with canopy volume and yield: moving toward high-density systems for macadamia. *HortScience*, 54: 596 – 602.

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Wang, M, Cribb, B., Clarke, A., Hanan, J., 2016. A Generic Individual-Based Spatially Explicit Model as a Novel Tool for Investigating Insect-Plant Interactions: A Case Study of the Behavioural Ecology of Frugivorous Tephritidae. *PLOS ONE*, 11 3: e0151777.doi:10.1371/journal.pone.0151777

Wilkie, J.D., Conway J., Griffin, J., Toegel, H., 2018. Relationships between canopy size, light interception and productivity in conventional avocado planting systems. *The Journal of Horticultural Science and Biotechnology*, DOI:10.1080/14620316.2018.1544469

Theses

Ahsan, M. U., 2019. Molecular regulation of phase transition and flowering in tropical/subtropical tree crops. PhD Thesis. University of Queensland, Brisbane, Australia, pp. 160.

Le Guicher. G. 2019. Analysis of vegetative growth and reproduction in mango and effects of the culture system. Masters Thesis. University of Bordeaux.

Mizani, A., 2019. Managing vigour, light, crop load and tree architecture in mango to maximize productivity and quality. PhD Thesis. University of Queensland, Brisbane, Australia, pp. 183.

Toegel, H., 2018. Relative timing of vegetative and reproductive growth and competition in macadamia. Honours Thesis. Central Queensland University, Bundaberg, Australia, pp. 47.

Toft, B., 2019. Phenotypic and genotypic diversity in macadamia canopy architecture, flowering and yield. PhD Thesis. University of Queensland, Brisbane, Australia, pp. 130.

Wang, M., 2018. Pattern-Oriented Modelling of biological Systems in Australian Orchards: Driving Research towards the Medawar Zone. PhD Thesis. University of Queensland, Brisbane, Australia, pp. 191.

Videos

The Small Tree - High Productivity Initiative video ‘Unlocking the secrets to high orchard productivity’, published 14 March 2017.

High density mango intensification: the basics. Full video & promo, published October 2019.

Field days

Avocado

1 April 2015, Avocado Study Group Project meeting, hosted at the Bundaberg Research Facility on 1st April 2015 including a presentation “Small Tree – High Productivity Initiative. An overview and some initial avocado results” by John Wilkie and field walk of the avocado Planting Systems Trial, 43 attendees,

11 August 2016, Avocado Planting Systems Trial field walk held in conjunction with the Avocado Industry run Central QLD Qualicado workshop, including presentation ‘Improving crop load in high density systems’ by H Hofman, 80 attendees.

17 August 2017, Avocado Planting Systems Trial and Rootstock Trial field walk was held in conjunction the Central Queensland Region Avocado Study Group meeting (AV14000) at the Bundaberg Research Facility, including presentation ‘Avocado field trials update August 2017’ by H Hofman, 81 attendees.

10 April 2019 - Avocado Planting Systems Trial and Rootstock Trial field day held at the Bundaberg Research Facility, including presentations ‘Avocado planting systems trial 2014-2018’ and ‘Rootstocks for high density’ by H Hofman, 63 attendees.

Mango

31 May 2014, Australian Mango Industry Association Field Day at Mango Planting Systems Trial at Walkamin Research Station.

28 October 2015, Mango Small Tree High Productivity Research Field Walk at the Walkamin Research Facility. Presentations included “An Overview of the ‘Small Tree High Productivity Initiative’ Ian Bally; “Vigour management” Paula Ibell; “Light relations in the orchard” Anahita Mizani; “Crop load manipulation and fruit quality” Ram Kolala followed by a field walk of the mango rootstock and Planting Systems Trial, 54 attendes.

29 November 2016, Mango Planting Systems Trial field walk held at the Walkamin Research Station.

23 November 2017, Mango Small Tree High Productivity Initiative Field Walk at the Walkamin Research Facility.

12 November 2018, Mango and avocado orchard intensification and robotics field day at the Walkamin Research Station, 80 – 100 attendees.

6 November 2019, Intensive Horticulture Bus Tour, attending the Walkamin Mango PST and RSK experiments, 27 attendees.

Macadamia

13 March 2014, Macadamia Industry MacGroup hosted at the Bundaberg Research Facility with a presentation on the research program by John Wilkie and field walk of the macadamia Planting Systems Trial.

27 April 2014, Macadamia Planting Systems Trial Advisory Group met at the Bundaberg Research Facility to discuss trial nutrition and tree pruning and training strategies.

16 July 2015 the Australian Macadamia Society facilitated ‘MacGroup’ field day viewed the STHPI Alloway macadamia ‘high-density remediation trial’ with a talk on the pruning method and trial results by John Wilkie.

17 November 2015 the Australian Macadamia Society facilitated ‘Nutrition investigative committee’ viewed the STHPI’s Macadamia Planting Systems Trial and the high-density remediation trial.

25 February 2016, Australian Macadamia Society’s MacGroup meeting held at the Bundaberg Research Facility inspected the Macadamia Planting Systems Trial after a presentation given by John Wilkie, 45 attendees.

9 March 2017, Macadamia Planting Systems Trial field walk was held in conjunction with the Australian Macadamia Society’s workshop held at the Bundaberg Research Facility.

1 March 2018, Macadamia Planting Systems Trial field walk at the Bundaberg Research Station, 90 attendees.

28 February 2019, Macadamia Planting Systems Trial field walk at the Bundaberg Research Station in conjunction with the Australian Macadamia Society Mac Group, 89 attendees.

Posters

Ahsan, M.U. 2018. “Rootstock and Scion interaction: microRNA perspective?”. PAG XXVI – Plant and Animal Genome Conference, January 13 – 17, 2018 San Diego, CA, USA.

Alam, M., Wilkie, J., Topp, B. 2016. “Early growth and graft success in macadamia seedling and cutting rootstocks”. II International Symposium Tropical Horticulture: Now is the Era for Tropical Horticulture, 20-25 November, Cairns, Qld, Australia.

Auzmendi, I., Mizani, A., Toft, B. D., Hanan, J., Ibell, P., Hofman, H., Bally, I., White, N., Wilkie, J. 2015.“Improving avocado, macadamia and mango productivity in Australia: Integration of field trials and functional structural plant modelling”. In: Tropical Agriculture Conference 2015 (TropAg2015). Brisbane (Australia).

Auzmendi, I., Wilkie, J., Hanan, J. 2017. “A virtual tree to improve our understanding of macadamia nut production”. Tropical Agriculture Conference 2017 (TropAg2017). Brisbane, Australia.

Auzmendi, I., Hanan, J. 2018. “Using L-studio to visualize data and modify plant architecture for agronomic purposes”. 6th International Symposium on Plant Growth Modeling, Simulation, Visualization and Applications (PMA2018), Hefei, China.

Hanan, J., White, N., Auzmendi, I., Griffin, J., Ibell, P., Bally, I., Wilkie, J. 2015. “Towards high productivity in tropical orchards: architectural representation and modelling intercepted light”. In: X International Symposium on Modelling in Fruit Research and Orchard Management. Montpellier, France.

Ibell, P. T., Wright, C., Kare, M., Bally, I. S. E. “How do alternate single leader, training systems influence tree growth and yields in an intensive mango production at early establishment?” Fruit Research and Orchard Management. Montpellier (France). ISHS Integrated Canopy, Rootstock and Environmental Physiology Symposium. Wentachee, USA.

Kare, M., Ibell, P. Wright, C., Wilkie, J., Bally, I. 2019. Light relations in intensive mango orchards. Trop Ag, Brisbane.

Mizani, A., Ibell, P., Bally, I., Kolala, R., Wright, C. 2015. The effects of terminals flowering percentage on postharvest fruit 2015, XI International Mango Symposium (poster).

Mizani, A., Bally, I., Wright, C. 2016. “Baseline light distribution in Kensington Pride Mango (*Mangifera indica* L.) tree canopies in North Queensland”. II International Symposium Tropical Horticulture: Now is the Era for Tropical Horticulture, 20-25 November 2016, Cairns, Qld, Australia.

Rigden, P., Carr, B., Wilkie, J., Hofman, H., Griffin, J., Toegel, H., Parfitt, C. 2017. “Small Tree - High Productivity Initiative, canopy development and early cropping in macadamia”. Presented at the November 2017 round of Australian Macadamia Society MacGroups.

Media

Radio

ABC Landline, An Australian Original, Helen Hofman interviewed by Pip Courtney on the new project, 1 June 2014. https://www.abc.net.au/local/archives/landline/content/2014/s4016259.htm

ABC Rural Radio, Professor Christine Beveridge (15 April 2014) Sugar to help redesign food crops. Interview with Eliza Rogers, http://www.abc.net.au/news/2014-04-15/sugar-shooting/5391010

ABC Rural Radio, More funding to get big yields from small trees, The Country Hour, by Eliza Rogers. 18 June 2014.

ABC Rural Radio February 2016 interview with John Wilkie about the macadamia component of the Small Tree – High Productivity Research Program

ABC Rural Radio, Helen Hofman was interviewed by an ABC reporter regarding the progress of the avocado crop load work being undertaken in the Small Tree - High Productivity Initiative. Bundaberg, 25 August 2016.

ABC Rural Radio, Paula Ibell was interviewed by Charlie McKillop ABC reporter "Bigger is not always better" about vigour control in Mango canopies the Small Tree - High Productivity Initiative. Mareeba, 2017.

Newspaper articles

Project to boost crops, Minister visits: $3M funding from Horticulture Australia, *News Mail*, 19 June 2014.

QLD horticulture project receives extra boost, *Blue's Country Magazine,* 24 June 2014.

Tree size matters, *News Mail Rural Weekly*. 4 July 2014.

Walkamin field day focusses on mangoes, *Tablelands Advertiser*, Mareeba QLD, 25 July 2014

Local specialists to give talks at macadamia conference, *News Mail,* 13 October 2014.

Research out on a limb, *Tablelands Advertiser,* Friday November 20 2015

Walkamin Field day, *Tablelands Advertiser,* Friday November 20 2015

Walkamin Field Day, *Cairns Post*, Rural report interview with Ian Bally, November 28 2016.

Food heroes head to Bundy, *Queensland Country Life*, 27 February 2017.

Farm walk will offer a glimpse into the "orchards of tomorrow", *Queensland Country Life*, 28 February 2017.

Macadamia industry shows off in-field research, *Good Fruit and Vegetables*, 10 March 2017.

Avocado our top crop, *The Tablelander*, 24 October 2017.

Farm focus for visiting researchers, *The Tablelands Advertiser*, 18 May 2018.

Mango small trees - high productivity, *The Tablelander*, 21 August 2019

Press Releases/Launch
Official opening and tree planting of the Mango Planting Systems Trial by Managing Director of AgriScience Queensland, DAFF, John Chapman, 18 December 2013.

Press release: "Sugar responsible for shooting", Christine Beveridge, 7 April 2014

Ministerial launch, the Honourable Dr John McVeigh, Queensland Minister for Agriculture officially launched the expanded HAL co-funded program in the macadamia Planting Systems Trial, 18 June 2014.

QAAFI Press Release: "Sweet Success for QAAFI Scientists" announcing successful ARC Discovery grant. Includes mention of the Small Tree High Productivity Initiative with quotes from our project team members, November 2014.

Taking productivity lessons from the apple industry, Australian Macadamia Society website, 19 July 2017.

CQU Data tool eases mango madness, Queensland Cyber Infrastructure Foundation, 14 September 2018

Television

Pip Courtney, ABC Landline reporter visited Bundaberg Research Facility and interviewed John Wilkie about the STHPI work, on 22 May 2016.

Seven News, Bundaberg. Macadamia growers have been shown the 'orchard of the future'.
Interviewees – Jolyon Burnett and John Wilkie. 9 March 2017.

WIN News, Toowoomba. Local macadamia growers have participated in industry discussions….
Interviewees - Jolyon Burnett and John Wilkie. 9 March 2017.

Seven News, Sunshine Coast. Macadamia growers have been shown the 'orchard of the future'….
Interviewees – Jolyon Burnett and John Wilkie. 9 March 2017.

WIN News, Cairns. "More than 150 macadamia growers have descended on the Wide Bay…".
Interviewees - Jolyon Burnett and John Wilkie. 9 March 2017.

Social Media
Big mango crop from intensive orchard management, Queensland Agriculture Facebook page, 4 April 2018.

Japanese politicians visit Small Tree High Productivity Big mango crop from intensive orchard management, Queensland Agriculture Facebook page, 1 May 2018.

Cairns Part II, University of Southern QLD Facebook page, 17 May 2018.

STHPI project related meetings

STHPI Team Webinar meeting:
29 January 2015. "Update presentations on progress at project centres".

2 April 2015. “Light Impact on Branching – The Case of the Rosebush” Nathalie Leduc, Institute of Research in Horticulture and Seeds, Angers, France.

19 May 2015. “Modelling final leaf length as a function of carbon availability during the elongation period” Inigo Auzmendi.

20 October 2015. “Studies on managing vigour, light, crop load and tree architecture in mango to maximise productivity and quality“ Anahita Mizani; “Horticultural manipulation of macadamia architecture: Understanding and controlling growth, canopy structure, flowering and fruit development” Ben Toft; “MicroRNA control of juvenility and phase change in tropical/subtropical tree crops - avocado, mango and macadamia” Mohammad Umair Ahsan; “Modelling of biological systems in Australian orchards – case study of avocados” Ming Wang.

14 July 2015 “Practicalities of measuring orchard light relations” John Wilkie; “Using QuasiMC for light modelling” Neil White; “X International Symposium on Modelling in Fruit Research and Orchard Management - Montpellier, France June 2 – June 5, 2015” Inigo Auzmendi and Jim Hanan.

18 August 2015. “Visit to CIRAD, Reunion Island by Ian Bally and Paula Ibell” Ian Bally); “Mango Canopy Architecture – Reunion Island Study tour May 2015” Paula Ibell; Meeting on mango modelling - Montpellier, France June 3, 2015” Jim Hanan and Inigo Auzmendi; “Analysing alternate bearing of tropical fruit crop through the costs of reproduction. The example of the mango tree” Mathilde Capelli.

3rd March 2016. “Sugar signalling in plant physiology and shoot branching“ Francois Barbier

13 May 2016. Proposals and designs for avocado and mango molecular physiology experiments suggestions and discussion” – Helen Hofman and Ian Bally. “Can a greater understanding of macadamia tree architecture lay the foundation for orchard productivity improvements?” – B. D. Toft, J. S. Hanan, B. Topp, I. Auzmendi and J. D. Wilkie. “Identifying vigour controlling rootstocks for mango” – A. Mizani, I. Bally, P. Ibell, C. Wright, C.Maddox and R. Kolala.

19 August 2016. “Improving crop load of ‘Hass’ avocado in high density systems” - H. Hofman, J. Wilkie, J. Griffin and R. Langenbaker. “Architectural analysis of vegetative and reproductive growth in Australian mangoes” – P. Ibell, F.Normand, C. Wright, N. White, I. Bally and R. Kolala.

7 October 2016. “Using Functional – Structural Modelling of Carbon Acquisition and Utilization to Understand Fruit size distribution in Tree Canopies” – Inigo Auzmendi and Jim Hanan. “Integrated canopy, rootstock and environmental physiology symposium study tour, Bologna, Italy 2016.” – Paula Ibell.

26 April 2017. “Small Trees High Productivity Initiative: Molecular Achievements” – Stacey Cook. STHPI video “Unlocking the secrets to high orchard productivity – The Small Tree High Productivity Initiative” – Peter Rigden.

6 July 2017. “Presentation of the molecular reports” - Rosanna Powell. “Small Tree High Productivity Initiative: The molecular side of the trees” – Francois Barbier

23 August 2017. “Modelling macadamia tree growth” – Inigo Auzmendi. ‘Economic Analysis for High and Medium vs Conventional Density Avocado Orchards’ – Peter Rigden

10 October 2017. ‘Small tree High Productivity Initiative - Avocado Planting Systems Trial update, October 2017’ - Helen Hofman.

19 October 2017**.** Some Factors affecting canopy development and early cropping in macadamia' – John Wilkie

25 October 2017. Small Tree High Productivity Initiative Research findings Update – Canopy Management and Light Relations – Kare Mahmud and Paula Ibell

7 March 2018. "Simulating growth and crop of tree canopies" Inigo Auzmendi. "Higher performance towards higher productivity" Liqi Han. "Modelling work update" Neil White. "Avocado annual growth module" Ming Wang. "Managing vigour, light, crop load and tree architecture in mango to maximise productivity and quality" Anahita Mizani

8 August 2018. "Macadamia Planting Systems Trial 2018 update", John Wilkie. "Avocado Planting Systems Trial 2018 update" Helen Hofman.

23 August 2018. "Observations of high density planting systems in South America and California" - Helen Hofman. "Mango planting systems trial update" - Paula Ibell

11 October 2018. "Modelling light in novel canopy architectures for increased productivity" - Neil White. "Investigating the effects of planting density and tree size on yield through functional-structural modelling" - Inigo Auzmendi

16 December 2019. STHPI end of project webinar series - Avocado rootstock and planting systems trial update - John Wilkie and Helen Hofman

18 December 2019. STHPI end of project webinar series - Macadamia rootstock and planting systems trial update - Mobashwer Alam and John Wilkie

20 December 2019. STHPI end of project webinar series - Mango rootstock and planting systems trial update - Ian Bally and Paula Ibell.

Avocado Planting Systems Trial Advisory Group:

August 2014. Avocado Planting Systems Trial Advisory Group met at the Bundaberg Research Facility to discuss trial design, nutrition and irrigation.

17 March 2015. Avocado Planting Systems Trial Advisory Group met at the Bundaberg Research Facility to discuss trial nutrition, irrigation and tree pruning and tree training.

20 November 2015. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss nutrition, irrigation and tree pruning and tree training.

25 May 2016. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss trial nutrition, irrigation, tree pruning and training.

24 November 2016. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss trial nutrition, irrigation, tree pruning and training.

30 August 2017. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss trial nutrition, irrigation, tree pruning and training.

30 August 2018. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss recent results, pruning and fertiliser.

8 October 2019. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss nutrition, irrigation, tree pruning and training.

Macadamia Planting Systems Trial Advisory Group:

27 April 2015. Met at the Bundaberg Research Facility to view the Planting Systems Trial block to discuss nutrition and tree training.

18 November 2014. Met at the Bundaberg Research Facility to view the Planting Systems Trial block

10 December 2015. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss nutrition, irrigation, tree pruning and training.

19 May 2016. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss trial nutrition, tree pruning and training.

24 November 2016. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss nutrition, irrigation, tree pruning and training.

13 July 2017. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss nutrition, irrigation, tree pruning and training.

17 April 2018. Met at the Bundaberg Research Facility to view the Planting Systems Trial block and discuss nutrition, irrigation, tree pruning and training.

Annual Project Advisory Group meeting

May 2014. Met at Ecosciences Precinct in Dutton Park, Brisbane. First meeting to discuss purpose of group and the purpose of the project.

May 2015. Met at Bundaberg Research Facility to review progress of the project, view major Bundberg trials and discuss next steps.

11 May 2016. Met at the University of Queensland to review progress over the previous 12 months, discuss interactions between activities and review the next steps and view the view labs and procedures used for molecular work.

19 May 2017. Met at the University of Queensland to review progress over the previous 12 months, discuss interactions between activities, a future project and funding options.

8 May 2018. Met at Ecosciences Precinct in Dutton Park, Brisbane. To review progress over the previous 12 months, discuss interactions between activities, a future project and funding options.

STHPI Annual Review meeting

12-13 November 2015. Met at the University of Queensland. Results from the previous 12 months for all elements of the STHPI work were presented and reviewed. Discussions centered on the development of molecular and physiological research in the remainder of the current project. 34 STHPI team members and guests (with relevant expertise) met.

26-27 October 2016. Held in Mareeba with approximately 30 attendees.

8 -9 November 2017. Held at The University of Queensland’s campus at St. Lucia. A total of 36 members of the STHPI team attended plus 6 guests from aligned organisations and stakeholders.

Appendix 2

Avocado Planting Systems Trial 2014-2019

H. Hofman, J. Wilkie, J. Griffin, C. Parfitt, H. Toegel

Introduction

The average yield for avocado (*Persea americana* Mill.) in Australia is 10-11 tonnes/ha, ranging from 4 tonnes/ha in the tri-State region to 20.5 tonnes/ha in north Queensland (Avocados Australia, 2018). Wolstenholme (1987) calculated a potential yield of 32.5 tonnes/ha, using the energy required to produce avocados, an oil intensive fruit. Avocados in Australia are generally planted in ≥10 m rows at ≥5 m spacing. High density plantings of avocado face many challenges, as reviewed in Menzel and Le Lagadec (2014).

We report here on our findings with an avocado planting systems trial in Bundaberg, Queensland, and on supplementary trials of possible strategies to increase crop load for avocado. We also review some of the variable factors in density/training treatments and rootstock treatments that may influence yield results and provide some recommendations for future research into high-density plantings.

Methods

Planting Systems Trial

Materials

We planted 'Hass' in July 2014 at the Bundaberg Research Facility in Bundaberg, Queensland, on a site with deep red volcanic soil. Bundaberg has a sub-tropical climate with an average rainfall of 1010 mm per year. Trees were planted in three density-training treatments (hereafter abbreviated to 'density treatments') and two rootstock treatments in a split plot design with density treatments as the main plots and rootstock treatments as the split-plots, with five replicates.

The row x tree spacing of the density treatments were: 9×5m spacing or 222 trees/ha, the industry standard for the region, hereafter referred to as 'low' density, 6×3m or 556 trees/ha ('medium' density), and 4.5×2m spacing or 1111 trees/ha ('high' density). Training of the density treatments was as follows: trees in the medium and high density treatments were pruned to a central leader in an approximate cone shape to improve canopy light distribution; and the trees in the high density treatment were also tied to a 4m single-plane trellis. We did not prune the trellised trees into a single plane but we used the trellis for limb bending and tying in the early years in an attempt to reduce vegetative growth and improve branch distribution. We did not use limb bending and tying after the third growth season. The trees in the low density treatment were not trained to any shape nor pruned (except for 'skirting' to facilitate sprinkler efficiency) until winter 2018 (four years old) when one or two major limbs were removed from the centre of the tree to increase light penetration.

Trees were grafted onto two rootstocks, 'Velvick' and 'Ashdot'. 'Velvick' is a vigorous rootstock and was considered to be the industry standard in the region at the time of planting. 'Ashdot', an Israeli rootstock, was chosen because it was reported to produce 'dwarfed' trees in a trial in central Queensland, and had the highest yield efficiency of rootstocks in that trial for 'Hass' scions (Le Lagadec, 2010).

Rows were oriented north-south and were mounded 40-60 cm.

We applied sprays of plant growth regulators (growth retardants from 2015 on all treatments at the same rates to reduce flush growth in spring. In 2018 we also sprayed in summer (Table 1).

Table 1 Plant growth regulator applications

Date of application	Product name	Active ingredient	Rate
3/09/2015	Sunny	50g/L uniconazole-P	500mL/100L
13/09/2016	"	"	500mL/100L
1/09/2017	AuStar	250g/L paclobutrazol	500mL/100L
13/09/2017	"	"	500mL/100L
7/09/2018	"	"	500mL/100L
25/09/2018	"	"	500mL/100L
30/11/2018	"	"	500mL/100L

The trees were irrigated with micro-sprinklers using an irrigation system that allowed each density to receive different amounts of irrigation. We monitored soil moisture using capacitance probes and manual assessment under randomly selected trees. We applied irrigation to maintain optimum soil moisture to each density.

We used soil and leaf testing, industry recommendations (Newett et al., 2001) and local industry knowledge to determine nutrition requirements. We based the area per tree for fertiliser application on canopy size, folowing the principle that fertiliser should be applied out to the drip line of the canopy.

We applied phosphorus, zinc and calcium (as gypsum) as required on a per square metre basis at the same rates throughout all density treatments, to maintain optimum levels within the soil. Boron was also applied on a per square metre basis using industry recommendations based on soil type that we modified over the years of the trial based on soil and leaf testing. Density treatments received the same rate/m^2 but the treated area per tree differed. Boron applications were generally 0.9g Boron/m^2 and split into approximately six applications.

We determined nitrogen and potassium rates using a combination of soil and leaf testing, expected crop load, industry recommendations (Newett et al., 2001)and local industry advice. In the first and second years after planting, all trees in the trial received the same rate per tree, as tree sizes between density treatments were similar. From the third year after planting, we varied the nitrogen and potassium rates per tree due to differences in tree size. The nitrogen and potassium fertiliser rates for each density treatment are presented in Table 2.

Table 2 Nitrogen and potassium fertiliser rates per tree and per hectare 2014/15 to 2018/19

		2014/15	2015/16	2016/17	2017/18	2018/19
Nutrient	Treatment	Nutrient rate/tree (g)				
Nitrogen	Low	140	127	207	387	761
	Medium	140	127	203	190	304
	High	140	127	160	108	152
Potassium	Low	160	30	234	342	711
	Medium	160	30	232	169	282
	High	160	30	180	97	142
		Nutrient rate/ha (kg)				
Nitrogen	Low	31	28	46	86	169
	Medium	78	71	112	106	169
	High	156	141	180	120	169
Potassium	Low	36	7	52	76	158
	Medium	89	17	129	94	157
	High	178	33	200	108	158

Canopy measurements

Canopy volumes (V) were calculated for all densities using the formula for an irregular ellipsoid:

$$V= (pi \times x \times y \times z)/6$$

where x is along row canopy width, y is across row canopy width and z is tree height less the height of any pruned 'skirt'. Scion and rootstock cross sectional area (CSA) were calculated from circumference or diameter measurements at 20cm above or below the graft union, except where this coincided with a branch on a scion, when the nearest representative position was measured.

Canopy measurements in this report were taken at harvest each year (May). Yield efficiency was calculated using these harvest measurements.

Tree health

Tree health was subjectively rated in November of 2017 and again in 2018 using a visual scale of 0 (healthy) to 10 (extreme leaf loss). Ratings of ≥5 triggered preventative or curative treatments for *Phytopthora* root rot.

Flowering terminal counts and fruit set data

Using sampled branches on one tree per plot (i.e. five replicates of each density/rootstock combination), each year we counted flowering terminals, fruit at the end of the spring flush and fruit at harvest.

We did counts on for the whole tree in 2015/16 and 2016/17. In 2017/18 as the trees were by then too large, counts were done on 12 branches per tree, four from the lower canopy, four from the middle canopy and four from the upper canopy. In 2018/19, due to resource constraints, we reduced this to three branches per tree in the lower canopy.

Counts of spring fruit set included identifying whether fruit had set on determinate or indeterminate shoots. An indeterminate shoot is one that develops an inflorescence with a vegetative tip that emerges from the apex over the spring period. A determinate shoot has an inflorescence but no vegetative shoot. In 2018 only, we identified whether flowering terminals were indeterminate or determinate inflorescences.

Fruit size and distribution

At harvest in 2018 and 2019 we used the branches from the flowering terminal and fruit set counts to assess fruit size distribution of treatments. We used fruit weight to estimate 'fruit count' (FC) sizes, that is, the number of fruit that fit into a standard 5.5 kg marketing tray, using the ranges shown in Table 3.

Table 3 Weight ranges used to estimate fruit size in terms of fruit counts (FC) per tray

Weight range (g)	Estimated fruit count per 5.5 kg tray
≥425	FC12
368 to 424	FC14
325 to 367	FC16
290 to 324	FC18
263 to 289	FC20
240 to 262	FC22
220 to 239	FC24
204 to 219	FC26
190 to 203	FC28
176 to 189	FC30
<=175	Small

Assessment of yield and quality of harvested fruit

Fruit were picked and weighed at commercial maturity (minimum dry matter percentage of 23%). From each tree, 50 fruit were randomly selected and weighed to determine average fruit weight. External quality (incidence and extent of blemish, rots, pest damage and so on) was assessed on all 50 fruit and an estimate made of whether the fruit was Class 1, 2, 3 or 'reject' according to the Australian Avocado Grading Guidelines (poster produced 2018 by Avocados Australia Ltd and Agri-Science Queensland, DEEDI).

Three fruit from each tree were randomly selected and the dry matter percentage was determined, by using 15g of flesh from each fruit weighed before and after drying at approximately 65°C for >48 hours. In 2016, 2017 and 2018 a sample of 20 fruit randomly selected from each plot were assessed for incidence of disease. We did not treat these fruit with any post-harvest fungicide.

Assessment of disease incidence of harvested fruit

At harvest in 2015/16, 2016/17 and 2017/18, 20 fruit per plot were randomly selected, taking roughly equal numbers from each tree in the plot, transported without refrigeration to QAAFI laboratories in Brisbane and assessed for disease incidence using standard protocols for avocados. None of the fruit received the post-harvest cool storage or any fungicide treatment that would be applied to commercial consignments, so the reported incidence data is high and results should not be compared to any commercial data. Each piece of fruit was assessed when deemed to be 'eating soft' for percentage of surface area affected by 'side' anthracnose and percentage of fruit volume affected by stem end rot. Less than 4% anthracnose is considered negligible, as is less than 3% stem end rot. From this 'severity' data, disease incidence was calculated. An approximation of 'marketability' was calculated based on the incidence of anthracnose and stem end rot combined.

Branch distribution

In January 2019 we counted and measured second order branches (i.e. emanating from the trunk) on one tree per plot (i.e. five replicates per density/rootstock combination). For the low density treatment, trees typically had three or four main spreading branches coming from the trunk, so the most vigorous and upright was selected as the ‘central leader’, and the remaining branches treated as second order branches.

Pruning

Trees in the medium and high density treatments were pruned in winter each year to shape them into a central leader, control height and keep the trees roughly within their planting space. All pruning was selective removal of branches, not hedging or topping. The diameter of pruned branches were recorded after pruning. Only branches >10mm in diameter were recorded. In general, pruning effort and severity did not differ much between the medium and high density treatments, except in 2017/18 when trees in the medium density treatment had a higher number of cuts per tree and higher combined CSA of branches (Table 4). In the summer of 2016/17 and 2017/18, the trees in the high density treatment were also ‘topped’ to try and limit vertical growth (Table 5). In 2018/19, a small amount of topping was done at the end of spring flush but not repeated in summer (pruning data not collected).

Until 2018, trees in the low density treatment were not pruned except for ‘skirting’ the lower canopy to facilitate irrigation efficiency. Skirting effort was high in 2017 but not in other years: in that year the number of branches cut for skirting on the low density treatment was not significantly different from the number of branches cut from trees in the medium density treatment (Table 4). In September 2018, according to standard local practice, several large limbs (on average 3.6 per tree) were removed from trees in the low density treatment to open up the inner canopy to light and to help control height.

Table 4 Winter pruning cuts by treatment 2016/17 to 2018/19

		No. of cuts per tree			Mean diameter of cuts (mm)			Total CSA of cut branches (cm^2)		
		2016/17	2017/18	2018/19	2016/17	2017/18	2018/19	2016/17	2017/18^2	2018/19
Treatment	Grand mean	22.4	13.2	7.0	15.4	19.3	34.8	50.4	48.1	50.6
Density	High	21.7	10.3 a	8.4 b	16.0	20.3 b	22.7 a	52.3	41.4 a	41.5
	Medium	23.0	14.0 b	8.9 b	14.8	20.8 b	26.9 a	48.5	60.4 b	48.8
	Low	--	15.2 b	3.6 a	--	16.9 a	54.9 b	--	42.3 a	61.4
	P	*0.183*	*0.016*	*<.001*	*0.204*	*0.038*	*<.001*	*0.506*	*0.001*	*0.081*
	Ese	*0.6*	*0.932*	*0.291*	*0.6*	*0.95*	*3.19*	*3.6*	*2.53*	*5.39*
Rootstock	Ashdot	19.6 a	12.9	6.9	16.4 b	18.7	31.6	50.1	42.7 a	43.9 a
	Velvick	25.2 b	13.4	7.0	14.3a	19.9	38.1	50.6	53.5 b	57.3 b
	P	*0.023*	*0.61*	*0.878*	*0.012*	*0.206*	*0.068*	*0.883*	*0.05*	*0.007*
	Ese	*1.4*	*0.7*	*0.4*	*0.5*	*0.6*	*2.3*	*2.3*	*3.5*	*2.9*
Density Rootstock*	*P*	*0.191*	*0.431*	*0.909*	*0.081*	*0.395*	*0.171*	*0.297*	*0.826*	*0.528*

Data excludes summer pruning (see Table 5)

Table 5 Summer pruning cuts (topping) treatment high density pruning by rootstock treatment 2016/17 and 2017/18 Click or tap here to enter text.

	No. of cuts per tree		Mean diameter of cuts (mm)		Total CSA of cut branches (cm^2)	
Treatment	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
Grand mean	3.1	8.0	24.0	23.8	18.8	40.4
Ashdot	3.3	7.3	21.8	22.4	17.4	33.3
Velvick	2.9	8.7	26.1	25.3	20.2	47.4

Architecture study

Five trees from each of the rootstock/density combinations were selected for detailed ‘architectural’ analysis. Using one branch per tree in 2015/16 and 2017/18, and two in 2016/17, we recorded the length, orientation, angle, node number, estimated time of growth (month), maturity, diameter and whether branching was proleptic or sylleptic on every growth unit of the selected branches three times per year: during flowering, after the spring flush and after the summer flushes each year. Flowering terminals and axillaries were recorded in spring, and fruit at the end of spring and in summer. In 2016/17 and 2017/18 we also counted leaves remaining on each growth unit. We also recorded the node numbers from which branches, flowers and fruit emerged, although this of necessity was restricted to visible nodes and excluded the ring of invisible nodes in the intercalary ring at the base of a proleptic growth unit.

Light interception study

Orchard light interception was estimated by measuring photosynthetically active radiation (PAR), that is, light in the 400 to 700 nm band, reaching the orchard floor. In 2015 we used an AccuPAR ceptometer (Decagon Devices Inc.) to measure light reaching the orchard floor; and from 2016 we used a purpose-built trolley with individual sensors (Apogee SQ-100 PAR point sensors, Apogee Instruments, Inc.) inserted in a horizontal boom stretching across the interrow at 50 cm above the ground. The point sensors were connected to a datalogger CR1000 (Campbell Scientific Inc.)

The ceptometer (AccuPAR LP-80, Decagon Devices Inc.) used had an 80 cm blade with sensors every 10 cm. We made ceptometer measurements perpendicular to the tree row and spaced at 1 m intervals in transects along the tree row, with transects extending out from the tree row on both sides to the mid-point between rows. We took 'above-canopy' ceptometer measurements in full sun conditions immediately before or after the below canopy measurements for each plot.

Sensors on the boom of the trolley were spaced at 30cm intervals and recorded readings every 6 seconds. When pulled at walking speed, this recorded approximately 10 readings per metre. We took an 'above canopy' reading using the light trolley away from the canopy immediately before or after each plot measurement.

We measured light in early summer each year (December/January). In 2014/15 and 2015/16, measurements were made three times in the one day under clear sky conditions at +/- 2 hours (sun position mid) and 4 hours (sun position low) before or after solar noon and at solar noon (all times within +/- 20 minutes). In subsequent years, measurements were made three times in the one day under clear sky conditions at +/- 1.5 and 3 hours before or after solar noon and at solar noon (all times within +/- 20 minutes).

For each measurement, we calculated light interception as the proportion of PAR removed between the above canopy PAR measurement and the measurement at the orchard floor. For each measurement time, we averaged the individual light interception (LI) measurements for the entire plot. We calculated total light interception for each plot using the equation:

$$\text{Total light interception} = (\text{LI sun low} + \text{LI sun mid} + \text{LI solar noon} + \text{LI sun mid} + \text{LI sun low})/5$$

Light distribution studies

In our first foray into the pattern and effects of light distribution in the three canopies in 2017/18 we used three single-tree replicates of each density/rootstock combination, using different trees for a study of canopy peripheries ('periphery study') and a second study along a transect within the canopy ('transect study').

We measured PAR with a ceptometer (AccuPAR LP-80, Decagon Devices Inc.) with an 80 cm blade with 8 sensors. PAR was measured in July and August after winter pruning (only trees in the medium and high density treatments were pruned). PAR was measured on the eastern side of the tree at solar noon (+/- 20 minutes), solar noon minus two hours (+/- 20 minutes) (eastern side illuminated) and solar noon plus two hours (+/- 20 minutes) (eastern side shaded). Readings for each tree were immediately preceded and succeeded by an 'above canopy' reading measured nearby in the open.

For the periphery study 2017/18, PAR was measured in 50 cm intervals, beginning 50 cm above the ground and ending at a height of 300 cm, with the centre of the ceptometer aligned with the centre of the trunk. We counted fruit and terminals where they were within 25 cm above or below each ceptometer reading x 80 cm (the width of the ceptometer) to a depth of 50 cm from the surface of the canopy.

For the transect study 2017/18, a frame with internal dimensions of 50 x 50 x 50 cm was erected at 1 metre height above ground (i.e. 1-1.5 metres) on the eastern side of the tree from the trunk eastwards to the edge of the canopy. PAR was measured at 50 cm increments at the top of the frame, beginning at 25 cm from the trunk of the tree. Fruit, terminals and leaves were counted within each 50 cm cube centring around each PAR measurement position.

In each band of the periphery study and in each transect cube, we tagged three randomly selected shoots during winter (before flowering had commenced). On each tagged shoot we measured the length and diameter of the parent GU (i.e. last summer flush), intensity of terminal flower (i.e. no of secondary axes), flowering stage of the terminal and also of the axillaries on the parent GU and length of the vegetative shoot (terminal only i.e. axillary branching not measured). This was done every fortnight from 15/8/17 to 26/9/17. Flowering stages were as described by Salazar-Garcia et al. (1998). We also measured the number and size of fruit (terminal and axillary) fortnightly from 4/10/17 until 31/1/18.

In 2018/19 we changed the data collection methodology to see if we could increase clarity in the relationships between light and our key variables. We changed the times of the readings to solar noon and 3 hours before and after solar noon (+/- 20 minutes each) and measured once in winter (August) and again in spring after the spring flush (November). In spring (but not in winter), we were able to measure a single time using diffuse light rather than full sunlight as the weather was consistently cloudy. Wunsche et al. (1995) found that a single measurement in diffuse light correlated well with several readings in sunlight. We studied only one rootstock ('Velvick') but increased the number of replicates from three to five. For the transect study, we increased the numbers of terminals tagged in each cube from three to five. For the periphery study, instead of measuring light only once in each 50 cm band with a ceptometer, we tagged 30 terminals and measured PAR just above the tip of each terminal with a single light sensor. The tagged terminals were located on the eastern side of the tree, within a one metre band, from the bottom to the top of the canopy or as high as we could safely reach from the cherry picker. We measured height and distance from the level of the trunk centre in addition to the usual measurements.

Crop load trials

Avocado trees have a low percentage fruit set per flowering terminal and a high fruit drop over summer. We conducted a range of crop load trials to try to boost avocado crop load, using methods reported as successful in published literature that might also be practical in a commercial high density orchard.

Flowering and crop load trial 2013/14

In this trial we thinned from 0 to 95% of inflorescences and measured resulting crop load. For details see Hofman et al. (2018).

Tipping and fruiting sites trial 2014/15-2015/16

In this trial we partially or fully 'tipped' the vegetative terminals of indeterminate inflorescences and/or applied 'Cytolin' (® Sumitomo Chemicals, 19g/L 6-benzyladenine and 19g/L gibberellins 4 +7) as a spray treatment at 15 or 20 ml/L after the spring flush. The objective of the tipping treatments was to reduce competition for resources between fruit set and vegetative growth, and the objective of the 'Cytolin' treatments was to increase branching sites (see discussion on 'Number of fruiting sites' and 'Fruit-shoot competition' in the section 'Review of factors that may have affected yield').

Avocado is largely a terminal flowering tree and thus the availability of fruiting sites is important in increasing yield. Cytokinins are known to overcome acrotony and apical dominance by stimulating the growth of axillary buds (Buban, 2000). This effect is used in crops such as apple to increase branching in young trees. Thorp and Sedgley (1993b) found that 'Cytolin' applied at the end of extension of the spring flush increased sylleptic axillary shoot growth and subsequent fruit set. For details of this trial, see Hofman et al. (2018).

Summer fruit drop architecture study 2015/16

A possible area for yield gain is to reduce the drop of young fruitlets, particularly the second period of drop in early summer that is characteristic of some cultivars including 'Hass'. While the spring fruit drop is the largest in numerical terms, summer drop can be very considerable and represents a significant mass of loss of dry matter. Reports of 35-66% drop can be found in the literature ((Lahav and Zamet, 1999; Salazar-Garcia et al., 2006). To date, no pattern has been found to the 'choice' of fruit that drop in summer. Dixon et al. (2006) concluded it was "an apparently random pattern" (p. 47).

To explore architectural factors that may be associated with summer fruit drop, we selected four branches on four sides of each of four 4-year-old Hass trees on 'Velvick' rootstock on a commercial farm in Childers in central Queensland. On average, branches had a basal diameter of 24mm, that is, a branch cross sectional area ('BCSA') of 4.9 cm. Fruit on these branches, in total 253 fruit, were tagged and numbered on 9/12/2015. Drop and diameters of the fruit and pedicel were recorded with digital callipers approximately every two weeks from 21/12/15 until 28/1/2016. Progressively over this period, we recorded the length, orientation, angle, node number, number of leaves, estimated time of growth, maturity, diameter and whether branching was proleptic or sylleptic on every growth unit of the selected branches. Temperature and humidity were recorded over the period using a TinyTag data logger in a mini Stevenson screen installed at the beginning of the adjacent row. This data was then analysed to see if we could find any structural factors influencing summer fruit drop.

'Cytolin' trials 2015/16 to 2017/18

Cytolin (® Sumitomo Chemicals, 19g/L 6-benzyladenine and 19g/L gibberellins $_{4+7}$) is a plant growth regulator that has been used successfully in several tree crops to increase sylleptic branching. Over three years we tested the use of 'Cytolin' applied at the end of extension of the spring flush to increase sylleptic axillary shoot branching in the guard trees of the Planting Systems Trial. We acknowledge that guard trees are not representative of trees within an orchard, but these experiments were intended as preliminary trials. Our trials varied in approach: in 2015/16 we sprayed individual shoots on young trees; in 2016/17 we sprayed whole trees (2 year old, 'Velvick' rootstocks, low density treatments, five single tree replicates); and in 2017/18 we sprayed whole trees in both the low and high densities ('Velvick' rootstocks, five single tree replicates). Trees were sprayed with 40 ml 'Cytolin' per litre of water, plus 0.33 ml/L 'Spread Wet'. We monitored fruit set through tagged shoots in all trials. In 2017, 2018 and 2019 we recorded whole tree yield.

Fruit thinning trials 2015/16

Early thinning of fruit in some tree crops can increase final yield by reducing the competition between fruit for resources. Early in the summer of 2015 we thinned fruit on five 3-year-old 'Hass' on ‘Velvick’ trees in a commercial orchard in Childers. Treatments were applied to one limb per tree. On each tree, one limb was thinned by removing 30% of fruit, another by removing 50% of fruit and a third by retaining one fruit on those inflorescences that had set more than one fruit. Treatments were compared to an unthinned control limb. In a similar trial, we applied treatments to whole trees (6 per treatment) in the guard rows of the Planting Systems Trial, thinning them evenly across all branches by removing 25% or 50% of fruit of each branch total. The objective was to see if any rates of thinning would reduce summer drop and result in an overall yield improvement.

Flowering intensity trials 2017/18 and 2018/19

Our data suggests that while fruit set is better on terminal inflorescences, axillary inflorescences contributed 20-30% of final fruit on our young trees (Table 36). Thorp (1992) found greater fruit set on ‘compound inflorescences’ (i.e. shoots) with four or more axillary inflorescences. In vigorous shoots on young trees, many viable axillary buds form (visible as white, plump buds) but only the most apical tend to ‘release’: many remain quiescent and eventually just abscise. We hypothesised that plant growth regulators could be used to increase axillary bud release and increase fruit set. Lovatt (2010, 2011) sprayed or trunk injected cytokinin products alone or combined with tri-iodobenzoic acid (TIBA), an auxin transport inhibitor, to increase spring bud break. Results varied with experiment but overall TIBA alone or in combination with cytokinin increased floral shoots and, in some cases, yield in the ‘off’ year.

In addition, we hypothesised that plant growth regulators could also be used to manipulate expansion of the floral and/or vegetative shoot and thus the balance of competition between vegetative growth and fruit set. Salazar Garcia and Lovatt (1995; 1998) found that, if a floral shoot was already differentiated, the apical inflorescences of shoots sprayed with GA_3 developed in advance of inflorescences on branches not treated with GA_3. The GA_3 also caused precocious development of the leaves of indeterminate inflorescences.

The aim of our trials was to increase flowering intensity through the use of plant growth regulators to increase bud release on axillary flowers. A secondary objective was to encourage earlier development of vegetative shoots in indeterminate inflorescences with the hypothesis that this might reduce the competition between fruit set and vegetative growth at mid-bloom.

Our trials were on medium density guard trees in the Planting Systems Trial (five replicates in each rootstock). In 2017/18 we sprayed one branch per tree on the SE side of the tree. There were five treatments as follows: GA_3 at 200mg per litre in July or August; Cytolin® spray July at 30ml/L in July or August or TIBA at 0.5g/L in August (dissolved in 4 g/L bicarbonate of soda). Cytolin ® is a product from Sumitomo Chemicals, and consists of 19g/L 6-benzyladenine and 19g/L gibberellins 4 +7. The GA_3 was applied as ProGibb®, with pH adjusted to 5 to 5.5. Sprays were to the point of run off. A non-ionic spreader at 1 mL per 10 L solution was added to all sprays. We left one tree per plot unsprayed as a control. Before the first treatment was sprayed, we tagged three shoots on each branch to monitor growth and fruit set.

In 2018/19 we applied four treatments to whole trees, again using guard trees at medium density, on both ‘Ashdot’ and ‘Velvick’ rootstocks (five replicates of each) i.e. a single spray of Cytolin or TIBA on 21 June 2018 or 31 July 2018 at the same rates as above, with a control treatment left unsprayed. Trees were sprayed to the point of run off to a height of 2.5 m (i.e. tops were not sprayed). Twelve shoots were tagged around the tree before treatments commenced for monitoring of growth and fruit set. At harvest time in 2019, we collected and weighed all the fruit from each tree.

Girdling trial 2018/19

Girdling is the removal of a thin strip of bark that interrupts the movement in the phloem until healed, but not movement in the xylem. Girdling (also called 'ringing' or 'cincturing') of avocados has been demonstrated to affect flowering intensity, timing and fruit set depending on timing.

Girdling of branches in winter has been shown to increase flowering intensity (Gardiazabal et al., 1995) and yield (Davie, 1997; Gregoriou, 1989; Hackney et al., 1995; Ticho, 1971; Trouchoulias and O'Neill, 1976). Lynce-Duque (2015) reports increased number of determinate flowers in addition to indeterminate flowers. Girdling at flowering (a more common timing) has been shown to increase fruit set and yield (Davie, 1997; Espindola et al., 2007; Hodgson and Cameron, 1937; Kohne, 1992). Girdling 60 days after full bloom increased fruit size (Flores Vivar and Escobedo Alvarez, 2015). Girdling after flowering (December) caused out of season flowering but increased fruit size in work by Davie et al. (Davie et al., 1995a; Davie et al., 1995b).

In order to test the effect of girdling on fruit set and retention in high density systems, we undertook a trial in a block of 'Hass' on 'Velvick' rootstocks planted at Childers in a 3 x 7m spacing. We applied treatments to single tree plots in eight blocked replicates. Treatments were the girdling of 1/3, 1/2 or 2/3 of larger branches in either winter or in spring after fruit set. Control trees had no girdling. Early in winter, we pruned all trees, including the control trees, to a vase shape by taking out one or two limbs from the centre of the tree. The aim of the winter girdling was to increase flowering intensity on the girdled limbs; the aim of the spring girdling (23 and 24/10/2018) was to increase fruit set. We tagged shoots on both girdled and ungirdled limbs to monitor fruit set and retention, and the yields of whole trees were collected at harvest in May 2019.

Analyses and reporting

Data for all trials were analysed using Genstat (18.2, Copyright 2016, VSN International Ltd).

Most analyses for the Planting Systems Trial applied two-way analysis of variance for a split plot design (densityxrootstock). Significantly different means were identified using Fisher's protected least significant difference test at α=0.05, that is, at the 95% confidence level. The convention of indicating 'significantly different' means by use of a differing suffix 'a', 'b', 'c' etc is used throughout this report, even when comparing only two means. In this convention, treatment means followed by the same letter, or no letter at all, are not significantly different from each other at the 95% confidence level. In this report we have reported the 'P' value, that is, the probability that the differences in the treatment means are due to the variation in the sample rather than to treatment differences. Error is reported as estimated standard error of the means or 'ese', that is, an estimate of how far the sample mean of the data is likely to be from the true population mean.

Simple linear regression was also used for some analyses.

For most variables there were no significant interactions between density and rootstock treatments at the 95% confidence level. We therefore have reported the *P* value for the interaction but not the means for the six different density/rootstock treatments, except where the *P* value was less than 0.05. An exception is the yield data shown in Table 12 as the density/rootstock combinations may be of interest to some readers even though the differences between them were not significant.

Results

Density effects on growth and canopy volume

The support of the trellis meant trees in the high density treatment initially grew rapidly in height. By harvest 2018, there was no significant difference between mean tree heights of the three density treatments, despite pruning of the tops of trees in the high density treatment (Table 6). By harvest 2019 the trees at high density were on average 5.4 m high compared to 5.0m for the trees at low density (ns). This testifies to the rampant growth in the tops of trees when 'topped'.

Scion and rootstock cross sectional areas and canopy volume per tree show significant differences between density treatments (Table 7, Table 8).

Trees in the high and medium density treatments 'filled' their allocated space rapidly. Canopy volume per hectare has not increased much for the high and medium density treatments since 2016/17, whereas the low density treatment shows a continuing increase in canopy volume per hectare over the four years (Table 6). The increase in 2018/19 for the high densities to 19700 m^3 per ha is due largely to height increases in the summer flush. By 2018/19 there were no significant differences between density treatments in mean canopy volume per hectare (Table 7).

Table 6 Mean tree height (cm) at time of harvest by density and rootstock treatments 2015/16 to 2018/19

		2015/16	2016/17	2017/18	2018/19
Treatment	Grand mean	320	416	486	521
Density	High	367 b	437 b	492	539
	Medium	289 a	398 a	481	528
	Low	305 a	412 ab	484	495
	P	*0.007*	*0.034*	*0.917*	*0.142*
	Ese	*13*	*9*	*18*	*143*
Rootstock	Ashdot	297 a	389 a	457 a	500 a
	Velvick	344 b	442 b	515 b	542 b
	P	*0.001*	*<.001*	*0.003*	*0.012*
	Ese	*8*	*7*	*11*	*102*
Density Rootstock*	*P*	*0.197*	*0.486*	*0.285*	*0.390*

Table 7 Mean canopy volume at time of harvest by density and rootstock treatments 2015/16 to 2018/19

		Canopy volume per tree (m^3)				Canopy volume per ha (m^3/ha)			
		2015/16	2016/17[1]	2017/18[1]	2018/19[1]	2015/16	2016/17[1]	2017/18[1]	2018/19[1]
Treatment	Grand mean	13.7	29	41	40.9	8452	12399	15556	18129
Density	High	13.9	17.5 a	20.1 a	25.4 a	15471 b	14746 b	16483 b	19746
	Medium	11.6	27.2 b	37.4 b	45.7 b	6450 a	13109 b	16554 b	18826
	Low	15.5	42.4 c	65.5 c	83.7 c	3434 a	9341 a	13630 a	15816
	P	*0.082*	*<.001*	*<.001*	*<.001*	*<.001*	*<.001*	*0.049*	*0.105*
	Ese	*1.0*	*2.6*	*4.3*	*4.7*	*1200*	*563*	*786*	*1182*
Rootstock	Ashdot	12.4 a	26.3 a	37 a	47.7	7618 a	11402 a	14437 a	17188
	Velvick	15. 0 b	31.8 b	45 b	55.5	9286 b	13395 b	16675 b	19070
	P	*0.048*	*0.019*	*0.046*	*0.118*	*0.048*	*0.003*	*0.032*	*0.058*
	Ese	*0.8*	*1.4*	*2.5*	*3.2*	*535*	*373*	*651*	*634*
Density Rootstock*	*P*	*0.997*	*0.378*	*0.304*	*0.805*	*0.442*	*0.919*	*0.559*	*0.678*

[1] Canopy volumes per tree use actual tree dimensions even where interlocking; volumes per hectare were calculated using planting space where canopies were interlocking

Table 8 Mean rootstock and scion cross sectional area by density and rootstock treatments 2015/16 to 2018/19

		Rootstock CSA (cm^2)				Scion CSA (cm^2)			
		2015/16	2016/17	2017/18	2018/19	2015/16	2016/17	2017/18	2018/19
Treatment	Grand mean	80	143	225	272	75	150	231	278
Density	High	70 a	112 a	155 a	176 a	66 a	110 a	149 a	173 a
	Medium	75 a	131 a	203 a	241 b	66 a	130 a	204 b	244 b
	Low	94 b	185 b	318 b	400 c	93 b	209 b	339 c	417 c
	P	*0.007*	*<.001*	*<.001*	*<.001*	*0.001*	*<.001*	*<.001*	*<.001*
	Ese	*4*	*9*	*16*	*19*	*4*	*8*	*14*	*19*
Rootstock	Ashdot	72 a	120 a	197 a	245 a	74	141	220	269
	Velvick	87 b	165 b	254 b	300 b	76	158	241	287
	P	*<.001*	*<.001*	*<.001*	*0.007*	*0.734*	*0.196*	*0.217*	*0.297*
	Ese	*3*	*6*	*9*	*12*	*4*	*8*	*12*	*12*
Density rootstock*	*P*	*0.977*	*0.582*	*0.777*	*0.856*	*0.236*	*0.681*	*0.646*	*0.425*

Table 9 Mean ratio of rootstock: scion cross sectional areas by density and rootstock treatments 2015/16 to 2018/19

		2015/16	2016/17^1	2017/18^1	2018/19^1
Treatment	Grand mean	1.057	0.984	0.991	0.988
Density	High	1.057	1.027 b	1.044	1.024
	Medium	1.127	1.024 b	0.988	0.982
	Low	1.042	0.899 a	0.941	0.959
	P	*0.313*	*0.024*	*0.157*	*0.211*
	Ese	*0.0394*	*0.0295*	*0.0338*	*0.0239*
Rootstock	Ashdot	0.998 a	0.893 a	0.921 a	0.929 a
	Velvick	1.153 b	1.074 b	1.061 b	1.048 b
	P	*0.013*	*<.001*	*<.001*	*<.001*
	Ese	*0.0377*	*0.0217*	*0.0197*	*0.0190*
Density rootstock*	*P*	*0.103*	*0.086*	*0.215*	*0.149*

There were no significant differences between densities in tree health rating in either 2017 or 2018 (*P*=0.135 and 0.125 respectively). Average ratings were 0.45 and 2.01 in these years respectively.

Timing of growth

Total annual growth in terms of length of vegetative growth per cm^2 of branch CSA as measured in our architecture studies showed there was no difference between density treatments except in 2015/16. In that year, sample branches in the medium density treatment had more growth than those in the high density treatment in summer (Table 10).

However, while treatment means are not significantly different for most measurements, there appear to be some differences between treatments in the timing of growth, particularly in terms of new branching. In 2015/16 and 2017/18 total growth in the low density and, to a lesser extent in the medium density treatments, was stronger in the summer flush than the spring flush, but not in 2016/17, both in terms of total length of growth (Table 10) and numbers of new branches (Table 11). Possibly the carbon accumulated by new vigorous summer flush in these years contributed to strong spring growth in the alternate year. Studies show avocado leaves only maintain high photosynthesis rates early in their life -- peaking at 50 days of age in a study by Liu et al. (2002) and peaking at 2 months' age but stable up to 4 months' age in a study by Medina-Torres et al. (2011). Timing of new growth could thus affect the quantum of carbohydrate stores in the density treatments.

In the high density treatment, there was an opposite trend: vegetative growth was concentrated in the spring flush in 2015/16 and 2017/18, but concentrated in the summer flush in 2016/17, possibly because winter pruning stimulated relatively more spring flush growth than summer growth. Why this did not occur in 2016/17 is unclear: it is possible that there is a biennial pattern here -- perhaps triggered by pruning of a certain severity -- that is not obvious in yield (see data on biennial bearing index in 'Density effects on yield').

In both spring and summer, new branching was generally the largest proportion of new growth for all density treatments (Table 10). This has implications for the availability of fruiting sites: see discussion and data on branching in the section 'Canopy complexity' below.

Table 10 Sum of length of total growth (cm) per cm^2 of branch CSA by type of growth unit and density treatment in spring and summer 2015/16-2017/18

	Spring					Summer			Year
Treatment	Terminal indeterminate inflorescences	Axillary indeterminate inflorescences	New vegetative branches	Continuing growth units	Total	New branches	Continuing growth units	Total	Total
	2015/16								
Grand mean	10.8	14.5	22.5	na	47.9	67.4	50.1	117	165
High density	11.9	14.5	32.2	na	58.6	43.0 a	46.4	89 a	148 a
Med density	9.8	23.1	32.4	na	65.3	87.8 b	54.5	142 b	208 b
Low density	10.8	6	3.1	na	19.9	71.4 ab	49.3	121 ab	141 a
P	*0.804*	*0.167*	*0.077*		*0.065*	*0.032*	*0.475*	*0.033*	*0.035*
ese	*2.26*	*5.68*	*8.91*		*12.39*	*9.69*	*4.54*	*11.4*	*16*
	2016/17								
Grand mean	13.3	7.83	41.5	2.49	64.3	41.1	24.1	65.2	129.6
High density	13.28	3.5	27.5	1.57	45.8	52.1	19.5	71.5	117.4
Med density	11.69	12.29	57.1	4.05	85.2	29	18.7	47.7	132.9
Low density	14.92	7.71	40	1.84	62	42.4	34.1	76.5	138.4
P	*0.422*	*0.054*	*0.226*	*0.112*	*0.112*	*0.085*	*0.052*	*0.115*	*0.692*
ese	*1.65*	*2.122*	*11.1*	*0.797*	*11.6*	*6.28*	*4.14*	*9.08*	*17.6*
	2017/18								
Grand mean	19.3	1.89	13.9	1.18	36.3	51.7	25.6	77.3	113.6
High density	17.9	1.65	29	2.26	50.8	36.5	23	59.5	110.3
Med density	21.4	1.51	10.1	0.45	33.5	50.9	26.5	77.4	110.8
Low density	18.7	2.52	2.6	0.83	24.7	67.6	27.3	94.9	119.6
P	*0.443*	*0.585*	*0.101*	*0.060*	*0.106*	*0.075*	*0.733*	*0.172*	*0.923*
ese	*1.93*	*0.724*	*7.71*	*0.473*	*7.65*	*8.16*	*4.02*	*11.92*	*18.46*

na not recorded in sufficient numbers in this period

Table 11 Numbers of new branches per cm^2 of branch CSA by density treatment in spring and summer 2015/16- 2017/18

	2015/16			*2016/17*			*2017/18*		
Density	Spring	Summer	Total	Spring	Summer	Total	Spring	Summer	Total
Grand mean	2.86	4.87	7.73	5.57	3.13	8.7	2.1	3.63	5.73
High	3.59	3.13 a	6.72	5.17	3.47	8.64	3	2.46 a	5.46
Medium	3.23	5.62 b	8.85	6.23	2.37	8.6	1.75	3.81 ab	5.57
Low	1.75	5.86 b	7.61	5.3	3.57	8.86	1.54	4.62 b	6.16
P	*0.2*	*0.009*	*0.294*	*0.742*	*0.21*	*0.988*	*0.29*	*0.024*	*0.868*
ese	*0.694*	*0.503*	*0.894*	*1.038*	*0.48*	*1.319*	*0.654*	*0.439*	*1.002*

'Spring' Includes axillary indeterminate inflorescences. 'CSA': cross sectional area

Density effects on yield

Yield per tree decreased with increasing density after the first crop, as would be expected given canopy size (Table 12).

Increased planting density did not compensate to produce higher yields per hectare after the first year. In the first cropping year (2015/16) yields per hectare were highest for the high density treatment, at 7.3 tonnes per ha compared to 1.8 for the medium density treatment and 1.6 for the low density treatment (*P*<.001) (Table 12). In the second and third cropping year, however, yields for all three densities were not significantly different at an average 7.9 tonnes/ha in 2016/17 and 13.01 tonnes/ha in 2017/18 (*P*=0.314 and 0.696 respectively). In the fourth cropping year (2018/19), yields for the high density treatment were 11.3 tonnes/ha compared to 16.0 tonnes/ha for the medium density treatment and 19.6 tonnes/ha for the low density treatment (*P*=0.004).

We calculated the alternate bearing index of trees for the last two years of yield using the formula (year 1 crop - year 2 crop)/(year 1 crop +year 2 crop). A figure of 0 means no variability in yield between the two years; 1 is highly biennial. There were no significant density treatment differences in the alternate bearing index (*P*=0.442). The overall mean of all treatments was 0.181.

Table 12 Mean yield per tree and per hectare by density and rootstock treatments 2015/16 to 2018/19

		Yield per tree (kg)				Yield per ha (tonnes/ha)			
		2015/16	2016/17	2017/18	2018/19	2015/16	2016/17	2017/18	2018/19
Treatment	Grand mean	5.72	17.4	31.8	42.2	3.63	7.9	13.0	15.6
Density	High	6.54	8 a	11.1 a	10.2 a	7.33 b	8.9	12.28	11.3 a
	Medium	3.19	14.7 a	24.1 b	28.9 b	1.85 a	8.2	13.38	16.0 b
	Low	7.43	29.5 b	60.2 c	87.7 c	1.71 a	6.6	13.37	19.6 b
	P	*0.073*	*0.001*	*<.001*	*<.001*	*<.001*	*0.314*	*0.696*	*0.004*
	Ese	*1.161*	*2.69*	*2.95*	*3.47*	*0.73*	*1.0*	*1.0*	*1.2*
Rootstock	Ashdot	5.93	20.2 b	37.3 b	46.3b	3.6	8.9	15.1 b	17.0b
	Velvick	5.51	14.6 a	26.2 a	38.2a	3.5	6.9	10.9 a	14.2a
	P	*0.682*	*0.019*	*<.001*	*0.009*	*0.881*	*0.063*	*0.002*	*0.004*
	Ese	*0.71*	*1.47*	*1.76*	*1.84*	*0.48*	*0.68*	*0.76*	*0.56*
Density rootstock*	High/Ashdot	6.5	8.7	12.8ab	11.8 a	7.2	9.7	14.2	13.1
	Med/ Ashdot	3.3	16.1	27.2c	28.8 b	1.8	9.0	15.1	16.0
	Low/Ashdot	8.0	35.9	72.0e	98.2 d	1.8	9.0	16.0	21.8
	High/Velvick	6.6	7.3	9.3a	8.6 a	7.3	8.2	10.3	9.5
	Med/Velvick	3.1	13.3	20.9bc	28.9 b	1.7	7.4	11.6	16.0
	Low/Velvick	6.9	23.2	48.4d	77.2 c	1.5	5.2	10.8	17.1
	P	*0.888*	*0.093*	*0.013*	*0.013*	*0.985*	*0.829*	*0.786*	*0.073*
	Ese		*3.2*	*3.7*	*4.1*		*1.3*	*1.4*	*1.4*

Density effects on fruit quality

Fruit size

There were no density treatment differences in mean fruit weight (Table 13). There was a weak trend to larger fruit size from the high density treatment but data is inconclusive (see discussion in the section ‘Fruit-shoot competition: the influence of canopy width’, including Figure 16 and Figure 17.

Table 13 Mean fruit weight (g) 2015/16 to 2018/19

		2015/16	2016/17	2017/18	2018/19
Treatment	Grand mean	291	281	290	272
Density	High	284	279	293	281
	Medium	290	283	294	276
	Low	298	281	282	272
	P	*0.383*	*0.916*	*0.236*	*0.113*
	Ese	*6.6*	*6.5*	*4.8*	*2.8*
Rootstock	Ashdot	300 b	300 b	298 b	283 b
	Velvick	281a	261 a	282 a	270 a
	P	*0.034*	*<.001*	*0.002*	*0.006*
	Ese	*5.5*	*4.6*	*3.0*	*2.9*
Density Rootstock*	*P*	*0.373*	*0.507*	*0.148*	*0.558*

Dry matter

After the first harvest, dry matter percentages did not differ by density treatment (Table 14).

Table 14 Treatment effects on dry matter percentage of fruit 2015/16 to 2018/19

		2015/16	2016/17	2017/18	2018/19
Treatment	Grand mean	26.1%	24.5%	25.2%	24.2%
Density	High	25.2% a	24.4%	24.9%	24.6%
	Medium	27.0% c	24.0%	25.0%	23.7%
	Low	26.1% b	25.0%	25.7%	24.3%
	P	*0.004*	*0.101*	*0.118*	*0.195*
	ese	*0.26%*	*0.25%*	*0.25%*	*0.33%*
Rootstock	Ashdot	26.9% b	24.9% b	25.4%	24.4%
	Velvick	25.4% a	24.1% a	25.1%	23.9%
	P	*<.001*	*0.037*	*0.400*	*0.104*
	ese	*0.24%*	*0.26%*	*0.23%*	*0.18%*
Density Rootstock*	*P*	*0.344*	*0.464*	*0.833*	*0.349*

External blemish

In 2017 and 2018 there was a higher percentage of fruit in the low density treatment downgraded to Grade 2 compared to the high and medium density treatments (Table 15). This was largely due to damage by pests including thrips and *Monolepta* beetles. This result may be due to better pesticide spray penetration into the 'thinner' canopies of the medium and high densities.

In 2019 the high density treatment had the lowest percentage of Grade 1 fruit (although ns, *P*=0.107) due mostly to a higher percentage of rejections (*P*=0.005) rather than downgrading to Grade 2. The main area of difference in reasons for rejection of fruit was residues from copper fungicide sprays. Copper residue was evident on 8.5% of high density fruit, 6.1% of medium density fruit and 1.3% of low density fruit. This again probably reflected spray penetration and this rejection rate would be avoidable with better timing of sprays, rather than being an issue intrinsic to high density systems.

The percentage of fruit affected by sun damage did not differ significantly between densities. The mean was 3.1% of fruit in all grades in 2017 (*P*= 0.934), 1.8% in 2018 (*P*=0.982) and 3.4% in 2019 (*P*=0.216).

Table 15 Percentage of fruit grades by external blemish by treatment 2016/17 to 2018/19

		2016/17				2017/18				2018/19			
	Grade:	1	2	3	Reject	1	2	3	Reject	1	2	3	Reject
Treatment	Grand mean	74.8	17.1	4.5	3.7	58.1	13.0	11.8	17.2	30.6	28.5	14.9	25.9
Density	High	77.6	13.7 a	4.8	4.0	63.6	9.2 a	11.4	15.8	25.7	28.7	14.9	30.8 b
	Med	76.1	16.6 ab	4.3	3.1	59.0	13.2 ab	11.2	16.6	30.5	29.6	15.0	25.0 a
	Low	70.9	20.9 b	4.3	3.9	51.6	16.7 b	12.6	19.1	35.8	27.2	14.9	22.0 a
	P	*0.111*	*0.037*	*0.879*	*0.802*	*0.087*	*0.048*	*0.8*	*0.545*	*0.11*	*0.78*	*1*	*0.005*
	ese	*2.1*	*1.6*	*0.8*	*1.0*	*3.3*	*1.8*	*1.6*	*2.1*	*2.9*	*2.3*	*2.0*	*1.3*
Rootstock	Ashdot	76.1	16.8	4.2	2.9	61.2b	11.6b	10.6	16.6	31.6	29.1	14.1	25.2
	Velvick	73.6	17.3	4.7	4.4	54.9a	14.5a	12.9	17.8	29.7	27.8	15.8	26.7
	P	*0.451*	*0.798*	*0.7*	*0.192*	*0.018*	*0.026*	*0.19*	*0.547*	*0.417*	*0.418*	*0.11*	*0.342*
	ese	*2.2*	*1.4*	*0.8*	*0.7*	*1.6*	*0.8*	*1.1*	*1.4*	*1.6*	*1.1*	*0.7*	*1.0*
*Density * Rootstock*	*P*	*0.227*	*0.144*	*0.494*	*0.451*	*0.312*	*0.849*	*0.735*	*0.590*	*0.460*	*0.336*	*0.490*	*0.576*

Disease

There were no significant differences between density treatment means in terms of incidence of anthracnose, stem end rot or of marketability from a disease perspective in any year, with the exception of a higher level of incidence of stem end rot in the first harvest in 2015/16 for the high and, to a lesser extent, medium densities (Table 16). This may have been due to sampling of fruit that were touching the ground due to the small numbers of fruit harvested from some young trees in that year. It may also have been due to the effect of regrowth after pruning which was very vigorous in the first year, and may reduce fruit quality due to competition for resources. Leonardi (2005) for example, found pruning too soon after harvest (during warmer autumnal weather) encouraged regrowth and detrimentally affected fruit quality compared to later pruning.

Table 16 Post harvest disease assessment 2015/16-2017/18

		Anthracnose incidence (% of fruit)			Stem end rot (% of fruit)			Marketability (% of fruit)		
		15/16	16/17	17/18	15/16	16/17	17/18	15/16	16/17	17/18[2]
Treatment	Grand mean	49.3	20.6	95.5	29.5	26.2	86.1	49.8	66.5	2.7
Density	High	50.2	21.1	95.5	44.2 b	29.3	88	42.7	63.5	2
	Medium	54.6	18.1	96.0	28.4 ab	30	88.7	45.2	63.3	3
	Low	43.0	22.5	95.0	16 a	19.3	81.5	61.6	72.8	3
	P	*0.8*	*0.847*	*0.879*	*0.034*	*0.215*	*0.162*	*0.434*	*0.282*	*0.759*
	ese	*12.18*	*5.54*	*1.31*	*6.13*	*4.39*	*2.61*	*10.66*	*4.43*	*1.08*
Rootstock	Ashdot	50.6	26.8 b	95.3	27.1	27.2	87.8	50.6	64.4	2.3
	Velvick	48.0	14.3 a	95.7	31.9	25.2	84.3	49.1	68.7	3
	P	*0.69*	*0.004*	*0.85*	*0.421*	*0.525*	*0.157*	*0.818*	*0.290*	*0.717*
	ese	*4.46*	*2.47*	*1.349*	*4.03*	*2.2*	*1.61*	*4.68*	*2.72*	*1.269*
Density Rootstock*	*P*	*0.436*	*0.166*	*0.813*	*0.714*	*0.093*	*0.169*	*0.704*	*0.021*[1]	*0.873*

[1] The interaction between density and rootstock is significant at α =0.05. Treatment means were high density Ashdot 56% a, high density Velvick 71% bc, low density Ashdot 79.6% c, low density Velvick 66% abc, medium density Ashdot 57.7% ab, medium density Velvick 69% abc. [2] Low marketability was due to very high incidence of disease incidence across all treatments. We have no explanation for this.

Rootstock effects on growth and canopy volume

Since planting, the height of the trees on 'Ashdot' rootstocks has been from 86% (2016) to 92% (2019) of trees on 'Velvick' rootstocks; and canopy volumes of trees on 'Ashdot' rootstocks have been 82% (2016) to 86% (2019) of the trees on 'Velvick' rootstocks (Table 6, Table 7).

Mean scion trunk CSAs did not differ significantly between rootstocks, but rootstock CSAs of trees on ‘Ashdot’ rootstocks were 75-85% of trees on ‘Velvick’ rootstocks (Table 8). Rootstock/scion CSA ratios were on average < 1 for trees on ‘Ashdot’ rootstocks every year (i.e trees had a smaller rootstock than the scion) and >1 for trees on ‘Velvick’ rootstocks each year (

Table 9).

It is not clear why ‘Ashdot’ produces smaller canopies: the two rootstocks show similar growth characteristics. Our architecture data shows no rootstock treatment differences in average length of growth units (Table 17), total length of growth per cm^2 of branch CSA (Table 19) or number of new growth units per branch CSA (Table 18). The only significantly different pattern in new growth units was that trees on ‘Ashdot’ showed higher numbers of axillary indeterminate inflorescences per cm^2 of branch CSA in 16/17 and 17/18 (*P*=0.087 and 0.027 respectively) (Table 18).

Table 17 Mean length (cm) of new growth units by type by rootstock treatment 2015/16-2017/18

	Spring				Summer	
Treatment	Terminal indeterminate inflorescence	Axillary indeterminate inflorescence	New vegetative branch	Continuing growth unit	New branch	Continuing growth unit
	2015/16					
Ashdot	9.6 b	10.8	16.3	na	14.41	10.07
Velvick	6.96 a	6.3	18.9	na	13.43	9.76
P	*0.032*	*0.288*	*0.570*	*na*	*0.325*	*0.723*
ese	*0.748*	*2.81*	*3.01*	*na*	*0.676*	*0.612*
	2016/17					
Ashdot	9.9 b	8.8	9.37	6.21	12.62	7.41
Velvick	6.6 a	8.8	8.3	5.14	13.24	6.24
P	*0.035*	*0.978*	*0.209*	*0.253*	*0.611*	*0.183*
ese	*0.973*	*1.61*	*0.57*	*0.62*	*0.844*	*0.588*
	2017/18					
Ashdot	6.57	na	6.74	6.12	16	8.23
Velvick	5.22	na	5.83	7.24	13.8	6.7
P	*0.129*	*na*	*0.424*	*0.103*	*0.433*	*0.076*
ese	*0.587*	*na*	*0.771*	*0.344*	*1.99*	*0.555*

na not recorded in sufficient numbers in this period. ‘Continuing’ means growth units extending in the same axis as the parent growth unit.

Table 18 Mean number of new growth units per cm² of branch CSA by type and rootstock treatment in spring and summer 2015/16-2017/18

	Spring					Summer			Year
Treat-ment	Terminal indeterminate inflorescences	Axillary indeterminate inflorescences	New vegetative branches	Continuing vegetative growth units	Total spring[1]	New branches	Continuing vegetative growth units	Total summer	Total
	2015/16								
Ashdot	1.24	1.71	1.19	nr	4.14	4.79	4.49	9.29	13.4
Velvick	1.46	1.73	1.08	nr	4.27	4.95	5.84	10.79	15.1
P	*0.577*	*0.958*	*0.851*		*0.891*	*0.888*	*0.211*	*0.456*	*0.494*
ese	*0.264*	*0.283*	*0.417*		*0.62*	*0.748*	*0.723*	*1.378*	*1.63*
	2016/17								
Ashdot	1.54	1.23	5.20	0.498	8.46	3.25	3.51	6.76	15.23
Velvick	2.02	0.82	3.88	0.456	7.18	3.02	3.27	6.29	13.47
P	*0.181*	*0.087*	*0.147*	*0.697*	*0.168*	*0.707*	*0.647*	*0.659*	*0.299*
ese	*0.241*	*0.155*	*0.6*	*0.0746*	*0.618*	*0.435*	*0.363*	*0.747*	*1.146*
	2017/18								
Ashdot	2.94	0.80 b	2.14	0.208	6.09	3.25	3.16	6.41	12.49
Velvick	4.31	0.19 a	1.06	0.336	5.9	4.01	3.85	7.87	13.77
P	*0.064*	*0.027*	*0.099*	*0.441*	*0.84*	*0.135*	*0.352*	*0.151*	*0.458*
ese	*0.476*	*0.172*	*0.425*	*0.1139*	*0.641*	*0.339*	*0.505*	*0.674*	*1.175*

[1] excludes determinate inflorescences 'nr': not recorded. 'Continuing' means growth units extending in the same axis as the parent growth unit.

Table 19 Sum of length of total growth (cm) per cm² of branch CSA by type of growth unit and rootstock treatment in spring and summer 2015/16-2017/18

	Spring					Summer			Year
	Indeterminate inflorescences		Vegetative growth units		Total spring	Vegetative growth units		Total summer	Total year
Treat-ment	Terminal	Axillary	New branches	Continuing		New branches	Continuing		
	2015/16								
Ashdot	11.1	17	23.8	10.1	51.9	69.0	43.4	112.0	164.0
Velvick	10.6	12	21.3	9.8	43.9	65.8	56.7	122.0	166.0
P	*0.857*	*0.505*	*0.833*	*0.723*	*0.616*	*0.82*	*0.209*	*0.654*	*0.951*
ese	*1.9*	*5.2*	*8.3*	*0.6*	*11.1*	*9.9*	*7.1*	*15.4*	*21.4*
	2016/17								
Ashdot	9.9 b	9.6 b	48.9	2.71	73.1	41.1	26.9	68.0	141.1
Velvick	6.6 a	6.1 a	34.2	2.27	55.6	41.2	21.2	62.5	118.1
P	*0.035*	*0.022*	*0.109*	*0.643*	*0.062*	*0.986*	*0.314*	*0.712*	*0.235*
ese	*0.9*	*0.9*	*6*	*0.6*	*6*	*7.5*	*3.8*	*10.4*	*13.0*
	2017/18								
Ashdot	16.8	3.4 b	21.5	0.8	42.5	49.5	24.3	73.8	116.3
Velvick	21.9	0.4 a	6.3	1.5	30.1	53.8	27	80.8	110.9
P	*0.12*	*0.009*	*0.054*	*0.46*	*0.137*	*0.662*	*0.635*	*0.59*	*0.766*
ese	*2.2*	*0.7*	*5.0*	*0.6*	*5.5*	*6.8*	*4.0*	*9*	*12.4*

'Continuing' means growth units extending in the same axis as the parent growth unit.

The proportion of branching that was sylleptic (an indication of vigorous growth) rather than proleptic was generally less for 'Ashdot' than 'Velvick' in most flushes but the difference between treatment means was only significant in spring 2016 (Table 20).

Table 20 Proportion of new branches that were sylleptic by rootstock treatment and period of growth 2015/16-2017/18

	2015/16		2016/17		2017/18	
Treatment	Spring	Summer	Spring	Summer	Spring	Summer
Ashdot	15%	18%	10% a	18%	24%	29%
Velvick	19%	19%	19% b	12%	36%	44%
P	*0.564*	*0.801*	*0.029*	*0.47*	*0.198*	*0.626*
ese	*6%*	*3%*	*2%*	*5%*	*6%*	*20%*

Table 21 Characteristics of growth units by density and rootstock treatment 2017/18

Treatment		No. of branches/ BCSA	Mean length of growth units	Mean no. of nodes per GU	Mean angle of growth of GUs(° from vertical)	% of annual vegetative growth (GUs) that grew in spring flush 2017/18
	Grand mean	5.2	10.2	6.9	105.6	39.3%
Density	High	5.1	10.0	7.0	105.1	45.3%
	Medium	5.2	10.5	7.0	100.7	34.5%
	Low	5.4	10.2	6.9	111.0	38.2%
	P	*0.98*	*0.815*	*0.826*	*0.065*	*0.533*
	ese	*1.0*	*0.6*	*0.2*	*2.6*	*6.6%*
Rootstock	Ashdot	5.4	10.9	7.0	106.0	40.6%
	Velvick	5.1	9.6	6.9	105.2	38.1%
	P	*0.74*	*0.055*	*0.368*	*0.875*	*0.662*
	ese	*0.6*	*0.4*	*0.1*	*3.4*	*4.0%*
Density Rootstock*	*P*	*0.195*	*0.565*	*0.994*	*0.915*	*0.532*

Rootstock effects on tree health

We have not had any tree deaths to date in the trial, but our tree health ratings showed some differences in tree health between the two rootstocks. In these ratings, 0 is healthy and 10 is drastic loss of leaf. In 2017 'Ashdot' rated on average 0.7 compared to 0.202 for 'Velvick' (*P*= 0.021), and in 2018 'Ashdot' rated 2.67 compared to 1.35 for 'Velvick' (*P*<.001). Note that these ratings were done in November when a heavy crop load can cause a large percentage of leaf drop which is recovered in the summer flush. This may have disproportionately affected the 'Ashdot' ratings and the canopy may have subsequently recovered. In 2018 a total of 10 of the 150 trees on 'Ashdot' rootstocks (6.7%) rated ≥5, the trigger for preventative or curative treatments for *Phytophthora* root rot. This compares to 1 for trees on 'Velvick' rootstocks, or 0.067%. it appears therefore that 'Ashddot' may require more vigilance and management of *Phytophthora* risk.

Rootstock effects on yield and yield efficiency

After the first year, yield per tree for 'Ashdot' rootstocks has been consistently higher than 'Velvick' rootstocks across all densities, at 138% in 2016/17 (*P*=0.019), 142% in 2017/18 (*P*<.001) and 121% in 2018/19 (*P*=0.009)(Table 12).

Because the canopy size is smaller, yield efficiency was considerably higher for trees on 'Ashdot' than on 'Velvick' rootstocks, at 163% in 2016/17 (*P*=0.0632), 170% in 2017/18 (*P*<.001) and 140% in 2018/19 (*P*=0.001) (Table 24). Note that Le Lagadec (2010) recorded high yield efficiency from trees on 'Ashdot' rootstocks but observed biennial bearing tendencies and some tree decline after six years.

We calculated the alternate bearing index of trees for the last two years of yield using the formula (year 1 crop - year 2 crop)/(year 1 crop +year 2 crop). While the 'Ashdot' mean was slightly lower (i.e. less alternate bearing) at 0.159 than 'Velvick' at 0.203, the difference was not significant at the 95% level (*P*=0.128).

Rootstock effects on fruit quality

Fruit size

Trees on 'Ashdot' rootstocks had greater mean fruit weights than trees on 'Velvick' rootstocks in all years (Table 13). Figure 1 and Figure 2 show the distribution by size for 2018 and 2019 (from 12 and six sample branches per tree respectively). Differences between rootstock treatments were more marked in the 2018 harvest. This may have been due to seasonal differences: the tendency for trees on 'Ashdot' to produce determinate flowers may be more of an advantage in some years than others (see discussion below in 'Fruit-shoot competition: the influence of seasonal conditions on fruit set').

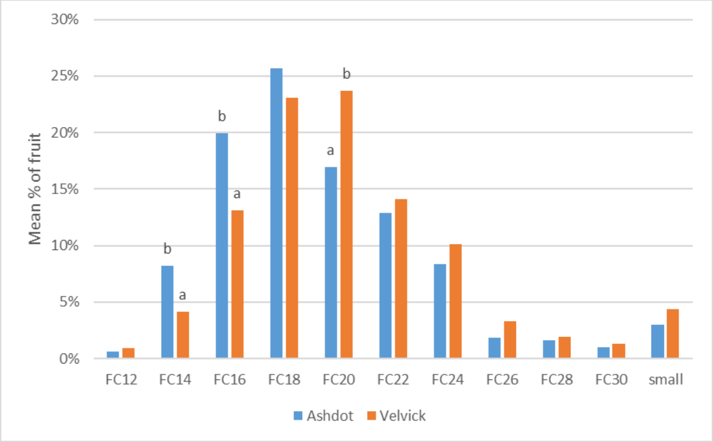

Figure 1 Fruit size distribution as a percentage of all sampled fruit by rootstock treatment 2018
FC=fruit count (the approximate number of fruit in a 5.5 kg tray). Differences between treatments were not significant at the 95% confidence level except for the fruit sizes marked by a and b.

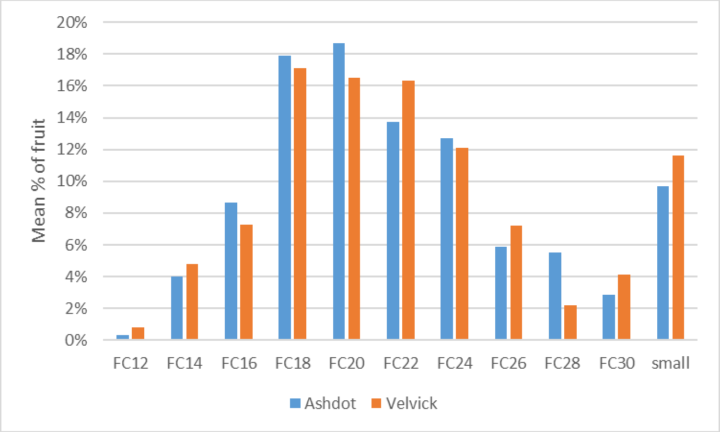

Figure 2 Fruit size distribution as a percentage of all sampled fruit by rootstock treatment 2019
FC=fruit count (the approximate number of fruit in a 5.5 kg tray). Differences between treatments were not significant at the 95% confidence level for any fruit size.

Dry matter

Fruit from trees on 'Ashdot' rootstocks had higher dry matter percentage at harvest in the first two cropping years, but not in the third and fourth year (Table 14).

External blemish

In 2017 and 2019 there were no significant differences between rootstocks in percentage of fruit graded according to external blemish (Table 15). In 2018 we assessed the trees on 'Velvick' rootstocks as having 55% of fruit in Grade 1 compared to 61% of the trees on 'Ashdot' rootstocks simple(*P*=0.018) with consequently higher numbers for 'Velvick' in the Grade 2 category (*P*=0.026) The main reason for downgrading was pest damage.

Disease

There were no significant differences in any year between rootstock treatment means in terms of incidence of anthracnose, stem end rot or marketability from a disease perspective, with the exception of a higher level of anthracnose incidence in the first crop (2015/16) for trees on 'Ashdot' rootstocks (Table 16).

Results of crop load trials

None of the management strategies tested in any of the crop load trials produced a significantly improved yield. The following summarises the key findings from each trial:

Flowering and crop load trial 2013/14

Yield per tree increased with inflorescence number (r^2 = 0.66, *P*<0.001). Yield efficiency (kg/m^3) increased with inflorescence density (inflorescences/m^3 canopy, r^2 = 0.59, *P*<0.001). For details see Hofman et al. (2018). This result indicates that flowering intensity can limit avocado yield, at least below a certain inflorescence density.

Tipping and fruiting sites trial 2014/15-2015/16

The first year of the tipping and fruiting sites trial showed no significant differences between treatments; in the second year, fruit set at the end of spring was significantly higher than the control for the weekly partial tipping treatment and the treatment in which we applied 'Cytolin' plus a one-off partial tipping. However, by the end of summer there was no difference in fruit retention between treatments. In both years, the 'Cytolin' application failed to produce a significantly different mean number of potential fruiting sites compared to the control. There was no significant difference in yield per tree or yield efficiency between treatments in either year of the trial. For details, see Hofman et al. (2018).

'Cytolin' trials 2015/16 to 2017/18

In 2015/16, spray treatment significantly increased the number of sylleptic branches in both the sprayed flush and the subsequent flush, but not the number of proleptic branches in the subsequent flush (*P*=0.018 and <.001 respectively). The mean length of the growth unit in the primary axis of the shoots, as well as the length of the first growth unit of sylleptic branches in the sprayed flush, was greater for treated shoots than control shoots (*P*=0.007 and 0.002 respectively).

In 2016/17 we monitored both 'simple' shoots (single axis, non-branching) and 'vigorous' shoots (already branching at the time of treatment). Simple shoots that were sprayed had longer growth units (20.3cm compared to 9.6 cm, *P*=0.009), but did not increase branching. In vigorous shoots, treatment increased the number of sylleptic branches in the flush after spraying, although to less than one sylleptic branch per pre-existing shoot axis (*P*=0.038). The more dramatic effect, however, was to increase the length of the primary axis and proleptic branches. The mean length of the growth unit of the primary axis in the subsequent flush almost doubled, from 13.9 to 26.4 cm (*P*=0.026); the mean length of proleptic branches growing in the sprayed flush increased from 9.2 to 13.1cm (*P*=0.017), and the mean length of proleptic branches growing in this flush from 10.9 to 20.4 cm (*P*=0.004).

Despite the effects on growth, there was no significant difference in yield per tree between treated trees and control trees in any year (*P*=0.290, 0.901, 0.721 for 2016/17, 2017/18 and 2018/19 respectively).

Overall, these results suggest that the 'Cytolin' spray treatments induced some increase in branching but also induced an unwelcome length of growth in the subsequent flush, and that this growth may have reduced growth in subsequent flushes. Gains in sylleptic branching did not translate into any discernible increase in yield.

Summer fruit drop architecture study 2015/16

This study showed that determinate fruit dropped at a slightly higher rate (62%, n=137) than indeterminate fruit (55%, n=116). Fruit that were smaller were more likely to drop (Figure 3). The rate of fruit growth of individual fruit started to decline two weeks or more before a fruit dropped (Figure 3).

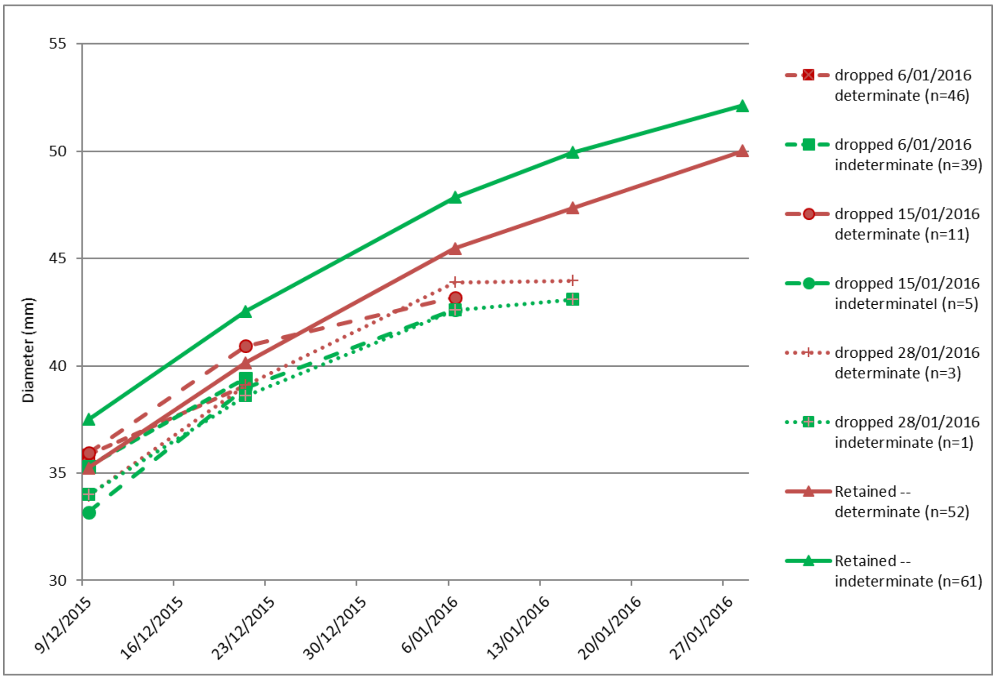

Figure 3 Summer fruit drop architecture study: mean growth of dropped and retained fruit from determinate and indeterminate inflorescences 2015/16

Graph does not show 25 fruit from determinate inflorescences and 10 fruit from indeterminate inflorescences which measured on average 37.0mm and 36.3mm respectively on 9/12/2015, and which dropped before measurement on 16/12/2015.

Temperature appeared to have no direct effect on the pattern of fruit drop over summer (Figure 4). This confirms findings of the lack of temperature influence by Wolstenholme et al. (1990), Garner et al. (2011) and Garner and Lovatt (2008), although Wolstenholme et al. (1990) recorded a second very small peak of fruit drop in late summer (around 30 January) which was correlated with high temperatures particular to the season of their study.

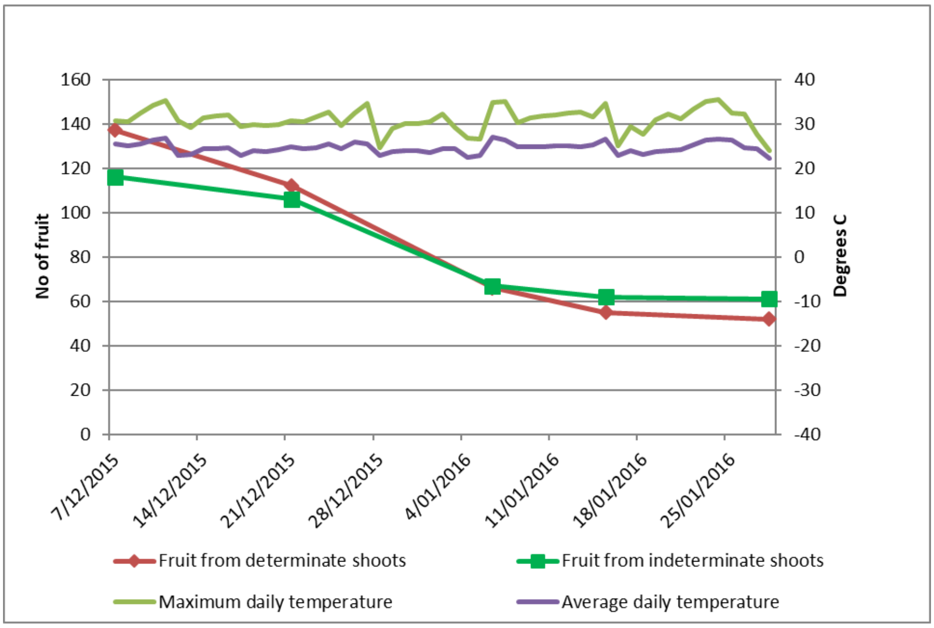

Figure 4 Summer fruit drop architecture study: Fruit retention (total sample numbers) and daily maximum and average temperatures (°C)

Fruit set and retention were correlated with greater branch angle, but this may be an effect of the weight of fruit rather than a cause. We did not find any evidence that fruit in clusters were more likely to drop, nor fruit on sylleptic shoots as opposed to proleptic shoots (data not shown). Position in the canopy, length of parent growth unit and the number of summer grown leaves did not appear to have any influence (data not shown).

The main attribute that appeared to have any effect was the number of pre-existing leaves on the branch. The relationships between the number of leaves per branch in the each growth season (summer 2014, spring 2015 and summer 2015) and the number of fruit retained on a branch suggests that summer 2014 leaves and spring 2015 leaves best helped explain fruit retention, with r^2 values of 0.5877 ($P<0.001$) and 0.3909 ($P=0.009$) respectively (n=16) (Figure 5). The variability in number of leaves per retained fruit on the sampled branches, along with the retention of a large proportion of determinate fruit over summer, may suggest that assimilates are shared between branches and/or that branches have a significant store of assimilates.

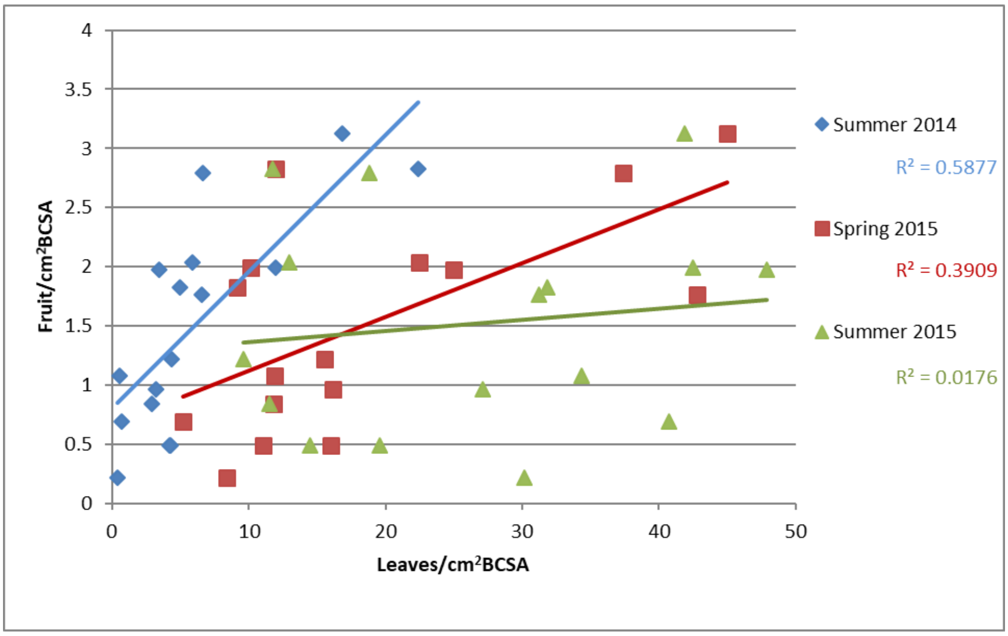

Figure 5 Summer fruit drop architecture study: Fruit retained to harvest in 2015 and leaves grown in summer 2014, spring 2015 and summer 2015 (per cm^2 branch CSA)

The results of this study suggest that shoot growth in summer is not a factor in stimulating summer drop: but that the presence of fruit on a branch may depress summer vegetative growth, most likely by supressing the release of the vegetative bud on a bearing shoot. Analysis on a *shoot* basis confirmed that the presence of a fruit tended to repress summer vegetative growth (at least in a heavy crop load year). While summer growth on non-bearing shoots was on average somewhat longer, had slightly more leaves and had more branching than growth on bearing shoots, the main effect was to repress summer flushes entirely (Table 22). However, on 29% of non-bearing shoots there was no bud release and on ~40% of bearing shoots there was bud release. This suggests that either the suppressive effect of fruit is earlier than the time we began measuring (at the end of spring) and/or the effect is not purely localized but signals or resources are shared across branches/trees.

Table 22 Summer fruit drop architecture study: summer growth 2015/16 by fruit fate

	Shoots with no summer growth		Shoots with summer growth				
Fruit fate	n	% of shoots	n	New summer GUs per shoot	Mean length of growth	No, of summer leaves per shoot	New axes (branches per shoot)
Fruit set in spring but dropped in summer	49	59%	21	1.5	9.2	8.2	0.48
Fruit retained to harvest	54	56%	27	1.5	10.9	8.1	0.59
Non-bearing shoots	132	29%	120	1.6	12.9	9.5	0.76

Overall, this study suggested that there appears to be little potential for reducing summer drop by direct architectural manipulation, although this does not preclude the indirect effect of manipulating canopy architecture on photosynthetic capacity and carbohydrate availability.

Fruit thinning trials 2015/16

In both trials, there were no significant differences between thinning treatments and the control treatment in number of harvested fruit per branch or per trunk CSA (*P*=0.24 in Planting Systems Trial; *P*=0.365 at Childers trial).

Flowering intensity trials 2017/18 and 2018/19

In 2018/2019 the number of axillary buds that developed into inflorescences was very low for all trees (0.31 per shoot compared to 1.5 in 2017/2018) and did not differ by treatment (*P*=0.403). Similarly there were no treatment differences in the number of sylleptic shoots that grew in spring or the number of axillary vegetative shoots (*P*= 0.191, 0.843 respectively). Yield did not differ by treatment (*P*=0.934). One treatment/rootstock combination (August Cytolin /Ashdot rootstocks) had significantly higher yield than most other rootstocks (*P*=0.012), but we suspect this is an artificial factor of the variability between trees.

Girdling trial 2018/19:

There was slightly better initial fruit set on limbs girdled in winter than on non-girdled limbs (1.3 fruit per tag v. 1.0 fruit per tag) but differences were not significant at the 95% confidence level (*P*=0.089)(Table 23). The winter girdled limbs had a slightly stronger propensity to produce determinate inflorescences, that is, 53% of inflorescences on girdled limbs were determinate compared to 43% on non-girdled limbs (*P*=0.065). On a whole-tree basis, however, there seemed to be a balancing mechanism across winter girdled and non-girdled branches in the tree because there was no significant difference between treatments in average number of fruit per tagged shoot on a whole tree basis (*P*=0.998).

Girdling of limbs in spring did not have a consistent effect on fruit retention (Table 23).

Overall, yield per tree did not vary significantly by treatment (*P*=0.368), that is, girdling did not prove to be an effective mechanism for increasing crop load. Note that trees in this trial were only three years old with a strong propensity to vegetative growth: it is possible that girdling may be a more effective strategy in colder climates and/or on older trees.

Table 23 Fruit set and yield by treatment at girdling trial 2018/19

		Spring set (fruit per shoot)		% spring fruit set on determinate shoots		Summer set (fruit per shoot)		Yield per tree (kg)
Time of girdling/ Prop'n of limbs girdled		Girdled	Not girdled	Girdled	Not girdled	Girdled	Not girdled	
Control/	0	--	1.009	--	42.6	--	0.27	20
Winter girdling/	1/2	1.293	0.997	51.5	42.3	0.289	0.459	21.8
	1/3	1.194	1.157	50.9	48.9	0.331	0.346	27.7
	2/3	1.514	0.872	56.9	36.6	0.294	0.373	20.9
Spring girdling/	1/2	--	--	--	--	0.343	0.492	22.3
	1/3	--	--	--	--	0.317	0.288	19.7
	2/3	--	--	--	--	0.353	0.338	17.5
P			*0.098*		*0.065*		*0.946*	*0.368*

Conclusions:

A confounding factor in the lack of success of our crop load trials is that in most trials we were using young trees, which have a decided inclination towards vegetative growth. However, we believe it was appropriate to use young material as early yield or precocious bearing is a key theoretical economic benefit in high density planting.

Overall, the internal mechanism in the avocado for balancing crop load proved highly resistant to manipulation by thinning, plant growth regulators or girdling strategies. This suggests a limitation of current and/or stored carbohydrates. We hypothesize that stored carbohydrates may play a greater role than current production of carbohydrates. Along with Wolstenholme and Whiley (1997), we also speculate that allocation of carbohydrates to stores is a priority for the tree that competes with allocation to fruit retention. We also suspect that root area is a major factor in the ability of trees planted at high density to support fruit, as discussed below.

Review of factors that may have affected yield in the Planting Systems Trial

In this discussion we review data we collected in the Planting Systems Trial and the crop load trials on the factors that may have limited yield in the higher density treatments and/or contributed to higher yield from trees on 'Ashdot' rootstocks compared to 'Velvick' rootstocks.

We have structured this discussion using a simple conceptual model that suggests that marketable yield per hectare is a function of planting density x fruiting sites/tree x flowering factors x % fruit set and retention x fruit quality (including size). The key factors in each of these components, many of which are interrelated, are shown in Figure 6. We do not claim that the factors discussed are all-inclusive or applicable in all situations, for example, pollination is an important limiting factor in cooler climates but did not appear to be implicated in treatment differences in our trial.

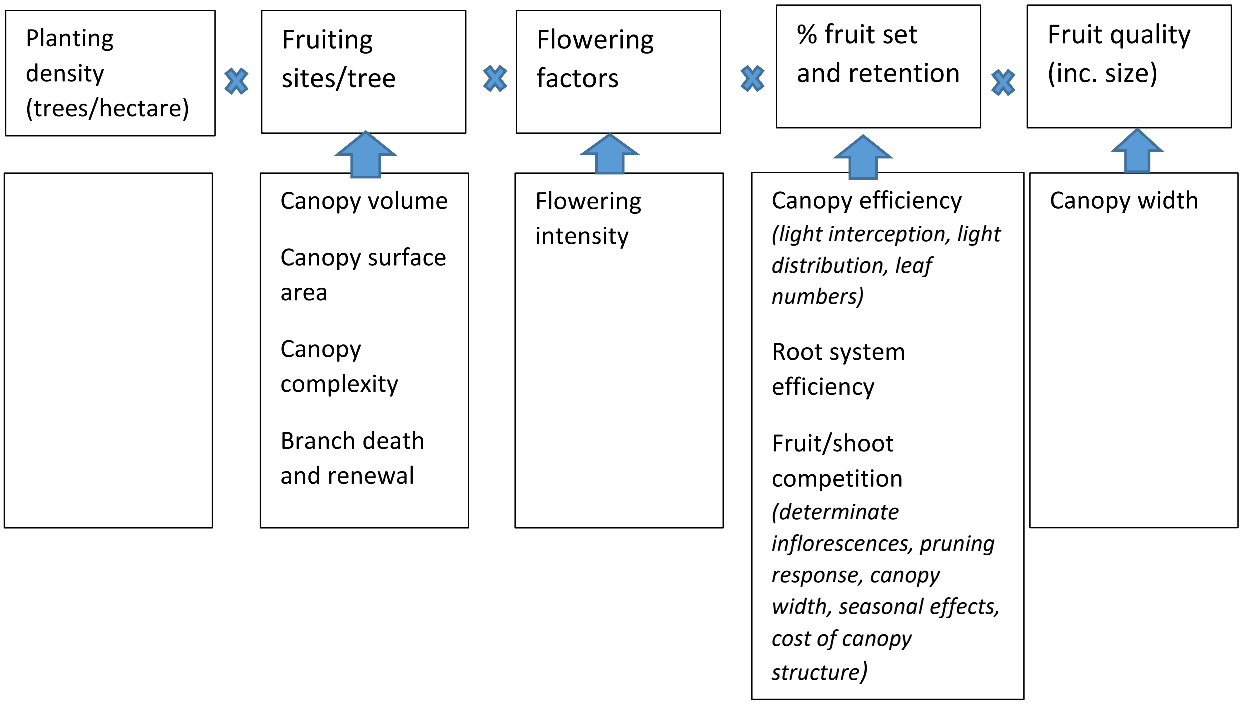

Figure 1 Conceptual model of some possible explanatory factors for yield results in the Avocado Planting Systems Trial

Number of fruiting sites

As a predominantly terminal bearer, limitations of the canopy in terms of numbers of fruiting sites can be a yield limiting factor for avocado. We have compared canopy volume, canopy surface area and canopy complexity for the three density treatments.

Canopy volume

Since 2016/17, canopy volume per hectare has been greater for the high density treatment than other density treatments until 2018/19 when there was no significant difference between the density treatments (Table 7). If yield were simply a function of canopy volume, the high densities should have yielded more fruit per hectare until that year. However, since 2017/18, the high density treatment had lower yield efficiency (kg of fruit per m^3 of estimated canopy volume) (P=0.006), and in 2018/19, the medium density treatment also had significantly lower yield efficiency (P<0.001)(Table 24). Note that canopy volume calculations do not take into account gaps in the canopy: see discussion below on ‘Canopy complexity’.

Table 24 Yield efficiency per m^3 of canopy 2015/16 to 2018/19 and per m^2 of canopy surface area 2017/18 and 2018/19 by density and rootstock treatments

		By canopy volume (kg/m^3)				By canopy surface area (kg/m^2)	
Treatment		2015/16	2016/17	2017/18	2018/19	2017/18[1]	2018/19[1]
	Grand mean	0.409	0.616	0.764	0.729	0.733	
Density	High	0.487	0.490	0.592 a	0.432 a	0.538 a	0.463 a
	Medium	0.274	0.577	0.662 a	0.651 b	0.688 a	0.782 b
	Low	0.467	0.781	1.038 b	1.104 c	0.972 b	1.232 c
	P	*0.119*	*0.171*	*0.006*	*<.001*	*0.002*	*<.001*
	ese	*0.0704*	*0.1004*	*0.0735*	*0.0434*	*0.058*	*0.053*
Rootstock	Ashdot	0.468	0.764 b	0.963 b	0.847 b	0.883 b	0.926 b
	Velvick	0.351	0.468 a	0.565 a	0.611 a	0.582 a	0.725 a
	P	*0.175*	*0.006*	*<.001*	*0.001*	*<.001*	*<.001*
	ese	*0.0574*	*0.0632*	*0.0572*	*0.0387*	*0.045*	*0.032*
Density Rootstock*	P	*0.919*	*0.238*	*0.123*	*0.153*	*0.153*	*0.048*[2]

[1] Surface area was calculated using planting space where canopies were interlocking; and assuming, based on light distribution studies, of a vertical 'wall' to 2m for high density plots and 2.5m for medium density plots. [2] The interaction between density and rootstock is significant at α =0.05. Treatment means were high density Velvick 0.376 a, high density Ashdot 0.55 b, medium density Velvick 0.753 c, medium density Ashdot 0.812 c, low density Velvick 1.047 d, low density Ashdot 1.417 e.

Table 25 Yield efficiency by trunk cross sectional area (kg/cm^2) 2015/16 to 2018/19

		Yield/rootstock CSA at harvest				Yield/scion CSA at harvest			
Treatment		**2015/16**	**2016/17**	**2017/18**	**2018/19**	**2015/16**	**2016/17**	**2017/18**	**2018/19**
	Grand mean	0.0719	0.1226	0.1357	0.1399	0.075	0.1117	0.1274	0.1325
Density	High	0.0953 b	0.0772 a	0.0791 a	0.0632 a	0.0989 b	0.0762	0.0805 a	0.0624 a
	Medium	0.0432 a	0.1161 ab	0.1249 b	0.1241 b	0.0482 a	0.1147	0.1208 b	0.1205 b
	Low	0.0772 ab	0.1745 b	0.2031 c	0.2325 c	0.0778 ab	0.1443	0.1809 c	0.2145 c
	P	*0.046*	*0.03*	*<.001*	*<.001*	*0.04*	*0.055*	*<.001*	*<.001*
	ese	*0.01231*	*0.02067*	*0.01153*	*0.00906*	*0.01145*	*0.01657*	*0.00962*	*0.00750*
Rootstock	Ashdot	0.0815	0.1603 b	0.1773 b	0.1702 b	0.0797	0.1334 b	0.1556 b	0.1499 b
	Velvick	0.0623	0.0849 a	0.094 a	0.1097 a	0.0702	0.0901 a	0.0992 a	0.1150 a
	P	*0.163*	*0.001*	*<.001*	*0.001*	*0.444*	*0.008*	*0.002*	*0.005*
	ese	*0.00914*	*0.01245*	*0.01162*	*0.01042*	*0.00852*	*0.00965*	*0.01005*	*0.00723*
*Density*Rootstock*	*P*	*0.881*	*0.109*	*0.163*	*0.091*	*0.815*	*0.56*	*0.696*	*0.352*

CSA cross sectional area

Canopy surface area

Canopy surface area is a potential explanatory factor for yield because avocados are terminal bearers. However, our calculations suggest that in both 2017/18 and 2018/19 yield per m^2 of surface canopy was lower for the higher densities, suggesting this is not a significant explanatory factor (Table 24). Note that canopy surface area calculations do not take into account gaps in the canopy: see discussion below on 'Canopy complexity'.

Canopy complexity

Canopy volume and surface area calculations provide the external dimensions of the canopy but do not account for gaps in the canopy or for 'empty' centres. We have used branch numbers to provide an indication of canopy structure and complexity. As well as indicating relative canopy consistency, canopy complexity determines the availability of fruiting sites in this terminal bearing species. Our work in both architecture analyses and flowering terminal counts suggests that the number of flowering terminals is, for all practical purposes, equal to the number of axes or branches, that is, almost every terminal will produce an inflorescence.

As trees matured, some of the trees in the high density treatment showed limited 2nd order branching (that is, branching from the trunk) due to the loss of weaker branches at the upper levels of each growth spurt. A branch count in 2019 showed trees in the high density treatment had lower numbers of branches from 2 to 4 metres but the density treatment means were not significantly different (branches above 5m were not counted) (Figure 7). However, as can be seen from the total sum of CSA of branches at each interval, trees in the medium and low density treatments showed significantly larger and stronger2nd order branches lower in the canopy than in the high density treatment (Figure 8). This simple measure suggests that the high densities had poorer canopy complexity and consistency.

There were no significant differences between the two rootstock treatments in branching distribution, measured as number of branches from each metre of the trunk, except at the 100-200 cm level (Figure 7). However, in terms of total branch cross sectional area at each level the ‘Ashdot’ rootstock treatment showed significantly stronger (larger) second order branches at the 0-100 cm level, but weaker (smaller) at the 100-200 cm level (Figure 8).

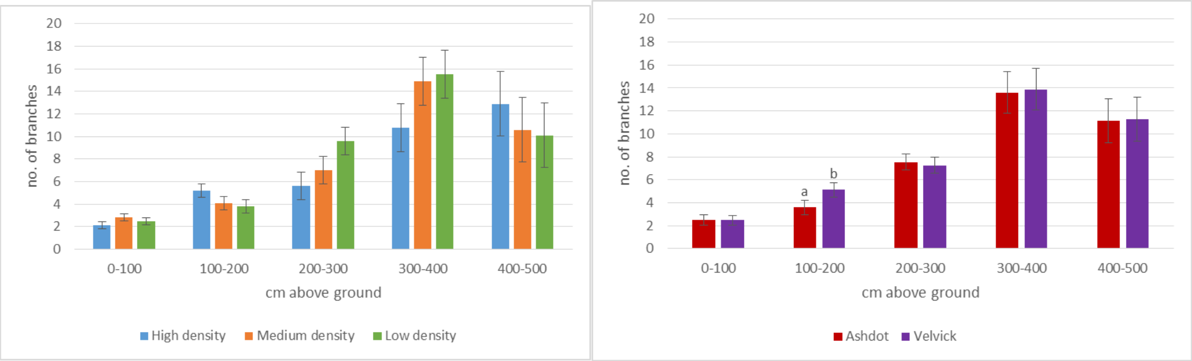

Figure 7 Mean number of 2nd order branches by height above ground and density treatment (left) and rootstock treatment (right) in January 2019
Error bars show ± standard errors of the means. Only treatment means with different letters within the one height band are significantly different at the 95% confidence level.

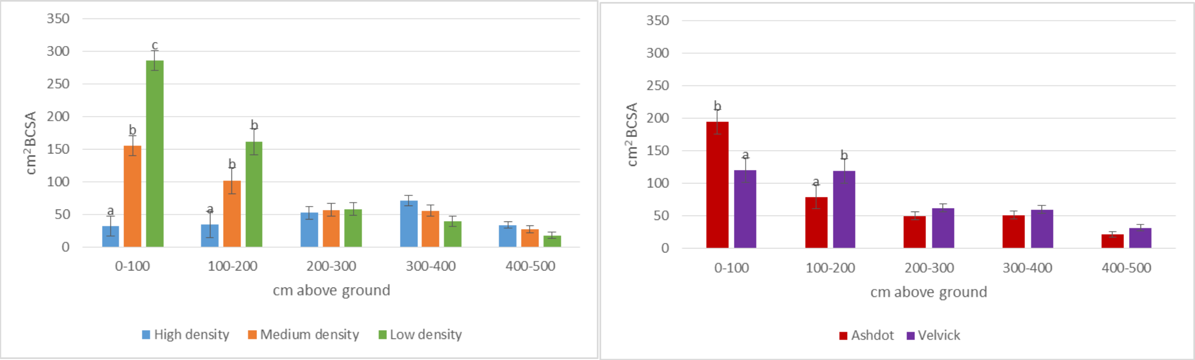

Figure 8 Mean sum of CSA of 2nd order branches by height above ground and density treatment (left) and rootstock treatment (right) in January 2019
Error bars show ± standard errors of the means. Only treatment means with different letters within the one height band are significantly different at the 95% confidence level.

The number of flowering terminals (FTs) per cm^2 of branch CSA in our flowering terminal counts tended to be similar in each year for all density treatments (the significant differences in 2016/17 are numerically very small). In 2018/19 some differences appeared to be emerging with a higher number of FTs per cm^2 of branch CSA for the low density treatment (*P*=0.055) (Table 30).

We estimated numbers of FTs per tree and per hectare for the 2018/19 year using branch CSAs for all branches on sample trees (Table 26). Because the trees in the medium and low density treatments had thicker branches than those in the high density treatment (Figure 8), they had greater numbers of FTs per tree. However, on a per hectare basis, the increased planting density means there were significantly more FTs per hectare for the medium and high density treatments than the low density treatments even in 2018/19 (Table 26).

The thicker branches on the lower part of trees on 'Ashdot' meant that in our estimates of total FTs per tree, the trees on 'Ashdot' rootstocks showed a 20% increase on 'Velvick' rootstocks, but differences between the two treatment means were not significant (*P*=0.259)(Table 26).

Table 26 Fruiting site estimates by treatment 2018/19

Treatment		Total BCSA/tree	Mean FT/cm^2 BCSA[1]	FT/tree	FT/ha ('000)
	Grand mean	391	6.49	2561	1254
Density	High	224 a	6.28	1402 a	1557 b
	Medium	385 b	5.77	2431 b	1351 b
	Low	564 c	7.41	3849 c	855 a
	P	*<.001*	*0.055*	*<.001*	*<.001*
	ese	*23.1*	*0.406*	*155.7*	*67*
Rootstock	Ashdot	386	6.41	2780	1324
	Velvick	396	6.56	2341	1185
	P	*0.783*	*0.813*	*0.095*	*0.259*
	ese	*25.2*	*0.44*	*171.2*	*83*
Density Rootstock*	*P*	*0.580*	*0.444*	*0.292*	*0.213*

'BCSA': branch cross sectional area 'FT': flowering terminal [1] For FT data for all years, see Table 27

Our architecture data also shows that there were no density or rootstock treatment differences in number of new branches per cm^2 of branch CSA grown each year (combined spring and summer flushes) (Table 11). As noted above there appeared to be some differences in the timing of growth (see section headed 'Timing of growth'). Branching in the high density treatment was significantly less than branching in the low density treatment in *summer* in 2015/16 and 2017/18 but not in 2016/17. Trees on 'Ashdot' rootstocks had more branches per branch CSA in spring 2017/18 (*P*=0.034) and possibly spring 2016/17 (*P*=0.059): in all other periods branching was not significantly different from trees on 'Velvick' rootstocks.

Architecture analysis indicates that the majority (~50%) of FTs are on single growth unit branches, as are spring set fruit (Table 27). In 2016/17 and 2017/18 the high densities had a higher proportion of FTs on older terminals (≥3 growth units) but as these longer axes tended to have slightly higher relative fruit set rates (Table 27), this tendency did not appear to explain density differences in crop loads.

The patterns of flowering on one, two or three growth unit branches does not differ significantly for trees on 'Ashdot' rootstocks compared to trees on 'Velvick' rootstocks (Table 27).

Table 27 Proportion of all flowering terminals and fruit bearing terminals by number of growth units in the axis

		2015/16			2016/17			2017/18		
Treatment	No of GUs in axis	1	2	≥3	1	2	≥3	1	2	≥3
		% of all flowering terminals								
	Grand mean	51.0%	26.8%	22.1%	48.7%	31.0%	20.4%	50.6%	25.8%	23.6%
Density	High	47.4%	26.5%	26.1%	34.5% a	36.9%	28.7% b	50.5%	20.1%	29.4% b
	Medium	55.2%	23.8%	21.1%	57.5% b	27.0%	15.5% a	57.0%	27.6%	15.5% a
	Low	50.5%	30.3%	19.2%	54.1% b	29.0%	16.9% a	44.3%	29.7%	25.9% b
	P	*0.62*	*0.573*	*0.431*	*0.004*	*0.111*	*0.017*	*0.122*	*0.104*	*0.028*
	ese	*5.5%*	*4.2%*	*3.7%*	*3.6%*	*3.1%*	*2.7%*	*3.8%*	*2.9%*	*3.0%*
Rootstock	Ashdot	55.5%	25.9%	18.6%	49.8%	31.6%	18.6%	55.5%	22.5%	22.0%
	Velvick	46.6%	27.8%	25.6%	47.5%	30.4%	22.1%	45.8%	29.1%	25.2%
	P	*0.125*	*0.733*	*0.139*	*0.642*	*0.802*	*0.387*	*0.062*	*0.110*	*0.489*
	ese	*3.8%*	*3.8%*	*3.1%*	*3.4%*	*3.3%*	*2.8%*	*3.3%*	*2.7%*	*3.1%*
Density Rootstock*	*P*	*0.295*	*0.755*	*0.569*	*0.167*	*0.292*	*0.019*	*0.335*	*0.282*	*0.305*
		% of fruit-setting flowering terminals								
	Grand mean	50.0%	20.0%	30.5%	35.1%	35.8%	28.4%	61.5%	16.3%	22.2%
		*	*	*				*	*	*
Density	High	41.0%	28.6%	30.4%	19.4% a	47.6%	31.0%	66.2%	9.0%	24.7%
	Medium	67.0%	0.0%	33.3%	45.0% b	24.1%	30.8%	56.6%	23.2%	20.2%
	Low	41.0%	31.3%	27.7%	40.7% b	35.8%	23.5%	61.8%	16.6%	21.6%
	P	*0.578*	*0.085*	*0.965*	*0.031*	*0.096*	*0.614*	*0.835*	*0.381*	*0.887*
	ese	*19.3%*	*9.2%*	*14.9%*	*5.9%*	*6.6%*	*6.0%*	*11.2%*	*6.7%*	*6.6%*
Rootstock	Ashdot	52.0%	15.6%	32.3%	34.2%	32.2%	32.3%	62.6%	16.2%	21.2%
	Velvick	47.0%	24.3%	28.7%	36.0%	39.5%	24.5%	60.5%	16.4%	23.1%
	P	*0.815*	*0.676*	*0.836*	*0.870*	*0.471*	*0.500*	*0.853*	*0.982*	*0.778*
	ese	*14.7%*	*13.7%*	*11.6%*	*7.6%*	*7.0%*	*8.0%*	*7.6%*	*4.3%*	*4.6%*
Density Rootstock*	*P*	*0.985*	*0.951*	*0.976*	*0.328*	*0.345*	*0.383*	*0.155*	*0.238*	*0.231*
		% of each flowering terminal type that set fruit								
	Grand mean	19.6%	13.4%	24.9%	16.5%	25.2%	33.6%	18.4%	11.7%	21.2%
Density	High	30.2%	26.0%	40.2%	9.7%	28.0%	29.1%	18.9%	9.7%	18.4%
	Medium	13.2%	0.0%	7.0%	18.0%	15.0%	37.4%	14.4%	14.0%	23.2%
	Low	15.4%	14.4%	27.5%	21.9%	32.7%	34.3%	22.1%	11.6%	22.1%
	P	*0.42*	*0.154*	*0.311*	*0.226*	*0.161*	*0.65*	*0.491*	*0.760*	*0.685*
	ese	*9.4%*	*8.4%*	*14.4%*	*4.6%*	*6.0%*	*6.2%*	*4.4%*	*4.1%*	*3.9%*
Rootstock	Ashdot	20.9%	14.5%	23.4%	16.6%	24.0%	35.2%	25.5%b	18.0%b	27.6%
	Velvick	18.3%	12.4%	26.3%	16.4%	26.4%	32.0%	11.4%a	5.5%a	14.9%
	P	*0.783*	*0.754*	*0.844*	*0.973*	*0.702*	*0.836*	*0.018*	*0.044*	*0.162*
	ese	*6.6%*	*4.8%*	*10.2%*	*3.9%*	*4.5%*	*10.6%*	*3.6%*	*3.9%*	*6.0%*
Density Rootstock*	*P*	*0.868*	*0.067*	*0.565*	*0.260*	*0.120*	*0.734*	*0.430*	*0.816*	*0.756*

Includes axillary fruit set; * missing values where sample branches did not set fruit; ese not adjusted for missing values

Taking all these trends into account, it appears therefore that canopy complexity, while contributing to yield differences, is not the main explanation for lower yields per hectare for the higher densities, or higher yields for the trees on 'Ashdot' rootstocks in the Planting Systems Trial.

Branch death and renewal

Although canopy complexity may not be the main factor behind yield variations, the simpler structure of the central leader training appears to have some disadvantages in terms of branch death and renewal which affects yields. In 2018, we calculated the percentage of sample branches that died during the growing season: the overall average was 16.6%. Some of this death is due to the avocado's natural habit of abscising smaller branches that are shaded and/or did not set fruit (Mickelbart et al., 2012; Scholefield et al., 1985); but at least 50% of branch death could be attributed to attack by scolytid beetles and the accompanying fungal infection. While this rate did not vary between densities, the tree structure in the high and medium density treatments is much simpler, relying on branches radiating from a single central trunk, so the lost branches in the higher densities had a greater impact on fruit load than in the low density. The estimated percentage of fruit in spring on branches that later died in the low density plots was 4%, in the medium density plots 6%, and the high density plots 15% (Table 28).

Table 28 2017/18 Branch death on sampled branches by treatment

Treatment		% of branches that died or partly died	% of flowering terminals on branches that died	% of spring-set fruit on branches that died
	Grand mean	16.6%	10.7%	8.5%
Density	High	25.8% c	17.6% b	15.4% b
	Medium	18.3% b	10.2% ab	6.4% a
	Low	5.7% a	4.3% a	3.7% a
	P	*<.001*	*0.01*	*0.01*
	ese	*2.2%*	*2.3%*	*2.1%*
Rootstock	Ashdot	15.5%	10.0%	8.8%
	Velvick	17.8%	11.4%	8.2%
	P	*0.597*	*0.623*	*0.828*
	ese	*3.0%*	*1.9%*	*1.8%*
Density Rootstock*	*P*	*0.383*	*0.202*	*0.143*

Flowering factors

Flowering intensity

Low flowering intensity has been identified as a limit to yield in avocado, often associated with the ‘off’ years in alternate bearing cycles (Salazar-Garcia et al., 1998; Dixon et al., 2007; Garner and Lovatt, 2008). However, our experience in this trial -- under central Queensland conditions – was that flowering intensity was more than sufficient. It did not appear to be a factor in the reduced crop load for the higher densities in the Planting Systems Trial: there were no differences between density treatments in the mean number of flowering terminals per cm^2 of branch CSA in any year (Table 30).

Axillary flowering is also a component of flowering intensity and is more common on terminals with very high light levels and in younger trees (Table 29). The proportion of axillary flowers was slightly lower for the high density treatment, but differences only came close to ‘significant’ (*P*=0.051) in 2016/17 when 32% of high density flowering was axillary compared to 51% and 57% on the medium and high density treatments respectively. The ’Ashdot’ treatment had a higher proportion of axillary flowering than the ‘Velvick” treatment in 2016/17 and 2017/18.

Fruit set from axillary determinate inflorescences was comparable to terminal determinate inflorescences in 2015/16 but declined as the trees matured (Table 36).

In conclusion, flowering intensity does not seem to be a significant contributor to treatment differences in yield. Note that we had no success in increasing flowering intensity or yield in our crop load trials through the use of selected plant growth regulators (see the section ‘Results of crop load trials’).

Determinate terminals

The fate of a flowering terminal as determinate or indeterminate affects fruit set and yield: this is discussed below under ‘Fruit set and retention’.

Table 29 Flowering intensity and proportion of axillary and determinate inflorescences by density and rootstock treatment 2015/16-2017/18

Treatment		Inflorescences/cm² BCSA			Proportion axillary			Proportion determinate		
		15/16	16/17	17/18	15/16	16/17	17/18	15/16	16/17	17/18
	Grand mean	5.26	6.86	6.55	60.3%	46.5%	14.8%	30.0%	48.7%	29.3%
Density	High	5.28	5.15	6.77	55.8%	31.7%	11.7%	27.8%	33.4% a	32.2%
	Medium	3.77	5.62	6.54	62.2%	50.7%	16.1%	23.8%	46.1% a	32.5%
	Low	6.73	10.05	6.77	62.8%	57.2%	16.5%	38.4%	66.5% b	23.2%
	P	*0.317*	*0.053*	*0.948*	*0.503*	*0.051*	*0.393*	*0.478*	*0.015*	*0.576*
	ese	*1.28*	*1.30*	*0.95*	*4.5%*	*6.3%*	*2.6%*	*8.2%*	*6.5%*	*6.9%*
Rootstock	Ashdot	5.82	7.35	7.68 b	63.9%	55.0% b	22.6% b	41.0% b	59.4% b	45.2%b
	Velvick	4.69	6.53	5.41 a	56.6%	38.1% a	7.0% a	19.0% a	38.0% a	13.4%a
	P	*0.278*	*0.513*	*0.033*	*0.311*	*0.006*	*0.006*	*0.048*	*0.047*	*<.001*
	ese	*0.70*	*0.86*	*0.67*	*4.8%*	*3.6%*	*3.3%*	*7.1%*	*6.8%*	*5.0%*
Density Rootstock*	*P*	*0.934*	*0.073*	*0.544*	*0.488*	*0.979*	*0.536*	*0.954*	*0.662*	*0.523*

Note that inflorescences can be both determinate and axillary

Fruit set and retention

Differences in spring fruit set and retention over the summer drop appear to be two of the more significant factors in yield limitation in the higher densities.

The high density treatment had lower spring-set fruit per cm² of branch CSA in 2017/18 (*P*=0.001) and in 2018/19 (although just ns, *P*=0.052). In the last two cropping years the number of summer-retained fruit per cm² of branch CSA was highest for the low density treatments, lowest for the high density treatment, with medium density midway between the two (*P*<.001 both years) (Table 30). The number of spring-set and summer-retained fruit per flowering terminal shows a similar pattern (Table 37).

The 'Ashdot' rootstock treatment tended to have higher spring-fruit set and summer-retention than the 'Velvick' on average, in terms of both fruit per cm² of branch CSA and per terminal, but differences were not significant in most years (Table 30, Table 31). Given the clear yield differences, we believe that this 'tendency' was true on average but highly variable from tree to tree.

Table 30 Flowering terminals and fruit set and retention per cm² of branch CSA by treatment 2015/16 to 2018/19

		Flowering terminals				Spring-set fruit				Summer-retained fruit			
Treat-ment		2015/16 [1]	2016/17	2017/18	2018/19	2015/16 [1]	2016/17	2017/18	2018/19	2015/16 [1]	2016/17	2017/18[2]	2018/19
	Grand mean	2.811	3.47	5.11	6.49	0.66	0.93	0.97	1.97	0.52	0.18	0.63	0.53
Density	High	2.965	3.52ab	5.2	6.28	0.94	0.89	0.65a	1.90	0.68	0.09a	0.35 a	0.24 a
	Med.	2.845	3.16a	4.81	5.77	0.34	0.8	0.93b	1.43	0.30	0.20ab	0.64 b	0.44 b
	Low	2.623	3.74b	5.33	7.41	0.70	1.1	1.32c	2.57	0.59	0.25b	0.90 c	0.90 c
	P	*0.61*	*0.04*	*0.428*	*0.055*	*0.148*	*0.487*	*0.001*	*0.052*	*0.146*	*0.044*	*<.001*	*<.001*
	ese	*0.24*	*0.13*	*0.28*	*0.41*	*0.19*	*0.172*	*0.08*	*0.28*	*0.13*	*0.04*	*0.03*	*0.06*
Root-stock	Ashdot	2.994	3.41	4.87	6.41	0.77	1.02	1.09	2.22	0.58	0.19	0.66	0.59
	Velvick	2.628	3.53	5.36	6.56	0.56	0.84	0.85	1.71	0.46	0.17	0.60	0.46
	P	*0.086*	*0.656*	*0.121*	*0.813*	*0.297*	*0.391*	*0.150*	*0.165*	*0.481*	*0.697*	*0.468*	*0.253*
	ese	*0.14*	*0.18*	*0.21*	*0.44*	*0.13*	*0.14*	*0.11*	*0.24*	*0.12*	*0.03*	*0.05*	*0.08*
*Density *Root-stock*	*P*	*0.122*	*0.178*	*0.763*	*0.444*	*0.205*	*0.249*	*0.912*	*0.321*	*0.696*	*0.479*	*0.817*	*0.475*

[1] excludes pruned branches [2] excludes branches dead by summer

Table 31 Fruit set and retention per flowering terminal by treatment 2015/16 to 2018/19

		Spring-set fruit /flowering terminal				Summer-retained fruit /flowering terminal				Summer-retained fruit/spring-set fruit (%)			
Treat-ment		2015/16	2016/17	2017/18	2018/19	2015/16	2016/17	2017/18[1]	2018/19[1]	2015/16	2016/17	2017/18 [1]	2018/19[1]
	Grand mean	0.29	0.27	0.20	0.31	0.18	0.15	0.12	0.08	85.8	60.9	63.4	32.6
Density	High	0.11	0.25	0.13a	0.32	0.22	0.12	0.08a	0.04 a	83.3	55.4	52.4	17.5 a
	Medium	0.25	0.25	0.20b	0.24	0.10	0.17	0.13 b	0.08 b	87.7	69.9	68.3	38.4 b
	Low	0.23	0.32	0.26c	0.36	0.21	0.17	0.16c	0.13 c	86.3	57.4	69.5	41.8 b
	P	*0.056*	*0.609*	*<.001*	*0.298*	*0.055*	*0.434*	*<.001*	*<.001*	*0.866*	*0.256*	*0.069*	*0.018*
	ese	0.04	0.05	0.01	0.05	0.03	0.04	0.004	0.008	5.9	6.1	4.9	5.0
Rootstock	Ashdot	0.20	0.31	0.23	0.33	0.19	0.17	0.14b	0.09	84.1	58.9	65.0	27.8
	Velvick	0.33	0.23	0.16	0.28	0.17	0.14	0.10a	0.07	87.5	62.9	61.8	37.3
	P	*0.651*	*0.139*	*0.104*	*0.353*	*0.807*	*0.282*	*0.047*	*0.156*	*0.415*	*0.553*	*0.558*	*0.151*
	ese	0.07	0.03	0.03	0.04	0.04	0.02	0.01	0.008	2.9	4.7	3.8	4.4
Density Rootstock*	*P*	*0.701*	*0.308*	*0.732*	*0.138*	*0.996*	*0.504*	*0.611*	*0.928*	*0.011*	*0.578*	*0.249*	*0.046* [2]

[1] Excludes branches dead by summer [2] The interaction between density and rootstock is significant at α =0.05. Treatment means were: high density Velvick 10.5% a; medium density Ashdot 23.9% ab, high density Ashdot 24.5% ab; low density Ashdot 34.8% bc, low density Velvick 48.7% c, medium density Velvick 52.8% c

The reasons for lower fruit set and retention in the high density treatment are unclear but appear to be a complex combination of factors which reflect the limitations of both planting density and the central leader training. Some of the potential factors are discussed below and include canopy efficiency, root system efficiency and fruit-shoot competition.

Canopy efficiency

We have reviewed treatment differences in canopy efficiency terms of light interception, light distribution and leaf numbers.

Light interception

Our investigations of light interception in conventionally planted orchards of a range of tree sizes indicated that yield is maximised until approximately 80-84% light interception (see Wilkie et al., 2019). There was a significant interaction between year of measurement and density treatment ($P < 0.0001$).

Our measurements of light interception in the Planting Systems Trial over the life of the project indicate that the high density treatment maximised light interception earlier in the life of the orchard than the medium and low density treatments, and reached a maximum of a little over 60% in 2017/18 (Figure 9). In 2018/19 the low density treatment had significantly lower total light interception than the medium and high density treatments and there was no significant difference between the high and medium density treatments. There were no significant effects of rootstock on total light interception ($P>0.05$).

We conclude that light interception is not the main contributor to lower yields for the higher density treatments.

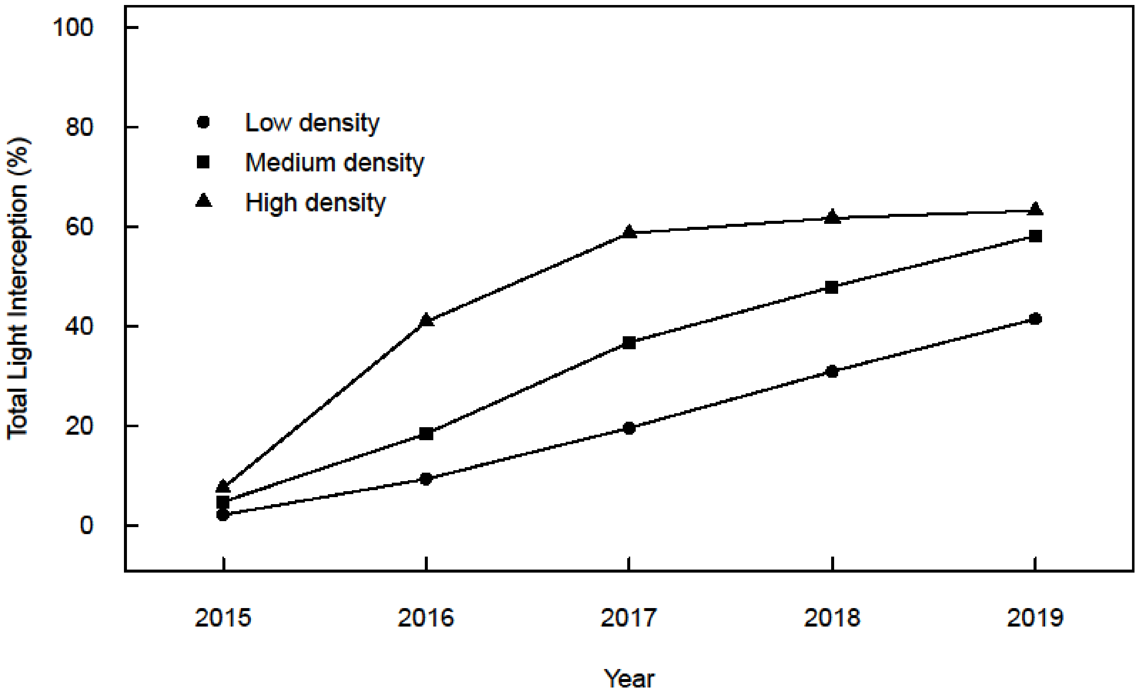

Figure 9 The effect of tree density on total light interception over time (P < 0.0001). The LSDs to determine significant differences between density treatments in the same year are 0.93, 5.7, 5.4, 5.1 and 5.6 in 2015, 2016, 2017, 2018 and 2019, respectively. The LSD to determine significant differences between density treatments across years is 4.5. There were also significant effects of density ($P < 0.0001$) and year ($P < 0.0001$) but not for rootstock ($P > 0.05$) or the interaction between year and rootstock ($P > 0.05$) or between year, density and rootstock ($P > 0.05$).

Light distribution

During the time covered in this report, trees in the low density treatment in most replicates were not yet touching or crowding and their canopy surfaces were thus well-illuminated. In the periphery light studies, there were no significant density treatments differences in 2017/18 or 2018/ (Figure 9, Figure 10). Some shading mid-canopy from growth in the tops and shoulders was evident in all densities.

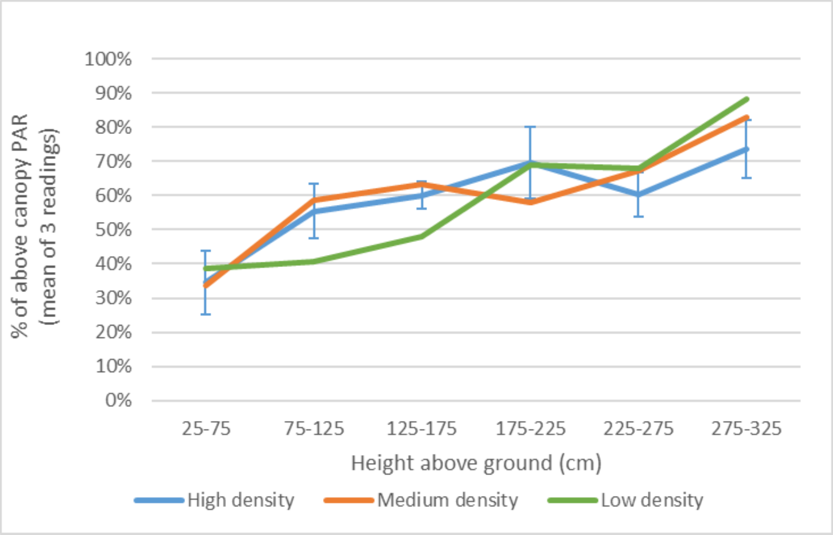

Figure 10 Mean % of above-canopy PAR measured in 50 cm intervals above ground in light periphery study 2017/18

Lines show the average of 6 trees (3 from each rootstock) for each density. Error bars show 1 standard deviation for 'high density' treatment only. ($P_{density}$=0.923, P_{band} <0.001, $P_{density*band}$=0.145) ('PAR': photosynthetically active radiation.

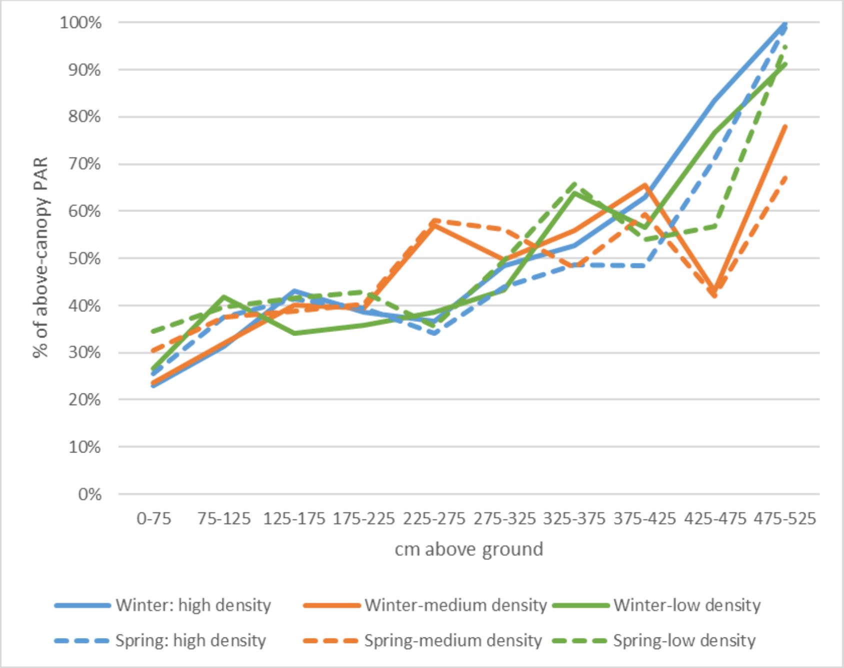

Figure 11 Mean % of above-canopy PAR by height above ground summarised in 50cm bands in light periphery study 2018/19

Lines show the predicted values from Genstat regression models using data from 5 trees ('Velvick' rootstocks only) for each density treatment. Winter = mean of three readings in full sun conditions. Spring = one reading under diffuse light conditions. In winter, $P_{density}$=0.614, P_{band} <0.001, $P_{density*band}$=0.103). In spring ($P_{density}$=0.109, P_{band} <0.001, $P_{density*band}$=0.091). 'PAR': photosynthetically active radiation.

In the light transect study, PAR levels differed through the canopy with the width of the canopy, but on average, all three densities show good PAR levels on the 50cm band around the periphery but low PAR levels through the canopy (Figure 12). In 2018, the high density treatment showed better PAR levels at 50cm from the trunk than the other density treatments. Hadari (2004) suggests that avocado leaves receiving less than 20% of ambient light are non-functioning. If this is the case, the majority of the high density canopy is above this level, whereas a majority of the medium and low densities canopies are below this level. Our measured levels for the high densities seem to be slightly better than that found by Hadari (2004) who found that light >20% ambience only penetrated 50cm into 'single leader' trees'. Note however that avocado leaves function well at low light levels. Light saturation has been recorded at ~25% of full sunlight (Scholefield et al., 1980), at ~33% (Bower, 1978) and at ~50% (Wolstenholme and Whiley, 1999).

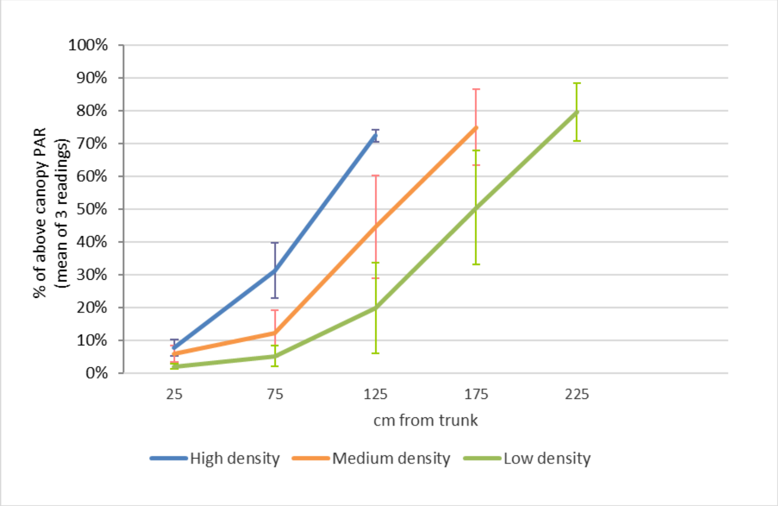

Figure 12 Mean % of above-canopy PAR in 50 cm intervals through the canopy winter 2017
Lines show simple means of n=6 for each treatment (3 of each rootstock). Error bars show 1 standard deviation. 'PAR': photosynthetically active radiation.

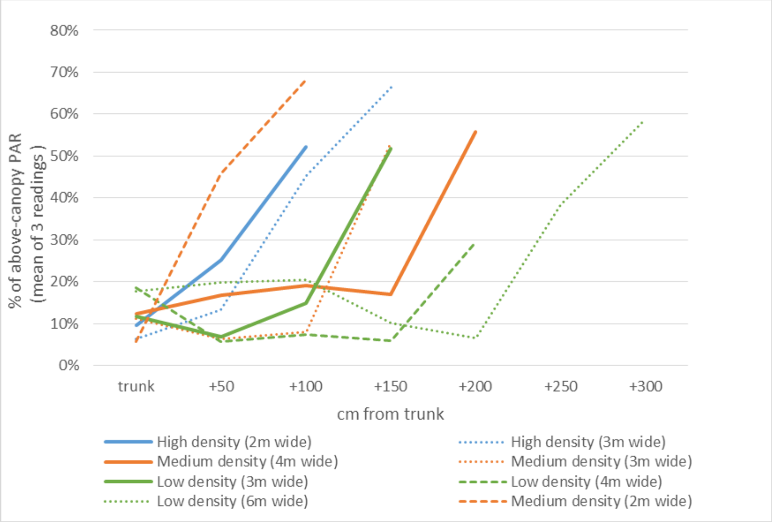

Figure 13 Mean % of above-canopy PAR in 50 cm intervals through the canopy in spring 2018
Solid lines show simple means of trees of the same canopy width (n=3 for high and medium densities, n=4 for high density). Dotted or dashed lines show single trees: we have presented these separately because their canopy width was atypical for that density. All trees on 'Velvick' rootstocks. 'PAR': photosynthetically active radiation.

These measurements suggest that the PAR levels *along* the canopy periphery were similar for all density treatments, and that levels *through* the canopy were better for the high and medium density treatments than the low density treatments, so neither factor contributed to lower fruit set and retention rates in the higher density treatments.

In any case, the results of our studies suggest that low levels of light have little direct effect on avocado fruit set at the shoot level (Figure 14 and Figure 15). This is, of course, contingent on fruiting sites being present i.e. where lack of light has led to branch death or lack of shoots clearly there will be no fruit.

In 2017/18 both the periphery and transect studies showed that PAR levels in our trial had a positive but weak linear correlation with several variables. These variables included the number of axillary inflorescences per shoot (a measure of flowering intensity), the time of floral expansion and time of anthesis and the length of vegetative growth of the shoot emerging from indeterminate inflorescences (Table 32). The relationship between PAR levels and flowering stage was consistent across all densities (see 2017/18 data in Figure 13). However, there were no such relationships between PAR levels and fruit set or retention in 2017/18 (Figure 14) or 2018/19 (Figure 15). In 2017/18 there was a weak linear relationship for the low density treatment (r^2=0.3114) (Figure 14) but it is an artefact of a single high data point. In 2018/19 there was clearly no relationship for any density treatment (Figure 15).

Table 32 Regression analysis (simple linear regression) in light studies 2017/18 and 2018/19 (all treatments combined)

	Periphery light study				Transect light study			
Explanatory variable	Winter % PAR		Parent GU diameter		Winter % PAR		Parent GU diameter	
Response variable	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
2017/18								
Parent GU diameter (mm)	0.345	*<.001*	--	--	0.144	*0.002*	--	--
Parent GU length (cm)	0	*0.83*	0.15	*<.001*	0.025	*0.131*	0.172	*<.001*
No. of axillary inflorescences/shoot	0.222	*<.001*	0.404	*<.001*	0.142	*0.003*	0.11	*0.008*
Terminal flowering stage on 15/08/2017	0.325	*<.001*	0.171	*<.001*	0.378	*<.001*	0.055	*0.046*
Terminal flowering stage at midbloom (12/09/2017)	0.07	*0.004*	0.018	*0.089*	0.092	*0.014*	#	*0.694*
Mean length of inf. vegetative shoots (31/10/2017)	0.186	*<.001*	0.418	*<.001*	0.162	*0.003*	0.289	*<.001*
No. of fruit per shoot on 31/10/2017	0.049	*0.013*	0.076	*0.003*	0.102	*0.010*	0.011	*0.209*
2018/19								
Parent GU diameter (mm)	0.191	*<.001*	--	--	0.05	0.052	--	--
parent GU length (cm)	#	--	0.286	*<.001*	#	--	0.303	*<.001*
No of axillary inflorescences	0.37	*<.001*	0.436	*<.001*	0.107	0.007	#	--
Terminal flowering stage on 22/8/2018	0.016	*0.076*	#	--	0.307	<.001	0.041	*0.072*
Terminal flowering stage midbloom 10-12/9/2018	*not recorded*				0.119	0.005	0.186	*<.001*
Mean length of inf. vegetative shoots (30/10/2018)	0.267	*<.001*	0.165	*<.001*	0.05	0.052	0.086	*0.015*
No of fruit per shoot (30/10/2018)	0.093	*<.001*	0.135	*<.001*	#	--	0.106	*0.008*

Residual variance exceeds variance of response variable. 'Winter % PAR' means PAR as a % of above canopy PAR, measured in winter each year.

In 2018/19, there were similar linear relationships between PAR levels and the number of axillary inflorescences per shoot and the vegetative growth of the indeterminate inflorescences, but the relationship between floral development and PAR was only evident in the transect study. Again in this year there was no direct relationship between PAR levels and fruit set.

Several variables were more strongly related to the diameter of the parent growth unit than to PAR levels: this includes the number of axillary inflorescences per shoot in 2017/18 and in 2018/19, and vegetative growth in 2017/18 (Table 32).

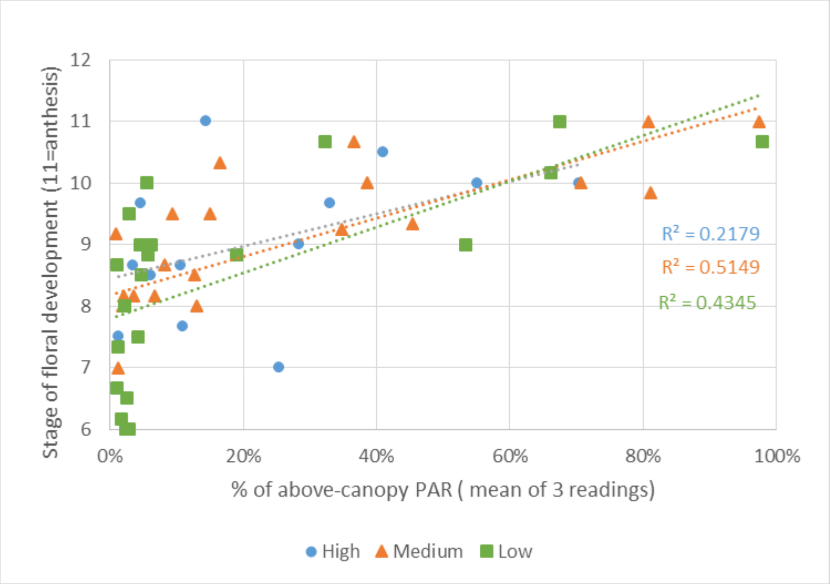

Figure 14 Transect light study 2017/18 Mean terminal flowering stage on 23/8/17 (mean of 3 shoots) by % of above canopy PAR by density treatment

Stages are as described by Salazar-Garcia et al. (1998). In addition, we have used stage '11' to indicate anthesis.

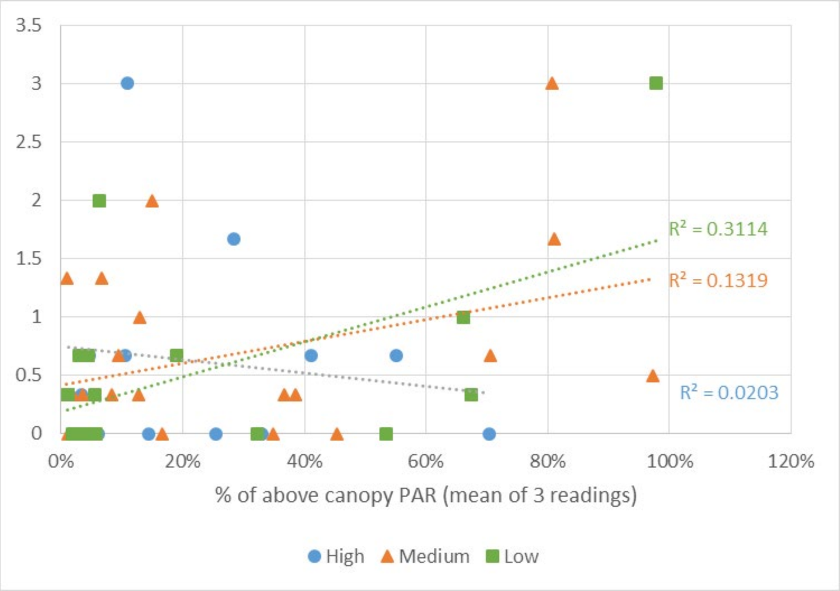

Figure 15 Transect light study 2017/18 Mean fruit set per shoot on 19/10/2017 by % of above canopy PAR by density treatment

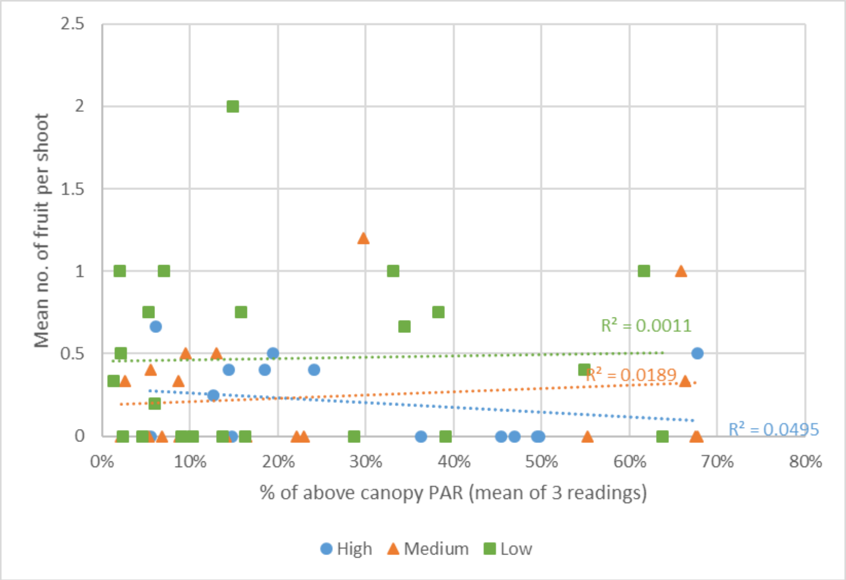

Figure 16 Transect light study 2018/19 Mean fruit set per shoot on 30/10/2018 by % of above canopy PAR (winter readings) by density treatment

We hypothesize that fruit set inside the canopy is not disadvantaged by the low levels of light because of the transport of resources between branches. In fact, we suggest that fruit that set in shaded parts of the tree may have some advantages, including the protective effect of the canopy, the reduced rate of vegetative growth of the indeterminate shoot which may compete with fruit set, and the later timing of set (due to later anthesis) meaning set is at a time when the competition for resources is less fierce.

Leaf numbers

Avocados have short-lived leaves (10-12 months) so the pattern of leaf drop and retention could conceivably affect fruit retention. Our architecture study identified leaves that remained on each growth unit at flowering, in spring and in summer. Because we identified the time of growth of each growth unit, we were able to analyse the number of 'old' and new leaves remaining at these times to see if there were density or rootstock treatment differences that might explain yield differences. On a cm^2 of branch CSA basis, there were generally no treatment differences in remaining number of leaves in the two years in which we counted leaves (2016/17 and 2017/18) at any period and of any leaf-age (data not shown). The exception was 2016/17 when the medium density treatment and the 'Ashdot' rootstock treatment had fewer 'old' leaves/cm^2 branch CSA at flowering (HD 32.8 b, MD 19.5 a, LD 27.5 b, P_{density}=0.011; Ashdot 23.4, Velvick 29.8, $P_{\text{rootstock}}$ =0.046). The reasons for this are unclear. Table 33 shows the trends in leaf loss per cm^2 of branch CSA for both years (average of all treatments).

Table 33 Number of leaves per cm^2 of branch CSA 2016/17 and 2017/18

	At flowering	Spring		Summer			
	Old leaves	Old leaves	Spring-grown leaves	Old leaves	Spring-grown leaves	Summer-grown leaves	Total leaves at harvest
2016/17	26.6	2.8	43.0	0.13	21.8	31.0	52.9
2017/18	46.4	25.4	29.2	2.04	13.3	39.5	55.2

'Old leaves' are leaves grown in the previous year's growth season. 'Summer-grown leaves' includes growth recorded as expanding in late spring plus summer flush leaves.

In terms of leaves per fruit, there was substantial tree to tree and branch to branch variability, so significant differences between density and rootstock treatments are few. Overall, leaf: fruit ratio was high but aligned with the ratio of 104:1 calculated by Thorp (1992) and 84:1 by Kohne (1989). The trend was to higher ratios for the higher densities (Table 34). Trees on 'Ashdot' rootstocks show a trend of fewer leaves per fruit than trees on 'Velvick' rootstocks at most time points (Table 34). This most likely reflects the determinate flowering pattern, as discussed in the section 'Fruit-shoot competition'. There was a difference between the two years in leaf: fruit ratio, which is curious in the light of a lack of an alternate bearing pattern. It appears also that the medium density treatment was out of 'sync' with the other two density treatments, having more leaves per fruit in 2016/17 and less in 2018/19, possibly due to pruning in 2016/17.

Table 34 Leaf:fruit ratio 2016/17 and 2017/18

		Per spring set fruit		Per harvested fruit				
Treatment		Old leaves	Spring grown leaves	Old leaves present at the beginning of spring^1	Old leaves present at the beginning of summer ^{1}	Spring grown leaves^1	Summer grown leaves^1	Total leaves remaining at harvest ^{1}
		2016/17						
Density	Grand mean	46.9	89	65.6	7.4	129	94	159
	High	61.8	74.0 ab	63.3	7.6	84	55	100
	Medium	57.5	153.9 b	95.1	9	245	163	288
	Low	24.3	38.8 a	37.9	5.6	59	63	89
	P	*0.105*	*0.042*	*0.509*	*0.877*	*0.262*	*0.404*	*0.283*
	ese	*12.78*	*29.7*	*33.51*	*4.61*	*80.2*	*59.6*	*92*
Rootstock	Ashdot	38.2	85	46.4 a	2.2 a	101	56	96
	Velvick	55.5	92	84.7 b	12.6 b	158	132	223
	P	*0.332*	*0.873*	*0.009*	*0.004*	*0.15*	*0.173*	*0.072*
	ese	*12.13*	*29.7*	*8.13*	*1.92*	*25.6*	*36.6*	*44.1*
Density Rootstock*	*P*	*0.718*	*0.841*	*0.041*^{2}	*0.025*^{2}	*0.041*^{2}	*0.056*	*0.020*^{2}
		2017/18						
	Grand mean	79	14.3	93	55	56.3	80	113
Density	High	79	15.2	116	56.9	84.9	86	133
	Medium	89	11.2	55	38.8	30.7	65	85
	Low	68	16.5	107	69.4	53.4	88	121
	P	*0.756*	*0.821*	*0.194*	*0.438*	*0.085*	*0.735*	*0.566*
	ese	*20.3*	*6.07*	*22.6*	*15.93*	*14.42*	*23.4*	*31.8*
Rootstock	Ashdot	39 a	9.4	71	42.9	45.2	49	70 a
	Velvick	118 b	19.2	114	67.2	67.5	111	156 b
	P	*0.021*	*0.149*	*0.13*	*0.225*	*0.189*	*0.062*	*0.037*
	ese	*20*	*4.42*	*17.5*	*12.71*	*10.67*	*19.1*	*23.1*
Density Rootstock*	*P*	*0.515*	*0.905*	*0.785*	*0.909*	*0.652*	*0.609*	*0.465*

[1] Excludes three missing values in 2016/17 and seven in 2017/18 due to no fruit on sample branches. Ese not adjusted for missing values [2] Note significant differences in density/rootstock means for leaves per harvested fruit in 16/17. In this year, there were a very high number of leaves per fruit for the medium density 'Velvick' compared to other density/rootstock combinations, which did not differ significantly from each other. This may have been due to a pruning response.

Root system efficiency

One possible factor in explaining yield variation by density is the effect of area available for roots ('root area'). Avocados have shallow and relatively inefficient fibrous feeder roots (Lahav and Whiley, 2013). Therefore it is conceivable that a limited root system in the higher densities may be inadequate to supply water and/or nutrients during flowering, fruit-set or retention. Silber et al. (2012) found in a lysimeter trial with 'Hass' that limited container size and water deficits had a much stronger negative effect on yield than it did on vegetative growth. We have no data on the extent or density of roots in our trial but we have calculated a theoretical root area using the following assumptions:

- root area does not cross planting space between trees
- root area along the row is limited to the canopy width or the planting distance, whichever is the smaller
- root area across rows is limited to the canopy width or the row width minus the width of the alleyway (2m), whichever is the smaller
- rooting depth for all densities is the same
- the calculation of root area does not differ by rootstock

Our estimations, while extremely rubbery, show that in 2017/18, when yield per hectare did not differ by density, root area for the high and low densities were not significantly different, and yield for root area was the same for all three densities (Table 35). In 2019, the yield per unit of root area was higher for the low density treatment, and an increase on the previous year; the high density treatment had lower yield per root area and a decrease from the previous year. Changes in canopy volume may be an interaction here; also possibly decreasing root efficiency where roots are crowded.

Table 35 Estimated feeder root area 2017/18 by density treatment

Treatment		Estimated root area/tree (m^2)		Estimated root space/ha (m^2/ha)		Yield 2018/ estimated root space (kg/m^2)	
		2018	2019	2018	2019	2018	2019
	Grand mean	14.08	15.3	5926	6203	2.204	2.48
Density	High	5.0 a	5.0 a	5556 a	5541 a	2.21	2.02 a
	Medium	12.9 b	11.9 b	6581 b	6593 b	2.03	2.42 ab
	Low	25.4 c	29.1 c	5641 a	6473 b	2.38	3.00 b
	P	*<.001*	*<.001*	*0.007*	*0.004*	*0.345*	2.02 a
	ese	*0.8*	*0.8*	*181*	*168*	*0.16*	0.18

Root limitations may also affect levels of hormone production as discussed below in the section 'Fruit-shoot competition: the role of determinate inflorescences'.

Fruit-shoot competition for resources

For many tree crops, fruit set, fruit retention, fruit growth and/or fruit quality can be limited by a insufficient supply of resources, including carbohydrates, minerals, water and phytohormones. The terms 'competition' and 'allocation' are commonly used to describe the variability of supply of finite resources to different plant organs, even though these terms imply a more active process than can be possible. Our main interest in vigour-control trials is the competition between vegetative and reproductive organs, or 'fruit-shoot' competition.

There is substantial evidence for fruit:shoot competition in spring for avocado, including (a) studies demonstrating better spring fruit set on determinate shoots (Salazar-Garcia and Lovatt, 1998; Thorp et al., 1994) although Evans et al. (2010) found no difference for Hass in New Zealand and Dixon et al. (2007) found set increased on determinate shoots only in an 'on-year'; (b) studies demonstrating reduction of vegetative growth with paclobutrazol or uniconazole at mid-bloom increases yield and/or fruit size (Gardiazabal et al., 1995 ; Kohne and Kremer-Kohne, 1987), and (c) studies demonstrating improved spring fruit set when vegetative shoots are removed (Biran, 1979; Cutting and Bower, 1990; Zilkah et al., 1987).

There are some reports that the gains made in fruit set in spring by these strategies are mitigated by increased drop in summer, resulting in no increase to final yield (Cutting and Bower, 1990; Wolstenholme et al., 1990). Our own study in the tipping and fruiting sites trial also found that gains in initial fruit set from vegetative 'tipping' did not deliver a final yield improvement (Hofman et al., 2018). The mechanism for this adjustment is unclear but hypotheses include unspecified signals reflecting limitations to stored and current carbohydrates, or competition between fruit growth and a proactive allocation to carbon stores.

The existence or importance of fruit:shoot competition during the critical summer drop period (November to January) is much less clear than for the spring growth period. Our architecture study of summer drop on unpruned trees at Childers found that while the presence of fruit on a *shoot* tended to suppress summer growth by preventing bud release, on a *branch* basis the growth of summer leaves did not appear to compete with fruit retention. Other factors such as hormonal action and/or active allocation of carbohydrate to storage, roots, and shoot preformation may be more important in determining summer fruit retention.

Fruit-shoot competition: the role of determinate inflorescences in yield

Our architectural studies and flowering terminal data showed clear differences in the likelihood of fruit set from indeterminate and determinate inflorescences, and from terminal and axillary inflorescences. Indeterminate inflorescences are those with a vegetative shoot emerging from the tip, determinate inflorescences have no vegetative tip.

In each of the three years of the architectural study, determinate terminal inflorescences had higher rates of fruit set and retention than indeterminate inflorescences, despite also experiencing the highest drop rate (Table 36). We hypothesise that the terminal determinate spring fruit set is higher due to reduced competition from vegetative growth at the shoot level.

Table 36 Percent of inflorescences and fruit set and drop per inflorescence by inflorescence type (mean of all treatments)

Inflorescence type	% of inflorescences			Spring-set fruit/ inflorescence ^{3}			Summer-retained fruit / inflorescence ^{3}			% summer dropped fruit/ spring-set fruit		
	2015/16	2016/17	2017/18	2015/16	2016/17	2017/18^{2}	2015/16	2016/17	2017/18^{1,2}	2015/16^{1}	2016/17^{1}	2017/18^{1,2}
Terminal determinate	7.2%	18.6%	21.9%	0.262	0.295	0.273	0.242	0.184	0.138	22.7%	43.5%	53.1%
Terminal indeterminate	32.6%	34.8%	63.4%	0.175	0.153	0.682	0.110	0.116	0.087	36.5%	27.3%	41.1%
Axillary determinate	22.8%	29.1%	7.5%	0.16	0.072	0.030	0.013	0.058	0.044	12.5%	33.3%	45.5%
Axillary indeterminate	37.5%	17.6%	7.3%	0.051	0.026	0.015	0.037	0.016	0.024	36.4%	33.3%	40.0%
Mean all types	--	--	--	0.096	0.139	0.165	0.071	0.091	0.090	31.2%	35.4%	46.9%

Estimated mean of all density and rootstock treatments in analysis of variance except where noted as ^{1} which are simple means only due to large numbers of zero values ^{2} Not adjusted for missing values from four branches which had no fruit ^{3} Can be more than one fruit per inflorescence

The proportion of terminal flowering that was determinate appears to have been lower in 2018/19 than in other years. We do not have architectural data for that year but our counts of flowering terminals (i.e. excludes axillary flowering) showed 11% were determinate, compared to 26% in 2017/18, 35% in 2016/17 and 18% in 2015/16.

In our architecture studies there were no clear density treatment differences in the proportion of determinate inflorescences, but clear rootstock treatment differences (Table 29). There were higher proportions of determinate inflorescences (includes both axillary and terminal inflorescences) on trees on 'Ashdot' rootstocks compared to 'Velvick' in all three years of study.

Our flowering terminal counts show that the percentage of spring set fruit on determinate inflorescences for trees on 'Ashdot' rootstocks was higher than 'Velvick' in all years but differences were only significant in 2015/16 and 2016/17 (Table 37).

Table 37 Percentage of spring fruit set on determinate terminal inflorescences

		2015/16	2016/17	2017/18	2018/19
Treatment	Grand mean	46.7%	57.3%	53.9%	18.7%
Density	High	39.6%	51.4%	57.2%	20.1%
	Medium	50.6%	53.6%	51.4%	23.0%
	Low	50.0%	66.9%	53.0%	13.0%
	P	*0.703*	*0.079*	*0.816*	*0.340*
	ese	*10.2%*	*4.5%*	*6.6%*	*4.6%*
Rootstock	Ashdot	58.5% b	69.2% b	61.2%	23.6%
	Velvick	34.9% a	45.4% a	46.6%	13.8%
	P	*0.036*	*0.032*	*0.067*	*0.158*
	ese	*6.9%*	*7.0%*	*5.1%*	*4.6%*
Density* Rootstock	*P*	*0.353*	*0.970*	*0.567*	*0.938*

The lack of statistical significance in the data on determinate flowering, despite large differences between treatment means, reflects the wide tree to tree variation within treatments in determinate flowering tendency. Determinate flowering is highly variable from tree to tree and sometimes also from branch to branch within a tree.

Our hypothesis is that determinate flowering terminals may be more common where there are compromised roots or where there are vascular restrictions between roots and individual branches. We looked at the ratio of rootstock to scion cross-sectional areas as evidence of poorer growth in root systems. On average, this ratio was smaller for trees on 'Ashdot' rootstocks than trees on 'Velvick' rootstocks in all years, with values commonly less than 1, that is, showing an overgrowth of the scion compared to the rootstock (Table 8). We hypothesise that the 'failure' of flowering shoots to develop a vegetative apex may be due to a reduced production of cytokinins in the roots. Cytokinins have important roles in shoot development and cellular differentiation. Growing root tips are the main source of cytokinins. Inadequate cytokinin supply from the roots may mean either that the vegetative meristems (which are distal to the floral meristems) do not form when the bud is formed at the end of the previous growth flush, or the vegetative meristems form but do not develop further when the floral shoot emerges.

Determinate flowering has also been associated with warmer winters: the hypothesis is that inadequate chilling results in incomplete inflorescence/shoot development. This may explain the drop in 2018/19 in determinate inflorescences: of the four seasons reported, the coldest winter was in 2018/19. An alternative explanation is that determinate inflorescences are less likely in an 'on' crop year (Salazar-Garcia et al., 1998).

Overall it appears that the determinate flowering pattern may have contributed to improved crop loads for 'Ashdot' compared to 'Velvick'. Whether this persists as the trees mature is not known.

Fruit-shoot competition: the effect of pruning

Pruning is necessary to reduce crowding and to ensure light penetration into the canopy. High density avocado orchards left unpruned decline in yield once canopies begin to crowd (e.g. Razeto et al., 1995).

The growth habit of our 'Hass' trees was that initial growth formed a central leader, with lateral branches decreasing in size towards the apex of the growth spurt. However, in the next period of growth, and in subsequent flushes, lateral shoots grew vigorously and quickly competed with the central leader. Maintaining a central leader shape in the higher densities, and fostering light inside the canopy, required substantial selective pruning during the years of this report. Where pruning produced light gaps in the canopy, the trees frequently and rapidly sent up highly vigorous watershoots and/or strong sylleptic growth (see also Gardiazabal and Mena, 2011). In addition, the tops of trees developed rapidly, forming a shading 'umbrella' over canopy below (see Figure 9 and Figure 10). Stassen and Snijder (1996) also found that the improvements to light levels from pruning in winter were short-lived, with light levels at 1 m from the edge of the canopy reverting to almost the same pre-pruning levels by summer.

It is difficult to assess the effects of pruning on yield from our data as relationships are indirect and may extend over several growing seasons. The reasons for detrimental effects of pruning could include diverting carbohydrate stores from fruit development to vegetative growth and/ or producing ethylene in response to stress that results in fruit abscission: the former seems most likely given the timing of pruning. It is difficult to measure pruning regrowth as it is unevenly distributed around the tree. The regrowth pattern is affected by light availability and also the availability of nodes for shoot emergence, and tended in the high and medium densities in our trial to be concentrated at the tops of the trees. To assess the effects of pruning we thus have relied on calculating the total branch CSA of pruning cuts per tree and comparing it to yield.

We have calculated correlation coefficients (r) between the total CSA of pruning cuts per tree and yield per tree in the year of pruning as well as yield a year later for the density treatments (Table 38). Note that there would have been an additional pruning during the 'year later' harvest which may confound results. Statistical confidence (Ps) in these calculations is extremely low and the trends outlined in the following paragraphs can only be considered hypothetical.

In 2016/17 pruning of the high and medium densities had little correlation with yield in the year of pruning but there seemed to be a weak negative correlation with yield in the harvest a year later (2017/18). In 2017/18 the negative correlation between pruning and yield in the high densities seems to have reversed, that is, correlations were more consistently negative in the year of pruning than a year later. It is possible that this is related to an underlying biennial pattern in carbohydrate storage even though there appeared to be only a weak alternate bearing pattern.

Pruning in the medium densities in 2017/18 had little correlation with yield in that year but tended towards a positive correlation with yield a year later. The difference between the two densities may be because pruning in the high densities tended to be dominated by controlling vigorous growth at the top of the tree, with perhaps little positive impact on light levels lower down in the tree; whereas pruning in the medium densities tended to be less focused on controlling tree height and more on 'opening up' the canopy by selective limb removal to improve light levels.

The number of pruning cuts on trees on 'Ashdot' rootstocks compared to 'Velvick' was not different in any year of reporting, but in 2017/18 and 2018/19 the total CSA of pruned branches for 'Ashdot' was ~ 20% less than that for ‘Velvick’ (Table 4). This reflects the smaller size of trees on ‘Ashdot’, but may also have meant less negative impact on yield.

This discussion illustrates that selective pruning is a ‘two-edged sword’: it has positive, if short-lived, impacts in improving light distribution through the canopy and in controlling tree size, but negative impacts in stimulating further vegetative growth which may act to reduce yield by consuming stores and current supply of carbohydrates. This is shown in pruning studies by Thorp and Stowell (2001) in which reducing the number of main scaffold branches from 8-12 to 6-8 increased productivity of the remaining branches by improving light distribution but yield per tree was similar to that of unpruned trees in two or three years and reduced in a third year.

Table 38 Correlation coeffcients (r) between the sum of CSAs of pruned branches per tree and yield per tree (kg) in the year of pruning and in the year following pruning 2016/17-2018/19

Treat-ment	*Time of pruning*	2016/17				2017/18				2018/19	
		Year of pruning		Following year		Year of pruning		Following year		Year of pruning	
		r	P	r	P	r	P	r	P	r	P
High	Winter	0.172	*0.402*	-0.351	*0.079*	-0.141	*0.466*	0.031	*0.873*	-0.13	*0.508*
	Summer	0.127	*0.537*	-0.270	*0.183*	-0.277	*0.146*	-0.211	*0.271*	--	--
	Summer + winter	0.181	*0.377*	-0.379	*0.059*	-0.243	*0.203*	-0.117	*0.547*	--	--
Medium	Winter	-0.144	*0.447*	-0.270	*0.149*	0.026	*0.892*	0.336	*0.070*	0.24	*0.194*
Low^1	Winter	--		--		-0.011	0.965	0.111	0.643	0.23	*0.870*

Data includes both ‘Velvick’ and ‘Ashdot’ rootstocks. n=29 trees for the high density treatment, 30 for medium density and 20 for low density. *P* values show two-sided tests of correlations different from zero. ^{1} In 2017/18 winter pruning was restricted to skirting of lower branches.

Fruit-shoot competition: the influence of canopy width

The 2017/18 light transect study suggests that the wider canopies in the low and medium density treatments provide greater variability in time of fruit set, with mean fruit size for the high density treatment on 24/10/17 being 23.5mm whereas for the low density treatment it was 16.2mm. Our transect light studies show that light strongly affects floral development (Figure 13).

We hypothesized that the later anthesis inside the canopy of the medium and high density treatment trees meant fruit set there is less affected by the slower vegetative growth of the indeterminate shoot, as well as setting when the competition for resources on a whole tree basis is declining. The distribution of individual fruit weights measured on 12 sample branches per replicate at harvest in 2018 and six branches in 2019 shows a trend to a higher percentage of larger fruit for the high density treatment but with few significant differences in percentage in each class between densities (Figure 16, Figure 17). In 2019 we also measured distance of harvested fruit from the nearest main leader on six branches per tree. This data suggests that, for all density treatments, small fruit are not concentrated in the inside of the tree, but are found at all distances from the trunk (Table 39).

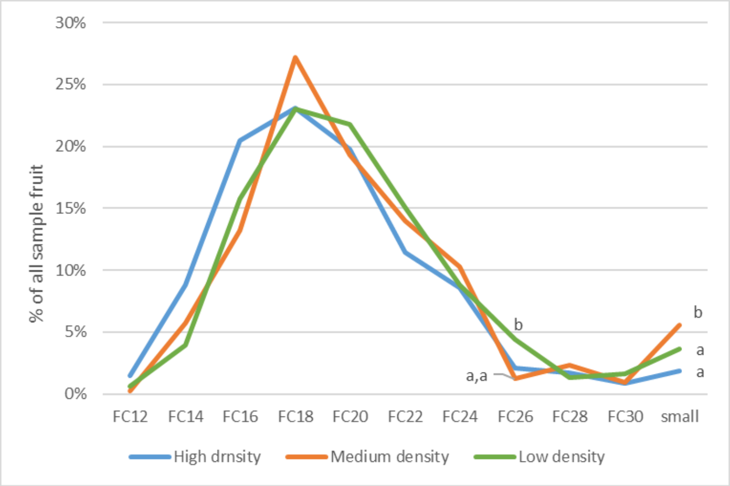

Figure 17 Fruit size distribution as a percentage of all sampled fruit by density treatment 2018
FC=fruit count (the approximate number of fruit in a 5.5 kg tray). Differences between treatments were not significant at the 95% confidence level except for FC26 (*P*=0.020) and 'small' fruit (<FC30) (*P*=0.006)

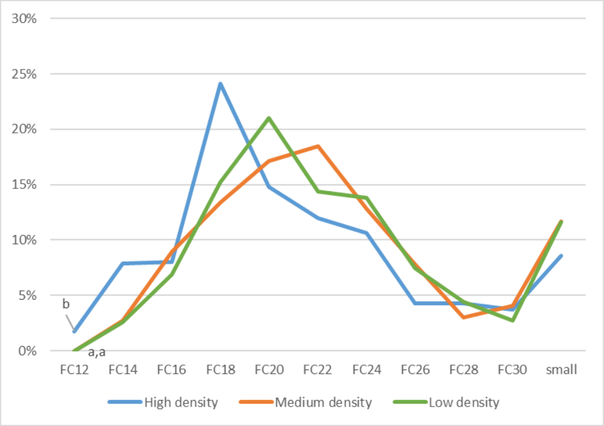

Figure 18 Fruit size distribution as a percentage of all sampled fruit (6 branches per tree) by density treatment 2019
FC=fruit count (the approximate number of fruit in a 5.5 kg tray). Differences between treatments were not significant at the 95% confidence level except for FC12 (*P*=0.027)

Table 39 Distribution of fruit sizes by distance from trunk or closest leader by density treatment 2019

Treatment/Distance from leader	FCs12-16	FCs18-22	FCs24-28	FC30+	n
Low density					
0-100cm	9%	54%	24%	14%	221
100-200cm	10%	47%	28%	15%	428
200-300cm	8%	51%	21%	20%	60
>300cm	10%	50%	30%	10%	10
Medium density					
0 -100cm	12%	52%	23%	14%	189
100-200cm	8%	45%	28%	20%	173
200-300cm	25%	42%	17%	17%	12
>300cm	na	na	na	na	na
High density					
0 -100cm	14%	51%	20%	15%	139
100-200cm	26%	52%	22%	0%	27
200-300cm	na	na	na	na	na
n>300cm	na	na	na	na	na

na not applicable

Fruit-shoot competition: the influence of seasonal conditions on fruit set

The data in Table 30 and Table 31 show seasonal differences in fruit set and retention. While some of this may be due to a biennial bearing pattern that reflects cycles in carbohydrate stores, we suggest that weather conditions during flowering and early fruit set may be a significant factor. In 2017/18 fruit per FT was very low (mean of 0.195 compared to 0.273 in 16/17 and 0.287 in 18/19)(Table 37). This may have been due to higher than average spring temperatures, which may have exacerbated vegetative flush growth to the detriment of fruit set (Figure 18). In 2018/19, spring fruit set was very high, due in part, we believe, to cooler late winter and early spring minimum temperatures (Figure 18). Note that in this year fruit drop over summer was very high (on average 67.4%). This seasonal variation may also have affected the densities in different ways, due to tree structure, as outlined below.

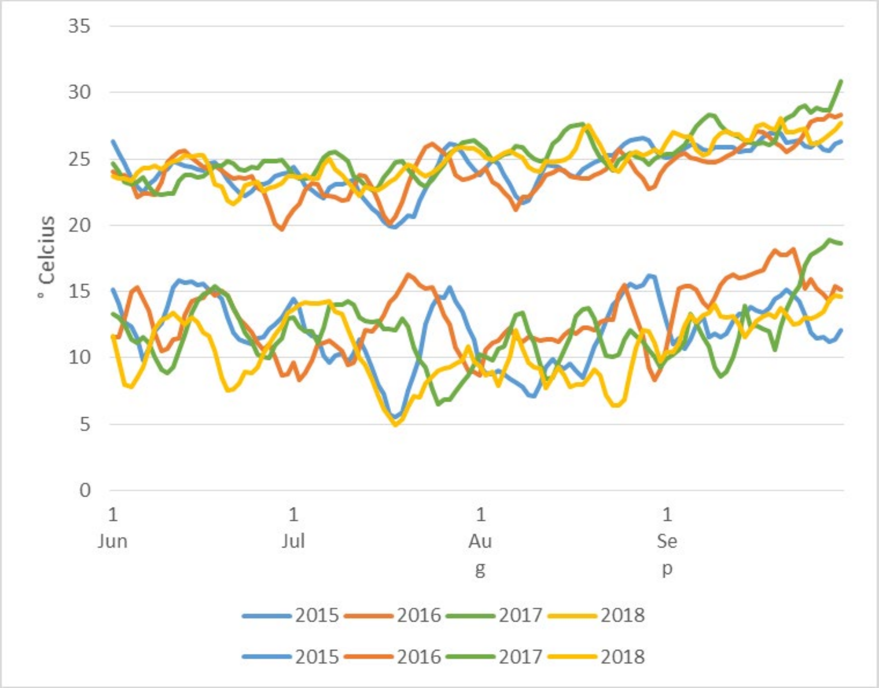

Figure 19 Minimum and maximum daily temperatures for Bundaberg Airport 1 June to 30 September (5 days smoothing) 2015-2018

Bureau of Meteorology data, station no. 38128.

Fruit-shoot competition: allocation to canopy structure

The costs of construction and respiration of support tissue for trees (roots, trunk and structural branches) is not well understood and difficult to measure. Givnish (1988) suggests the costs are significant, particularly for tall trees. Shade-tolerant trees tend to have a greater canopy diameter/height ratio than shade-intolerant trees (data from F.A. Hunt, quoted in Givnish 1988). It is possible that the 'cost' advantage initially gained by the artificial support of the trellis in our high density treatment was lost as the trees competed for height and vegetative growth at the tops. This is an area of study that may be best explored in the future by functional structural plant models.

Conclusions and recommendations for future research

Our high and medium density planting treatments to date have failed to deliver increases in productivity per hectare compared to a conventional low density planting.

Structural issues have contributed but do not appear to be the main factor in poor yields. In avocado, there are high levels of branch death and limited branch renewal inside the canopy, so branch structure is easily weakened by pruning, low light levels, growth spurts and pest damage. Snijder and Stassen (1998) reported that central leader training matched or increased yield compared to control trees, but in our trial the central leader shaping appears to have produced a poor canopy structure in terms of weaker branches and increased branch death. Regardless, our data and calculations suggest that fruiting sites and flowering should have been sufficient for increased yields per hectare from higher densities. While structure may not be the main factor in low yields, improving branching and canopy filling should contribute to better yields through increased canopy efficiency and numbers of fruiting sites. It remains to be explored whether tree structure can be substantially improved by more vigilant training in early years, and by pruning techniques that minimise structural damage (see discussion in ‘Avocado rootstocks’ chapter).

Our experience suggests, however, that central leader shaping appears to have exacerbated exuberant regrowth at the tops and shoulders of trees planted at higher densities, increasing shoot/fruit competition as well as increasing the amount of shading lower in the canopy. ‘Hass’ has a natural tendency to develop three to four spreading leaders (Thorp and Sedgley, 1993a). Pruning to a central leader shape meant removal of more material than might be needed for more upright varieties. Future high density trials for ‘Hass’ should include trials of multi-leader structures as an alternative to the deficiencies of central leader structures. Multi-leader systems, planted at 2.5m x 2.5m or 3m x 3m, are reported to have high yields in Chile, where growers have abandoned the central leader pyramidal shaping initially used (Gardiazabal and Mena, 2011). Systems that stimulate constant regeneration of short fruiting branches, notably two-dimensional trellis systems, should also be tested. These systems may have additional advantages in maximising light distribution and in improved canopy:root area ratios.

While tree structure has been less than optimal, the main factor limiting yield appears to have been rates of spring fruit set and fruit retention over summer. The poorer fruit set and retention in the higher density treatments does not appear to be due to lack of light through the canopy. The effects of competition for resources between fruit development and vegetative growth (‘fruit-shoot competition’) in spring may be a significant factor, particularly from regrowth in spring after winter pruning. This competition may be exacerbated by a tendency for anthesis timing to be more compressed in well-illuminated canopies, but supporting data on fruit size distributions are inconclusive. It may also be exacerbated in years where warmer spring temperatures advance vegetative growth relative to the timing of anthesis and fruit set.

While we cannot demonstrate direct relationships, pruning seems to be an important contributor to low levels of fruit set and retention, most probably through the scarcity of resources for fruit development due to the demands of vegetative regrowth. Our experience suggests that high density plantings of avocado are unlikely to be successful unless vigour can be better managed, at least in sub-tropical climates such as ours. In the absence of a dwarfing or vigour-controlling rootstock, the potential for reducing fruit/shoot competition through changing pruning times, through changes to nutrition and/or through changes to PGR (plant growth regulator) strategies needs to be explored.

Of these options, it may be that new PGR strategies could be the best for providing vigour control without the negative impacts of pruning. The use of soil drenches for avocado plantings less than 2.5m high of the PGR ‘AuStar’ (active ingredient paclobutrazol) has recently been added to this product’s registration, and future research needs to include this management option. This chemical also reportedly encourages drooping or weeping branches rather than vertical growth, an added advantage (Mitchell, 2019).

It is also possible that root systems in high density plantings are too limited to support the same rate of fruit set and retention as low density plantings: this area remains to be studied.

We also need a better understanding of the mechanisms that determine summer drop in avocado. We have been able to 'rule out' several factors i.e. canopy volume, surface area, and light distribution. Another hypothesis is that summer fruit drop is due to the preferential allocation of photosynthates to storage to support the next annual flowering and fruiting cycle rather than supporting the current crop (Wolstenholme and Whiley, 1997). We need to see if we can manipulate carbon storage with PGR, pruning and nutrition strategies. We suggest that understanding and managing summer fruit drop is a priority for future research including improving our understanding of carbohydrate storage and allocation needs to accompany and guide this research. We hope that the HortInnovation project AV16005 provides some insights on this issue.

Comparing the results of the two rootstock treatments indicate the potential for the choice of rootstock to contribute to the success of high density plantings. While 'Ashdot' cannot be considered to be dwarfing, trees on this rootstock had slower canopy growth in the years of our trial. In all cropping years, trees on 'Ashdot' rootstocks performed significantly better in terms of yield, with larger fruit and with no detrimental effect on fruit quality. Superior yields appear to be partly due to reduced vegetative growth, less sylleptic growth and a slightly improved branch structure. However, the main contributor appears to be a strongly determinate flowering pattern. The reason for the higher proportion of determinate flowering in one rootstock over the other is unclear, and it remains to be determined whether this trait persists as the trees age and whether or not this trait can be exploited in breeding new rootstocks. We need to better understand the phenomenon of determinate flowering in avocado, and explore methods for manipulating it. In addition, we need to monitor the comparative performance, including tree health, of the two rootstocks over the long term. Less vigorous scions than 'Hass', or scions with different branching, leaf density and/or fruiting habits, may also be more successful in high density plantings, and the range of scions tested should be increased in future high density research.

Definitions and abbreviations

Alternate bearing -	tendency for a tree to produce a greater than average crop one year, and a lower than average crop the following year. Also *biennial bearing*
BCSA -	branch cross sectional area
Biennial bearing -	see *alternate bearing*
CSA -	cross sectional area
Determinate -	inflorescences that do not produce a vegetative growth unit from their apex. Compare *indeterminate*
ese -	estimated standard error of the mean, that is, an estimate of how far the sample mean of the data is likely to be from the true population mean.
FC -	fruit count, that is, the approximate number of fruit of that size that will fit in a tray of ~ 5.5 kg of fruit

FT -	flowering terminal
Growth unit -	the section of a branch that grows in a single flush
Indeterminate -	inflorescences that produce a vegetative growth unit from their apex. Compare *determinate*
P or *P* value -	the probability that the differences in the treatment means are due to the variation in the sample rather than to treatment differences. A P of 0.05 or less is used as the standard for declaring that treatment means are significantly different.
PAR -	photosynthetically active radiation, that is, the wavelengths of light that are used in photosynthesis (400-700 nm)
PGR -	plant growth regulator
Proleptic -	growth of the first growth unit of a branch in a later flush than the parent growth unit. Compare *sylleptic*
PST -	Planting Systems Trial
QAAFI -	Queensland Alliance for Agriculture and Food Innovation
Selective pruning -	removal of some limbs and/or branches as distinct from ‘hedging’ or unselectively trimming the periphery of the canopy
Sylleptic -	growth of the first growth unit of a branch in the same flush as the parent growth unit. Compare *proleptic*

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Appendix 3

Avocado rootstocks for high density systems

H. Hofman, J. Wilkie, J. Griffin, C. Parfitt, H. Toegel

Introduction

An important component of the STHPI was screening domestic and international avocado rootstocks for vigour management and productivity in a high-density planting. Vigour-controlling rootstocks are generally considered to be an important ingredient in high density tree cropping systems and some consider it vital to success with avocado (Menzel and Le Lagadec, 2014; Thorp and Hallet, 1999). Finding a proven dwarfing rootstock would be a 'game changer' for the industry (Van Rooyen et al., 2015, p. 492). Without vigour-controlling rootstocks it will be difficult to achieve the same magnitude of productivity gains as the apple and stone fruit industries.

At present there are no proven dwarfing rootstocks for avocado, domestically or internationally. In 2017, as part of this project, we reviewed the availability of vigour-controlling rootstocks internationally through both published reports and through seeking advice from international researchers. Avocado rootstock research has been conducted internationally since the 1940s, with the largest programs conducted in California and Israel. Gene pool orchards have been established in Mexico, Guatemala, Costa Rica, Chile and Israel (Ben-Ya'acov and Barrientos-Priego, 2003; Castro et al., 2007) and there is also an extensive collection in California. However, most rootstock selection programs have not included vigour control as an objective, focusing rather on objectives such as increasing productivity, overcoming susceptibility to infection by *Phytophthora cinnamomi,* and coping with stressful and saline environments

Most rootstocks that have become available internationally in the last few decades have already been trialled in Australia in conventionally spaced plantings by researchers and growers (Le Lagadec, 2010; Whiley et al., 2013): none are reported to exert significant vigour control.

Internationally, there has been little progress in selection or breeding of a dwarfing rootstock. Several rootstocks that were reported as dwarfing have been failures due to premature tree decline (Ben-Ya'acov and Michelson, 1995; Crane et al., 2013). Reports of promising dwarfing rootstocks that subsequently disappear from discussions are common, for example, 'VC241', 'Witney' and 'Frolic' (Whitney et al 2005, 'Benjamina' (Ben-Ya'acov et al 1993), 'MT4' (Berg & Whitsell 1962), 'Ryan' (Roe & Morudu 2000).

There is no reported success in a dwarfing rootstock from the extensive program run by the University of California. In 2012 the University released 'Steddom' (PP24), and reported that, while not dwarfing, it was 'relatively slow growing but heavy yielding' (Douhan, 2009) and therefore 'may be an excellent choice for high density or hedgerow avocado plantings' (Crane et al., 2013, p. 213). However, Guirado et al. (2015) report that in their rootstock trials with 'Hass' on a range of rootstocks 'Steddom' was 'intermediate' in terms of trunk cross-sectional area and yield.

Chile and South Africa have rootstock selection programs but researchers in these countries advised that in their selections to date they have not found any dwarfing or semi-dwarfing rootstocks (M. Castro Valdebenito, pers. comm. 16 November 2016; S. Kohne, pers. comm. 11 November 2016 respectively).

Out of the extensive program conducted in Israel, 'Ashdot 17' remains the only recommended vigour-reducing rootstock for 'Hass' (H Cohen, pers. comm., 21 January 2017). Our experience with this rootstock is outlined in this report and in the report on the Planting Systems Trial.

A remaining hope for a truly dwarfing rootstock may be in Mexico. Mexico began a germplasm collection in the 1980s focusing on actively sourcing wild species from the countries of origin of the avocado in order to help conserve the avocado gene pool (Ben-Ya'acov and Michelson, 1995). Dr Alejandro Barrientos-Priego and his team at the Universidad Autonoma Chapingo in Mexico report there is some potential for dwarfing rootstocks from this program, but the work is in an early stage i.e. recovering of rootstocks prior to cloning and then field evaluation (A.F. Barrientos Priego, pers. comm. 6 December 2016). Of particular interest is the species *Persea parvifolia* Will., which Dr Barrientos Priego considers to be a possible ancestor of the avocado (Barrientos-Priego et al., 2016).

In South Africa, both Westfalia and AllesBeste, the two largest producers, have shifted their approach to planting more compact and regular bearing scion cultivars grafted on precocious, high-yielding clonal rootstocks. (A.I. De Villiers and A.A. Ernst, pers. Comm. 19 and 23 January 2017 respectively). The hypothesis is that early bearing will retard vegetative growth. Management practices are also important aspects of their high density systems: this includes planting on ridges to limit root growth, and application of plant growth regulators.

The key cultivars used in South Africa are 'Gem'® (Westfalia), which is reportedly 1/3 smaller than 'Hass'; and 'Maluma Hass' (Allesbeste). 'Gem' is a consistent and heavy bearer where trialled in California and South Africa (Arpaia, 2005; Bruwer and Van Rooyen, 2007). It is reportedly of 'vigorous moderately-spreading upright growth habit' with 'leaves displayed in a greater quantity than the 'Hass' variety' (US Patent application USPP14239 P3). 'Maluma Hass' is reportedly an upright grower, with a natural central leader, strong lateral branching and sylleptic growth pattern meaning excellent light penetration (Ernst, 2007; Ernst and Ernst, 2011). It is reported as less vigorous than 'Hass', and more precocious (early to fruit) than 'Hass'.

Israel and Spain are also following this approach: the Israeli avocado breeding program that started in the early seventies has recently been revived with a focus on new 'Hass-like' scion cultivars that are more productive and with large fruit size, as well as early and late harvested cultivars (Irihimovitch et al., 2015) . Research in Spain by the Universidad Miguel Hernández Elche, Alicante, has also shifted to scions with reduced canopy volume (Martínez Font et al., 2015).

We report here on trials of domestically available rootstocks for 'Hass' in order to see if any were more or less suitable for high density planting.

Avocado high density rootstock trial

Methodology

This trial was established in two 'phases' due to different supply times for the rootstocks. We planted both phases in the same block of red volcanic soil at the Bundaberg Research Facility on the Central Queensland coast. Rootstocks, scions and training techniques differed between the two phases as a result of lessons learnt from previous research, including results of the Avocado Planting Systems Trial. In both phases, we planted trees at 2 m spacing in rows 4.5 m apart, the same spacing as the high-density treatment in the Avocado Planting Systems Trial, and trellising for both phases was a single-plane trellis with wires at 50 cm intervals to 3 m.

Phase one planting (May 2016)

Material

We planted 'Hass' on the following rootstocks:

- 'Zutano' ('179') (from California, Mexican x Guatemalan hybrid
- 'BW2' (Australia, believed to be West Indian x Guatemalan hybrid) (seedling) (BW refers to 'Birdwood')
- 'BW181' (Australia, believed to be West Indian x Guatemalan hybrid) (seedling)

- ‘Ashdot’ (Israel, West Indian) (seedling)
- ‘Dusa (Merensky 2 cv.)’ (from South Africa, unknown origin) (clonal)
- ‘Latas (Merensky 1 cv.)’ (from South Africa, Mexican race) (clonal)
- ‘Bounty’ (clonal)
- ‘Velvick’ (from Australia, believed to be either West Indian or a West Indian x Guatemalan race hybrid) (seedling)
- ‘Reed’ (130) (from California, Guatemalan race) (seedling)

Note that the ‘Hass’ on ‘Bounty’ plants that were supplied to us showed two distinct phenotypes in early growth: one indistinguishable from ‘Hass’ on other rootstocks, and the other with slow growth, sparse foliage and narrow greyish leaves. The latter phenotype also flowered at different times and had intermittent blooms in 2017/18. We hypothesise that a treatment in the nursery, possibly as a heavy application of plant growth regulators, may have caused these anomalies. In addition, one individual had clearly not been grafted with ‘Hass’ and growth was solely from the ‘Bounty’ rootstock: this individual was excluded from analyses. In short, while data from 11 ‘Bounty’ replicates are included in the analyses below, we have little confidence that the ‘Bounty’ results are typical of this rootstock.

Pruning and training

We planted trees as single tree plots in 12 replicates of the nine rootstock treatments in four rows, with guard trees at the end of each row and a guard row on each side. Trees were pruned to a three-dimensional central leader shaping, with the leader and some branches attached to the wire. We lightly pruned trees to maintain this shape, approximately monthly once growth commenced, until they reached ~ 2m. By winter 2018, most trees were filling their allotted space and we undertook a major pruning. This was followed by a less rigorous pruning in late spring (early September) to maintain height at 3.5m and remove vigorous vertical water shoots. In addition, there was a light pruning in late summer to keep alleyways free.

Our pruning objectives were informed by lessons learnt from the poor branching complexity and branch death in the Avocado Planting Systems Trial. We aimed to develop trees with more complex structure and stronger branches. Table 1 shows branch numbers in April 2019 at metre intervals on the trunks (mean of five replicates per rootstock in each trial) for the two trial systems for the two rootstocks that these trials have in common, ‘Ashdot’ and ‘Velvick’. This demonstrates we were successful in improving branch structure at that date, although it is likely there will be some branch death as the High Density Rootstock Trial trees age. Note that in both trials there was little difference between the two rootstocks in branch distribution.

Table 1 Mean number of second order branches by height of trunk in two trials April 2019

Height of branching from trunk (0=ground)	Avocado Planting Systems Trial high density treatment			Avocado High Density Rootstock Trial Phase 1		
	Ashdot	Velvick	Total	Ashdot	Velvick	Total
0-100 cm	1.4*	2.8*	2.1	5.0	3.8	4.4
100-200 cm	4.0	6.4	5.2	8.2	10.0	9.1
200-300 cm	6.2	5.0	5.6	14.0	15.4	14.7
300-400 cm	10.8	10.8	10.8	7.6	10.0	8.8
400-500 cm	16.8	12.4	14.6	na	na	na
Total	36.6	35.8	36.6	34.8	39.2	37

n=5 per rootstock

* Indicates significant differences between rootstocks within the one trial at P≤.05. Other rootstock means within trials were not significantly different.

We used plant growth regulator applications to attempt to reduce both summer and spring flushes (Table 2).

Table 2 Plant growth regulator applications in Avocado High Density Rootstock Trial Phase 1

Date of application	Product name	Active ingredient	Rate
10/8/2017	Sunny	50g/L uniconazole-P	500mL/100L
3/9/2017	"	"	250mL/100L
3/1/2018	"	"	250mL/100L
1/09/2018	AuStar	250g/L paclobutrazol	500mL/100L
25/09/2018	"	"	500mL/100L
30/11/2018	"	"	500mL/100L

Measurements

We recorded canopy and trunk dimensions at the time of harvest each year (May), using the same methods outlined for the Avocado Planting Systems Trial.

At flowering in 2018 (31/8/2018), we recorded terminal type (determinate or indeterminate) and inflorescence intensity (number of secondary axes on the terminal inflorescence) on a stratified random sample of 12 shoots per tree on 12 replicates per treatment. The sample was stratified to six shoots on each side of the trellis wire (~east and ~west). We also visually rated each tree canopy on both sides in a scale from 0 (no vegetative flush) to 10 (strong vegetative flush) on the same date.

Fruit were harvested at maturity (>23% dry matter) in 2018 and 2019 and counted and weighed on a full tree basis. At harvest in 2019, we randomly selected two fruit from the west and two from the east side of the tree on six replicates and used these for dry matter assessment using the same methods outlined for the Avocado Planting Systems Trial.

Analyses

Data were analysed using Genstat (18.2, Copyright 2016, VSN International Ltd) using analysis of variance to separate means. Significantly different means were identified using Fisher's protected least significant difference test at α=0.05, that is, at the 95% confidence level. The convention of indicating 'significantly different' means by use of a differing suffix 'a', 'b', 'c' etc is used throughout this report. In this convention, treatment means followed by the same letter, or no letter at all, are not significantly different from each other at the 95% confidence level. In this report we have reported the 'P' value, that is, the probability that the differences in the treatment means are due to the variation in the sample rather than to treatment differences. Error is reported as estimated standard error of the means or 'ese', that is, an estimate of how far the sample mean of the data is likely to be from the true population mean.

Phase One results to date

Growth

In Phase 1 trees, some of the trees on 'Ashdot' rootstock showed less growth by harvest in 2018 but differences between treatments in canopy size were not significant, partly due to pruning (Table 3).

Table 3 Trunk cross sectional area 2017/18 and 2018/19 in Avocado High Density Rootstock Trial Phase 1

	Canopy volume[1]	Rootstock CSA[2]		Scion CSA		Rootstock/scion CSA	
Treatment	2017/18	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
Grand mean	17.0	72	103	67	100	0.96	1.04
Zutano[3]	17.1	84 a	118 de	65 a	93 b	0.79 a	1.27 e
BW2	15.9	75 ab	106 cd	66 ab	96 bc	0.87 ab	1.10 cd
181	17.2	75 bcd	111 cde	70 bcd	107 cd	0.95 bcd	1.06 cd
Ashdot	15.2	69 cd	102 cd	66 cd	100 bc	0.99 cd	1.01 bc
Dusa	16.6	54 e	79 ab	60 e	91 b	1.13 e	0.88 ab
Latas	17.9	71 de	93 bc	71 de	109 cd	1.02 de	0.86 a
Velvick	18.3	83 bcd	119 de	77 bcd	114 d	0.94 bcd	1.05 cd
Reed	17.3	75 abc	126 e	67 abc	109 cd	0.89 abc	1.15 de
Bounty[4]	17.3	59 de	76 a	59 de	77 a	1.03 de	1.02 cd
P	*0.203*	*<.001*	*<.001*	*0.006*	*<.001*	*<.001*	*<.001*
ese	*0.8*	*4*	*6*	*3*	*5*	*0.04*	0.05

[1] As we pruned all trees to the same dimensions in winter 2018, canopy volume is not shown for 2018/19

[2] Where graft union is below ground, rootstock CSA is assumed to equal scion CSA

[3] One replant was excluded from analysis in 2017/18 but due to rapid growth was included in 2018/19 analysis.

[4] See note above in 'Methodology: Material' re 'Bounty' results.

Pruning intensity in terms of numbers of pruning cuts ≥10mm diameter per tree and the total CSA of these cuts shows pruning was less necessary for 'Ashdot' and most necessary for 'Velvick'(Table 4).

Table 4 Pruning intensity by treatment in Avocado High Density Rootstock Trial Phase 1 2017/18 and 2018/19

Treatment	No. of cuts/tree ≥10mm diameter		Total CSA of pruned branches ≥10mm diameter (cm²)	
	2017/18	2018/19	2017/18	2018/19
Grand mean	18.2	15.6	39.1	32.7
Zutano	18.5 bc	16.8 bc	37.8 abc	37.4 bc
BW2	21.6 c	16.3 bc	47.0 c	30.2 b
181	19.7 bc	17.1 bc	46.8 c	34.5 bc
Ashdot	12.8 a	13.9 ab	30.0 a	29.5 ab
Dusa	18.7 bc	15.7 bc	39 abc	33.0 bc
Latas	19.6 bc	18.1 bc	44.1 bc	37.7 bc
Velvick	20.7 bc	19.0 c	49.5 c	44.4 c
Reed	15.5 ab	14.6 bc	32.3 ab	29.6 ab
Bounty[1]	11.9 a	9.1 a	25.5 a	18.4 a
P	*0.006*	*0.011*	*0.005*	*0.004*
ese	*2.0*	*1.8*	*4.9*	*4.1*

[1] See note above in 'Methodology: Material' re 'Bounty' results

Flowering and flush patterns

As in the Avocado Planting Systems Trial, the 'Ashdot' trees in the Phase 1 planting showed a significantly higher proportion of determinate flowering than all other rootstocks in 2018/19 (Table 5). Flowering intensity was also comparatively high and spring vegetative growth low.

Table 5 Avocado High Density Rootstock Trial Phase 1: Spring flowering and flush observations 2018/19

Treatment	% of terminals determinate	Mean no. of 2ndary axes on terminal inflorescence	Spring vegetative growth rating (mean of east and west canopies
Grand mean	13%	6.3	2.9
Zutano[1]	10% ab	**5.5 ab**	**3.3 b**
BW2	18% b	**6.1 abc**	**2.3 ab**
181	9% ab	**6.2 abc**	**3.1 b**
Ashdot	30% c	**7.3 d**	**2.4 ab**
Dusa	11% ab	**6.7 cd**	**2.7 ab**
Latas	13% ab	**6.4 bcd**	**3 b**
Velvick	9% ab	**5.4 a**	**3.3 b**
Reed	15% b	**6.5 cd**	**1.8 a**
Bounty[2]	3% a	**6.3 bc**	**4.5 c**
P	*<.001*	*0.003*	*<.001*
ese	*4%*	0.3	0.4

[1] Includes one replant

[2] See note above in 'Methodology: Material' re 'Bounty' results. This data excludes one replicate in which the 'Hass' graft was not attempted or unsuccessful.

Yield and fruit quality

To date we have harvested two crops from the Phase 1 planting (2018, 2019). Yield did not differ significantly between rootstock treatments in the first year (2017/18), a result not unexpected from young trees. In the second year, 2018/19, 'Ashdot' significantly outperformed all other rootstocks (P<0.001) (Table 6). Tree to tree variation in yield was low for 'Ashdot' and 'Dusa' and very high for 'Reed'.

Table 6 Avocado High Density Rootstock Trial Phase 1: Yield 2017/18 and 2018/19

	Mean yield per tree (kg)		Yield per tree variation (cv)		Mean yield per ha (tonnes)	
Treatment	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
Grand mean	3.51	6.72			3.9	7.5
Zutano[1]	3.50	6.21 ab	83%	82%	3.9	6.9 ab
BW2	3.15	8.23 b	84%	41%	3.5	9.1 b
181	3.10	6.24 ab	61%	57%	3.4	6.9 ab
Ashdot	4.43	13.30 c	49%	37%	4.9	14.8 c
Dusa	4.72	4.44 a	48%	68%	5.2	4.9 a
Latas	3.57	5.90 ab	57%	70%	4.0	6.6 ab
Velvick	4.29	5.08 a	73%	68%	4.8	5.6 a
Reed	2.73	4.54 a	115%	59%	3.0	5.0 a
Bounty[2]	2.08	6.53 ab	62%	44%	0.2	7.3 ab
P	*0.095*	*<.001*			*0.095*	*<.001*
ese	*0.65*	*0.94*			*0.7*	*1.0*

[1] One replant was excluded from analysis in 2017/18 but due to rapid growth was included in 2018/19 analysis.

[2] See note above in 'Methodology: Material' re 'Bounty' results

Fruit size and dry matter percentage at harvest did not differ between treatments with the exception of '181' and 'Bounty' (see note on 'Bounty' in Methodology: Materials above) (Table 7).

Table 7 Avocado High Density Rootstock Trial Fruit Phase 1: Fruit size 2017/18 and 2018/19 and dry matter percentage 2018/19

	Mean fruit weight (g)		Dry matter percentage
Treatment	2017/18	2018/19	2018/19
Grand mean	286	261	23.3%
Zutano[1]	284	266 b	23.1%
BW2	272	258 ab	23.1%
181	283	243 a	23.6%
Ashdot	305	266 b	24.0%
Dusa	293	268 b	23.0%
Latas	282	267 b	23.0%
Velvick	278	267 b	23.2%
Reed	300	267 b	23.0%
Bounty[2]	283	244 a	23.9%
P	*0.128*	*0.006*	*0.597*
ese	*8.2*	5.9	0.046%

[1]One replant was excluded from analysis in 2017/18 but due to rapid growth was included in 2018/19 analysis.

[2]See note above in 'Methodology: Material' re 'Bounty' results

Phase Two Planting (January 2018)

In the second phase of planting, we included the following rootstock treatments:

- Hass/A10 (clonal)
- Hass/Reed (clonal)
- Hass/Velvick (seedling)
- Hass/Velvick (clonal)
- Hass/Dusa (clonal)
- Hass/Zutano (clonal)

Note the comparison of clonal and seedling rootstocks on 'Velvick'.

We also included as scions 'Gem'® and 'Maluma Hass'. 'Gem' scions were on 'Zutano' seedling rootstocks and 'Maluma' scions on 'Velvick' seedling rootstocks.

In this phase, we imposed two training treatments in a factorial design. The first factor was 'training system' with two treatments, and the second factor was 'rootstock and/or scion' treatments.

The two training treatments were a three- dimensional (3-D) central leader system, as used in Phase 1 and in the Avocado Planting Systems Trial, and a two-dimensional (2-D) system in which second order branches (those from the trunk) are espaliered from a central trunk along the wires.

The inclusion of a 2D treatment in this phase of the trial was in response to the emerging poor results from the 3-D system in the Avocado Planting Systems Trial, as well as significant interest from industry in trialling 2-D systems. We hypothesize that 2-D systems have the potential to improve yields compared to the central leader 3-D shaping because of improved light distribution through the whole canopy as well as better ratio of canopy: feeder root area. In addition, exposing horizontal branches along the wire should encourage renewal of short branches for fruiting after pruning as light levels there can be maintained at a higher level than the inside of a 3D canopy (see discussion in the Avocado Planting Systems Trial chapter).

We removed flowers from the Phase 2 trees in spring 2018 as the trees were not fully established, so there was no crop from these trees in 2019. Therefore, at the time of reporting we have no yield results for this trial.

Discussion and recommendations

In the Phase 1 trial in 2018/19, trees on 'Ashdot' rootstocks yielded 14.8 tonnes/ha compared to 4.9 to 7.3 tonnes/ha for trees on other rootstocks. 'Ashdot' was also one of the more consistent performers, that is, yields from tree to tree were less variable, with a coefficient of variation of 37% compared to 'Velvick' at 68% and 'Zutano' at 82%.

As in the Avocado Planting Systems Trial, the trees on 'Ashdot' rootstocks in the Phase 1 planting showed a significantly higher proportion of determinate flowering than all other rootstocks in 2018/19 (Table 5). Trees on 'Ashdot' rootstocks tended to have a higher flowering intensity and less spring vegetative growth than other rootstocks (Table 5) but the differences in these characteristics are not as extreme as the differences in determinate flowering pattern.

We discussed the possible factors behind this higher productivity in the report on the Avocado Planting Systems Trial. Our hypothesis that less root growth leads to more determinate flowering and better fruit set is not, however, supported by our measurements of rootstock and scion CSAs in this trial (Table 3). The ratio of rootstock to scion CSAs for 'Ashdot' was <1 in 2017/18 but not in 2018/19, and several other rootstocks had lower ratios in that year.

Overgrowth of the scion above the graft union has been associated with dwarfing (Olmstead et al., 2010 [sweet cherry]) but more often is considered a sign of 'incompatibility' (Ben-Ya'acov and Michelson, 1995; Whiley, 1994). There seems to be little to suggest that rootstocks that habitually foster overgrowth of scions in avocado display any reduction in vigour. Mickelbart et al. (2007) report differences in overgrowth of 'Hass' on a range of rootstocks but no effect on canopy volume. Similarly, other reported tendencies of specific avocado rootstocks to overgrowth of the scion were not associated with a reduction in vigour (Whiley and Anderson, 2002). Of interest in this context is Whiley's finding (1994) that there was more overgrowth for 'Hass' on cloned 'Velvick' rootstocks than on seedling 'Velvick' rootstocks, along with higher scion starch concentrations, with a higher percentage of determinate inflorescences (which generally have higher fruit set), but not with reduced vegetative vigour. There is some anecdotal reporting that rootstock overgrowth is associated with biennial bearing in avocado (e.g. Wolstenholme, 2009), which might conceivably be linked to vigour, but there does not appear to be any published data to support this.

Overall, the average yield for this phase of the trial from all rootstocks was 7.5 tonnes/ha in 2018/19. These yields (except for 'Ashdot') show little improvement on the results in the Avocado Planting Systems Trial where the 'Velvick' trees yielded 6.9 tonnes/ha in their second year after planting (2016/17). In terms of our objective to increase yields by a better branch structure and more rigorous pruning to maintain shape, this is a disappointing result considering the trees are already at the maximum size and height for our planned regime.

In the case of 'Ashdot', the high density trees in the Phase 1 trial yielded 14.8 tonnes/ha compared to 8.9 tonnes/ha in the Avocado Planting Systems Trial in their second year. Whether this significant increase to yields for this rootstock is due to our changed pruning and training regime and not simply to different seasonal conditions remains to be seen in future years. However, the lack of improvement to 'Velvick' yields from this regime compared to the Avocado Planting Systems Trial leads us to suspect that the 'Ashdot' gain may be at least partly because these trees required less pruning in 2017/18 and 2018/19 (see Table 4) and therefore did not suffer the same adverse effects of pruning as other more vigorous rootstocks.

While it is too early for a final judgment, it is no surprise that the rootstocks we have tested in this trial have not delivered vigour control or productivity gains in a high density environment. They have been available to Australian growers for several years without any reports of vigour-controlling tendencies. To date, 'Ashdot' appears the best of the trialled rootstocks for plantings of 'Hass' in a high density system. It should be noted that 'Ashdot' is considered to be prone to biennial bearing, sensitive to stress and requiring of careful management (Le Lagadec, 2010).

Our experience to date suggests that a vigour-controlling rootstock is paramount in increasing avocado yields from high density plantings in the high-vigour climates of Australia. Slow progress to a dwarfing rootstock for avocado internationally appears to be due in part to the limited range in existing genotypes as well as a lack of understanding of the physiological mechanisms that underlie dwarfing potential. In these circumstances, the focus of research may best be in developing and maintaining international collaborative approaches rather than investment in a breeding program.

As discussed in the report on the Avocado Planting Systems Trial, future high density avocado research should include exploration of the use of compact scions. Research should include assessment of relative yield efficiency in Australian conditions compared to the existing cultivars, and differences in fruit quality, consumer acceptance, pest management and post-harvest management.

The relatively better performance from 'Ashdot' rootstocks in our trials to date appears to be due not only to reduced vegetative growth but also to a determinate flowering pattern. This characteristic, whether it has a genetic and/or environmental base, would be worth further examination in breeding or genotyping research.

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Glossary and abbreviations

biennial bearing	tendency for a tree to produce a greater than average crop one year, and a lower than average crop the following year.
CSA -	cross sectional area
cv -	coefficient of variation. The standard deviation of the sample mean, divided by the mean. This provides a standardised indication of variability when comparing samples with different means.
Determinate -	inflorescences that do not produce a vegetative growth unit from their apex. Compare *indeterminate*
ese -	estimated standard error of the mean, that is, an estimate of how far the sample mean of the data is likely to be from the true population mean.
Indeterminate -	inflorescences that produce a vegetative growth unit from their apex. Compare *determinate*
P or *P* value -	the probability that the differences in the treatment means are due to the variation in the sample rather than to treatment differences. A P of 0.05 or less is

used as the standard for declaring that treatment means are significantly different.

Proleptic - growth of the first growth unit of a branch in a later flush than the parent growth unit. Compare *sylleptic*

Sylleptic - growth of the first growth unit of a branch in the same flush as the parent growth unit. Compare *proleptic*

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Appendix 4
Understanding the bases of early orchard productivity in macadamia: the effect of tree density, tree training and cultivar on orchard development and yield

Wilkie JD, Griffin J, Hofman H, Parfitt SC, Toegel H, Toft B, Wright C

Summary

Macadamia orchards are traditionally planted in low density, large tree orchards (c.a. 7 m x 4 m to 10 m x 5 m) because of their vegetative vigour. The trees are large at maturity and the crowding that occurs as orchards age leads to declining yields. In addition, the low planting density results in long delays until the orchards produce their maximum yields.

A number of temperate tree crop industries, such as apples, have successfully adopted high density planting. The work here reports on the early orchard life of a macadamia Planting Systems Trial where a conventional low tree density is compared with more intensive tree densities for two commonly planted scion cultivars. Central leader tree training systems are also compared with conventional tree training techniques. The purpose was to test the hypothesis that increased tree density leads to higher early yield and tree training affects early tree development and precocity.

Increased tree density led to increased canopy volume/ha, total light interception and yield/ha in both cultivars. ‘A203’ was more precocious than ‘741’, due to earlier and more intense flowering and continued to have greater yield than ‘741’ through this entire early orchard phase of the trial. The effects of tree training were less pronounced than that for cultivar and tree density, with examples of increased canopy development in central leader trained trees but intermittent effects on raceme density and yield efficiency.

Early yield/ha of macadamia is limited by total light interception and raceme density. Increased tree density leads to greater canopy volume/ha and consequently greater total light interception, which provides a greater yield potential during early orchard life. The high raceme density of the precocious cultivar ‘A203’ was able to convert the greater yield potential of the high density systems into greater yield/ha.

Introduction

Macadamia (*Macadamia integrifolia*, *M. tetraphylla* and M. *integrifolia* x *tetraphylla*) is an evergreen, recurrent flushing tree crop native to eastern Australia and is cultivated in subtropical locations for its edible kernel. Macadamia was domesticated relatively recently and has had relatively little selection or breeding from wild populations to the currently cultivated genotypes. Macadamia production systems are also relatively poorly researched, particularly when compared with some temperate tree crops. Macadamia is thus an underdeveloped tree crop.

Macadamia trees grow vigorously in orchard environments and are large at maturity. As a consequence, macadamia orchards tend to be planted at relatively low tree densities with common commercial tree densities ranging from 200 trees/ha (10 m x 5 m row and tree spacing, respectively) to 357 trees/ha (7 m x 4 m). At these conventional tree densities, the time to maximum yield/ha has been reported to range from 11 to 12 years (Stephenson *et al.*, 2011) and greater than 15 years (Mayer *et al*., 2006).

Tree crop orchard systems are characterised by little or no yield for some years after planting, depending on the crop. This is followed by a strong increase in yield/ha until it tapers before the maximum is reached and then plateaus. The delay between planting and maximum yield/ha is partly due to changing total light interception as orchards develop (Tustin *et al*., 2001; Robinson *et al*., 1991a). For some crops it can also be partly due to poor flowering or fruit set in young trees. As the orchard develops in the years following planting, leaf area and canopy volume/ha increase.

Total light interception refers to the percentage of photosynthetically active radiation (PAR) falling on an area of orchard that is absorbed by the canopy as opposed to falling on the ground between the trees or being reflecting off the tree canopies. Total light interception is related curvilinearly to leaf area and canopy volume, with strong increases in total light interception as canopy volume/ha increases from low levels, with smaller increases as orchard canopies begin to fill their allotted space and crowd (Wilkie *et al*., 2018; McFadyen *et al*., 2004; Wagenmakers and Callesen 1995).

Total light interception is well known to be a strong driver of yield in crops in general (Monteith, 1977) and also of tree crops (Robinson and Lakso, 1991, Wunsche *et al*., 1996). For the well-studied temperate tree crop apple, yield/ha increases linearly with total light interception, up to approximately 60% to 70%, with some reports of a subsequent decline in yield/ha as total light interception increases above 85% (Wunsche *et al*., 1996). For conventional macadamia orchards, yield/ha increases with canopy volume and total light interception up to approximately 30 000 m^3 to 40 000 m^3 canopy volume/ha and 85% to 95% total light interception, and then declines as the canopy crowds further (McFadyen *et al*., 2004; Olesen *et al*., 2007). Thus, the time to reach maximum yield/ha after planting is at least partly related to the time to reach optimum levels of total light interception.

The manner in which light is distributed through the canopy is also an important determinant of fruit yield and quality. For apple, low light levels within the canopy lead to decreased floral initiation (Cain, 1971), reduced fruit set, lower rates of fruit growth (Byers *et al*., 1990; Bepete and Lakso 1998), poor fruit colour (Barritt *et al*., 1997) and reduced allocation of assimilates to fruit. In general, apple is a shade sensitive crop. Part of the reason for shade sensitivity is the requirement for high within canopy light levels for fruit quality. However, the tendency for apple fruit to source assimilates from photosynthesis locally is also likely to contribute. Generally, as total light interception increases, within canopy shading increases, so the relatively low reported total light interception optimum for apple of 60% to 70%, and 85% for the commencement of yield decline, are likely due to the sensitivity of processes related to the development of crop load.

Within canopy light distribution is also important for macadamia, although there are indications that macadamia is more shade tolerant than apple. Leaf density, shoot growth and fruit set decline as within canopy light levels decrease and flower raceme density is reduced in the most heavily shaded portions of the canopy (Olesen *et al*., 2011). There are also anecdotal reports of the formation of voids in the most shaded portions of crowded macadamia orchards in which no growth occurs at all (Huett, 2004). Even so, macadamia appears to be relatively shade tolerant, with the high reported total light interception of 85% to 95% for maximum yield, and declining yields above these levels of light interception (McFadyen *et al*., 2004; Olesen *et al*., 2007).

For a range of tree crops, low yields during early orchard life can also be due to limited flowering. In mango, early tree training increased early flowering and yield by increasing the density of terminal shoots that subsequently flowered (Oosthuyse *et al*., 1995). For pear, heading the primary axis of trees following grafting resulted in increased branching from the resulting leader in the second year after grafting and increased flowering the third year after grafting due to an increased number of suitable shoots for flowering and a greater proportion of buds becoming floral (Seleznyova *et al*., 2013). For apple, early yield can also be affected by early flowering (Webster 1995). Dwarfing apple rootstocks such as 'M.9' can induce greater floral densities in the scion (van Hooijdonk *et al*., 2009) but vigorous rootstocks such as 'MM.106' induce greater canopy growth, and so early yield per tree may not be significantly affected. Early tree training and tree manipulations such as limb bending also affect flowering in apple (Robbie *et al*., 1993). Early yield in macadamia is also limited by the extent of early flowering (McFadyen *et al*., 2016). The first flowering of macadamia in northern NSW for cultivar '246' and '816' grafted on to seedling rootstocks was three and four years after planting, respectively (McFadyen *et al*., 2016). Excessive tree training and pruning in the years after planting can reduce early flowering and yield (McFadyen *et al*., 2016; Olesen *et al*., 2011). There may be opportunities to improve early flowering and yield through architectural manipulations in macadamia.

The fruit production of various orchard planting systems are the result of an interaction of a number of variables. Some of these variables, such as row and tree spacing, as well as rootstock and scion cultivar, are set when the orchard is established. Other variables, such as canopy management strategies (tree training and pruning) and crop load management, may be altered, at least to some extent, throughout the life of the orchard.

Over the latter part of the 20th century, apple orchard planting systems underwent radical transformations from low density, large tree systems planted on vigorous rootstocks, to high density, small tree systems planted on dwarfing rootstocks (Tustin *et al*., 2001; Robinson and Lakso, 1991). For apple, high density orchard systems provide a range of benefits. One of the primary benefits is that higher tree densities lead to greater total light interception and thus higher yield during early orchard life. Another benefit is that the narrower row spacing and consequently narrower canopies lead to a more even distribution of light throughout the orchard (Wagenmakers and Callesen, 1995). The combination of narrow row spacing and canopies can allow high levels of light interception while maintaining low levels of within canopy shading, consequently resulting in high yields of high quality fruit (Palmer *et al*., 1992). The dwarfing rootstocks used in intensive apple orchard plantings lead to greater partitioning of assimilates to fruit production and minimise vegetative growth, the requirement of pruning and the propensity for orchard crowding.

There are two main purposes to this work. First, to determine if intensive planting systems can be used to increase early yield in macadamia and to this end we compare a range of plant densities, pruning systems and scion cultivars. Second, improve the understanding of the underlying physiological drivers of early orchard productivity in macadamia.

Materials and Methods

Site, preparation and agronomy

The trial was undertaken at the Bundaberg Research Facility, Bundaberg, QLD, Australia (24°51'00.6''S, 152°24'01.2"E, elevation 27 m) on a well-drained red ferrosol soil. The land has a long history of cultivation, much of it being sugar cane cropping. The final sugar cane crop was harvested in spring 2013, the soil was ripped and rotary hoed and then beds for the tree rows constructed that were approximately 100 mm high. Two experiments were planted side-by-side in the plot in late

January 2014 with all rows oriented north. The trees were purchased from a commercial macadamia nursery and were standard two-year-old grafted macadamia trees consisting of a scion cultivar grafted on to seedling ‘H2’ rootstock.

The trial was irrigated by a micro sprinkler system that allowed different volumes of water to be applied to each plant density treatment based on soil moisture status. Fertiliser requirements were based on soil and leaf analyses and industry best practice recommended by a trial advisory group of leading growers and consultants. In the first and second years after planting, each tree received 50 g and 75 g of nitrogen (N), respectively. Thereafter, the N applications increased by 50 g/tree/year until a maximum rate of 100 to 120 kg N/ha was reached. This meant that the treatments with greater tree density reached their maximum rate of N/ha earlier than treatments with lower tree densities.

Pest and disease activity was monitored regularly and pesticides applied when threshold levels of damage were observed. Prophylactic sprays for macadamia husk spot disease were applied twice during crop development in spring 2018.

Experiment 1: main trial

The main trial consisted of three treatment factors: tree density, tree pruning system and cultivar. There were three levels of tree density, including an industry standard low density (LD, 8 m x 4 m, 312.5 trees/ha), medium density (MD, 6 m x 3 m, 556.5 trees/ha) and high density (HD, 5 m x 2 m, 1000 trees/ha). For the LD, there was only one level of pruning treatment; the industry standard pruning (CONV) treatment. For the MD and HD, there were two levels of pruning treatment; the CONV pruning treatment and a central leader pruning (CL) treatment. For all tree density and pruning treatment combinations there were two cultivars: ‘Hidden Valley A203’ (A203) and ‘HAES 741’ (741).

The treatments described above were laid out using a split-split-plot design, with tree density at the main plot level, pruning treatment at the sub-plot level and cultivar at the sub-sub-plot level. The main plots (tree density plots) consisted of four rows of trees with the rows either five trees in length for the industry standard low density or 10 trees in length for the medium and high densities to accommodate the two tree training systems treatments. Within each tree density main plot, the two outer rows and end trees were buffers for the three tree data collection plots for each treatment combination. Each of the ten treatment combinations was replicated five times.

At planting, the shoots emerging from the grafted scion were thinned back to two vertical shoots per tree, which were decapitated 20 cm above the graft union. Two months after planting, once the trees were established, the weaker of the two vertical shoots was removed. Pruning treatment for CONV trees consisted of simulated horizontal and sometimes vertical hedging of the trees five times between planting and winter 2016 and then annually each winter up until and including 2018, except for ‘A203’, which was not pruned in winter 2018. The simulated hedging consisted of decapitating all shoots at a set height (horizontally) or at a set distance out from the tree row (vertically), often removing 20 cm to 30 cm of canopy. The MD CL tree training was similar to the traditionally recommended macadamia tree training (O’Hare *et al.* 2004), with vertically growing shoots that were competing with the central leader removed or decapitated to inhibit competition with the central leader. The central leader was decapitated 30 cm above the previous lateral shoot if there had been no branching. The HD CL tree training involved bending and tying of all lateral limbs emerging from the central leader so that they were oriented at or below the horizontal. Vigorous vertical shoots emerging from the bent lateral limbs were removed. Similar to the MD CL treatment, the central leader was decapitated if it had not branched within 30 cm of the previous lateral shoot.

Experiment 2: extra high density trellised system

The extra high density trellised system (HD-T) was planted at 4.5 m x 1.5 m (1481 trees/ha,) adjacent to the main trial. The trees were trained to a vertical trellis with six wires: the first wire 60 cm above the ground and the remaining wires 50 cm apart. At planting, the scion was thinned to the strongest central leader. Unlike the main trial, the trees were not decapitated at planting. The central leader of all trees was tied to the trellis wires as it grew.

From planting until harvest 2018, this trial tested the effect of cultivar and limb bending on early orchard development and productivity. The trial was laid out as a split plot, with each of the four replicates (data collection tree rows) split into two main plots, which were allocated to one of two cultivars: 'A203' or '741'. Each of these main plots was split into two two-tree sub-plots, which were allocated to one of two levels of bending treatment: an unbent control (unbent) and a limb bending (bent) treatment. For the unbent treatment, limbs emerging arising from the central leader were allowed to grow naturally until they extended more than 1.5 m in the inter-row, at which point they were shortened back to a side branch. For the bent treatment, lateral limbs emerging from the central leader that were oriented vertically were bent and tied down to at or below the horizontal. Following the harvest in 2018, the early orchard limb bending treatment in the trial ceased.

In 2014, 2015 and up until winter 2016, the pruning was undertaken two to three times per year and then again once in 2017. Tree height was pruned back to 4.2 m in winter 2017 and winter 2018 by selectively pruning the shoots back to a side branch.

Data collection

Unless specified otherwise, we applied the following date collection methods to both experiments.

Tree dimensions

Canopy dimensions (canopy height (h), from the bottom of the canopy to the top of the tree; canopy width between the row (x); canopy width along the row (y) up to a maximum of the within row tree spacing) were measured three times per year. Measurements were undertaken in autumn, spring and summer to coincide with crop maturity, flowering and total light interception measurements, respectively. The canopies were assumed to be the shape of an irregular ellipsoid (Charles-Edwards et al., 1986) and canopy volume (V) was calculated using the equation $V = (\pi \cdot h \cdot x \cdot y)/6$. Rootstock circumference was measured 20 cm above ground level at the same time the autumn and spring canopy dimensions were measured and used to calculate rootstock trunk cross-sectional area (RTCA).

Flowering, fruit set and yield

Representative trees from each treatment combination plot were selected for detailed crop load assessments. In 2015/16, 2016/17 and 2017/18 seasons, all measurements were undertaken on the entire tree. The basal diameter of all major limbs was measured at flowering time, in order to calculate limb cross-sectional area and the number of flower racemes on each of these limbs recorded. In February, leading up to fruit maturity and before mature fruit drop commenced, the limb cross-sectional area of these limbs was measured again and the number of fruit per limb recorded. In the 2018/19 season, the trees were too large to count racemes and fruit on the entire tree. The limb cross-sectional area was measured for all limbs, but flowering and fruit set measurements were undertaken on every third limb that was encountered working up from the base of the tree as it was assessed.

Yield was measured for all three data trees in each plot. A pre-harvest clean-up was undertaken by removing any nuts under the trees in early February of each year. One ground harvest was undertaken in March by picking up all nuts underneath the tree, which was followed by a strip harvest in late April, during which all nuts from the trees were removed. Nuts from both harvests were mechanically de-husked and passed over a sieve to remove nut-in-shell (NIS) less than 18 mm in diameter. The whole tree wet NIS yield was measured, then a 50 nuts sub-sample was taken and weighed, then dried in an oven for two days at 35°C, then two days at 45°C and then two days at 55°C. The sub-sample was then re-weighed. The dried sub-sample was assumed to have a moisture content (MC) of 1.5% and relative sub-sample weights before and after drying used to calculate the NIS yield per tree at 10% MC. The 50 nuts sub-samples from the three trees within each plot were combined and used for quality assessments to calculate the percentage of total kernel recovery, the percentage of sound kernel recovery (SKR) and sound kernel/tree.

Light interception and distribution

Total light interception was measured annually for each treatment combination plot during January and February from 2015 to 2019. Measurements were undertaken in clear blue sky conditions using the principles of Wunsche *et al*. (1995). In 2015 in the main trial, and in 2015 and 2016 in the HD-T, the measurements were undertaken using an AccuPar ceptometer (Decagon Devices Inc.). Below canopy ceptometer measurements were taken perpendicular to the tree row and spaced at 1 m intervals in transects along the tree, with transects extending out from the tree row on both sides from the tree row until all shadows from the tree canopies were measured (up to a maximum of the area allocated to the plot). Above canopy ceptometer measurements were taken in full sun conditions immediately before or after the below canopy measurements for each plot.

From 2016 in the main trial and 2017 in the HD-T, we used a purpose-built trolley with individual sensors (Apogee SQ-100 PAR point sensors, Apogee Instruments, Inc.) inserted in a horizontal boom stretching across the inter-row at 50 cm above the ground. The point sensors were connected to a datalogger CR1000 (Campbell Scientific Inc.). Sensors on the boom of the trolley were spaced at 30cm intervals and PAR levels recorded 10 times/second. The trolley was pulled through the trial plots at walking pace along both sides of the data tree rows. PAR measurements were used from sensors positioned near the trunk out into the inter-row so that all shadows from the tree canopy were covered, to a total width equalling the row width. We took an 'above canopy' reading using the light trolley away from the canopy in full sun immediately before or after each plot measurement.

Measurements were made at three times of the day for each plot. In 2015 and 2016 measurements were made +/- 2 hours (sun position mid) and 4 hours (sun position low) before or after solar noon and at solar noon (all times within +/- 20 minutes). In subsequent years, measurements were made at +/- 1.5 and 3 hours before or after solar noon and at solar noon (all times within +/- 20 minutes).

For each measurement, whether from the ceptometer or trolley, percentage light interception was calculated as the proportion of PAR removed between the above canopy measurement and the below canopy measurement. For each measurement time, we averaged the individual light interception (LI) measurements for the entire plot. We calculated total light interception for each plot using the equation:

Total light interception = (LI sun low + LI sun mid + LI solar noon + LI sun mid + LI sun low)/5

In winter 2017, following pruning of the trial, light distribution measurements were undertaken in the main trial and the trellised system on selected treatment combinations. In the main trial, one representative tree per plot of ‘A203’ LD CONV, HD CONV and HD CL treatments, and in the trellised system one tree per plot of ‘A203’ bent and unbent treatments were selected. An aluminium frame shaped as a rectangular prism running horizontally through the tree canopy perpendicular to the tree row surrounding the central leader of the tree, with a cross-section perpendicular to the tree row of 0.5 m x 0.5 m was erected. Ceptometer measurements were taken within the canopy every 50 cm out from the tree row to the east and west at a height of 2 m. Light measurements were made two hours before solar noon, at solar noon and two hours after solar noon, and the within canopy ceptometer measurements compared with an above canopy ceptometer reading to calculate the within canopy percentage light interception. The number of leaves within the 0.125 m^3 area bounded by the transect below, were counted at the time of PAR measurement, and the number of flowering racemes within the same area was counted during spring.

Statistical analyses

Data from the main trial and the trellised system were analysed in asreml-R using linear mixed-models, given their hierarchical experimental designs. The designed treatment factors and their interactions (tree density, tree training and cultivar) as well as the year after planting, were fixed effects and the blocking factors were random effects (replicate, main plot, sub-plot and sub-sub-plot). At times, continuous variables of interest were added as a covariate to the mixed model analyses. When mixed models indicated significance of main effects or interactions, differences between treatments were determined using a 95% least significant difference (LSD).

Results

Tree and canopy characteristics

Canopy volume/tree increased over the five years from planting ($P < 0.001$; Fig. 1). There was a significant interaction between year and tree density ($P < 0.001$), with canopy volume/tree increasing at a similar rate for all densities in the main trial until 2018 when canopy volume/tree of the LD and MD trees was significantly greater than that of the HD trees. In 2019, differences in canopy volume/tree between the densities became greater still, with the LD trees being significantly larger than the MD trees which were in turn significantly larger than the HD trees. There was an indication that the canopy volume/tree for the HD-T trees was greater than those in the main trial up until 2017, after which it appeared they were overtaken in canopy volume by all density treatments in the main trial. RTCA per tree followed similar trends to that of canopy volume per tree over the five years of measurements (Table 1), with significant increases in RTCA over time ($P < 0.001$) and a significant interaction between tree density and year after planting ($P < 0.001$). From 2018 onwards, there were also significant differences in RTCA between tree densities.

Table 1. The effect of tree density and year after planting on rootstock trunk cross-sectional area (RTCA) per tree and RTCA/ha

The interaction between tree density and year after planting was significant for RTCA/tree ($P < 0.001$) and RTCA/ha ($P < 0.001$). For RTCA/tree there were also significant effects of year ($P < 0.001$), cultivar ($P < 0.001$), and the interactions between year and cultivar ($P < 0.001$) and tree density and pruning treatment ($P < 0.001$). For RTCA/ha there were also significant effects of year ($P < 0.01$), cultivar ($P < 0.001$) and tree density ($P < 0.0001$), and a significant interaction between density and pruning treatment ($P < 0.001$).

Density	2015	2016	2017	2018	2019
			RTCA per tree		
LD	2.94	16.1	40.9	78.3	108.2
MD	3.06	17.4	41.7	75.7	101.7
HD	3.23	16.1	37.7	65.4	85.8
LSD	0.26	2.3	3.9	5.2	7.20
			RTCA per hectare		
LD	919	5 046	23 165	24 460	33 806
MD	1 702	9 670	12 785	42 083	56 512
HD	3 233	16 062	37 684	65 382	85 832
LSD	295	1 845	2 493	3 908	5 498

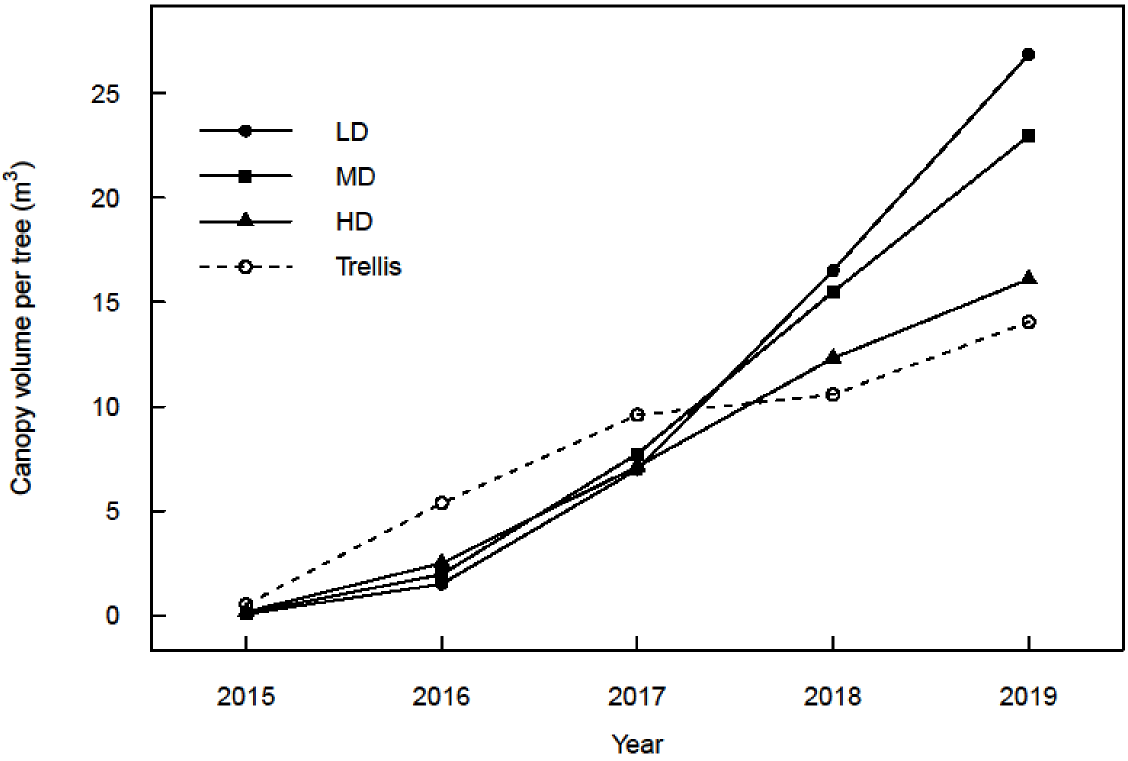

Figure 1. The effect of tree density on canopy volume/tree over time in the main trial and HD-T plot. The interaction between year and tree density ($P < 0.001$) in the main trial and year in the HD-T ($P < 0.001$) are presented. The LSDs to determine significant differences between treatments in the main trial are 0.044, 0.0339, 1.29, 1.76 and 2.38 in 2015, 2016, 2017, 2018 and 2019, respectively. The LSD to determine significant differences between years in the HD-T is 0.75. In the main trial there were also significant effects of year ($P < 0.001$) and cultivar ($P < 0.01$) and significant interactions between year and cultivar ($P < 0.001$), between density and pruning treatment ($P < 0.001$), between year, density and pruning treatment ($P < 0.001$), between year, cultivar and density ($P < 0.001$) and between year, cultivar, density and pruning treatment ($P < 0.05$).

Canopy volume/ha also increased over the five years from planting (*P* < 0.001; Fig. 2). Canopy volume/ha of the HD systems increased faster and was greater in 2019 than the MD systems, which were in turn greater than the LD systems. The canopy volume/ha of the HD-T system also increased over time from planting (*P* < 0.001) and has so far appeared to be greater than that of the systems in the main trial. RTCA/ha (Table 2) followed a similar trend to canopy volume/ha, with significant increases over time from planting (*P* < 0.001) and a significant interaction between year after planting and tree density (*P* < 0.001). The RTCA/tree of the HD systems was greater than that of the MD systems, which were in turn greater than that of the LD systems across all years of the trial.

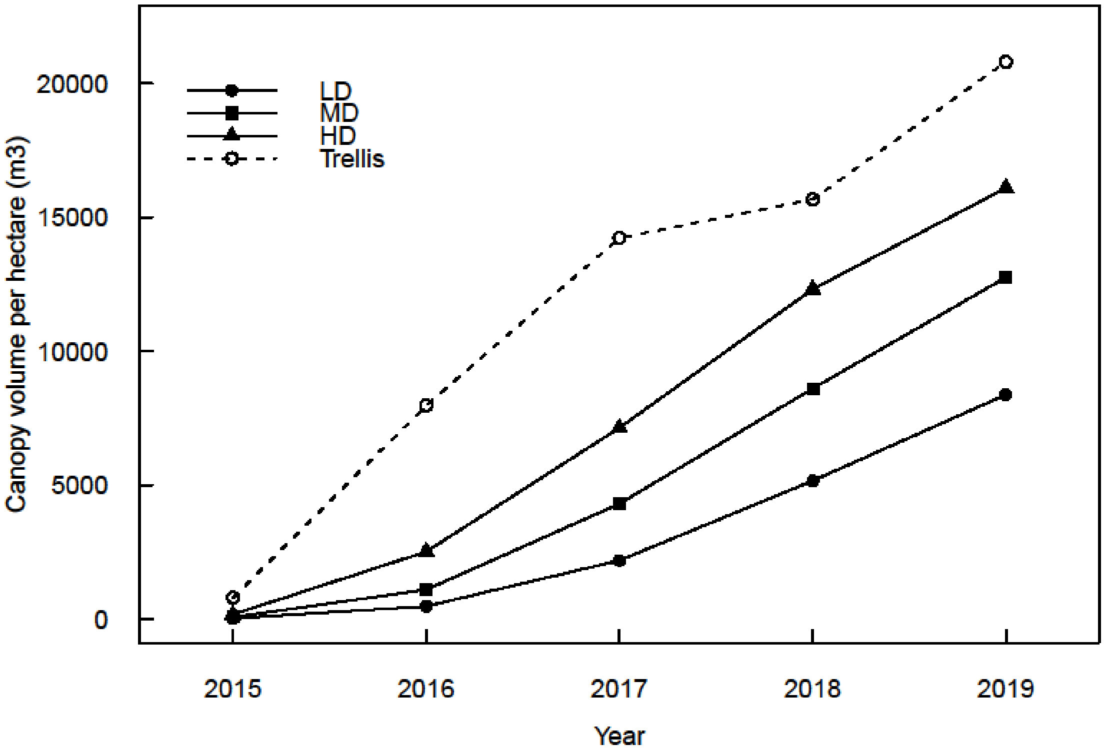

Figure 2. The effect of tree density on canopy volume/ha over time in the main trial and HD-T plot. The interaction between year and tree density (*P* < 0.001) in the main trial and year in the HD-T (*P* < 0.001) are presented. The LSDs to determine significant differences between treatments in the main trial are 43, 249, 853, 1 132 and 1,382 in 2015, 2016, 2017, 2018 and 2019, respectively. The LSD to determine significant differences between years in the HD-T is 1,106. In the main trial there were also significant effects of year (*P* < 0.001) and cultivar (*P* < 0.001) and significant interactions between year and cultivar (*P* < 0.001), between density and pruning treatment (*P* < 0.05), between year and density (*P* < 0.001), between year, density and pruning treatment (*P* < 0.001), between year, cultivar and density (*P* < 0.001) and between year, cultivar, density and pruning treatment (*P* < 0.05).

Light interception and distribution

Total light interception also increased over time (*P* < 0.001; Fig. 3). In the main trial, there was a significant interaction between year after planting and tree density (*P* < 0.001). The HD systems had significantly greater total light interception than the MD systems, which in turn were significantly greater than the LD systems in all years of measurement except in 2015, when there was no significant difference between MD and LD systems. In 2019 the total light interception for LD, MD and HD systems was 29, 49 and 69%, respectively. Total light interception in the HD-T systems also increased significantly with year after planting ($P < 0.001$) and appeared to be greater than the total light interception in the main trial in all years. In 2019, the total light interception in the HD-T system was 81%.

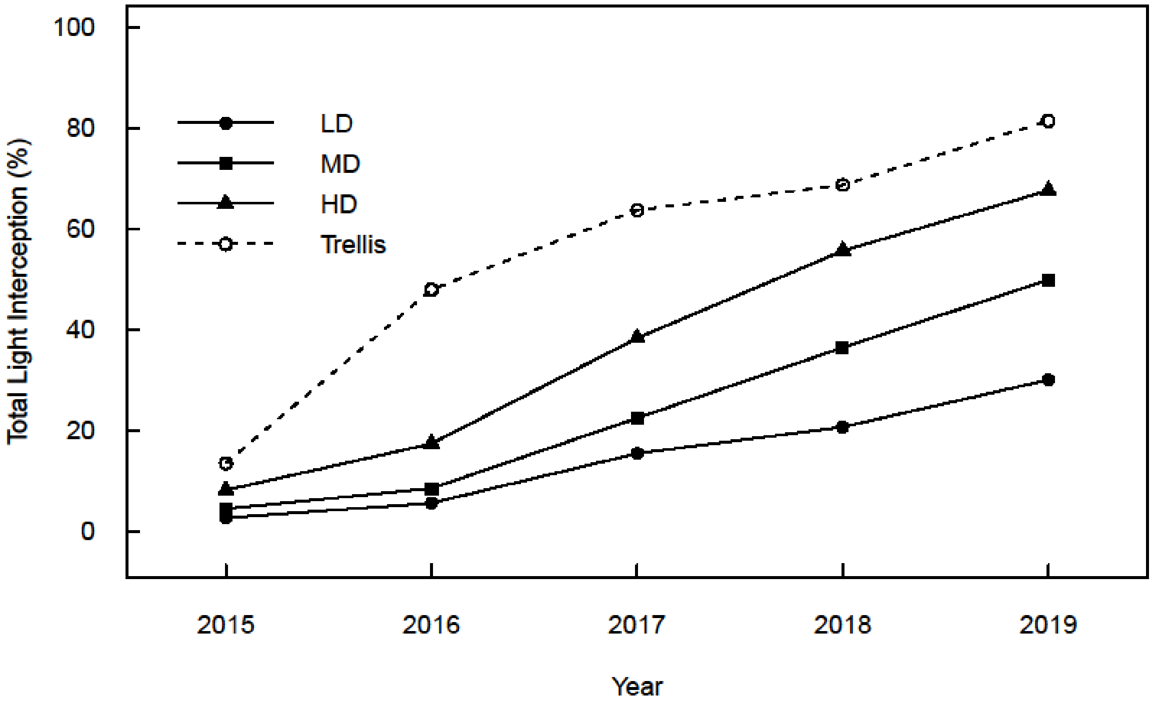

Figure 3. The effect of tree density on total light interception over time in the main trial and HD-T plot. The interaction between year and tree density ($P < 0.001$) in the main trial and year in the HD-T ($P < 0.001$) are presented. The LSDs to determine significant differences between treatments in the main trial are 2.05, 1.92, 3.69, 3.46 and 3.85 in 2015, 2016, 2017, 2018 and 2019, respectively. The LSD to determine significant differences between years in the HD-T is 5.46. In the main trial there were also significant effects of year ($P < 0.001$) and cultivar ($P < 0.001$) and significant interactions between year and cultivar ($P < 0.001$), between density and pruning treatment ($P < 0.05$), between year and density ($P < 0.001$) and between year, density and pruning treatment ($P < 0.05$).

Total light interception increased curvilinearly with canopy volume/hectare ($P < 0.001$; Fig. 4). At the lower levels of canopy volume/ha there were strong increases in total light interception, whereas at the higher levels of canopy volume/ha the rate of increase in total light interception was smaller. The relationship differed with tree density ($P < 0.0001$), with the amount of light intercepted at any given canopy volume per hectare greater for HD systems than MD systems, which were in turn greater than LD systems. For example at 10 000 m^3 canopy volume/ha the LD, MD and HD systems had a total light interception of 34, 41 and 48%, respectively.

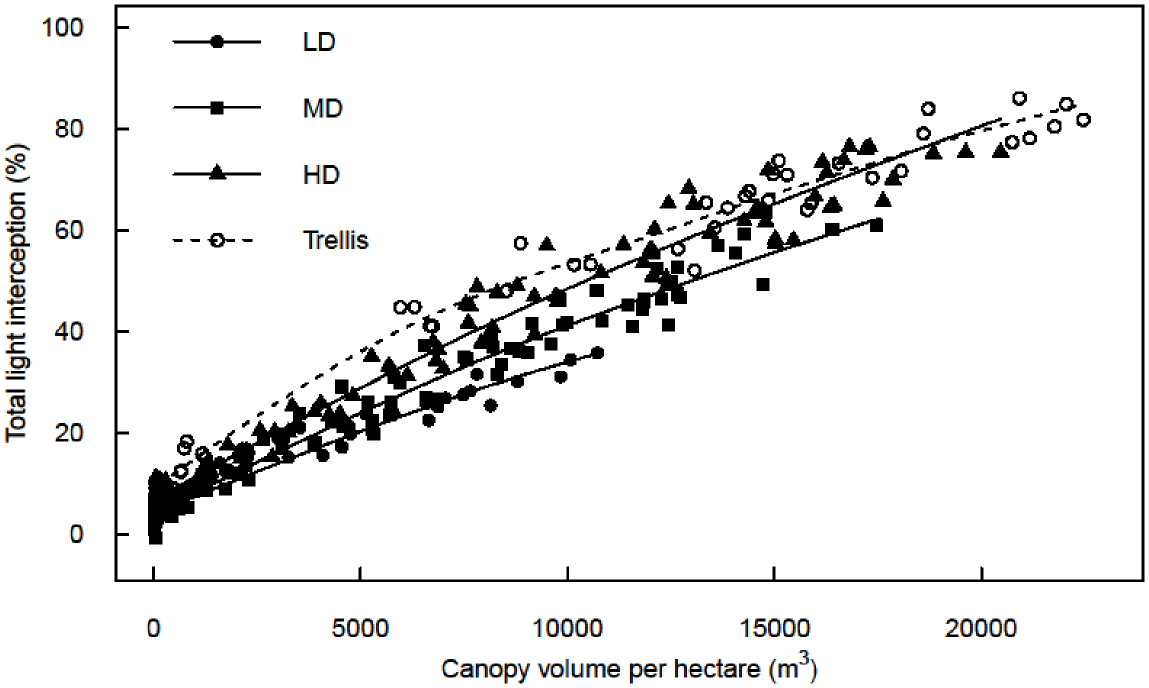

Figure 4. The relationship between canopy volume/ha and total light interception for the three tree density treatments in the main trial and for the one tree density in the trellised plot over five years of annual measurements. The interaction between canopy volume/ha and tree density ($P < 0.001$) in the main trial and the effect of canopy volume/ha in the HD-T ($P < 0.001$) are presented. The LSD to determine significant differences between tree density treatments in the main trial is 2.5. In the main trial there were also significant effects of canopy volume/ha ($P < 0.001$), cultivar ($P < 0.001$) and tree density ($P < 0.001$) and significant interactions between canopy volume/ha and cultivar ($P < 0.001$), between density and pruning treatment ($P < 0.001$), between canopy volume/ha, density and pruning treatment ($P < 0.001$) and between canopy volume/ha, density and pruning treatment ($P < 0.05$). In the HD-T, there was also a significant effect of cultivar ($P < 0.05$) and a significant interaction between canopy volume/ha and cultivar ($P < 0.01$).

The mean proportion of PAR intercepted by the canopy above the within canopy transects was affected by the density and tree training treatment combination at all three measurement times (Fig. 5; morning, $P < 0.05$; noon, $P < 0.05$; afternoon, $P < 0.01$). The statistical ranking for within canopy light availability for the three ‘A203’ treatment combinations was LD CONV and HD CL > HD CONV. There was also a significant effect of distance from the trunk on within canopy light levels for the morning ($P < 0.0001$) and afternoon ($P < 0.0001$) measurement times but not for solar noon ($P > 0.05$), with greater PAR levels reaching the measured transect as distance from the trunk increased.

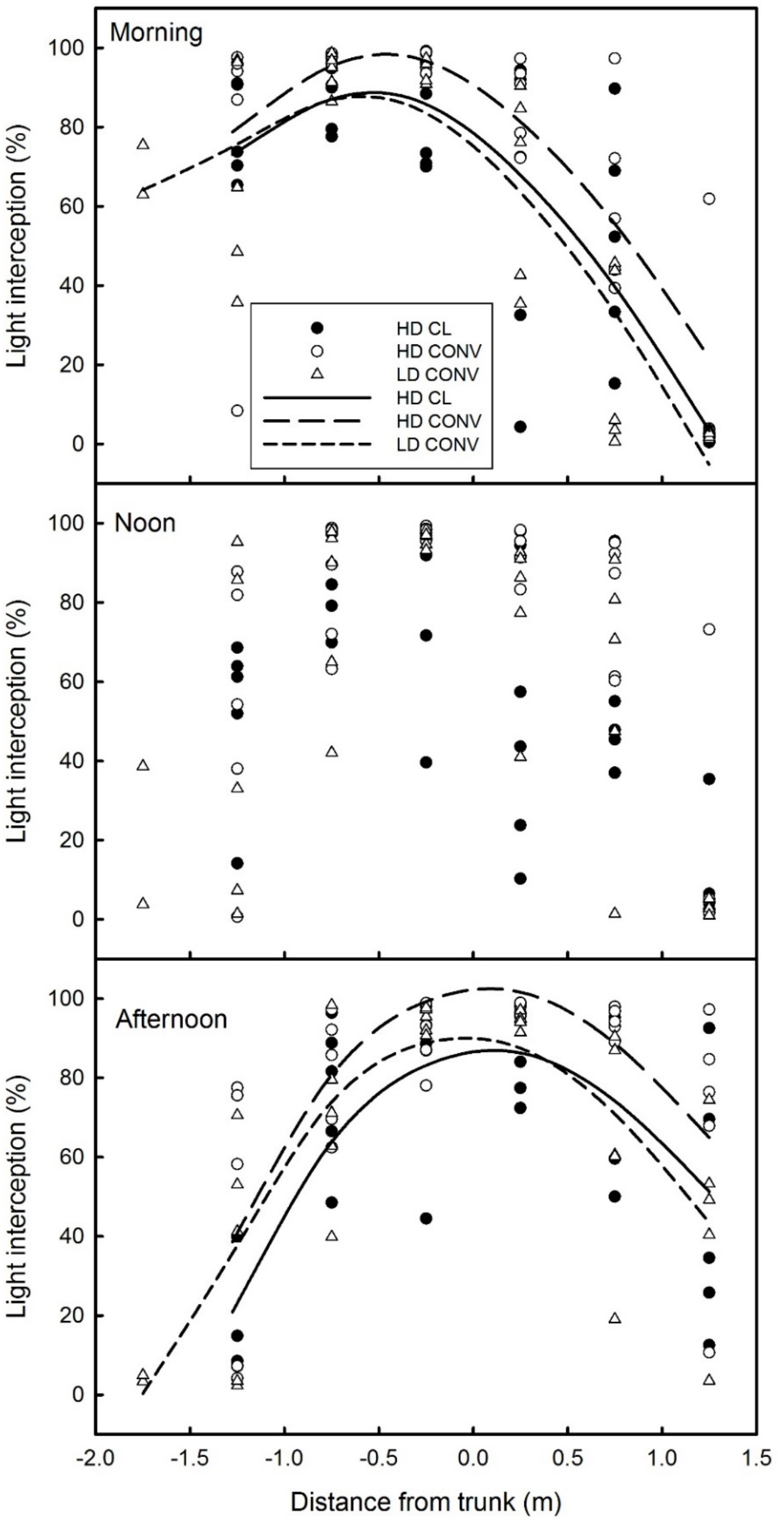

Figure 5. The effect of three ‘A203’ tree training and tree density treatment combinations on light distribution within the canopy in winter 2017 at the three times throughout the day that measurements were taken. For the morning measurements there were significant effects of treatment (*P* < 0.05) and distance from the trunk (*P* < 0.001) and the LSD for treatment differences is 11.9. For the solar noon measurements there was a significant effect of treatment (*P* < 0.05). For the afternoon measurements there were significant effects of treatment (*P* < 0.01) and distance from the trunk (*P* < 0.001) with the LSD for differences between treatments 9.6.

Yield

First flowering occurred in 2015 for ‘A203’ and 2016 for ‘741’ (Table 2) and flowering intensity appeared to increase in each successive year. ‘A203’ produced significantly more racemes/tree than ‘741 in each year. In spring 2017, there was a significant interaction between cultivar and tree density (*P* < 0.01) with no difference in racemes/tree for the tree densities in ‘741’ but for ‘A203’ the ranking was LD > MD > HD. Raceme density (racemes/m^3 canopy volume) was also significantly affected by cultivar in each year, with ‘A203’ having significantly greater raceme density than ‘741’ (Table 3). Raceme density was significantly affected by the interaction between tree density and pruning treatment in 2015 and 2018. In 2015, the CL treatments have greater raceme density than the CONV at their respective tree densities. In 2018, the HD CONV had significantly greater raceme density than the HD CL.

Table 2. The effect of tree density and cultivar on the number of flower racemes/tree in the main trial.

Analyses for each year were undertaken separately. There was a significant effect of cultivar (*P* < 0.001) in all years. In 2015, there was also significant interactions between density and pruning treatment (*P* < 0.01) and between cultivar, density and pruning treatment (*P* < 0.01). In 2017, there was also a significant effect of density (*P* < 0.05) and significant interaction between cultivar and density (*P* < 0.01).

	2015	2016	2017	2018
		Interaction between cultivar and tree density		
A203 LD	2.4 n.s.	128.4 n.s.	1 038	4 917 n.s.
A203 MD	8.0	123.9	688.1	4 943
A203 HD	10.8	122.2	450.7	3 489
741 LD	0	5.6	260.8	2 219
741 MD	0	3.7	225.5	1 754
741 HD	0.07	5.0	288.7	1 035
LSD	5.6	56.5	231	1 355

		Cultivar		
A203	8	120.12	663.1	4 356
741	0.03	4.6	257.8	1 559
LSD	3.04	30.6	115.1	742.2

Table 3. The effect of the interaction between tree density and tree training treatment and the effect of cultivar on raceme density (racemes/m^3 canopy volume) in the main trial.

Analyses for each year were undertaken separately. In 2015, data were log transformed (log transformed values in in parentheses), there was a significant effect of variety ($P < 0.001$), interaction between density and pruning treatment ($P < 0.01$) and interaction between cultivar, density and pruning treatment ($P < 0.01$). In 2017, there was a significant effect of cultivar ($P < 0.001$), density ($P < 0.05$) and interaction between cultivar and density ($P < 0.01$). In 2018,

	2015	2016	2017	2018
	Interaction between tree density and pruning treatment			
LD CONV	1.77 (0.57)	13.5 n.s.	51.0 n.s.	174.0
MD CONV	1.74 (0.56)	13.0	48.4	183.7
MD CL	6.13 (1.29)	8.19	31.5	152.5
HD CONV	2.76 (0.74)	11.85	42.4	226.0
HD CL	5.20 (1.53)	8.74	41.6	114.7
LSD	(0.625)	7.8	21.1	55.3
	Cultivar			
A203	7.15 (1.84)	20.31	54.6	231.1
741	0.04 (0.03)	1.81	31.4	109.2
LSD	(0.44)	5.22	13.8	37.5

The first NIS produced was in 2016 and 2017 for ‘A203’ and ‘741’, respectively, due to the timing of their first flowering. NIS yield per tree increased significantly with tree age ($P < 0.001$) with the yield/tree in 2019 ranging between 2.2 kg NIS and 9.4 kg NIS/tree for the different treatment combinations (Fig. 6). There were a range of significant effects including the third order interaction between year after planting, cultivar and tree density ($P < 0.001$). From 2017 onwards, when both cultivars had begun to produce NIS, the NIS yield/tree for ‘A203’ was greater than ‘741’ for all tree densities. For ‘A203’, yield/tree was similar for the tree densities in the main trial in 2016 and 2017. In 2018, the ranking for yield/tree was LD and MD > HD, and in 2019 was LD > MD > HD. For ‘741’, there were no differences in NIS yield per tree until 2019 when the LD and MD had greater yield/tree than the HD. For the HD-T there was a significant interaction between year after planting and cultivar. For ‘A203’, the general trend was for NIS yield per tree to increase over the course of the trial, except for a decline in 2018 relative to 2017 and 2019. The ‘A203’ HD-T NIS yield/tree appeared to be similar to the main trial HD in 2016 and 2017 and then lower in 2018 and 2019. The HD-T ‘741’ NIS yield/tree increased consistently across the course of the trial and appeared to be similar to the main trial ‘741’ HD in all years.

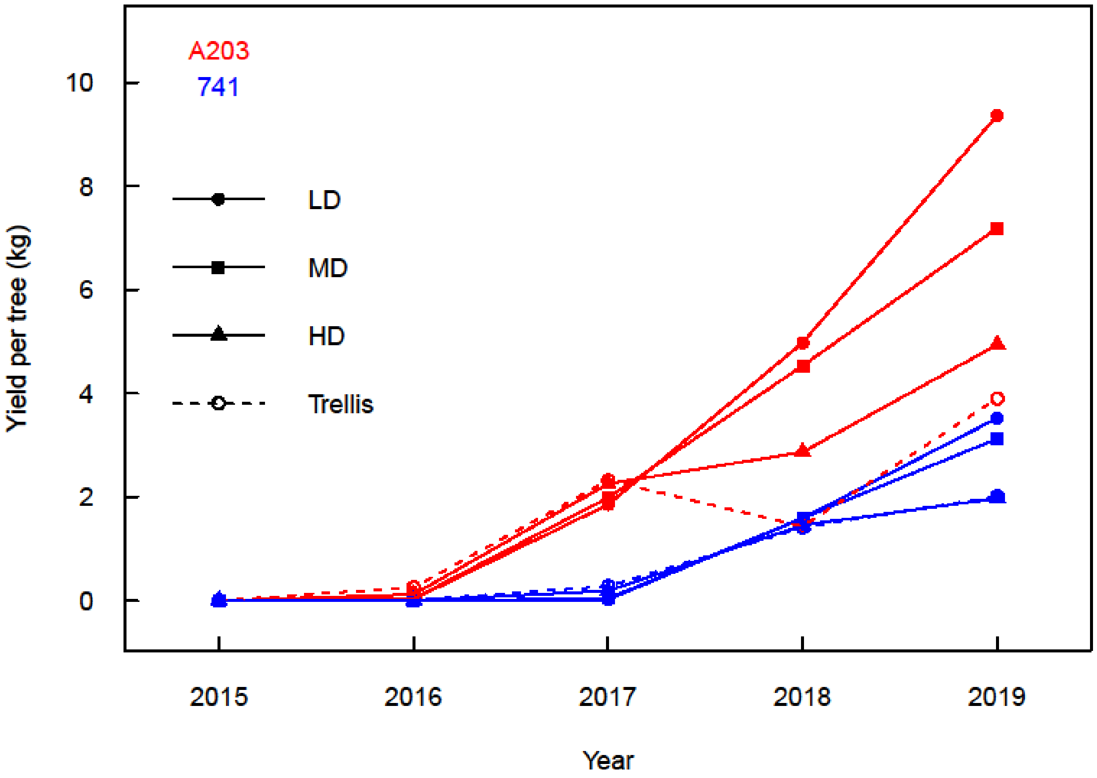

Figure 6. The effect of tree density and cultivar on NIS yield/tree over time in the main trial and trellised plot. The interaction between year, tree density and cultivar (*P* < 0.0001) in the main trial and between year and cultivar in the HD-T (*P* < 0.01) are presented. The LSDs to determine significant differences between treatments in the main trial are 0.06, 0.62, 0.72 and 1.00 in 2016, 2017, 2018 and 2019, respectively. The LSDs to determine significant differences between cultivars in the HD-T are 0.29, 0.59, 0.70 and 0.82 in 2016, 2017, 2018 and 2019, respectively. In the main trial there were also a significant effect of year (*P* < 0.001) and significant interactions between year and cultivar (*P* < 0.001), between year and density (*P* < 0.001) and between year, cultivar and density (*P* < 0.0001). For the HD-T, there was also a significant effect of year (*P* < 0.001).

For NIS yield/ha in the main trial, there were a range of significant effects including the third order interaction between year after planting, cultivar and tree density (*P* < 0.001). Yield/ha increased over the course of the trial once the respective cultivars began to crop (Fig. 7). Similar to NIS yield/tree, the NIS yield/ha for 'A203' was generally greater than for '741'. For example, in 2019 the yield/ha of all 'A203' systems was significantly greater than that of any of the '741' systems. Greater tree density generally led to greater NIS yield/ha for both cultivars by their second year of cropping. In 2017 and 2019, the NIS yield/ha ranking for 'A203' was HD > MD > LD and in 2018, it was HD and MD > LD. In 2018 the statistical ranking for NIS yield/ha for '741' was HD > MD and LD and in 2019, it was HD and MD > LD. There was also a significant interaction between year after planting and cultivar for the HD-T (*P* < 0.001; Fig. 7). The NIS yield/ha for the '741' HD-T increased relatively consistently following the onset of cropping and appeared to be greater than the main trial HD in each year. In contrast, the 'A203' HD increased strongly from 2016 to 2017, declined in 2018 and then increased to its greatest level of 5 780 kg NIS/ha in 2019. The 'A203' HD-T yield/ha appeared to be greater than that of the main trial HD in all years except 2018, where it appeared to be lower than that of the HD and MD.

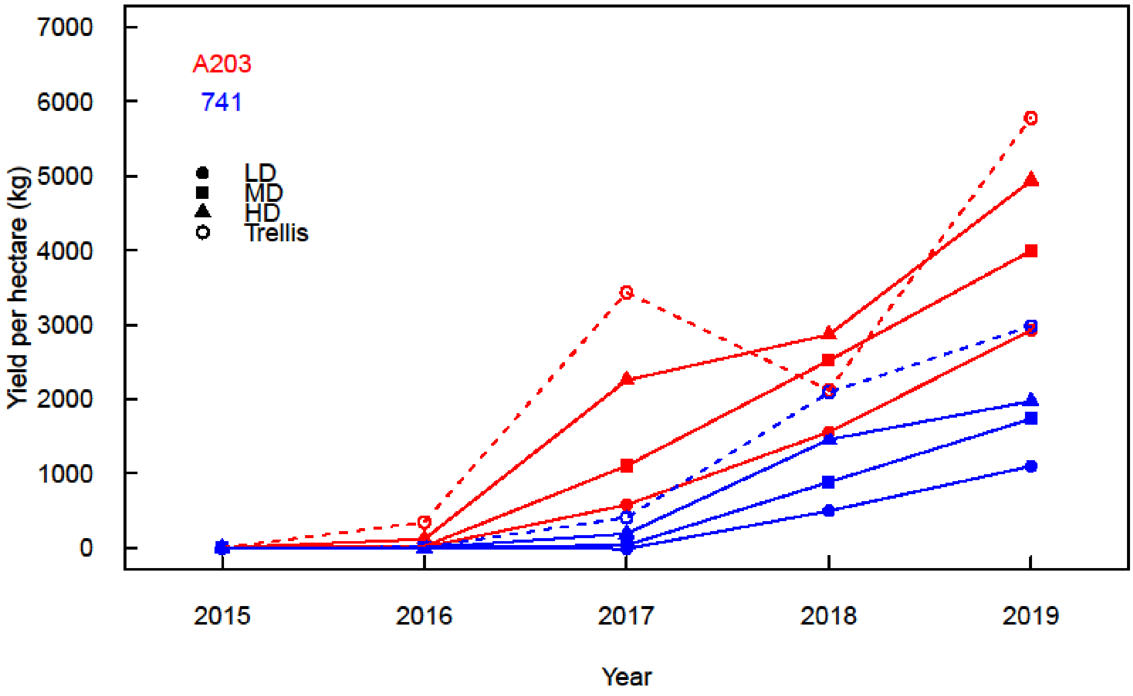

Figure 7. The effect of tree density and cultivar on NIS yield/ha over time in the main trial and trellised plot. The interaction between year, tree density and cultivar (*P* < 0.001) in the main trial and between year and cultivar in the HD-T (*P* < 0.001) are presented. The LSD to determine significant differences between treatments in the main trial is 407.5 and 615.6 in the HD-T. In the main trial there were also significant effects of year (*P* < 0.001), cultivar (*P* < 0.001) and density (*P* < 0.001) and significant interactions between year and cultivar (*P* < 0.001), between year and density (*P* < 0.001), between cultivar and density (*P* < 0.001) and between year, cultivar and density (*P* < 0.001). For the HD-T, there were also significant effects of year (*P* < 0.0001) and cultivar (*P* < 0.001).

Treatment effects on sound kernel/ha (Table 4) were similar to those of NIS yield/ha with a significant third order interaction of year after planting, cultivar and density (*P* < 0.001), amongst others. The magnitude of the differences between ‘A203’ and ‘741’ in sound kernel/ha tended to be slightly smaller than for NIS yield/ha due to the greater SKR of ‘741’ compared with ‘A203’ (Table 4).

Table 4. The effect of tree density, cultivar and year after planting on the production of sound kernel/ha and variety and year after planting on sound kernel recovery (%).

For sound kernel/ha there were significant effects of year ($P < 0.001$) and cultivar ($P < 0.05$) and significant interactions between year and cultivar ($P < 0.001$), between density and pruning treatment ($P < 0.01$), between year and density ($P < 0.001$), between cultivar, density and pruning treatment ($P < 0.01$) and between year, cultivar and density ($P < 0.001$). For sound kernel recovery (%) there significant effects of year ($P < 0.001$) and cultivar ($P < 0.001$) and a significant interaction between year and cultivar ($P < 0.001$). The LSD to determine significant differences of SKR between years and cultivars is 1.227.

	2015	2016	2017	2018	2019
			Sound kernel (kg/ha)		
A203 LD	0	2.6	190.8	505.7	1 013
A203 MD	0	5.6	354.8	823.3	1 384
A203 HD	0	34.8	757.3	964.1	1 662
741 LD	0	0	3.4	183.0	403.0
741 MD	0	0	11.6	320.9	639.4
741 HD	0	0	31.5	544.5	742.2
LSD	0	17.5	122.2	234	234
			Sound kernel recovery (%)		
A203		29.06	32.88	32.83	34.30
741			32.15	36.63	37.24

Yield efficiency (kg NIS/m^3 canopy volume) was significantly affected by year after planting ($P < 0.0001$), the interaction between year after planting and cultivar ($P < 0.001$) and the interaction between year after planting, tree density and pruning treatment ($P < 0.01$; Table 5). In 2017, the HD CONV yield efficiency was significantly greater than that of the LD CONV. There were no significant differences between treatments in 2018, although the HD CONV was non-significantly greater than the other treatments, and in 2019, the yield efficiency for the HD CONV was significantly greater than all treatments apart from the LD CONV.

Table 5. The effect of year after planting, tree density and pruning treatment on yield efficiency (kg NIS/m^3 canopy volume).

There was a significant effect of year ($P < 0.001$) and significant interactions between year and cultivar ($P < 0.001$) and between year, density and pruning treatment ($P < 0.01$).

Treatment	2015	2016	2017	2018	2019
LD CONV	0	0.006	0.064	0.166	0.218
MD CONV	0	0.002	0.100	0.177	0.207
MD CL	0	0.006	0.078	0.154	0.196
HD CONV	0	0.004	0.137	0.187	0.246
HD CL	0	0.019	0.110	0.144	0.170
LSD		0.012	0.07	0.05	0.03

NIS yield/ha was related to total light interception. In the 2016/17 season there was a strong linear increase in NIS yield/ha with total light interception for ‘A203’ (Fig. 8). The NIS yield/ha also increased with total light interception in ‘741’ but to a lesser extent than for ‘A203’. In 2017/18, NIS yield/ha increased almost linearly with total light interception up to a maximum yield/ha at approximately 60% total light interception and then appeared to decline slightly (Fig. 9). The NIS yield/ha for ‘A203’ was significantly greater than for ‘741’ at all levels of total light interception (*P* < 0.001). In the 2018/19 season, NIS yield/ha was linearly related to total light interception (Fig. 10; *P* < 0.001). The relationships differed with cultivar with the yield/ha for ‘A203’ greater than for ‘741’ at all levels of total light interception (*P* < 0.001) and the rate of increase in yield/ha with total light interception greater for ‘A203’ than for ‘741’.

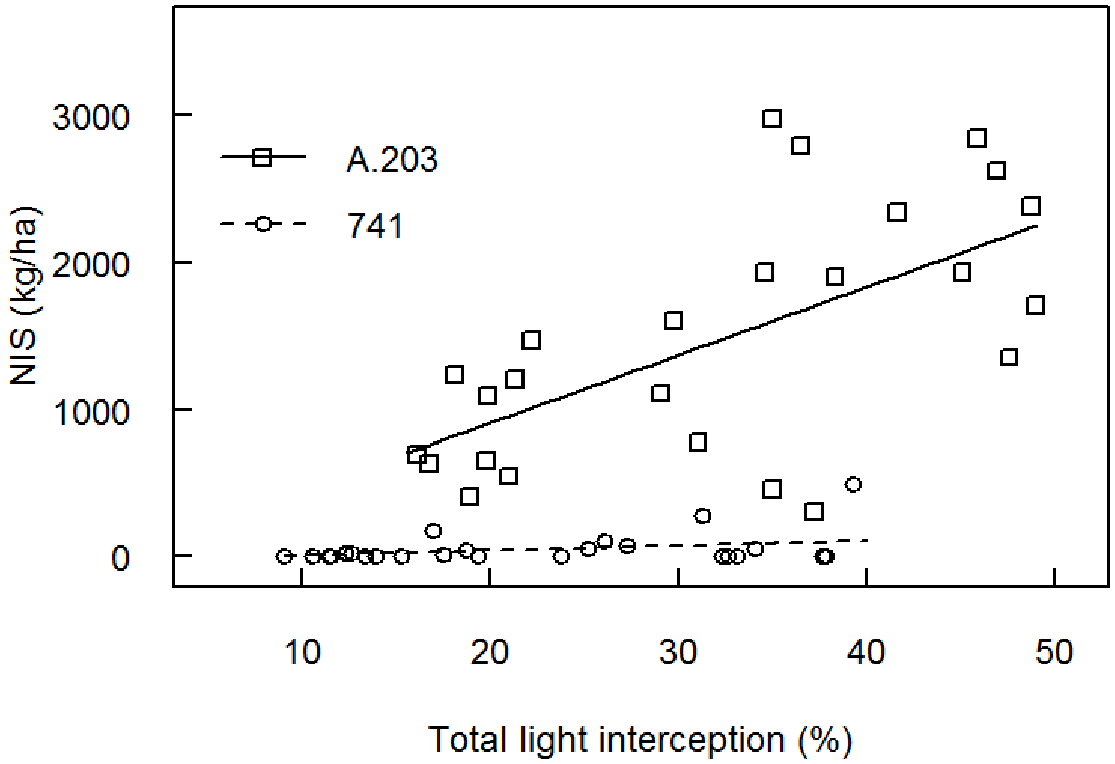

Figure 8. The effect of total light interception on NIS yield/ha in the 2016/17 season. There was a significant effect of total light interception on NIS yield/ha (*P* < 0.001), as well as a significant effect of cultivar (*P* < 0.0001) and interaction between total light interception and cultivar (*P* < 0.01). The LSD for differences between treatments is 534.

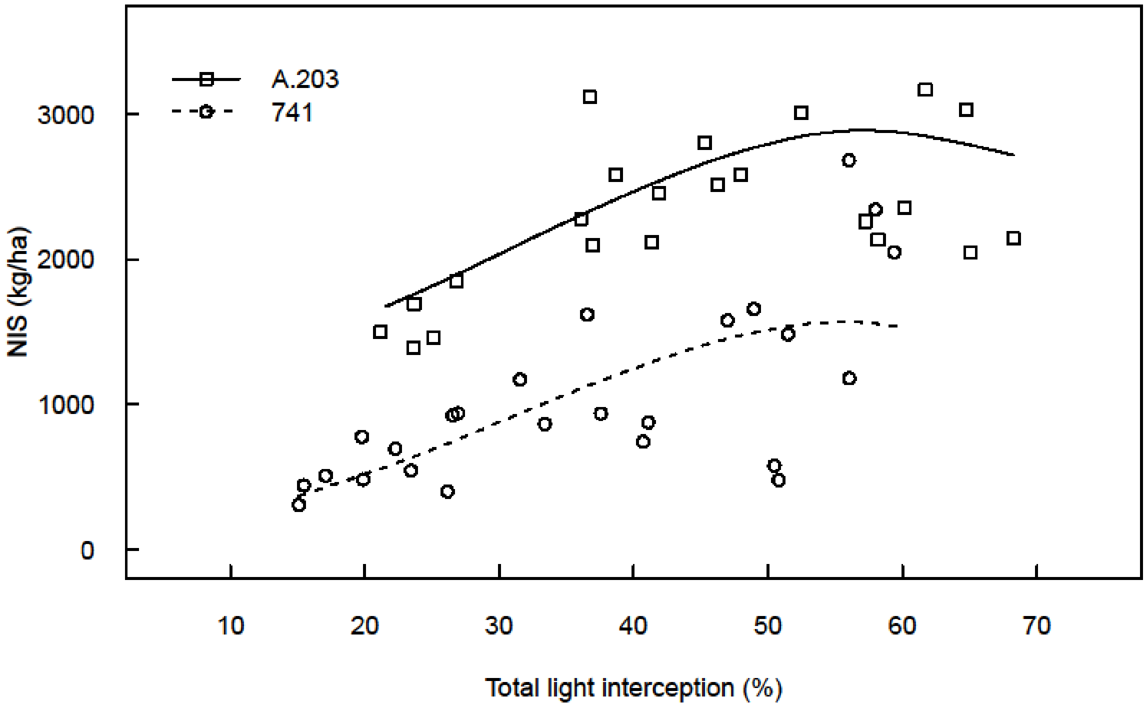

Figure 9. The effect of total light interception on NIS yield/ha in 2018. There was a significant effect of total light interception (*P* < 0.001) and cultivar (*P* < 0.001) on NIS yield/ha, with the LSD for differences between cultivars 520.

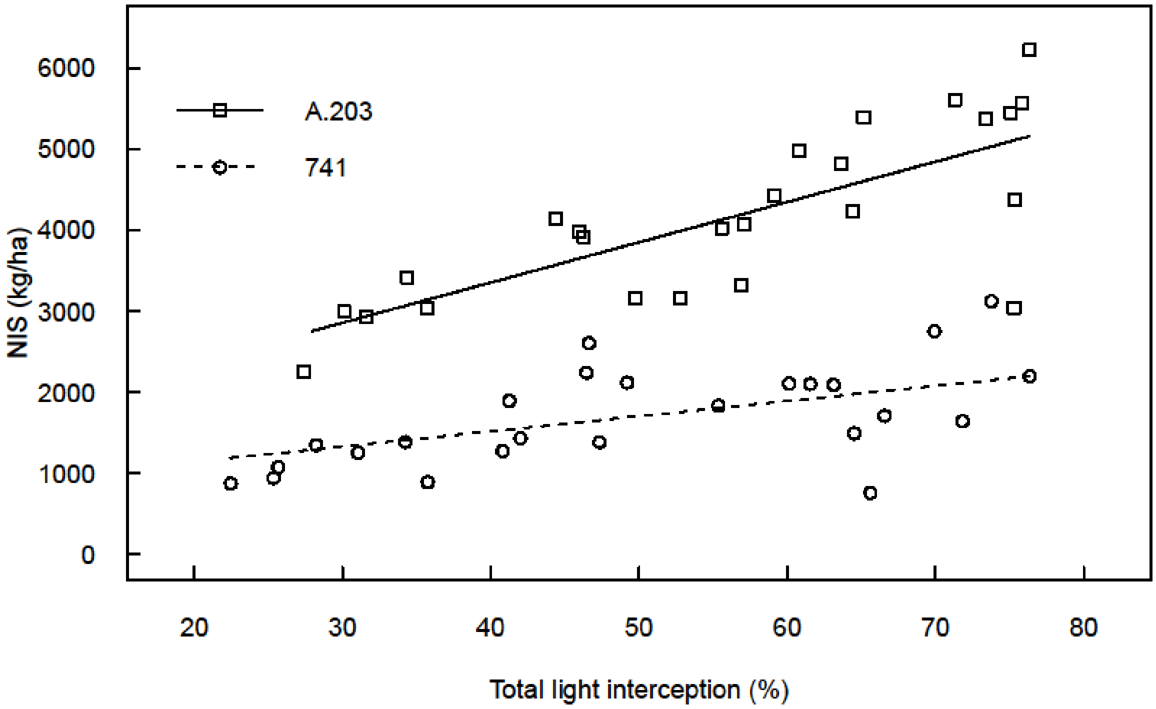

Figure 10. The effect of total light interception on NIS yield/ha in the 2018/19. There was a significant effect of total light interception on NIS yield/ha (*P* < 0.001), as well as a significant effect of cultivar (*P* < 0.001) and interaction between total light interception and cultivar (*P* < 0.001). The LSD for comparison between cultivars is 382.

Yield efficiency increased linearly with raceme density (racemes/m^3 canopy volume). In the 2016/17 season (Fig. 11), raceme density for '741' ranged from 0 to 8.8 and for 'A203', it ranged from 4.1 to 50.7 with the yield efficiency for 'A203' significantly greater than for '741' at any level of raceme density ($P < 0.001$). In 2017/18 (Fig. 12) the raceme density for '741' ranged from 2.74 to 80.9 and for 'A203', it ranged from 24.7 to 128.2 with the yield efficiency for 'A203' being significantly greater than that for '741' at any given level of raceme density. In 2018/19 (Fig. 13) the raceme density for '741' ranged from 25.5 to 315.2 and for 'A203', it ranged from 107.8 to 399.3 with the yield efficiency for 'A203' being significantly greater than that for '741' at any given level of raceme density.

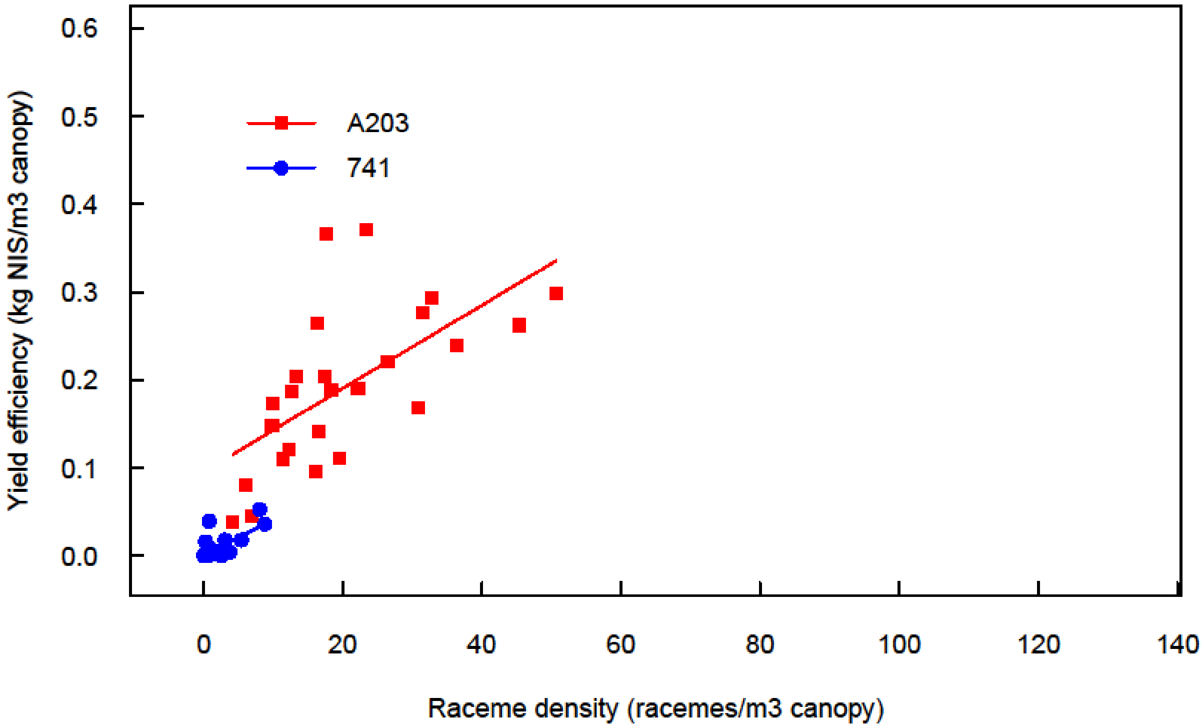

Figure 11. The effect of raceme density (racemes/m^3 canopy volume) on yield efficiency (NIS kg/m^3 canopy volume) in the 2016/17 season. LSD for comparison between cultivars in 0.045. There were significant effect of raceme density (P < 0.001), cultivar ($P < 0.001$) and density ($P < 0.05$).

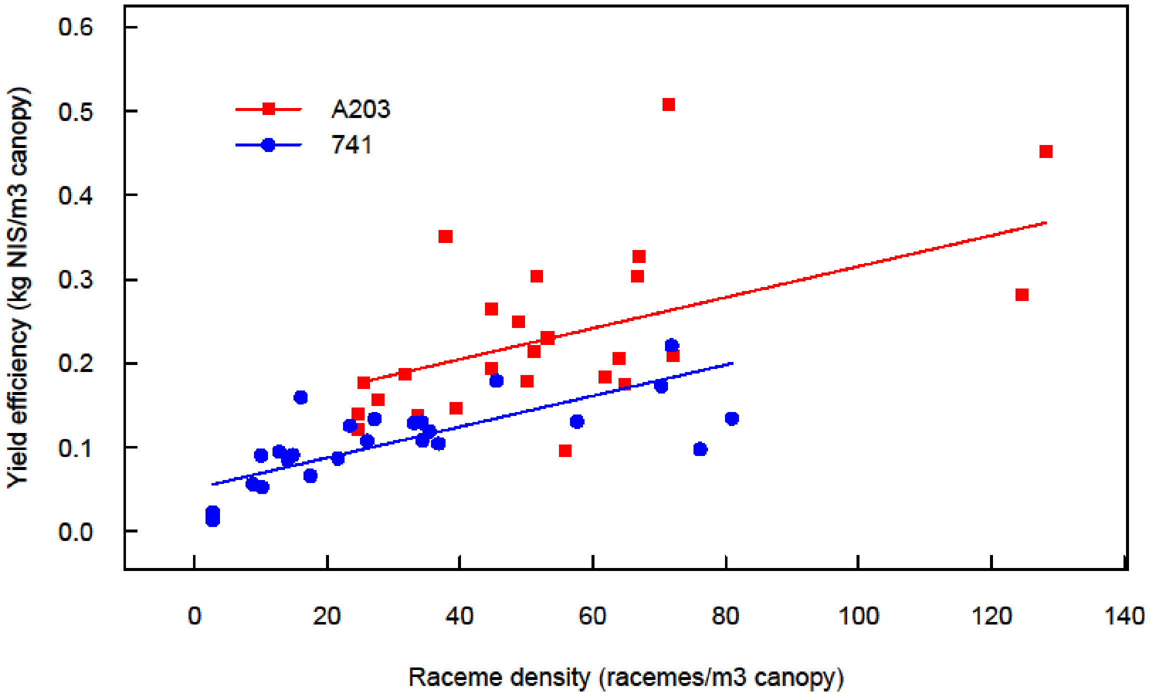

Figure 12. The effect of raceme density (racemes/m^3 canopy volume) on yield efficiency (NIS kg/m^3 canopy volume) in the 2017/18 season. There was a significant effect of raceme density ($P < 0.001$), cultivar ($P < 0.001$) and interaction between raceme density and cultivar ($P < 0.01$). The LSD for comparison between cultivars is 0.058.

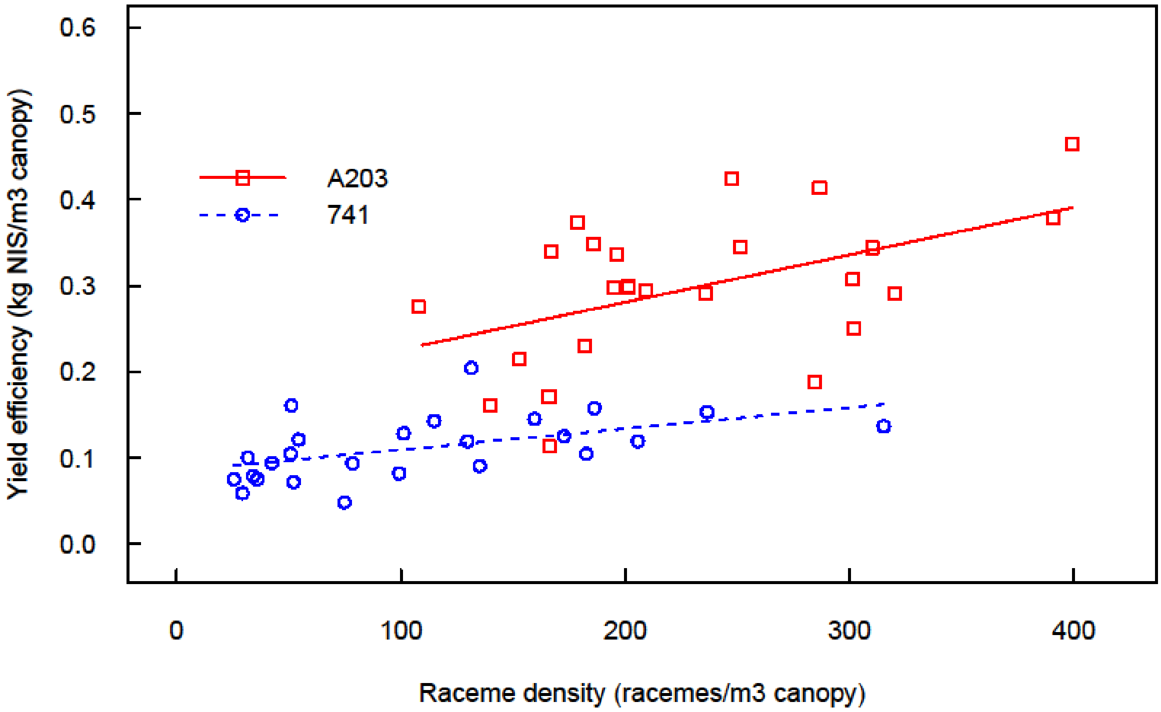

Figure 13. The effect of raceme density (racemes/m^3 canopy volume) on yield efficiency (NIS kg/m^3 canopy volume) in the 2018/19 season. There was a significant effect of raceme density ($P < 0.01$) and cultivar ($P < 0.001$) but the interaction between raceme density and cultivar was not significant ($P > 0.05$). The LSD for comparison between cultivars is 0.058.

Discussion

The work presented here is the canopy development, light relations, crop load and productivity during the early orchard life of a macadamia planting systems trial, defined by us in the macadamia context as being up to five years after planting. From the perspective of the planting systems variables we trialled, the yield/ha was strongly affected by tree density, cultivar, year after planting and their interactions, with much smaller impacts of the imposed tree training treatments. The work also provides an opportunity to understand the underlying physiological limitations to early orchard productivity in macadamia.

Canopy development and total light interception

The canopy volume/ha over the first five years of the trial was consistently greater for the higher tree densities. During the first three years of tree development, there was little impact of tree density on canopy volume/tree (Fig. 1) and canopy volume/ha was largely proportional to tree density (Fig. 2). From 2018, canopy volume/tree at the higher densities was restricted by inter-tree competition and pruning and by 2019 the canopy volume/ha of the LD compared with the HD had increased up to approximately 50% from approximately 30% in 2017. The restricted growth and smaller individual canopy size in high density planting systems is consistent with apple planting systems trials (Robinson *et al*., 1991a; Tustin *et al*., 2001).

Total light interception increased in all systems over the first five years and the final levels of total light interception were greater in the higher density systems (Fig. 3). This overarching impact of higher density orchard systems filling their allotted space and reaching their desired level of total light interception earlier in the life of the orchard than lower density systems has been widely reported (Tustin et al., Robinson and Lakso, 1991b) and is one of the fundamental benefits of increased tree density.

However, tree density is not the only factor affecting the development of total orchard light interception. A significant driver of total light interception is canopy volume/ha (Fig. 4; McFadyen *et al*., 2004; Wilkie *et al*., 2018), with almost linear increases in total light interception with canopy volume/ha during early orchard life. At higher levels of canopy volume/ha, the relationship becomes more curvilinear and total light interception begins to plateau due to shading within and between canopies (Wilkie et al., 2018). Further increases in canopy volume/ha beyond this point are likely to increase the heavily shaded portion of the canopy for little additional total light interception. For conventionally spaced macadamia and avocado orchards, this plateau has been reported to occur at approximately 30 000 m^3/ha (Wilkie *et al*., 2018; McFadyen *et al*., 2004). The almost linear increase in total light interception with canopy volume/ha in the systems in the main trial, indicates limited within canopy crowding.

Factors such as scion/rootstock combinations that affect canopy development are also likely to affect total light interception. For example, in the current work the ‘A203’ trees were larger than the ‘741’ trees in the main trial and the total light interception for ‘A203’ was also greater than for ‘741’ (data not presented). Rootstock cultivar in apple planting systems trials have often been reported to affect canopy development and light interception (Tustin et al., 2001).

In addition to the volume of canopy, total light interception is affected by the arrangement of the canopy across the orchard area. In the current work, tree density affected the relationship between canopy volume/ha and total light interception (Fig. 4), with the HD systems intercepting more PAR than the LD systems for any given canopy volume/ha. This is most likely due to the reduced row width in the HD systems leading to more even distribution of light interception across the orchard floor, similar to that reported by Wagenmakers (1995). Tree training systems such as 'V-Trellis' or 'Tatura Trellis' systems that spread the canopy volume into the inter-row also lead to improved distribution of PAR interception across the orchard area and lead to greater PAR interception per unit of canopy volume (Robinson *et al.*, 1991a; Robinson and Lakso, 1991b).

Flowering and yield

Yield limitations during this early orchard life period were due to limitations in the development of crop load (precocity) and limitations in total light interception.

Within each cultivar, the yield/ha in the higher density systems increased faster and the maximum yield/ha at five years after planting was greater than in the lower density treatments. The greater yield/ha of the higher density systems was due to the greater canopy volume/ha and total light interception in these systems. The strong increases in yield/ha with total light interception (Fig. 8 – 10) are consistent with the early orchard phase where the systems are filling their allocated space and has been reported previously for conventionally spaced macadamia (McFadyen *et al.*, 2004) and avocado orchards (Wilkie et al., 2018). This work confirms that planting at higher tree densities allows the limitation on yield/ha due to total light interception to be overcome earlier in the life of a macadamia orchard.

Despite the greater levels of total light interception in the HD systems in the main trial, there was little indication that the canopy had reached a point where its efficiency had begun to decline. First, the yield efficiency of the HD systems was similar to those of the MD and LD systems (Table 4), which had significantly lower canopy volume/ha and total light interception. Second, the relationship between total light interception and yield/ha in 2019 was linear across the range of total light interception observed, indicating that the conversion efficiency of PAR intercepted to NIS yield/ha remained the same in the systems intercepting more light. The curvilinear relationship between total light interception and yield/ha in 2018 may seem to contradict this, but the plateau may have been related to more severe pruning in the plots intercepting more light (larger trees) leading to competition between vegetative re-growth and fruit set (McFadyen et al 2011).

For conventionally spaced macadamia orchards, the maximum yield/ha is achieved at 85 to 95% total light interception (Olesen *et al.*, 2007; McFadyen *et al.*, 2004). The HD-T and HD systems reached approximately 80% and 60% total light interception in 2019, respectively, and so it is unlikely that any of the systems reached their maximum productivity in 2019.

As total light interception increases, light availability within the canopy can be reduced to the extent that crop load development processes are compromised and yield/ha can plateau or even decline as light interception increases further still (Wunsche and Lakso 2000; Wagenmakers and Callesen, 1995). These yield plateaus and potential declines have been observed in subtropical macadamia and avocado and are likely due to deleterious effects of an increased proportion of the canopy volume being highly shaded on crop load development processes (Olesen *et al.*, 2011; McFadyen *et al.*, 2004; Wilkie *et al.*, 2018). Planting systems that lead to more even interception of PAR across the orchard area, such as those with reduced row width and V-trellis systems, can allow greater total light interception without compromising within canopy light distribution (Palmer et al., 1992; Wagenmakers and Callesen, 1995). These systems appear to allow yield/ha to continue to increase with total light interception that would otherwise lead to limitations in within canopy light distribution. It is unclear whether the more intensive systems in the current study will achieve greater yield/ha than the conventionally spaced systems at the higher levels of total light interception.

The interception and utilisation of PAR is not the only limitation to productivity during early orchard life. The greater total light interception of the high density systems provided a greater yield potential than the lower density systems. However sufficient raceme production was required to convert this yield potential into NIS yield/ha.

Raceme production limited early yield in both cultivars throughout these first five years of the trial. The linear relationship between raceme density and yield efficiency in the 2016/17 (Fig. 11), 2017/18 (Fig. 12) and 2018/19 (Fig. 13) seasons indicates that greater raceme densities would have led to greater yield efficiency. The slope in the relationships between raceme density and yield efficiency appeared to decrease over the three seasons as the range of raceme densities produced by the trees increased. However, even in the 2018/19 season with the upper level of raceme densities observed for ‘A203’ almost 400 racemes/m^3, there was a 0.16 kg NIS/m^3 increase in yield efficiency from the lowest to highest raceme density. The limitation to early yield due to poor flowering is consistent with McFadyen *et al*. (2016), where the tree training treatments and cultivars with reduced flowering also had reduced yield.

There were large effects of cultivar on early orchard NIS yield. ‘A203’ produced their first commercially relevant crop earlier than ‘741’ at three and four years after planting, respectively, due to the timing of their first flowering (Table 2). Following the first crops, the NIS yield for ‘A203’ continued to be greater than that produced by ‘741’. For example in 2019, the ‘A203’ LD produced 9.4 kg NIS/tree and the ‘741’ LD produced 3.5 kg NIS/tree (Fig. 6). Part of the difference was due to the smaller canopy volume and subsequently lower total light interception of ‘741’ compared with ‘A203’ (data not presented), providing greater yield potential for ‘A203’. However, for any given level of total light interception, ‘A203’ had a greater yield/ha (Figs. 8 – 10), indicating that ‘A203’ was better able to convert the yield potential provided by the total light interception to NIS yield/ha than ‘741’. This was largely due to greater raceme densities in ‘A203’ compared with ‘741’, given that raceme density was a significant limitation to yield, as described above.

For any given level of raceme density, the yield efficiency of ‘A203’ was greater than for ‘741’. This indicates ‘A203’ was either able to retain more fruit/raceme, the fruit it retained were larger, or both, compared with ‘741’. Thus, there are aspects to the greater productivity of ‘A203’ that could not be explained by either flowering or total light interception and the reasons for this are unclear.

Tree training

The effect of tree training treatment on canopy development, light relations and productivity were relatively minor compared with those of cultivar and tree density.

During the first two years of the trial, the CL treatments tended to have a greater canopy volume than the CONV treatments within each density (data not shown). This was probably largely due to the repeated simulated mechanical hedging holding back the growth of the trees relative to the CL treatments where comparatively little pruning was undertaken. Additionally, for the HD CL systems, the limb bending served to increase the dimensions of the trees. The HD CL systems continued to have a greater canopy volume/ha than the HD CONV systems in the final three years of the trial, although the reasons for this are unclear, given the severity of pruning between the CONV and CL tree training treatments was more similar than during the first two years.

One of our main hypotheses for the tree training treatments was that limb bending would lead to more precocious flowering. In 2015, the ‘A203’ CL systems produced more racemes/tree (data not shown) and had a greater raceme density (Table 3) than their CONV counterparts but the HD CL (limb bending) did not produce more racemes/tree or have a greater raceme density than the MD CL (no limb bending). Therefore, it does not appear that limb bending in macadamia can improve early flowering, and the minor improvements in flowering in the CL systems did not persist in subsequent years.

No further crop load impacts of the tree training systems were observed until 2018/19. In that season, raceme density of the HD CONV systems was significantly greater than the HD CL and this led to differences in yield efficiency, with the HD CONV having significantly greater yield efficiency than the HD CL. Although there was no significant effect of the interaction between density and tree training in the multi-year analysis for NIS yield/tree, it is worth noting that the ‘A203’ HD CONV produced 5.3 T/ha NIS compared with 4.5 T/ha from the HD CL in 2019. The reasons for the improved raceme density and yield efficiency of the HD CONV relative to the HD CL is unclear. However the limb removal required to maintain the CL tree structure may have removed shoots that would otherwise have flowered, thus reducing the raceme density. Alternatively, the CL pruning may have improved light availability within the canopy to such an extent in 2017 (Fig. 5) that raceme production was reduced. Olesen et al. (2011) reported approximately 50% of racemes were produced in cultivar ‘849’ in portions of the canopy receiving 2% or less of above canopy PAR.

For this first five year period of the trial, the CONV tree training was based on mechanical hedging and resultant shoot decapitation. This approach has been shown to lead to vigorous vegetative regrowth with the potential for severe competition with the developing crop when the regrowth from pruning coincides with early fruit development (McFadyen *et al*., 2011). Additionally, selective limb removal has been reported to improve fruit set relative to mechanically hedged and topped trees (Appendix 5). In the current work there has been no evidence so far of competition from post hedging regrowth affecting fruit set and productivity.

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Appendix 5

Effects of selective limb removal, mechanical hedging and topping and plant growth regulators on yield and canopy development in high density macadamia

Wilkie JD, Hofman H, Griffin J

Introduction

Macadamia (*Macadamia integrifolia*, *M. tetraphylla* and *M integrifolia* x *M. tetraphylla*) is an evergreen tree native to subtropical eastern Australia. It is a relatively recently domesticated and underdeveloped crop that is cultivated in orchards in a range of subtropical and tropical locations around the world for its kernel.

Shoot growth in macadamia, as for many other subtropical tree crops, is through recurrent flushing. The time between successive flushing events is dependent on temperature and in subtropical environments, these flushes may occur at any time throughout the year (Wilkie et al., 2009). Each flushing event involves the release of terminal or axillary buds, the production of several nodes, the extension of internodes and then cessation of growth.

Commercial macadamia orchards are generally planted using low density, large tree systems. In Australia, plantings generally range from 357 trees/ha (7 m x 4 m) to 200 trees/ha (10 m x 5 m). These low density systems have the disadvantage that yield is delayed in early orchard life compared with more intensive plantings (Mayer et al., 2006), because yield in macadamia increases with canopy volume per hectare and total light interception (McFadyen et al., 2004). On the other hand, the time before canopy crowding and total light interception increases to such high levels that yield decline commences (ca. 85 to 94 % light interception; McFadyen et al., 2004; Olesen et al., 2007), is probably delayed. Canopy management operations to maintain tree size and prevent crowding are undertaken for a range of reasons in addition to the maintenance of yield, including: orchard access, the maintenance of vegetative ground cover to prevent erosion and improved spraying efficiency.

Mechanically hedging the sides of the canopy is a commonly undertaken canopy management practice in commercial macadamia orchards in Australia. Hedging is cheap relative to hand pruning. In the short term, hedging has been reported to lead to no yield penalty up to a moderate yield penalty compared with unpruned control trees, depending on the time of hedging and cultivar (McFadyen et al., 2005; McFadyen et al., 2012). In the longer term, side hedging has been reported to increase tree height and decrease yield compared with unpruned control trees (McFadyen et al., 2005), possibly due to the repeated hedging in the same position over several years leading to a depletion in available nodes for flowering.

Mechanically hedging the tops of canopies (topping) to control tree height is also undertaken. Topping has generally been reported to lead to severe yield reduction (McFadyen et al., 2011; McFadyen et al., 2013; Olesen et al., 2016). It has also been reported to lead to rapid increases in tree height in the subsequent years (McFadyen et al., 2013; Olesen et al., 2016).

Removal of selected limbs from the canopy manually (selective pruning) is another strategy to manage tree size. McFadyen et al. (2013) removed limbs in the upper canopy of crowding macadamias, with the aim of improving light penetration. The selective pruning resulted in increased yield compared with an unpruned control and an annual hedging treatment, in two out of seven years and five out of seven years, respectively. In a separate experiment, selective removal of limbs in the upper canopy or removal of a large codominant leader (both aimed at reducing tree height) decreased yield compared with an unpruned control in one of four years of the trial and increased yield compared with mechanical topping in three of four years of the trial (McFadyen et al., 2013).

There are several reasons for these yield penalties associated with pruning. First, yield in macadamia orchards increases with interception of photosynthetically active radiation (PAR), total light interception, up to 85 to 94% (McFadyen et al., 2004, Olesen et al., 2007), and so pruning that reduces leaf area and light interception may also reduce yield. Second, the removal of the outer canopy that occurs in mechanical hedging and topping exposes shade adapted leaves, that have a lower photosynthetic potential (Huett, 2004), which may reduce the amount of carbon assimilates produced by the canopy and thus energy available for fruiting. Third, hedging and topping resulting in vigorous and synchronised regrowth may result in reduced flower raceme production and yield, due to signals produced in the developing flush that inhibits floral initiation (Olesen, 2005; Wilkie et al., 2010). Fourth, pruning that results in vigorous regrowth around the time of early nut set may induce competition that leads to reduced fruit set and yield (McFadyen et al., 2011). For example, mechanical hedging in Australia in September (around the time of anthesis) generally led to greater yield loss than hedging in November or December, once fruit growth rates had increased (McFadyen et al., 2012).

Triazole growth regulators, paclobutrazol (PBZ) and uniconazole (UCZ), are gibberellin biosynthesis inhibitors (Rademacher, 1995), used in a range of tree crops to modify the balance between vegetative and reproductive growth. PBZ has been reported to promote flowering in lychee (Menzel and Simpson 1990), mango (Wilson 1992) and UCZ for macadamia (Nagao et al., 1999). PBZ applications reduced both shoot and root growth in apple when applied as both a foliar application or as a root drench (Lehman et al., 1990). Foliar applications of UCZ has been demonstrated to decrease post hedging shoot growth in avocado (Leonardi, 2000) and macadamia (Wilkie et al., 2010), and for avocado were at times able to reduce the yield penalty associated with the pruning. There is further scope to determine the potential for growth regulators to reduce canopy growth of macadamia in order to reduce the frequency and intensity of pruning and reductions in yield following pruning due to competition between regrowing shoots and fruit.

Here we compared combinations of mechanical hedging and topping, selective pruning, and growth regulator treatments over three cropping seasons to determine the effects on vegetative growth, flowering, fruit set and yield. Our first purpose was to develop improved canopy management practices for macadamia. Our second purpose was to improve understanding of the physiological factors limiting yield in macadamia.

Materials and Methods

Experimental site

The experiment was conducted at a commercial macadamia orchard near Bundaberg (24.85^{o}S, 152.5^{o}E) in eastern central Queensland, Australia, using trees of cultivar ‘A203’. The orchard planting configuration was five metres between rows and two metres between trees within rows (1000 trees/ha) with the rows oriented north-south and 10 consecutive rows of each cultivar. The orchard block was planted in 2004, and so in 2013, at the commencement of the experiment, the orchard was nine years of age.

Treatments and data collection

In July 2013, three consecutive tree rows within the orchard were selected. Twenty plots of 12 trees were then selected, which consisted of four consecutive trees in each of the three rows and directly adjacent to the trees in the neighbouring rows. All trees within the 12 tree plot had experimental treatments applied to them, however the two central trees within the middle row, which were fully buffered by the other trees within the plot, were the only trees from which data was collected. Plots were allocated to one of four treatments: mechanical hedging and topping (Hedged), mechanical hedging and topping plus uniconazole (Hedged + UCZ), selective pruning (Selective), and selective pruning plus uniconazole (Selective + UCZ).

Mechanical hedging and topping involved vertical mechanical hedging cuts to the sides of the canopy such that there was a 1.8m alley way following the hedging. The top of the canopy was mechanically hedged with pitched cuts at approximately 45^o such that that highest point along the centre of the tree canopy was approximately 5.5 m above the ground. Selective pruning was undertaken using a long handled chain saw and involved removing entire limbs or portions of limbs back to either a side branch or the trunk. Limbs were removed in order to reduce tree size (height and width) and shape (remove tree 'shoulders' to create a more pyramidal than rectangular shape) to be similar to those of the mechanically hedged and topped trees. However, in all years this was not quite achieved and tree height and width of the selectively pruned trees was approximately 10 cm to 20 cm greater than the hedged and topped trees.

UCZ was applied as the product Sunny ® at a concentration of 0.75 mL/Litre UCZ with the non ionic surfactant DuWet ® at 1 mL/Litre with a spray volume of approximately 1000 L/hectare, which corresponded to the commencement of spray run-off.

Mechanical hedging and topping and selective pruning was undertaken in July 2013, August 2014 and August 2015; winter being the conventional pruning time for many macadamia orchards in the Bundaberg region. The UCZ was applied in September 2013, September 2014 and September 2015; the purpose being to spray the re-growing shoots from the mechanically hedged and topped trees when they were 5 to 10 mm in length.

Four limbs per tree were tagged in each tree (one each in the upper and lower portions of the canopy on both the east and west sides) and the limb diameter measured at flower anthesis. Flower raceme numbers per limb were counted once at anthesis and fruit numbers per limb were counted four times throughout fruit development in each of the three seasons the experiment was undertaken. Total shoot length of all new shoot axes arising following pruning on 12 tagged terminal stems per tree (three stems each in the upper and lower portions of the canopy on both east and west sides) were measured during November and then in the following April or May, following harvest, in each of the seasons the experiment was undertaken. Canopy dimensions were measured twice each cropping season, once at flowering and once after harvest. Tree volume was calculated using the equation for an irregular ellipsoid, $V = (\pi \cdot x \cdot y \cdot z)/6$, where x is the difference between tree height and average skirt height, y is the maximum tree width across the row, and z is the tree width within the row (limited to the maximum of the within row tree spacing).

Yields were measured for each tree in 2014, 2015 and 2016 by harvesting mature fallen fruit from the ground and removing all fruit from each tree in the last week of March in each year. The husk were removed from the fruit and the remaining nut in shell (NIS) weighed. A subsample of 50 NIS per tree was randomly selected, dried and used to calculate the NIS moisture content and total kernel recovery (percentage of NIS that is kernel).

Total light interception was measured in each cropping year during the summer. Unfortunately, in the final cropping season the data set could not be completed due to weather and so the results for the first two seasons are presented. PAR interception was measured once during the day under diffuse light conditions using an 80 cm long Accupar ceptometer (Decagon Devices Inc.) for the below canopy measurements. Ceptometer measurements were made perpendicular to the tree row in transects with each measurement spaced at 25 cm intervals along the within row distance occupied by the two-tree data plot. Three 80 cm wide measurement transects extended out from both sides of the trunk. Simultaneous above-canopy measurements were taken using taken using an Apogee SQ-100 PAR point sensor (Apogee Instruments, INC.). For each measurement, light interception was calculated as the percentage of PAR removed between the above canopy measurement and the below canopy measurement. The total light interception for each plot was calculated as the average of the light interception of all measurements taken for the plot.

Statistical analyses

Data were either analysed using Genstat Version 16 or asreml-R Version 3. The NIS, kernel/ha, kernel recovery and flower raceme data sets were analysed using the ANOVA repeated measures technique, with repeated measurements over the three consecutive years the experiment was undertaken. The fruit set data sets were also analysed using the ANOVA repeated measures technique, but with repeated measurements over the four consecutive measurements per cropping season. The shoot growth and total light interception data sets were analysed using ANOVA, with each measurement occasion analysed separately. Data were transformed when they did not meet the assumptions of ANOVA.

Results

Mechanical hedging induced synchronised vegetative regrowth from the pruned stems. Shoot growth was greater ($P < 0.001$) from the shoots tagged high in the canopy compared with those tagged low in the canopy (27.1 cm vs 5.6 cm in autumn 2014; 19.5 cm vs 7.1 cm in autumn 2015; and 12.6 vs 4.2 cm in autumn 2016). Canopy management treatments also affected shoot growth (Table 1), with both hedged treatments having significantly greater shoot length than both selectively pruned treatments in the spring and autumn of the 2013/14 and 2014/15 cropping seasons. In spring 2015, the Hedged + UCZ treatment had significantly shorter shoots than the Hedged treatment and was not significantly different to the selectively pruned treatments.

Table 1. The effect of canopy management treatment, time within cropping season and cropping season on total shoot growth (cm) per tagged stem.

	Hedged	Hedged + UCZ	Selective	Selective + UCZ	*P*

2013/14					
Fruit set	9.64 *b*	10.22 *b*	3.67 *a*	4.21 *a*	< 0.05
Fruit maturity	28.2 *b*	22.5 *b*	7.8 *a*	7.0 *a*	< 0.001
2014/15					
Fruit set	8.21 *c*	3.26 *b*	0.62 *a*	0.97 *a*	< 0.001
Fruit maturity	23.4 *b*	16.1 *b*	6.7 *a*	7.0 *a*	< 0.001
2015/16					
Fruit set	9.15 *b*	5.08 *a*	4.18 *a*	2.71 *a*	< 0.05
Fruit maturity	11.71	8.77	7.44	5.70	n.s.

Fruit set was also significantly affected by limb height within the canopy ($P < 0.001$) in all three seasons, with the limbs high in the canopy holding on average 0.5448, 0.5344, 0.4687 fruit/cm^2 branch cross-sectional area (BCA) and limbs low in the canopy holding on average 0.1565, 0.164, 0.1775 fruit/cm^2 BCA in the 2013/14, 2014/15 and 2015/16 seasons, respectively. In all three cropping seasons the number of fruit/cm^2 BCA declined strongly over spring and early summer with little drop over the later summer months (data not shown). In the 2013/14 cropping season there was a significant interaction between canopy management treatment and date of measurement of fruit set ($P = 0.002$), however there were no significant differences in fruit set between the treatments at the final measurement date.

The canopy volume/ha after pruning was significantly greater in the selective pruning treatments compared with the hedged and topped treatments in the 2013/14 and 2015/16 seasons and when canopy volume was measurement following harvest, there was no significant effect of treatment on canopy volume/ha (Table 2). In 2014/15, there was no significant effect of treatment on canopy volume/ha following pruning but following harvest, the selective pruning treatments had significantly greater canopy volume/ha than the hedged and topped treatments.

Table 2. The effect of canopy management treatment, time within cropping season and cropping season on canopy volume/hectare (m^3).

	Hedged	Hedged + UCZ	Selective	Selective + UCZ	*P*
2013/14					
After pruning	17,233 b	17,370 b	19,500 a	19,970 a	< 0.05
Harvest	23,260	22,672	25,586	25,326	n.s.
2014/15					
After pruning	12,490	13,129	13,757	13,444	n.s.

Harvest	17,282 a	18,613 b	20,944 c	20,339 c	< 0.001
			2015/16		
After pruning	16,123 a	16,064 a	20,186 b	19,730 b	< 0.001
Harvest	22,584	22,351	22,015	21,100	n.s.

Across all cropping seasons, there were significant effects of canopy management treatment on NIS/ha, kernel/ha and total kernel recovery (Table 3). The Selective treatment had significantly greater NIS/ha than the Hedged treatment and the Selective + UCZ treatment. The Hedged + UCZ NIS/ha was non-significantly lower than that of the Selective treatment. Across the three cropping seasons the Selective treatment produced 1.98 tonnes kernel/ha, which was significantly greater than the Hedged and Selective + UCZ treatments and non-significantly greater than the Hedged + UCZ treatment. Total kernel recovery was significantly lower in the treatments that included UCZ than the Selective and Hedged treatments.

The interactions between cropping season and treatment were close to being statistically significant for NIS/ha (*P* = 0.089) and kernel/ha (*P* = 0.077) and so we have presented these interactions along with confidence intervals of the means to gain a more complete understanding of the effects (Table 4). NIS and kernel yield was greater in 2014/15 and 2015/16 than in 2013/14. The means and confidence intervals indicate that NIS/ha in 2013/14 for the Selective treatment was greater than the Hedged and Hedged + UCZ treatments; in 2014/15, the NIS/ha for the Selective treatment was greater than the Hedged + UCZ but not the Hedged treatment, due to high variability in the Hedged treatment; and in 2015/16, the NIS/ha for the Selective treatment and the Hedged + UCZ treatment were greater than the Hedged treatment. The means and confidence intervals indicate similar differences between treatments for kernel/ha.

Table 3. The effect of canopy management treatment on NIS yield per hectare (tonnes/ha), kernel per hectare (tonnes/ha) and kernel recovery (%) across the three years of the trial.

Treatment	NIS (tonnes/ha)	Kernel (tonnes/ha)	Kernel recovery (%)
Hedged	3.89 *a*	1.42 *a*	36.0 *b*
Hedged + UCZ	4.80 *bc*	1.70 *ab*	34.9 *a*
Selective	5.42 *c*	1.98 *b*	36.3 *b*
Selective + UCZ	4.57 *ab*	1.61 *a*	35.0 *a*
P	0.01	0.01	0.014

Table 4. The effect of canopy management treatment on the interaction between year and NIS yield per hectare (P = 0.089), kernel per hectare (P = 0.077) and kernel recovery (P = 0.038). Treatment means and confidence intervals of the means (parentheses) are presented.

Treatment	2013/14	2014/15	2015/16
		NIS (tonnes/ha)	
Hedged	2.37 (0.80)	4.88 (1.25)	4.42 (0.97)
Hedged + UCZ	3.09 (0.79)	5.07 (0.78)	6.25 (1.12)
Selective	4.44 (0.74)	6.00 (0.83)	5.81 (1.00)
Selective + UCZ	3.96 (0.89)	4.95 (1.21)	4.80 (1.77)
		Kernel (tonnes/ha)	
Hedged	0.82 (0.30)	1.87 (0.49)	1.57 (0.41)
Hedged + UCZ	1.03 (0.30)	1.84 (0.29)	2.21 (0.41)
Selective	1.58 (0.31)	2.26 (0.35)	2.10 (0.37)
Selective + UCZ	1.38 (0.36)	1.78 (0.46)	1.67 (0.65)
		Kernel recovery (%)	
Hedged	34.5 (1.57)	38.2 (0.89)	35.2 (1.70)
Hedged + UCZ	33.2 (1.49)	36.4 (1.36)	35.1 (1.52)
Selective	35.4 (1.38)	37.5 (0.91)	36.0 (0.84)
Selective + UCZ	34.6 (1.47)	36.0 (1.85)	34.3 (2.12)

Raceme production was significantly affected by the interaction between cropping season and tag height ($P < 0.001$) with significantly greater raceme production in the upper canopy than the lower canopy in two of three cropping seasons (Table 5). The interaction between cropping season and canopy management treatment was also significant ($P = 0.036$), with the main impact being a decrease in raceme production over the three cropping seasons of the trial from greater than 18.5 racemes/cm^2 BCA in 2013/14 to less than 4.7 racemes/cm^2 BCA in 2015/16. However, in 2013/14 there were differences between canopy management treatments with the Selective treatment producing more racemes/cm^2 BCA than all other treatments.

Table 5. The effect of canopy management treatment and cropping season and tag height and cropping season on raceme production on tagged limbs. Raceme production is presented as racemes per cm^2 branch cross-sectional area. There was significant interactions between canopy management treatment and cropping season (*P* = 0.036) and cropping season and height (*P* < 0.001). The LSD for the interactions between cropping season and canopy management treatment and between cropping season and tag height were 3.7 and 5.0, respectively.

Treatment	2013/14	2014/15	2015/16
		Canopy management treatment	
Hedged	18.5 cd	15.8 bc	2.95 a
Hedged + UCZ	20.2 d	12.1 b	4.27 a
Selective	24.7 e	14.9 b	3.55 a
Selective + UCZ	20.0 d	14.8 b	4.7 a
		Tag height	
High	25.2 d	19.0 c	5.0 ab
Low	16.5 c	9.8 b	2.7 a

Total light interception was measured in the 2013/14 and 2014/15 cropping seasons (Table 6). In 2013/14, the Selective and Selective + UCZ treatments had significantly greater total light interception than the Hedged and Hedged + UCZ treatments. In 2014/15, the Selective treatment had significantly greater total light interception than the Hedged and Hedged + UCZ treatments. There was no significant relationship between the total light interception in 2013/14 and the NIS yield/ha in 2014 or the total light interception in 2014/15 and the NIS yield/ha in 2015 (Fig. 1).

Table 6. The effect of canopy management treatment on total light interception.

Treatment	2013/14	2014/15
Hedged	86.1 a	86.5 a
Hedged + UCZ	84.9 a	85.9 a
Selective	90.1 b	88.9 b
Selective + UCZ	90.6 b	87.3 ab
P	< 0.01	< 0.05

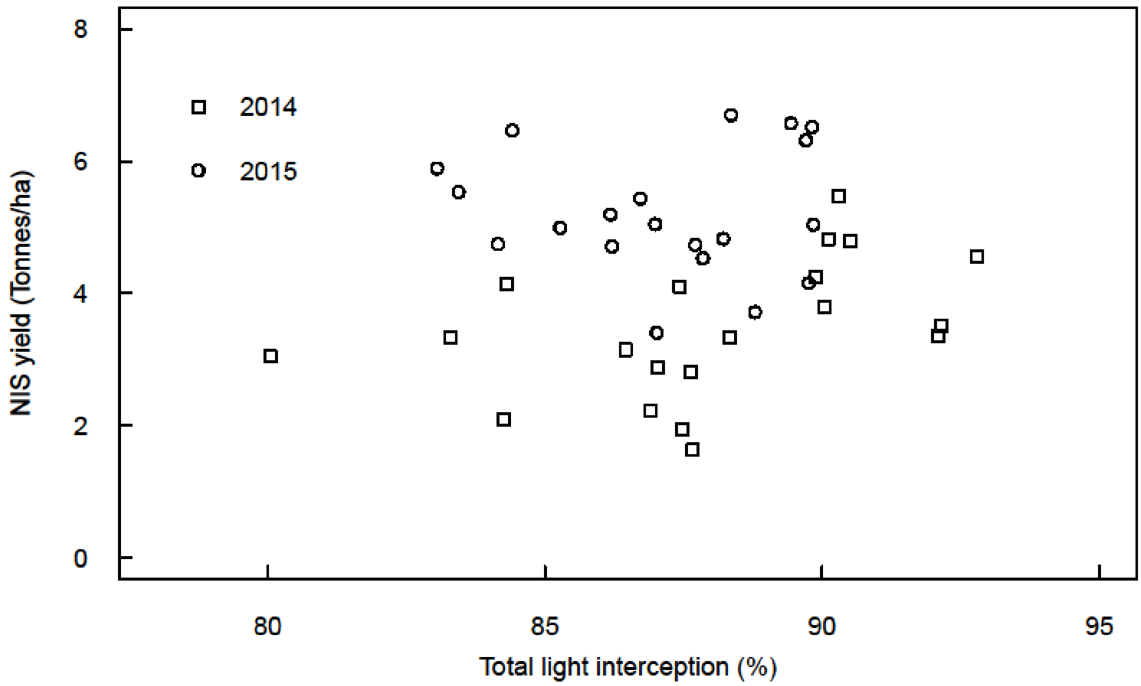

Figure 1. The relationship between total light interception and NIS yield/ha in 2013/14 and 2014/15. The relationship was non-significant (P > 0.05) in both seasons.

Discussion

Over the course of three consecutive cropping seasons, selective pruning was able to maintain canopy size within the high density orchard to acceptable levels and prevent additional crowding, while providing high levels of NIS production. When averaged across all cropping seasons, the Selective treatment produced 39% more NIS/ha than the Hedged treatment, with the difference being statistically significant and is consistent with the comparisons of selective pruning with hedging and topping reported by McFadyen et al. (2013).

Resources were allocated differently in the Hedged and Selective treatments. The shoot decapitation from the mechanical hedging led to significantly greater post-pruning shoot growth than the selective pruning (Table 1), where limbs were either removed entirely or back to a side branch. The greater re-growth in the Hedged treatment was apparent when measured at the end of the fruit set period and also when measured around fruit maturity, indicating that the excessive re-growth in the Hedged treatment occurred in a number of consecutive vegetative flushes. Vigorous shoot growth around the time of early fruit set, in response to hedging, has been shown to lead to competition for resources between vegetative growth and fruit development and subsequently reduce fruit set (McFadyen et al., 2012; Appendix 7). Competition for resources with the vigorous vegetative growth was likely a large contributing factor to the non-significant decreases in fruit set and significantly lower NIS and kernel yield observed in the Hedged treatment compared with the Selective treatment.

There was an indication that reduced competition between vegetative growth and fruit set was also responsible for the significantly greater NIS yield of the Hedged + UCZ treatment compared with the Hedged treatment (Table 4). The UCZ application reduced re-growth on the Hedged + UCZ compared with the Hedged treatment when measured at the end of fruit set in 2014 and 2015, with the means and confidence intervals indicating greater NIS yield/ha for the Hedged + UCZ compared with the Hedged treatment in 2016 but not 2015 (Table 4). UCZ has previously been reported to reduce post-hedging shoot growth in macadamia, although there was no effect of UCZ on yield (Wilkie et al. 2010). However, in the work of Wilkie et al. (2010), the hedging was undertaken earlier and so the post-hedging re-growth was less likely to coincide with fruit set.

Part of the reduction in yield in the Hedged treatment compared with the Selective treatment may also be due to reduced assimilate supply. The mechanical hedging and topping would have removed the outer layer of sun adapted leaves and exposed the less photosynthetically active shade adapted leaves from within the canopy. Huett (2004) reported that shade adapted leaves exposed to full sun operated at approximately 50% of the photosynthetic rate of sun adapted leaves one week after re-exposure and it took around four weeks for these leaves to reach the photosynthetic rate of sun adapted leaves. On the other hand, the selective pruning would have both retained some sun adapted leaves and exposed some shade adapted leaves from within the canopy. McFadyen et al. (2012) found that hedging in winter reduced the build-up of carbohydrate stores within the tree in the lead up to anthesis, probably partly due to reduced assimilate supply from the canopy and partly due to demand for assimilates by the post-pruning flush. Carbohydrate stores are likely to be affected to a lesser extent following selective pruning compared with hedging, due to less effect on the photosynthetic capacity of the canopy (described above) and less demand from the post-pruning vegetative flush. The overall effect for the current work is that the selectively pruned trees were likely to have had canopies capable of greater photosynthetic rates and greater levels of stored carbohydrates than hedged and topped trees in the weeks leading up to anthesis.

Total light interception is a strong driver of assimilate production and yield/ha (McFadyen et al., 2004; Olesen et al., 2007). In 2013 and 2015, the post-pruning canopy volume was significantly lower in the Hedged treatment compared with the Selective treatment (Table 2) and this reduced canopy volume resulted in reduced total light interception. The relationship between total light interception and NIS yield/ha (Figure 1), however, indicates that the lower total light interception in the Hedged treatment was not one of the reasons for its lower yield compared with the Selective treatment.

Fruit set declines in the more heavily shaded portions of macadamia canopies. (Olesen et al., 2011). This is supported by the significantly greater fruit set in the upper canopy limbs compared with the lower canopy limbs reported here (*Results*), as the upper canopy limbs would undoubtedly also be more highly irradiated than the lower canopy limbs. Poor light distribution has been proposed as one reason for declining yields in crowded macadamia orchards (McFadyen et al., 2004; Huett, 2004). The selective pruning and hedging and topping are likely to have had differing effects on light and leaf area distribution within the canopy but it is unclear whether this was a contributing factor to the differences in yield. By removing the outer layer of leaves, the hedging and topping would have increased the amount of light penetrating within the canopy, however, the vigorous re-growth on the canopy periphery probably resulted in the increased light penetration being short lived. The selective pruning probably also improved the transmission of PAR within the canopy through gaps created by removal of limbs. The effect of the selective pruning on light transmission was probably more persistent due to less vegetative re-growth (Table 1).

Alternate bearing cycles have been reported for macadamia, with heavy crop loads in one cropping season, reducing raceme production in the following cropping season and leading to reduced yield

(Wilkie, 2010). We found minor significant differences in raceme production between treatments in the first cropping season (Table 5). We also found large differences in raceme production between cropping seasons, with the final cropping season producing approximately five fold less racemes/cm^2 BCA than the first cropping season. Given that the final cropping season yields were greater than those in the first cropping season, it is unlikely that raceme production limited yield throughout the trial.

Relatively little research has been undertaken on vegetative growth limiting PGRs such as UCZ and PBZ in macadamia. The significantly greater NIS yield of the Hedged + UCZ compared with the Hedged treatment across the three cropping seasons of the trial (Table 3), however, indicates that there may be potential for commercial PGR applications aimed at reducing vegetative growth. It is important to note, however, that even though the overall NIS production of the Hedged + UCZ treatment was greater than the Hedged treatment across all cropping seasons, this was strongly affected by a large difference in the treatments in 2015/16 (Table 4). In this trial, the combination of hedging and UCZ improved NIS production and reduced shoot re-growth, but did not lead to an overall reduction in canopy dimensions at the end of the cropping season (Table 2). Whereas, Wilkie et al. (2010) reported hedging combined with UCZ did not affect yield but reduced shoot re-growth and resulted in an overall reduction in canopy dimensions at the end of the cropping season, compared with trees not treated with UCZ. The reduced yield for the Selective + UCZ compared with the Selective treatment (Table 3) appears to be inconsistent with the responses to UCZ for the hedged and topped treatments. There were also non-significant reductions in fruit set in the Selective + UCZ treatment relative to the Selective treatment, so the UCZ may have affected processes required in setting or retaining fruit. Plant growth regulators such as UCZ or PBZ should be further investigated as canopy management tools for macadamia production.

Implications for growers

The block of trees where the trial was conducted was nine years of age and had well and truly reached its maximum desirable canopy dimensions, with annual hedging and topping being undertaken to prevent crowding. The narrow row spacing coupled with the vigorous re-growth from the annual hedging and topping meant there was little alley-way remaining 12 months after hedging, probably partly due to the low crop loads being carried by the trees. The annual hedging and topping was being undertaken to prevent crowding and subsequent yield decline, but competition from the vigorous hedging re-growth was probably also reducing yield.

The selective pruning generally did not quite reduce the canopy dimensions to that of the hedging and topping, but due to less re-growth from the selective pruning the canopy dimensions were similar to those of the hedged and topped trees following harvest in two of the three cropping seasons. Although not measured in the trial, visual observations suggested that part of the increase in canopy dimensions in the selectively pruned trees was due to limb angle becoming more horizontal.

The selective pruning took approximately 5 minutes/tree to complete, equating to approximately 80 hours/ha. However, the pruning was being undertaken with a high level of care and consistency as it was for a research trial. It is likely that the operation would be able to be undertaken significantly faster by growers. Due to the relatively small individual tree size in the high density orchard, the limbs removed by the selective pruning were relatively small and were mulched on the ground by a tractor mounted mulcher. Much of the expense in commercial selective limb removal operations in conventionally spaced orchards with large crowded trees is from the chipping of the pruned limbs.

Over the course of the three year trial, the Selective treatment produced an average of 1.53 t/ha NIS (0.56 t/ha total kernel) more than the Hedged treatment. However, the selective pruning required to achieve this was time consuming, expensive and would require persistence over the long term by a grower to prevent canopy crowding. Additional management tools, such as growth regulator treatments or low vigour rootstocks, to slow re-growth and reduce the intensity and frequency of required pruning would improve the practicality of managing canopy crowding in high density macadamia orchards for growers.

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Appendix 6

Macadamia Rootstock Screening for high Performance: Genetic diversity , striking rate, early field growth and precocity

Mobashwer Alam, John Wilkie and Bruce Topp

Introduction

Rootstocks in tree crops like Macadamia play a vital role on the crop performance. As a part of a dual plant system, rootstocks translocate water and nutrients (Ferree and Carlson, 1987) to the above ground scion and thereby, regulate resource supply for growth and development of vegetative and reproductive parts. Having a strong tap root system with resistance to several biotic and abiotic factors, rootstocks increase adaptability to adverse environmental conditions. Studies in horticultural crops identified that rootstocks improve yield efficiency through reducing tree size and increasing precocity (Westwood, 1993). Till tody, very few investigations were made on macadamia rootstocks to increase production efficiency in Australia and limited to few genotypes only (Hardner and McConchie 2006; Hardner, 2004; and Neal et al., personal communication). As a part of “Transforming subtropical/tropical tree crop productivity” research project, this study designed on a diverse range of macadamia root stocks aiming to manage the vigour of the scion and increase production efficiency. In this report we presented the following aspects of this project:

- Graft success of macadamia seedling and cutting rootstocks.
- Establishment success in the field.
- Genetic diversity of the rootstocks,
- Early growth
- Precocity

Materials and Methods

Developing rootstock seedlings and cuttings

A total 30 genotypes were propagated for evaluation as rootstocks in this trial (Table 1). The genotypes consisted of 6 high performing rootstock cultivars; 3 elite cultivars with high breeding values for harvest index; 6 cultivars with high yield efficiency; 5 potential dwarf genotypes from Australian breeding program; 1 AVG resistant cultivar; 8 wild germplasm including 3 *Macadamia jansenii*, 3 *M. ternifolia* and 2 *M. tetraphylla*. Genotypes were propagated from seeds and cuttings during April to October 2014. Seedlings and cuttings were initially grown in the water controlled mist house and then transferred to the shed house for further development as detailed in Alam et al (2016).

Grafted tree development and management

Scions of HAES741 were whip-grafted on 20th June 2016 onto 245 seedlings and 188 cuttings Rootstocks of Macadamia. Initially two HAES741 cultivars, which were planted in December 1982 at the arboretum of Maroochy Research facility, Nambour, QLD, were cinctured in March 2016. On the same date of grafting, healthy and disease free grafting woods were collected from previously cinctured branches. Around 15-20 cm long scions were prepared by notifying the base as slanting cut and top as horizontal cut. Graft woods were sterilized with chlorinated water and kept wet by wrapping with water soaked towels. The top of the rootstocks were cut off at a point of approximately 25 cm above the soil and matched with the graft-woods of same thickness to help to get a good match between cambium layers of rootstock and scion. Sloping cut of about 30 mm long was made at the base of the scion and on the cut end of the rootstock using small wood plane. The wood plane was sterilized with alcohol at regular interval to avoid any contamination at the grafting region. The cut regions of rootstocks and scions were clamped together using cloth pegs to help hold the scion in place and were wrapped with tape from bottom upwards to produce an overlapping pattern. The scion and tape was painted with grafting mastic to prevent it drying out. The grafted plants were then transferred to shed house providing 30% shade with 4x15 minutes sprinkling per day. Plants were fertilized with soluble native fertiliser ('Searles Flourish') at 7 days intervals and were checked every second/third day to remove any new buds the rootstocks. Initial graft success was noted when the scion wood produces a new shoot and final graft success was counted before planting trial. All the plants were taken out of the shade house three weeks before planting to ensure proper hardening of the plants. Plants were watered two times a day during the hardening period.

Planting rootstock trial

The rootstock trial was planted on 4th April 2017 in a previously uncultivated paddock at Maroochy research facility, Nambour QLD. The soil of the land was prepared two month before planting and fertilized with recommended doses of nutrients after soil testing. The experiment was planted in an incomplete block design considering all the spatial variation across 8 rows, 20 columns and 5 blocks. 12 buffer plants were planted in the middle of the trial, where there was a "cricket pitch". The trail was planted as a 2 plant plots of grafted and non-grafted plants from same genetic source. Non-grafted trees of the pairs were planted in a zig-zag to make a staggered design. The trial consisted of 42 grafted cuttings and 106 grafted seedlings. In addition to the grafted trees, 4 cuttings of 'HAES741' were also included in the trial to make a comparison on growth and performance with the grafted scion. Second tree of the plots having 'HAES741' was filled with buffer plants. Buffer plants were planted around the trial to void any edge effect. Growth parameters of grafted and non-grafted trees will be evaluated in first 3-4 years; non-grafted ones from each pairs will be removed afterwards. The buffer plants paired with 'HAES741' will also be removed.

Table 1. Number of progeny in each of the open-pollinated families from diversified origin of macadamia genotypes used in the precocity trial. OP = open pollinated, HVP= Hidden Valley Plantation, Beerwah Australia; HAES= Hawaiian Agricultural Experiment Station, Hawaii, USA; Aus Heritage = Australian Heritage Cultivars , Aus Elite= Elite selections from the Australian macadamia breeding program, and Wild jansenii= *Macadamia jansenii* wild germplasm

Family	Source	Parentage	# Progeny
HAES246	HAES	*Macadamia integrifolia*	21
HAES344	HAES	*Macadamia integrifolia*	21
HAES788	HAES	*Macadamia integrifolia*	24
HAES791	HAES	*Macadamia integrifolia*	19
HAES814	HAES	*Macadamia integrifolia (?)*	21
A268	HVP	HAES344 OP	12
A376	HVP	-	21
A38	HVP	*Own Choice OP*	20
A4	HVP	Renown OP(x Own Choice)	19
A538	HVP	-	20
BAMAM02-6-3	Aus Elite	NG8xHAES762	21
BQBRS97-2-46	Aus Elite	HAES 246xA16	21
BQBRS97-6-16	Aus Elite	A16xHAES 814	21
BQBRS98-10-111	Aus Elite	HAES 246xA16	21
BQBRS98-10-93	Aus Elite	A16xHAES 781	11
BQBRS98-11-35	Aus Elite	HAES 849xDaddow	18
BQBRS98-11-80	Aus Elite	HAES 814xA16	5
BQBRS98-13-115	Aus Elite	HAES 842xDaddow	12
BQBRS98-14-25	Aus Elite	A16xHAES 814	18
BQBRS98-14-93	Aus Elite	DaddowxHAES 246	16
BQBRS98-15-37	Aus Elite	DaddowxA16	21
BQBRS98-16-41	Aus Elite	DaddowxA16	23
BQBRS98-6-73	Aus Elite	HAES 842xA16	23
BQBRS98-6-79	Aus Elite	A16xHAES 814	14
BQBRS98-7-109	Aus Elite	HAES 842xDaddow	18
BQBRS98-7-74	Aus Elite	DaddowxA4	14
BQBRS98-8-87	Aus Elite	HAES 816xA4	13
BQBRS98-9-72	Aus Elite	HAES 842xDaddow	21
M141	AUS Elite	-	20
D4	AUS Heritage	-	22
Daddow	AUS Heritage	-	21
Macadamia jansenii	Wild jansenii	*Macadamia jansenii*	12
Grand Total			584

Genetic diversity analysis

27 rootstocks were genotyped in a Diversity Array technology (DArT) platforms using 4174 DArTSeq based SNP markers (Kilian et al., 2012). Principal Coordinates Analysis (PCoA) and relationships based on marker data explain the genetic diversity among the genotypes. A genetic dissimilarity matrix was constructed using DARwin v. 6.0.13 (Perrier et al., 2003) software to identify the genetic relationships among the genotypes as illustrated in the

Neighbour-Join dendrogram. Clades strength in the dendrogram was tested by 20,000 bootstrap analyses. GenAlEx v. 6.5 was used to perform PCoA, which was based on the standardized covariance of genetic distances calculated for the markers under study, using 999 permutations.

Phenotypic measurements

We phenotyped the following growth characteristics in November 2017 and May 2018 (Table 2). We estimated the growth rate for all the parameters, which includes Total height increase (THI), canopy width increase (CWI), canopy depth increase (CDI), rootstock height increase (RHI), Rootstock trunk circumference increase (RCI), scion height increase (SHI), and scion trunk circumference increase (SCI). Flowering (FLW) was recorded every year. Total nut mass (TNM) was measured as an indicator of nut in shell yield.

We also phenotyped for flowering in August-September 2018 and 2019.

Data analysis

Genetic diversity was analysed using DARwin 6.0.13 software. Data on graft success, growth data were analysed using REML mixed model in Genstat19 (Payne, 2000) platform.

Table 2. Growth characteristics measured in the macadamia rootstock trial.

Traits of study	Units	Measurement period			
		Nov-17	May-18	May-19	May-20
Total height (THT)	cm	√	√	√	√
Canopy width along (CWA)	cm	√	√	√	√
Canopy depth across (CDA)	cm	√	√	√	√
Rootstock Height (RHT)	cm	√	√	√	√
Rootstock Trunk circumference(RTC)	cm	√	√	√	√
Scion height (SHT)	cm	√	√	√	√
Scion trunk circumference (STC)	cm	√	√	√	√
Flowering (FLW)	Y/N		√	√	√
Total nut mass (TNM)	g				√

Results and Discussion

Genetic diversity

A dendogram (Figure 1) was constructed using DARwin 6.0.13 to elucidate the genetic relationship among the macadamia rootstocks. A total of 4174 DArTseq based SNP markers were used to produce the dendogram. Genetic dissimilarity between pairs of rootstocks were estimated and was varied from 0.31 to 0.29 with an average of 0.18. Genetic dissimilarity was smallest among the accessions of Macadamia jansenii (M.jan1, M.jan2 and M. Jan3) and peaked between rootstock pairs 'A4' and "M.jan2", although the accessions of all *Macadamia jansenii* and *Macadamia ternifolia* showed greatest dissimilarity with all existing cultivars and elite genotypes used in this study.

In the dendogram, four main differentiating clusters of rootstocks were identified. First cluster contains all the wild germplasms. Cultivars "Beaumont", 'D4', "IMCDW" and 'A268' also formed cluster 1 and indicated close relationship with *M. tetraphylla* (M.tet1). Four dwarf rootstocks ("BDW1", "BDW2", "BDW3" and "BDW4") showed very close genetic relationships and formed the second cluster with "BHY1" and "BHY4". Existing high performing rootstock 'HAES842' and AVG resistant cultivar "Daddow" were clustered with Elite cultivars "BHI1", "BHI2", "BHY3" and "BHY5". The fourth cluster includes high performing macadamia rootstocks 'A4' and 'A16', and elite breeding lines "BHI3" and "BHY2".

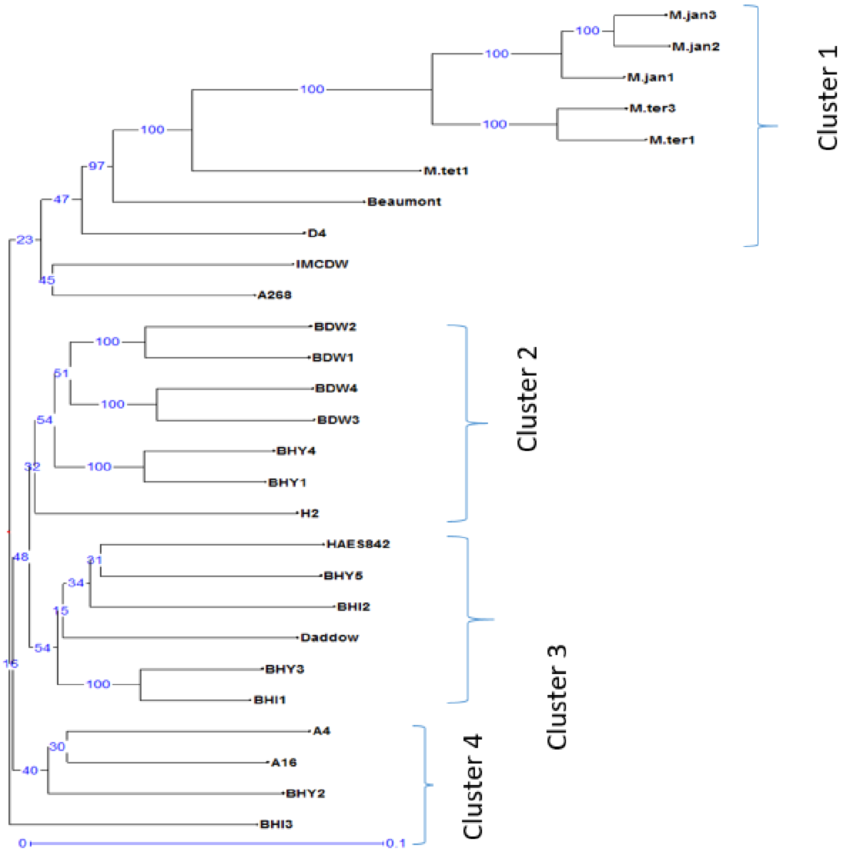

Figure 1. A dendogram showing genetic relationships among the macadamia rootstocks using 4174 DArTSeq based SNP markers.

Principal coordinate analysis (PCoA) was performed through similarity matrix. First two principal components generated two distinct axes explaining 58.1% and 6.1% of the total variance respectively (Figure 2). PCoA identified four distinct groups with two group having single representative only. All the commercial cultivars, elites for high selection index and high performance, and dwarves (Except IMCDW) formed one group. Accessions from wild *M. jansenii* and *M. ternifolia* formed a separate group. IMCDW and M.tet1 were distinctly separated from both groups. Results thus clearly identified a wide range of diversity in the rootstock trial.

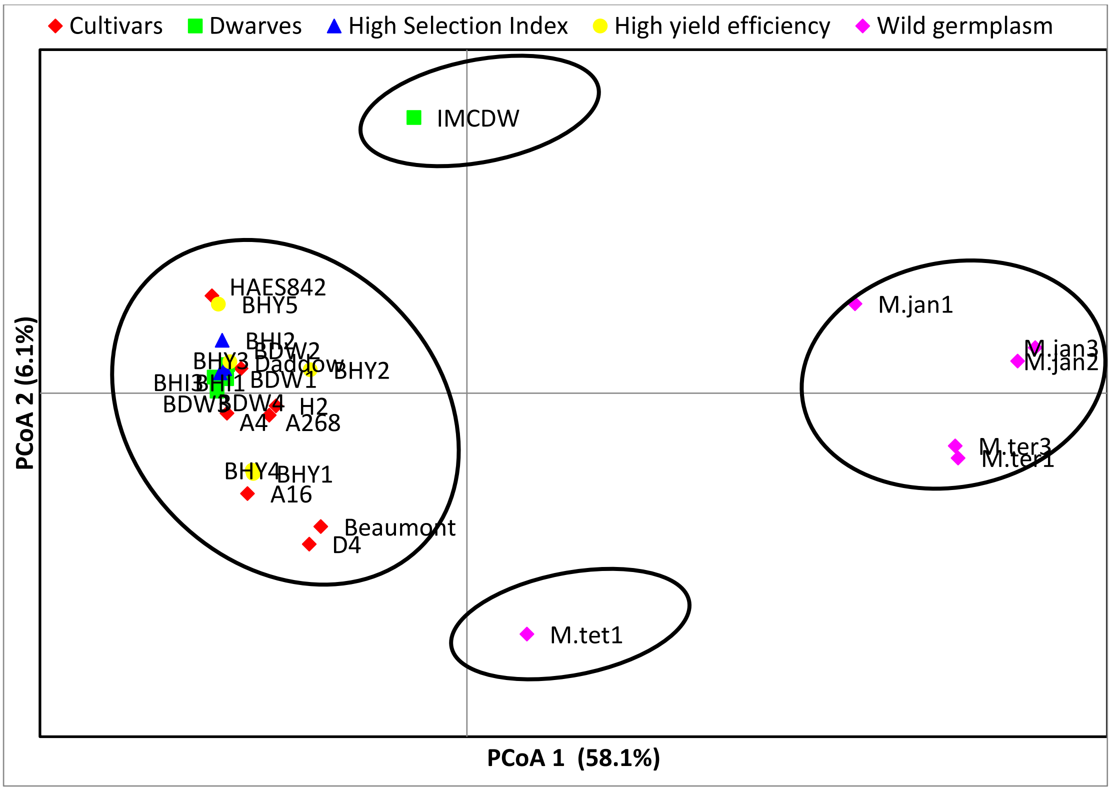

Figure 2. Principal Co-ordinate analysis of macadamia rootstocks using 4174 DArTSeq based SNP markers.

Graft success

An existing commercial cultivar 'HAES741' was grafted onto a total of 245 seedlings and 145 cutting rootstocks. There was significant variation in graft success of macadamia rootstock genotypes and methods of propagation (data not shown). The range of graft success varied from 0% to 100% in seedlings and 0% to 89% in cuttings (Figure 3). Over all, macadamia seedlings had greater graft success than that of cuttings. Variation between initial and final graft success was greater in cuttings than that of seedlings. Mostly dwarf genotypes and wild germplasm showed significant death of initially shooted grafted seedlings. While in cuttings, most of the genotypes had significant death of initially shooted grafted trees as observed by the reduction of graft success at final evaluation (%FGS).

In both seedlings and cuttings, Beaumont had greater graft success than other cultivars. Seedlings of "BHY1" and "M.tet2" were most compatible rootstocks with 'HAES741' due to their high rate of graft success in comparison with other seedling genotypes. Where as in cuttings, "BHI2", 'D4' and "Daddow" were on top for IGS and FGS. We previously reported that all these five genotypes had very high early vigour in terms of stem diameter. It is also to be noted that "M.tet2" was not tested cutting rootstock.

Among dwarves, BDW1 seedlings had higher rate of IGS (~80%) than "H2", whereas all the cuttings showed very low (0 to 34% for IGS and 0-12% for FGS) graft success. But FGS of "BDW1" seedlings was reduced to ~50%. Similarly, "IMCDW" had greater IGS (~70%) and lower FGS (~25%). Graft success was hugely truncated when 'HAES741' was grafted onto the seedlings and cuttings of Wild *M. jansenii* and *M ternifolia*. Though IGS of "M.tet1" seedlings was high (~60%), but was reduced significantly during final count (~25% FGS).

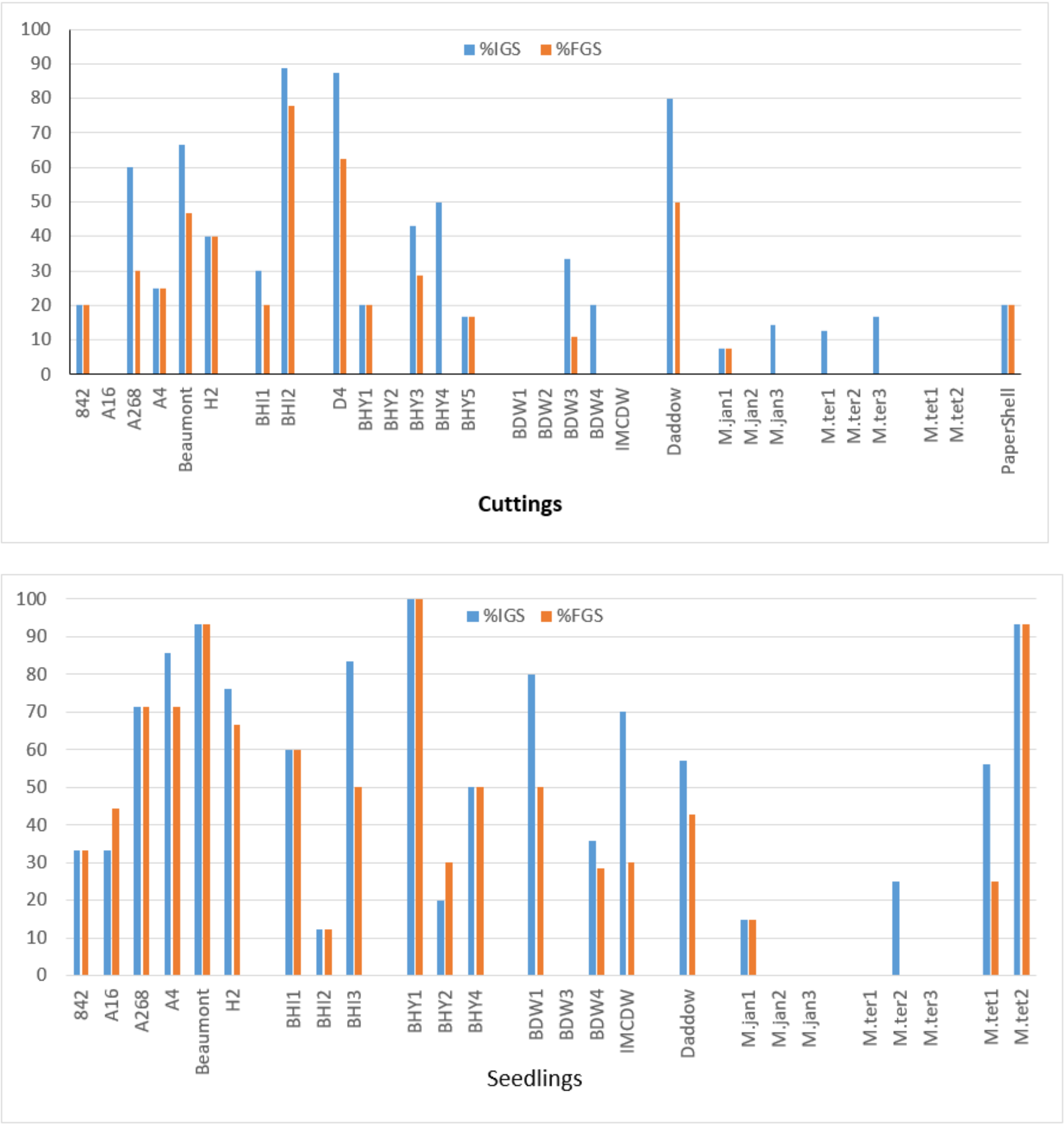

Figure 3. Initial and final graft success rate in macadamia seedling and cutting rootstocks.

Among the existing cultivars, seedlings and cuttings of A268 had greater graft success. Seedlings of D4 and Daddow and Cuttings of A4 also had higher rates of graft success. Among the cultivars, A268 seedlings and cuttings produced more grafted trees than H2; and cuttings of D4 and Daddow also had greater graft success rates than H2 for scion cultivar HAES741. It was observed that graft success was higher in genotypes with greater stem diameter increase rate (GRD), which was also supported by the finding of Mng'omba et al. (2010) in mango. The lower rate of graft success in cuttings may be due to their inferior root system than that of seedlings ((Bell and Bell, 1993).

Establishment success in the rootstock trial

Establishment success of the rootstock trial is presented in Table 3. Initial establishment success was evaluated six weeks after planting. Most of the genotypes had 100% established success in the field trial with an average of 99% in seedlings and 98% in cuttings. Only 1 grafted seedlings of 'HAES842' and an 'A268' grafted cutting were died.

Table 3 Summary of grafted tree plantation and tree establishment in macadamia rootstocks used in Small tree high productivity trial at Nambour (on 15/05/2018)

Rootstocks	Types	Seedlings			Cuttings		
		NGT	GTP	PTE	NGT	GTP	PTE
842	High Performing Rootstock Cultivars	9	3	67	10	2	100
A16		9	4	100	2	-	
A268		7	5	100	10	3	67
A4		14	10	100	4	1	100
Beaumont		15	13	100	15	7	100
H2		21	12	100	5	2	100
BHI1	High Index Value	10	6	100	10	2	100
BHI2		8	1	100	9	7	100
BHI3		6	3	100		-	
D4	ficiency		-	-	8	5	100
BHY1		10	10	100	10	2	100
BHY2		10	3	100		-	
BHY3			-	-	7	2	100
BHY4		2	1	100	4	-	
BHY5			-	-	6	1	100
BDW1	Potential Dwarves	10	5	100	2	-	
BDW2			-	-	8	-	
BDW3		2	-	-	9	1	100
BDW4		14	4	100	5	-	
IMCDW		10	3	100	3	-	
Daddow	AVG Resistant	7	3	100	10	5	100
M.jan1	Wild Species:	20	3	100	13	1	100
M.jan2	*M. jansenii*	8	-	-	4	-	-
M.jan3		10	-	-	7	-	-
M.ter1	Wild Species:	1	-	-	8	-	-
M.ter2	*M. ternifolia*	8	-	-	2	-	-
M.ter3		3	-	-	6	-	-
M.tet1	Wild Species:	16	4	100	6	-	-
M.tet2	*M. tetraphylla*	15	13	100		-	-
PaperShell	Others		-	-	5	1	100
Total		245	106	99	188	42	98

NGT= number of grafted tree, GTP= Grafted tree planted, PTE= Percent tree establishment.

Variability in growth parameters in grafted trees

Combined analysis of growth of grafted trees from November 2017 to May 2018 showed that there was significant genotypic variation in tree height increase. Significant variation between seedling and cuttings were observed for the growth of canopy width, canopy depth and shoot trunk circumference (Table 4).

Table 4. F-statistical probability estimated from REML mixed model

Components	THI	CWI	CDI	RHI	RCI	SHI	SCI
Genotype	0.039	0.168	0.858	0.372	0.248	0.929	0.189
Type (seedling vs cuttings)	0.146	0.01	0.004	0.102	0.913	0.112	0.009
Genotype x Type	0.935	0.367	0.353	0.824	0.798	0.921	0.574

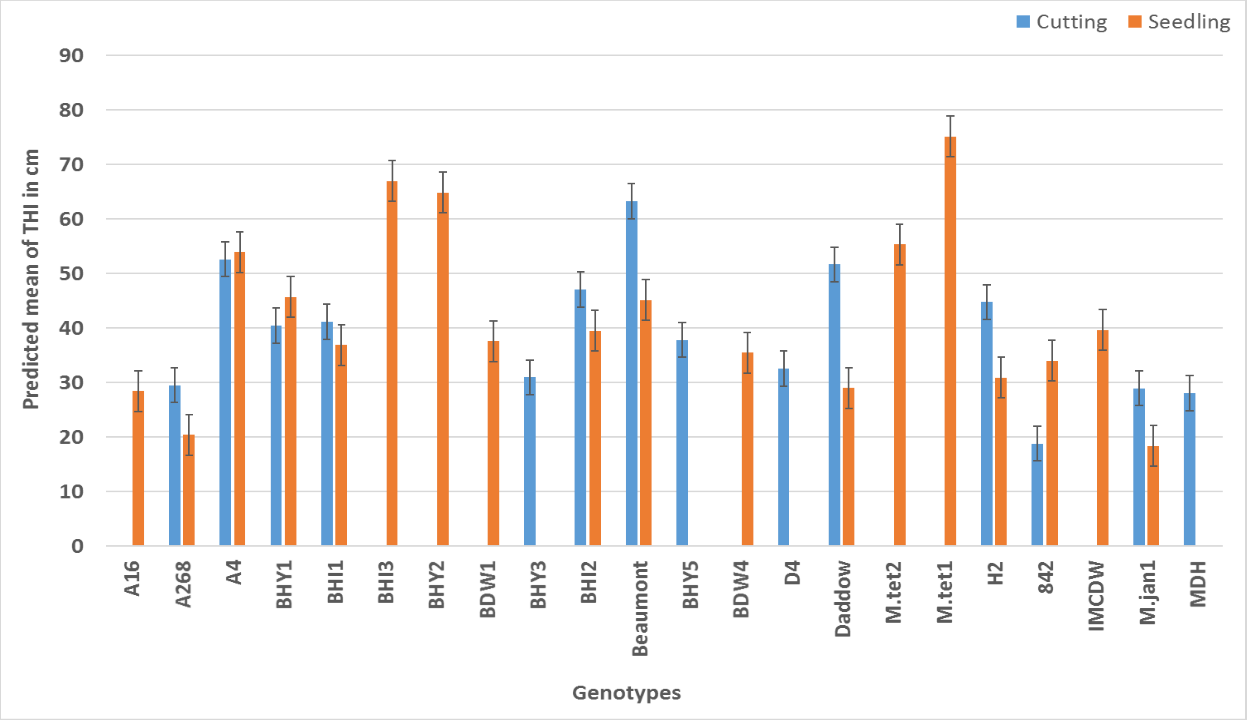

Figure 4. Variation in tree height increase (November 2017 to May 2018) in seedlings and cuttings of different genotypes

In total 21 genotypes were compared for growth parameters. Pairwise comparison between seedling and cuttings could be made only between 10 genotypes. THI varies from 19 cm to 63 cm in cutting and 18 to 75 cm in seedlings. Among seedlings, THI was greatest in “M.tet1” followed by “BHI3” and “BHY2”. THI was least in the seedlings of “M.jan1” followed by ‘A268’. In cuttings, grafted trees of “842” showed least vigour in THI and ‘Beaumont” was greatest.

Table 5. Summary of flowering data of grafted and ungrafted trees.

Type	Number Flowering 2018	Number Flowering 2019	Total Plants
Grafted	5	80	142
Scion (HAES741)	0	0	4
Ungrafted	8	50	144
Total	13	130	290

REML mixed model analysis shows that there was no significant difference among the genotype and types (cuttings vs seedlings). But significant genotype X type interaction indicated that seedlings of some genotypes can be precocious than cuttings, and vice versa (Table 6).

Table 6. Wald statistics of fixed components.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Genotype	35.52	23	1.54	56	0.09
Type	3.99	1	3.99	56	0.05
Genotype x Type	22.42	9	2.49	56	0.02

Table 7: Predicted means and standard errors (SE) of flowering propensity in the rootstock trial.

Genotype	Predicted mean		SE	
	Cutting	Seedling	Cutting	Seedling
MDH	0.77	*	0.51	*
BHY5	1.41	*	0.51	*
BHY3	1.42	*	0.37	*
D4	0.75	*	0.25	*
BHI2	0.80	1.77	0.22	0.53
Daddow	0.29	1.26	0.24	0.31
842	0.09	1.14	0.40	0.38
BHI1	0.11	1.12	0.37	0.22
BDW1	*	0.93	*	0.23
M.jan1	0.36	0.74	0.55	0.30
A268	0.91	0.74	0.37	0.27
BHI3	*	0.71	*	0.37
BHY2	*	0.71	*	0.38
BDW3	*	0.65	*	0.55
M.tet1	*	0.56	*	0.20
M.tet2	*	0.52	*	0.15
Beaumont	0.68	0.43	0.24	0.15
BDW4	*	0.42	*	0.27
A4	0.62	0.41	0.55	0.17
A16	*	0.38	*	0.27
BHY1	0.94	0.34	0.41	0.17
H2	0.97	0.30	0.38	0.16
IMCDW	*	0.30	*	0.30
BHY4	*	0.00	*	0.55

Variability in Flowering

In 2018, a total of 13 trees were flowered, of which five were grafted trees. Interestingly, all the grafted flowering trees have seedling rootstocks (BAMAM02-6-3, A268, A4, GTFRS00-23-30, and BALLO02-6-76) and non-grafted ones are cuttings of Beaumont and D4. In 2019, almost equal percentage of grafted and not grafted trees flowered (Table 5).

Predicted flowering propensity of each genotype is presented in Table 6. Flowering propensity in cuttings varies from 0.09 (HAES842) to 1.42 (BAMAM02-7-23). While, in seedlings, BAMAM02-9-28 (1.77) shows the greatest potentiality of precocity and BBAFF 15-24 was the least. Interestingly, seedlings of genotype BALLO02-6-76 were precocious than cuttings, as suggested m genotype x type interaction (Table 7).

Conclusion

This study identified that the graft success depends on the genetic background of the rootstocks and compatibility with the scion. In comparison, seedlings were found to be more successful in grafting than cuttings, although one genotype outperformed other existing high performing cultivars for graft success as cutting rootstock. Establishment success of the rootstock trial was also high. Inclusion of wild germplasm increased the diversity in the rootstock trial. “M.jan1”, ‘A268’ and ‘A16’ seedlings are promising to reduce early vigour in grafted trees. Cuttings of “842” had also reduced total height of the grafted trees. We also presented the variability in early flowering among the rootstock genotypes. To select superior rootstock for high performance, we still need to continue the evaluation of growth and productivity parameters for the next five years. So, the following tasks are yet to be done:

- Management of rootstock trial sites over the next five years.
- Characterising for growth, flowering and yield parameters.
- Superior rootstock selection for high performance.
- Identifying the mechanism of rootstock-scion interaction.

Appendix 7
The effect of the relative timing of growth on resource allocation in macadamia

Toegel H, Hanan J, Brown P, Wilkie JD

Abstract

Commercial macadamia orchards are routinely hedge pruned around anthesis in order to maintain long-term orchard health and manageability. This often results in a reduction in yield, which has been attributed to the concurrence of reproductive development and post-hedging vegetative growth, both competing for carbohydrates. We aimed to both improve our understanding of carbohydrate partitioning and to explore the possibility of manipulating both vegetative and reproductive growth in order to improve yield and reduce shoot growth. Our hypothesis was that the timing of growth of one organ, compared to others, determined whether it competed strongly. We tested this hypothesis with two trials, in which we applied staggered tip-pruning treatments. In one trial, we looked at vegetative growth and the competition between shoots. In the second trial, tip-pruning treatments were timed before, during and after anthesis. We examined the timing of the post-pruning flush, relative to reproductive growth, and the competition between them. There were strong treatment effects on shoot and fruit characteristics, leading to differences in fruit abscission and yield, as well as effects on the extent of shoot growth. These results suggest that partitioning of carbohydrates between simultaneously growing organs is governed by their relative growth rates, and that a deliberate and timely hedging regime could improve fruit retention and yield.

Introduction

Macadamia (*Macadamia integrifolia* Maiden and Betche, *M. tetraphylla* Johnson, and hybrids) is an evergreen tree native to subtropical, eastern Australia, cultivated for its nutritious nut and usually grown in hedgerows. Canopy crowding is a common phenomenon in macadamia orchards and causes problems such as restricted machinery access, reduced spray penetration and insufficient light levels for ground cover survival, increasing the risk of subsequent soil erosion. In the long term, crowding has also been shown to reduce yield (McFadyen *et al.* 2004). Canopy management usually consists of tree-size control by means of annual mechanical hedging, which addresses the aforementioned orchard management issues, but has the potential to lead to a decline in yield as well (McFadyen *et al.* 2005).

One of the factors determining yield is the availability of carbohydrates to developing fruit from storage reserves and current photosynthesis. As carbohydrates are required for all growth but are available only in finite amounts, they are partitioned to the growing organs. Partitioning of carbohydrates depends on its availability in leaves from current photosynthesis and from storage organs (source), the demand of growing organs (sink), and the transport paths that connect them (Minchin and Thorpe 1996). Different models have been developed trying to explain the mechanism of carbohydrate partitioning (e.g. Grossman and Dejong 1994; Marcelis 1996; Minchin and Thorpe 1996; Brown *et al.* 2019). These models have concentrated on sink activity as an indicator of ‘sink strength’ (a term used to describe a sink’s ability to attract photosynthate), often using the potential growth rate (i.e. the growth rate when supply is not limited) to model partitioning to sinks. Their focus, however, has been on predicting carbohydrate flow under different circumstances, rather than changing its outcome.

Given that flowering and early fruit development in macadamia coincide with spring vegetative growth, resource demand, including that of carbohydrates, is high during this time. The carbohydrate content of branches declines in this period (McFadyen *et al.* 2011), showing that storage carbohydrates are being used to satisfy demand from developing fruit and shoots that cannot be supplied by current photosynthates, and suggesting that organs may compete with each other for limited supplies. Competition has been shown to occur between different organ types, notably vegetative and reproductive organs, and between organs of the same type, such as fruit. For example in apple, trees with high crop loads had reduced fruit size compared with trees with low crop loads (Palmer 1992). Similarly in macadamia, heavily flowering trees had a lower fruit set per raceme than trees with few racemes (Wilkie 2010). Competition between different organ types has been demonstrated in multiple ways. Trees on which flowers or fruit had been thinned produced more vegetative growth than unthinned trees (Berman and Dejong 2003; Wilkie 2010). Conversely, when vegetative growth was reduced, such as through the use of growth retardants or the manual removal of new growth, fruit abscission tended to be lower (Iglesias *et al.* 2003; McFadyen *et al.* 2011). Generally, the competition between vegetative and reproductive organs can be described as an inverse relationship between their respective growths.

In macadamia, flowers, borne on racemes, reach anthesis in early spring. Fruit set is followed by two to three months of heavy fruitlet shedding, referred to as the premature fruit abscission period. Mature fruit abscise, with some differences between cultivars, from early autumn until late winter, and are regularly harvested throughout this period (Trueman 2013). Vegetative growth occurs in flushes throughout the year, with major flushes developing in spring and late summer (Stephenson *et al.* 1986). Hedging, usually performed in winter or early spring, influences the timing of the flush cycle by triggering shoot growth from the axillary buds behind the pruning cuts (Olesen *et al.* 2006).

As hedging affects vegetative growth, its timing can have an impact on partitioning of carbohydrates and competition between vegetative and reproductive organs. Studies have found a significant effect of the timing of hedging on fruit abscission and yield (Olesen 2005; Wilkie *et al.* 2010; McFadyen *et al.* 2012). When trees were hedged at anthesis, yield was lower than with other hedging times. In this case, post-hedging shoot growth coincided with early fruit development and it was deduced that fruit in this stage was less successful in the competition for carbohydrates than the simultaneously growing flush. When macadamia hedging was carried out in November (seven weeks after anthesis), thereby allowing fruit to develop further before the commencement of the post-hedging flush, yield was comparatively higher (McFadyen *et al.* 2012). Studies in both citrus (Mehouachi *et al.* 1995) and apple (Berüter and Droz 1991) have shown that young fruit is more likely to abscise due to a carbohydrate shortage than older fruit. These results are consistent with the assumption that an organ's ability to import carbohydrates changes during its development, with a lower ability early in its growth, followed by an increased ability to import carbohydrates once it is further developed.

Currently, the most common timing for hedging macadamia trees in Australia is in winter or early spring in the period between harvesting in one season and the beginning of flowering in the following season. However, this practice is mainly based on logistical considerations and, as mentioned above, may lead to a reduction in yield. Here, we aimed to both improve our understanding of carbohydrate partitioning in macadamia, and to explore the possibility of manipulating both vegetative and reproductive growth for the purpose of increasing yield and reducing shoot growth. The work consists of two trials, in which staggered tip-pruning was used to create a range of shoot maturities. Thus, we tested the hypothesis that organs in different growth stages exhibit different levels of competitive strength, and that this difference in competitive strength has direct consequences on the extent of shoot growth, fruit abscission and yield.

Materials and Methods

Trial 1: The effect of the relative timing of shoot growth on shoot characteristics.

This trial was conducted within an experimental orchard at the Bundaberg Research Facility in Bundaberg, Queensland, Australia (24.9°S, 152.4°E, elevation 29 m) on well-watered and well-fertilised trees. The selected trees of variety '741' were two years old at the time of the trial and were planted at 8 m between rows and 4 m between trees within the row. At the time, the trees were approximately 2.1 m tall and 1.2 m wide, with all surfaces of the canopy well irradiated.

The trial comprised two treatment factors, arranged as an unbalanced split plot design with five replicates. The first treatment factor, pruning intensity, consisted of two levels: partial pruning and complete pruning (all pruning was tip-pruning). On the partially pruned trees, only two shoots were tip-pruned; whereas on the completely pruned trees, all shoots were tip-pruned. The partially pruned trees were considered an experimental control, in which there was minimal resource restriction at the whole-tree level for shoot growth on the two pruned stems. The second factor, pruning time, had different numbers of levels for the two levels of pruning intensity. For the partially pruned trees, there was one level of pruning time: pruning on day 1. For the completely pruned trees, there were six levels of pruning time: pruning on day 1, 6, 9, 13, 16 and 20. Within each replicate, one of six trees was randomly allocated to the partial pruning level (control). On the remaining five trees, which were allocated to complete pruning, all but two shoots were pruned on day 1. The remaining two shoots were pruned either on day 6, 9, 13, 16 or 20. Thus, the pruning intensity factor was at the whole-plot level (trees) and the pruning time factor was at the sub-plot level (shoots within trees). The trial commenced on 20/01/2016 (day 1).

The purpose of pruning all but two shoots of the completely pruned trees on day 1 was to induce a highly synchronised flush across the tree and create a high level of competition for carbohydrates between the growing shoots. The remaining two shoots were pruned at different dates in order to create a variation in the timing of new shoot growth and thus in the growth rates on those two shoots, relative to the majority of shoots on each tree.

Tip-pruning involved the removal of approximately half of the most distal growth unit of the relevant number of vegetative axes within the canopies. A growth unit is the length of stem in one axis that grows in one flush. Tip-pruned shoots were tagged in order to follow the growth of the post-pruning flush. On the control trees, the two pruned shoots were tagged. On the completely pruned trees, the two later pruned shoots were tagged, as well as two randomly chosen shoots that had been pruned on day 1. The date of the first axillary bud burst following tip-pruning was recorded for each tagged shoot, and length and node number of that first shoot subsequently assessed twice weekly until shoot elongation ceased. The date of terminal bud burst from the second post-pruning flush on that first shoot was also recorded, at which time the shoot was harvested and its dry weight measured (shoots that had not commenced their second flush by 29/06/2016 were harvested on that date). Additionally, the length and node number of all other shoots of the first post-pruning flush of each tagged stem were measured. A relationship between shoot length and dry weight of the harvested mature shoots was used to calculate the total dry weight per tagged stem, based on the measured lengths. A separate relationship between length and dry weight was established for developing shoots by recording length and dry weight of five random shoots of the first post-pruning flush on each day that shoot lengths were measured. This relationship was used to calculate dry weight throughout shoot development. Linear regressions were used to calculate these relationships, with the length of the developing shoots quadratically transformed to account for curvature.

In order to account for differences in temperature throughout the trial, thermal time was used for the analyses. For the calculation of growing degree days (GDD) for each calendar day, a base temperature of 12°C (Trochoulias and Lahav 1983; Wilkie *et al.* 2009) was subtracted from the daily mean temperature (mean of maximum and minimum temperatures), using data obtained from an automatic weather station at the Bundaberg Research Facility.

The first part of the data analysis comprised nonlinear regressions describing the increase in length and shoot dry weight over thermal time. Logistic curves were fitted with ‘R’ (R Core Team 2017), using the equation $y = a/(1+e^{b(c-x)})$, with a, b and c as parameters, y as the measured shoot length or calculated shoot dry weight, and x as cumulative GDD since bud burst.

As the second part of the analysis, linear mixed models were fitted using GenStat (VSN International 2015) to determine the relationships between the delay in shoot bud burst from the first day of pruning in GDD and predicted final shoot dry weight (the nonlinear regression parameter a), as well as its maximum growth rate (calculated from the nonlinear regression: $a \cdot b/4$; all growth rates in the analyses are absolute growth rates). Linear mixed models were also used to identify treatment effects on multiple measured and calculated variables. Variables were at times natural log transformed to account for curvature in the response or unequal variance.

Certain measurements had to be excluded from statistical analyses. One shoot, pruned on day 13, was excluded from the regression because curves could not be fitted to it, as were all shoots on two trees that became diseased during the trial (pruned on days 9 and 20). Additionally, all control shoots were excluded from the variables ‘predicted final shoot dry weight’ and ‘maximum growth rate – shoot dry weight’. They grew considerably longer than the shoots used for calculating developing shoot dry weight, which made the calculations inaccurate for control shoots.

Trial 2: The effect of the relative timing of vegetative and reproductive growth on shoot and fruit characteristics.

This trial was based on the findings of trial 1 and shares some methodology. The main difference was the inclusion of fruit in the observations. It was conducted on trees of variety ‘A203’ at the Bundaberg Research Facility, which were planted at 8 m between rows and 4 m between trees within the row. At the time of the trial, the trees were three and a half years old and had not reached canopy closure within the rows, thus allowing even irradiation on all sides.

The trial was laid out as a randomised complete block design with five replicates and six treatments, including a control. Treatments were applied to single tree plots and consisted of successive tip-pruning before, during and after anthesis, which was estimated to occur on 26/09/2017. One tree per replicate was treated every two weeks, starting four weeks before, and ending four weeks after anthesis. The control trees remained unpruned. Tip-pruning involved the removal of approximately the one most distal growth unit from all shoot axes on the tree that extended to the periphery of the canopy. According to the estimated date of anthesis, the tip-pruning dates for the five treatments were 29/08/2017, 12/09/2017, 26/9/2017, 10/10/2017 and 24/10/2017. The treatments were categorised as weeks before (BA) and weeks after (AA) the estimated date of anthesis, i.e. 4BA, 2BA, A, 2AA, 4AA.

The purpose of tip-pruning at this time was to induce a strong vegetative flush that could potentially compete strongly with reproductive organs. The staggered timing of the different treatments generated a range of shoot maturities around the period of fruit set and early fruit development, with the intention of creating different combinations of developmental stages in shoots and fruit within individual trees.

Four pruned shoots (on the tip-pruned trees) or four terminal growth units (on the control trees) were randomly selected on each tree, in order to monitor their growth. The date of the first axillary bud burst after tip-pruning was recorded, and shoot length and node number of that first shoot subsequently assessed twice weekly, until shoot elongation ceased. On the control trees, the first emerging shoot out of any terminal or axillary bud of the selected growth unit was chosen for monitoring. Upon the commencement of the second post-pruning flush, shoots were measured and harvested according to the protocol in trial 1 (the date of the harvest of the shoots that had not started a second flush was 05/03/2018). Linear regressions were used to describe the relationships between length and dry weight, for both developing shoots and mature shoots, as in trial 1. To account for curvature in the relationship of the developing shoots, both variables were log transformed.

In addition to the selected shoots, the development of fruit was monitored. Twenty racemes per tree were selected around the time of anthesis and fruit number per raceme, as well as the diameter of three fruit per tree were recorded weekly from 09/10/2017 (two weeks after anthesis). These fruit measurements were taken fortnightly from 04/12/2017 until the end of the measurement period on 15/01/2018. A relationship between fruit diameter and fruit dry weight was developed by harvesting and measuring 10 fruit from adjacent non-trial trees at the same intervals as fruit measurements were taken on the trial trees. This relationship changed with time, which is why linear regressions were fitted separately for each measurement day, and fruit dry weights were calculated for each measurement day using that day's function.

Following fruit maturity, whole tree yield was measured. A first ground harvest was carried out on 14/03/2018 and the remaining fruit stripped off the trees and harvested on 18-19/04/2018. The fruit were dehusked after each harvest and their wet weight measured. A subsample of 50 nuts (that is, the kernel and shell of the fruit) of the combined crop of each tree was weighed, dried (at 35, 45 and 55°C for two days each) and weighed again. Thus, the weight of nut-in-shell at 10% moisture content (the industry standard) was calculated. Kernel recovery (kernel weight as a percentage of total nut-in-shell weight) was calculated by cracking the subsampled nuts and weighing the kernel. Canopy height (h), width along the row (x) and width across the row (y) were measured after harvest and a canopy volume (V) calculated for each tree, using the equation $V=(\pi \cdot h \cdot x \cdot y)/6$.

The results were analysed using 'R' (R Core Team 2017) and GenStat (VSN International 2015). First, nonlinear regressions were fitted, describing the relationships between cumulative growing degree days (calculated as in trial 1), and shoot and fruit development. Logistic curves were suitable for fitting to increases in shoot dry weight and fruit dry weight. For fruit abscission, exponential curves were fitted to describe fruit number per raceme over time. Using the parameters from the logistic functions, maximum growth rates were calculated for shoot length, shoot dry weight and fruit dry weight, and treatment effects were determined using analysis of variance. Additional variables analysed with analysis of variance were: individual fruit dry weight at the end of the measurement period, final shoot length, final shoot dry weight (all three predicted from the logistic functions), final fruit number per raceme, mean internode length and node number, as well as number of shoots and total calculated mature shoot dry weight per pruned stem. Analysis of variance was also used to determine treatment effects on the whole-tree variables yield, yield efficiency (yield per m^3 of canopy volume), kernel recovery and canopy volume.

Certain measurements had to be excluded from statistical analyses. In a severe storm during the trial, one control tree was seriously damaged and was excluded from all variables that were measured on a whole-tree basis. On other trees, several large limbs broke off and with them a total of 34 racemes, which were subsequently excluded from the variable 'final fruit number per raceme', as well as from the regression describing fruit abscission over time. Three of the selected shoots broke off during their growth and were excluded from all variables and regressions describing shoot growth and shoot characteristics. Additionally, all tagged control stems that did not produce any new growth (five out of 20) were excluded from individual shoot characteristics, but were included as zero for the per-stem variables such as 'total shoot dry weight per stem'. A number of trees of treatments 4BA (one tree), 2BA (two trees) and A (four trees) dropped all fruit on selected racemes and therefore made it impossible to measure fruit diameters. Data from early fruit growth before those fruit dropped was included in the regressions.

In the analyses of both trials, the significance threshold was set at $\alpha = 0.05$, and Fisher's Least Significant Difference Test was used to compare means.

Results

Trial 1: The effect of the relative timing of shoot growth on shoot characteristics.
The extent of shoot growth in the fully pruned trees generally decreased with the delay in bud burst from the first pruning date (Figure 1). Final shoot length and final shoot dry weight, as predicted by the respective regression parameters (α), decreased with the delay in bud burst from the first pruning date, and final shoot length was significantly higher in the controls (partially pruned trees) than in the fully pruned trees (Table 1). Final node number responded similarly, with the controls having significantly more nodes, and a decrease in node number with the delay in bud burst. The maximum absolute growth rate for shoot length, calculated from the regression parameters, was highest for the early treatments (days 1, 6, 9), and decreased with the delay in bud burst, with the controls similar to the two middle treatments (days 9, 13). The maximum absolute growth rate for shoot dry weight again tended to decrease with the delay in bud burst (Table 1). One out of 10 shoots tip-pruned on day 20 did not follow the pattern and grew both quickly and to a greater length when compared to the other shoots of the same treatment. It can be seen as an outlier in Figure 1 and it also raised the means of that treatment (Table 1).

The development of shoot dry weight over time was consistent with the logistic function, as can be seen in the shoot growth curves in Figure 2. This figure shows that shoot growth of the successive treatments was progressively slower and ended when shoot dry weight was lower, when compared to the shoots tip-pruned on day 1.

When considering the sum of all shoots emerging from the selected stems, the control trees tended to have grown to a similar extent as the early treatments. Their shoots were longer, as mentioned above, but fewer shoots emerged. Consequently, total calculated mature shoot dry weight per stem was similar for the controls and the early treatments (days 1, 6, 9), but again decreased with the delay in bud burst. Shoot number per pruned stem was significantly lower for trees tip-pruned on days 13 and 16 than for the early treatments. Mean internode length of all shoots was higher for the controls and the early treatments, and decreased with the delay in bud burst. The duration of shoot elongation was significantly higher for the control trees than the fully pruned trees. It was significantly lower for trees tip-pruned on days 9, 13 and 16, than for trees tip-pruned on days 1 and 20 (Table 1).

Trial 2: The effect of the relative timing of vegetative and reproductive growth on shoot and fruit characteristics.

There was a significant treatment effect on nut-in-shell yield per tree. Trees that were tip-pruned at anthesis on 26/9/2017 (treatment A) had the lowest yield. When treatments were applied earlier than this date (4BA, 2BA), or after this date (2AA, 4AA), trees produced higher yields, with treatment 4AA and the control having significantly higher yields than all other treatments (Table 2). There was a non-significant trend towards a smaller canopy volume in treatment 4AA, compared to the early treatments and the control ($P = 0.4$). Yield efficiency was again lowest for treatment A, and highest for treatment 4AA and the control. Final fruit number per raceme followed a similar but not identical trend as yield and yield efficiency, with treatments 2AA, 4AA and the control having a significantly higher final number of fruit per raceme than treatments 4BA, 2BA and A (Table 2).

In regard to fruit characteristics, the late treatments (2AA, 4AA) again differed significantly from the early treatments (4BA, 2BA, A). The predicted fruit dry weight at the end of the measurement period was higher for the early treatments, indicating larger fruit size, than for the late treatments (Table 2). However, kernel recovery was significantly lower for the early treatments than for treatments 2AA, 4AA and the control. The maximum fruit growth rate, which was calculated from the parameters of the logistic function and is therefore related to the predicted fruit dry weight, was highest for the treatments with a low final number of fruit per raceme and large final fruit size (4BA, 2BA, A). For treatments with a high final number of fruit per raceme and a small final fruit size, the maximum fruit growth rate was significantly lower (2AA, 4AA), with the control positioned in between (Table 2).

The development of fruit number per raceme over time was consistent with the exponential function (Figure 3a), and that of fruit dry weight over time was consistent with the logistic function (Figure 3b). In these figures, the developing separation is again noticable between the early treatments (low fruit number per raceme and high fruit dry weight), and the late treatments and control (high fruit number per raceme and low fruit dry weight).

The extent of vegetative growth tended to be greater in the treatments with fewer fruit per raceme (early treatments 4BA, 2BA, A) than in the treatments with a higher number of fruit per raceme (late treatments 2AA, 4AA and control). Accordingly, the predicted final shoot length and the predicted final shoot dry weight, which are based on the measurements of individual shoots, were significantly higher in treatments 4BA, 2BA and A, than in treatments 2AA, 4AA and the control (Table 3). When taking all shoots per stem into account rather than just the characteristics of individual shoots, the trends were similar. Total calculated mature shoot dry weight per stem was significantly lower for the control than the early treatments 4BA, 2BA and A. The late treatments 2AA and 4AA had a low total dry weight, similar to the control; however, they were not significantly different from treatment 2BA. The number of shoots per stem was significantly lower for the control trees than for all tip-pruned treatments (Table 3).

The development of shoot dry weight over time was consistent with the logistic function, as shown in the shoot growth curves in Figure 4. The curves of the early treatments (4BA, 2BA, A) are similar to each other. The shoot growth of treatments 2AA and 4AA was progressively slower and ended when their weight was lower, when compared to the early treatments, with the control similar to treatment 2AA.

There were significant treatment effects on other observed shoot characteristics, most importantly the internode length. The mean internode length of all shoots of each stem was significantly shorter in treatment 4AA than in all other treatments. There was also a significant treatment effect on final node number per individual shoot, with the early treatments having a higher number of nodes than the late treatments and the control. The maximum growth rate for shoot length, calculated from the regression parameters, was significantly lower in treatment 4AA than in the early treatments 4BA, 2BA and A (Table 3).

Figure 5 compares for each treatment the temporal development of fruit abscission and growth of shoots and fruit, relative to one another and to the timing of anthesis and tip-pruning. So far, the relationship between shoot and fruit development has been described as an inverse relationship between their respective growths: treatments with a high final fruit number per raceme had a low final shoot length, and vice versa. In this figure, the same concept is demonstrated in a different way: treatments in which the fruit growth rate had increased while the shoot growth rate was low had lower fruit abscission. Conversely, treatments in which the shoot growth rate had increased while the fruit growth rate was still low had higher fruit abscission. This indicates that the relative timing of fruit and shoot growth had a strong effect on fruit retention and ultimately on yield.

Table 1. The effect of pruning intensity and pruning time on various shoot characteristics in trial 1. Treatment effects were determined by fitting linear mixed models with pruning time nested within pruning intensity. Where variables had to be log-transformed for the analysis, back-transformed values are presented in parentheses. Control shoots are excluded from variables involving shoot dry weight, as their lengths were not converted to dry weights during the analysis.

Treatment	Predicted final shoot length (mm)	Maximum growth rate - shoot length (mm GDD^{-1})	Predicted final shoot dry weight (g)	Maximum growth rate - shoot dry weight (g GDD^{-1})	Final node number	Total calculated mature shoot dry weight per stem (g)	Mean internode length (mm)	Shoot number per stem	Duration of shoot elongation (GDD)
Day 1	5.25 c (191)	1.31 d	0.94 c (2.57)	0.023 e	1.74 d (5.68)	3.46 c (31.7)	32.4 d	6.66 bc	5.72 a (304)
Day 6	5.01 bc (150)	1.35 d	0.46 bc (1.58)	0.019 de	1.57 c (4.79)	3.21 c (24.7)	32.0 cd	7.05 bc	5.75 a (314)
Day 9	4.97 bc (144)	1.22 cd	0.39 bc (1.48)	0.015 cd	1.55 c (4.69)	3.25 c (25.7)	28.6 c	7.44 c	5.75 a (315)
Day 13	4.74 b (114)	0.91 b	-0.08 b (0.92)	0.010 bc	1.34 b (3.81)	2.39 b (10.9)	23.7 b	4.30 a	5.89 ab (362)
Day 16	3.91 a (50)	0.58 a	-1.71 a (0.18)	0.002 a	1.10 a (3.02)	1.51 a (4.5)	15.0 a	3.47 a	5.97 b (392)
Day 20	3.99 a (54)	0.36 a	-1.63 a (0.20)	0.005 ab	1.27 b (3.57)	1.99 ab (7.3)	17.3 a	5.15 ab	6.01 b (408)
Control	5.75 d (314)	1.00 bc			2.27 e (9.71)	3.78 c (43.8)	33.3 cd	5.60 abc	6.56 c (708)
SED	*0.18*	*0.12*	*0.33*	*0.003*	*0.08*	*0.30*	*2.1*	*1.05*	*0.09*
P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

Different letters represent significant differences within a column at $P < 0.05$. The average standard error of difference (SED) is for the interaction between pruning intensity and pruning time. All growth rates are absolute growth rates. GDD – growing degree days.

Table 2. The effect of differently timed tip-pruning treatments and an unpruned control treatment on various tree and fruit characteristics in trial 2. Treatment effects were determined using analysis of variance. Each value represents the mean of five replicates (i.e. five trees). Fruit characteristics were calculated from three fruit per tree; raceme characteristics were calculated from 20 racemes per tree.

Treatment	Nut-in-shell yield per tree (kg)	Canopy volume (m^3)	Kernel recovery (%)	Yield efficiency (kg m^{-3})	Predicted fruit dry weight at the end of the measurement period (g)	Maximum fruit growth rate (g GDD^{-1})	Final fruit number per raceme
4BA	2.40 bc	22.9	31.2 a	0.12 bc	12.2 bc	0.020 c	0.11 a
2BA	1.61 ab	21.7	30.6 a	0.08 ab	12.9 c	0.024 d	0.05 a
A	0.83 a	20.1	31.3 a	0.05 a	12.1 bc	0.019 bc	0.02 a
2AA	3.14 c	20.4	33.1 b	0.17 c	9.2 a	0.015 a	1.07 b
4AA	4.31 d	18.1	33.7 b	0.27 d	9.4 a	0.015 a	1.16 b
Control	5.13 d	21.4	34.3 b	0.27 d	9.9 ab	0.017 ab	1.03 b
SED	*0.46*	*2.24*	*0.76*	*0.03*	*1.13*	*0.001*	*0.25*
P	*<0.001*	*0.4*	*<0.001*	*<0.001*	*0.017*	*<0.001*	*<0.001*

Different letters represent significant differences within a column at $P < 0.05$. All growth rates are absolute growth rates. SED – standard error of difference. GDD – growing degree days.

Table 3. The effect of differently timed tip-pruning treatments and an unpruned control treatment on various shoot characteristics in trial 2. Treatment effects were determined using analysis of variance. Each value represents the mean of five replicates (i.e. five trees) and were calculated from four shoots or stems per tree.

Treatment	Predicted final shoot length (mm)	Maximum growth rate - shoot length (mm GDD^{-1})	Predicted final shoot dry weight (g)	Maximum growth rate - shoot dry weight (g GDD^{-1})	Final node number	Total calculated mature shoot dry weight per stem (g)	Mean internode length (mm)	Shoot number per stem
4BA	176 b	1.32 bc	1.64 b	0.014 bc	4.95 b	26.0 c	33.9 c	4.28 b
2BA	172 b	1.43 c	1.62 b	0.016 c	5.00 b	16.3 b	32.6 bc	3.41 b
A	158 b	1.36 bc	1.43 b	0.014 bc	4.50 b	23.9 c	34.3 c	4.25 b
2AA	108 a	1.00 ab	0.76 a	0.008 ab	3.70 a	15.3 ab	26.8 b	3.65 b
4AA	70 a	0.64 a	0.35 a	0.004 a	3.33 a	10.4 ab	19.4 a	3.40 b
Control	104 a	0.98 ab	0.72 a	0.008 a	3.20 a	8.4 a	30.7 bc	2.12 a
SED	*24*	*0.20*	*0.32*	*0.003*	*0.33*	*3.3*	*3.3*	*0.52*
P	*<0.001*	*0.007*	*0.002*	*0.004*	*<0.001*	*<0.001*	*0.002*	*0.006*

Different letters represent significant differences within a column at $P < 0.05$. All growth rates are absolute growth rates. SED – standard error of difference. GDD – growing degree days.

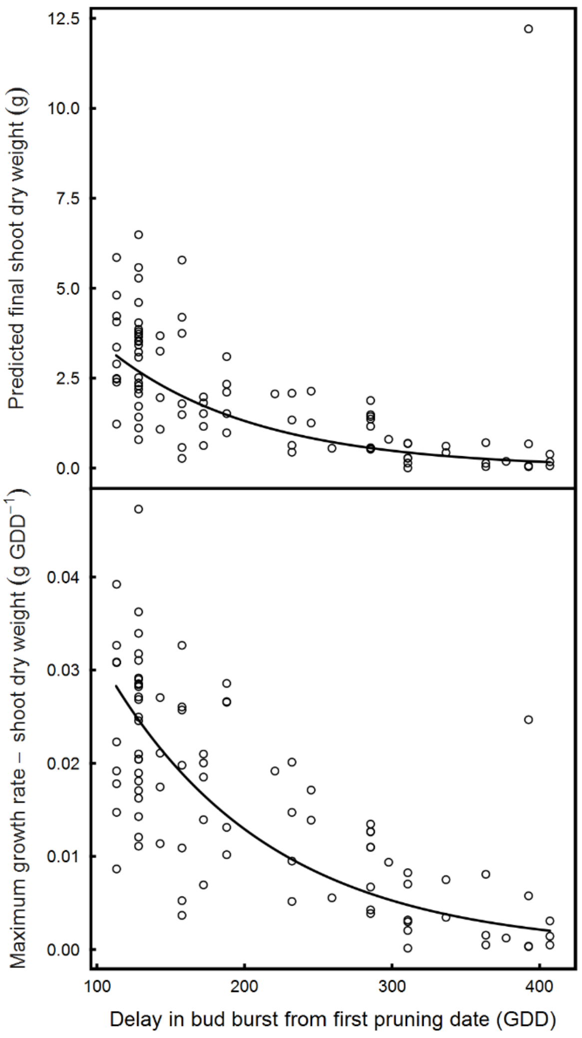

Figure 1. Predicted final shoot dry weight and maximum absolute growth rate for shoot dry weight in response to the timing of bud burst, relative to the first day of pruning (20/1/2016) in trial 1. Points represent all tagged shoots of each fully pruned tree. Lines represent the fitted exponential function.

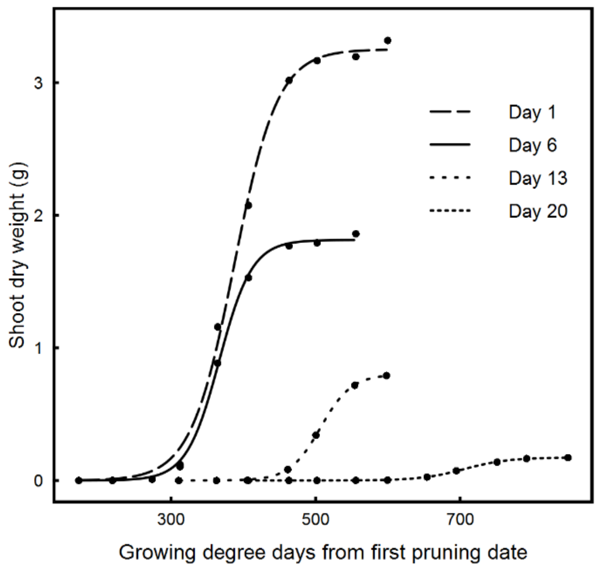

Figure 2. The accumulation of shoot dry weight over thermal time for shoots pruned on days 1, 6, 13 and 20 in trial 1. Points represent calculated shoot dry weight for the shoot with the median final shoot dry weight, and lines represent that shoot’s fitted logistic function.

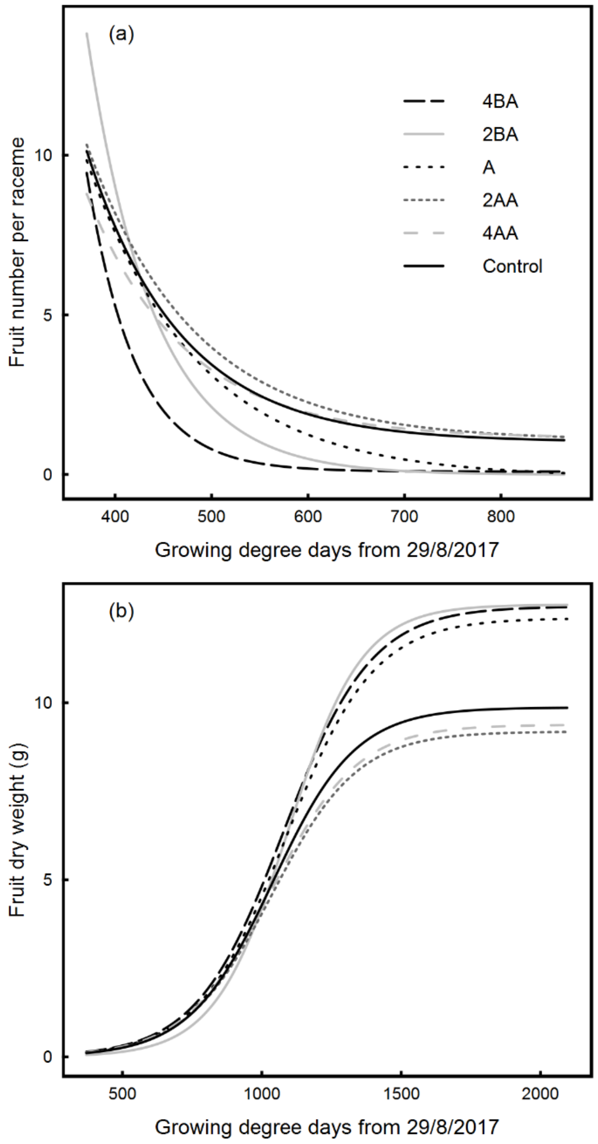

Figure 3. Fruit development over time, using thermal time from the date of the first tip-pruning treatment (29/08/2017) in trial 2. (a) Fruit abscission over time, using treatment means from five replicates (i.e. five trees) and 20 racemes per tree. Lines represent the fitted exponential function. (b) The accumulation of fruit dry weight over time, using treatment means from five replicates (i.e. five trees) and three fruit per tree. Lines represent the fitted logistic function.

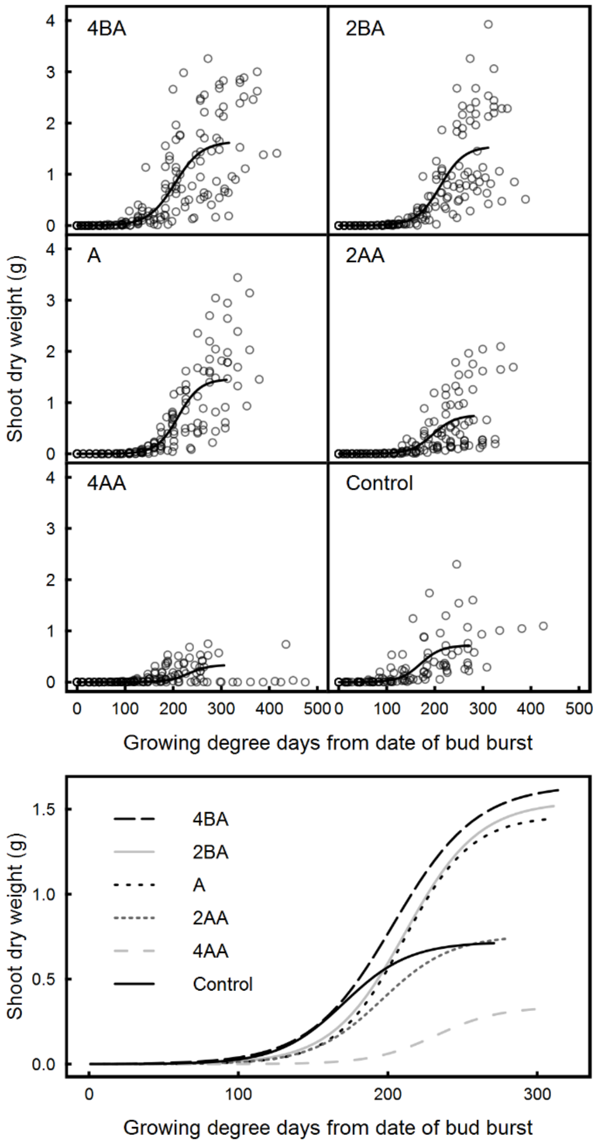

Figure 4. The accumulation of shoot dry weight over time, using thermal time from the date of bud burst in trial 2. Points represent the shoot dry weight of all selected shoots, calculated from biweekly shoot length measurements. Lines represent the fitted logistic function, based on treatment means from five replicates (i.e. five trees) and four shoots per tree.

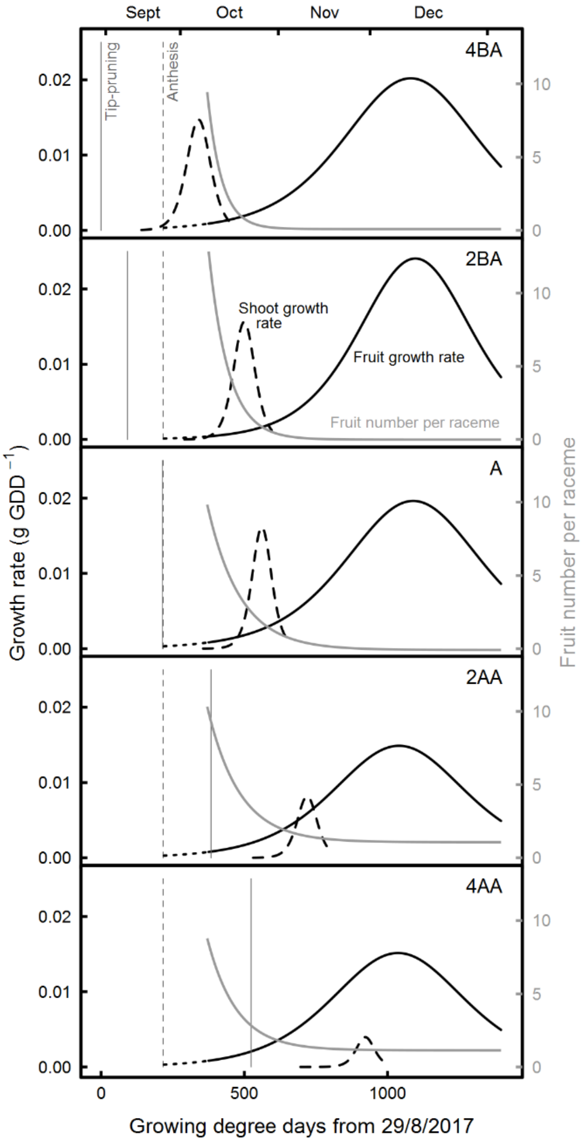

Figure 5. Relative timing of mean absolute growth rates of shoots and fruit, as well as fruit abscission, for each treatment over time, using thermal time from the date of the first tip-pruning treatment (29/08/2017) in trial 2. Shoot and fruit growth rate curves were calculated from the logistic functions of their respective dry weights. The fruit growth rate curve is based on fruit measurements starting on 09/10/2017. The dotted part of the curve represents the projected fruit growth rate before the commencement of fruit measurements. The fruit abscission curves represent the fitted exponential function. Vertical lines show the timing of anthesis and of each tip-pruning treatment. Control trees are not included, as the timing of their vegetative development was too erratic to make a mean curve meaningful.

Discussion

The simultaneous growth of individual organs led to competition for resources, which manifested itself in a number of ways. The competition affected the characteristics and development of both the organs more successful at securing resources, and the ones less successful. There were also differences in how it affected vegetative and reproductive organs. Fruit that had to compete with concurrent strong vegetative growth was more likely to abscise, as shown in the early treatments (4BA, 2BA, A) of trial 2. In these treatments, vegetative growth was strong and therefore strongly competing for carbohydrates, leading to a higher fruit abscission rate and subsequently a low final fruit number per raceme, as well as low yield. However, the effect was different in the treatments in which competition was mainly pertinent between individual fruit due to little shoot growth. Here, fruit tended to be smaller and have a lower growth rate. This effect was demonstrated in the late treatments (2AA, 4AA) and the control of trial 2, where fruit number per raceme was higher, but fruit size and maximum growth rate were reduced.

Shoots facing strong competition from either fruit or vegetative growth tended to be shorter and have a lower growth rate. This effect was apparent in the late treatments (days 13, 16, 20) of trial 1. The individual shoots of these treatments had to compete with strong vegetative growth on most other parts of the same tree from shoots pruned on day 1. Consequently, final shoot length, dry weight and node number were reduced, the maximum growth rate was lower, as was the total shoot dry weight per stem, when compared to the strongly competing shoots on the same trees (day 1). In trial 2, shoots competing with strong fruit growth were similarly affected by competition. A large number of fruit per raceme in the late treatments (2AA, 4AA) and the control provided strong competition for vegetative growth, again leading to shorter, slower growing shoots with fewer nodes, as well as less shoot dry weight per stem.

Greater levels of competition did not only affect shoot characteristics, but also the initiation and cessation of growth. In the late treatments (days 13, 16, 20) of trial 1, there was a trend for fewer buds to burst and grow into new shoots from the pruned stems, when compared to the early treatments. In previous studies, synchronised vegetative flushes growing around the time of floral initiation reduced raceme production, which is another form of axillary bud growth (Olesen 2005; Wilkie *et al.* 2010). The reduced raceme production was attributed to signals produced by the developing vegetative shoots. An alternative explanation is that strong shoot growth and competition for carbohydrates directly affected bud release through altered sugar signalling (Mason *et al.* 2014). In trial 1, strong competition also led to a shorter period of shoot elongation, as well as fewer initiated nodes in these treatments. Toft (2019) reported a strong correlation between node number per shoot and the duration of shoot elongation. These results suggest that high levels of competition are not only leading to less carbohydrate allocation during growth, but also premature cessation of growth.

Competition had the potential to affect growth in both trials, yet under certain circumstances organs were more successful in the competition for resources. In our trials, growth was staggered and the outcome of competition could be attributed to these differences in timings, as can be seen when examining their growth curves. The growth of both fruit and shoots were described with logistic curves, which describe the increase in dry weight over thermal time (Figure 2, Figure 3b, Figure 4). These curves illustrate that growth occurs in three phases: slow growth at the beginning is followed by rapid growth, which is again followed by slow growth. In both trials, there were a number of different combinations of slowly and rapidly growing shoots or shoots and fruit on individual trees (Figure 5). This enabled us to observe the timing of their growth relative to each other, and the effect of that relative timing on competition for carbohydrates.

The results of both trials indicate that the relative timing of growth is what determined how successfully an organ competed for carbohydrates. For example, in those treatments of trial 1, in which the majority of shoots had reached rapid growth at the time when the two later shoots were only beginning to grow, the final length of these two shoots was significantly reduced. Similarly, in those treatments of trial 2, in which rapid shoot growth coincided with early and slow fruit growth, fruit abscission was significantly higher than in treatments in which slow shoot growth coincided with rapid fruit growth. A different way of expressing this concept is to attribute the outcome of competition to the growth rate of an organ, relative to the growth rates of all other organs growing simultaneously.

In previous research, sink strength has been attributed to the growth rate as well, particularly in fruit (Grossman and Dejong 1994; Marcelis *et al.* 2004; Wubs *et al.* 2009; Brown *et al.* 2019). In sweet pepper plants, which continually produce flowers, rapidly growing fruit had a higher sink strength than simultaneously slow growing fruit, leading to the abscission of the latter (Marcelis *et al.* 2004). Similarly, carbohydrate shortage induced by shading, defoliating or girdling branches, rather than by competition, has also been shown to have a negative effect on young fruit, but not on older fruit, in apple (Berüter and Droz 1991) and citrus (Mehouachi *et al.* 1995). In general, plant organs in the earliest stages of growth seem to be more susceptible to carbohydrate shortage and competition. The results of the current study are consistent with this concept, but have additionally shown that the same treatment (tip-pruning) can both increase and decrease vegetative growth, based on its timing relative to other developments on the tree. What is important to note is that these increases and decreases in growth were deliberately induced and operated simultaneously on the fast and the slow growing organs, which has implications for practical canopy management.

Fruit has been considered a sink with a higher priority than vegetative growth (Wardlaw 1990). The current study, however, has found no evidence to support this concept, as the effect of increased or decreased growth occurred independent of organ type. For example, vegetative growth was clearly a more successful sink than fruit in the early treatments of trial 2. It is, however, still conceivable that fruit has a higher ability to mobilise distant carbohydrate sources than vegetative growth, as has been suggested in previous studies (Obeso 2002; McFadyen *et al.* 2011). In a trial that through hedging encouraged shoot growth only on the top half of macadamia trees, it was found that carbohydrates moved from the upper to the lower canopy in order to supply fruit, but did not do so towards growing shoots (McFadyen *et al.* 2011).

A previous study has found that hedging macadamia trees at a time that limits the simultaneous growth of shoots and fruit improved yield, compared to when shoot growth coincided with fruit set and early fruit development (McFadyen *et al.* 2012). The results of the current study are consistent with this concept. In addition, this study was able to time vegetative growth in such a way as to give the fruit a chance to outcompete shoots and thereby not only limit immature fruit abscission, but reduce vegetative growth as well. As crowding is a serious and widespread concern in macadamia orchards, finding a way of reducing shoot growth would be very beneficial.

From a canopy management perspective, which is particularly relevant to the macadamia industry, this work has confirmed the results from McFadyen *et al.* (2012). That study suggested that delaying mechanical hedging until several weeks after anthesis (around anthesis is the standard timing of macadamia hedging) can reduce the yield penalty associated with this hedging. By working with thermal time, the current study allows an estimation of a ‘best bet’ hedging time for other macadamia growing environments, which could then be tested experimentally. Additionally, it could be compared with an earlier hedging time, which is practised by some macadamia growers in the Bundaberg region, where these trials were undertaken. An additional aspect worth exploring is the long-term effect of a hedging treatment that reduces vegetative growth. This study did not measure treatment effects on a second season or of repeated annual applications, which should be addressed in future research. An additional area of interest is the potential of combining the understanding drawn from this study with other types of canopy management, such as selective pruning or the application of growth regulators.

Actively growing vegetative and reproductive plant organs are in competition for resources with each other in macadamia. The relative timing of their growth has been shown to affect this competition and can therefore change fruit retention and yield, as well as the extent of shoot growth. This study has provided an improved understanding of resource allocation in macadamia and highlights the fact that this allocation can be altered through a well-timed canopy management regime. These results may contribute to future research into macadamia physiology but have implications for orchard management as well. However, further research is necessary into hitherto unknown details, such as potential effects on long-term productivity.

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Appendix 3

A comparison of the responses of two macadamia cultivars to the use of selective limb removal to control tree height, with respect to yields, nut quality and insect damage

Trevor Olesen[a,b,c], David Robertson[a], Alister Janetzki[a], Tina Robertson[a], Stephen Morris[a], John Wilkie[d,*]

[a]NSW Department of Primary Industries, Wollongbar Primary Industries Institute, 1243 Bruxner Highway, Wollongbar, NSW 2477, Australia

[b]Retired

[c]Orcid number: 0000-0002-4500-1547

[d]Qld Department of Agriculture and Fisheries, c/o NSW Department of Primary Industries, Wollongbar Primary Industries Institute, 1243 Bruxner Highway, Wollongbar, NSW 2477, Australia

[*]Corresponding author at: Qld Department of Agriculture and Fisheries, c/o NSW Department of Primary Industries, Wollongbar Primary Industries Institute, 1243 Bruxner Highway, Wollongbar, NSW 2477, Australia. Tel.: +61 2 6629 1170; fax: +61 2 66291023.

E-mail addresses: trevorolesen@gmail.com (T. Olesen), david.robertson@dpi.nsw.gov.au (D. Robertson), alister.janetzki@dpi.nsw.gov.au (A. Janetzki), tina.robertson@dpi.nsw.gov.au (T. Robertson), stephen.morris@dpi.nsw.gov.au (S. Morris), john.wilkie@daf.qld.gov.au (J. Wilkie).

Abstract

Macadamia becomes increasingly difficult to manage the further the tree height extends beyond 6 metres. Selective limb removal of the taller branches at the centre of the canopy is a potential means of tree height control that may not reduce yield because the removal of such branches deep within the canopy has little effect on the light intercepted by the trees, and thus the amount of fruit the trees can carry through to harvest. This study tested two macadamia cultivars, '246' and '816', and two pruning strategies, with trees subjected to selective limb removal and control trees that were not pruned. Pruning commenced when the trees exceeded approximately 6 metres in height, and continued at intervals to restrict the trees to this height. The trees were monitored for four years with respect to yields and nut quality, and in the fourth year with respect to infestation by *Sigastus* sp. weevil. After the four years the pruned trees were 1.1 m shorter than the control trees. Across the four years the yields of the pruned trees were similar to those of the control trees. The yields of the '246' trees were higher than those of '816'. Both cultivars showed irregular bearing. The nuts of '816' were larger, had higher kernel recovery, and had a higher proportion of first grade

kernel and a lower proportion of unsound kernel than ‘246’. However, ‘816’ had a thinner shell and was more prone to insect damage. In contrast, the pruned ‘246’ trees had a lower incidence of kernel insect damage at harvest than the control ‘246’ trees. Late in the study, selective limb removal was used to reduce the height of all trees to approximately 6 m, including the control trees to test the benefits of early versus late intervention in tree height control. The yield of the subsequent harvest in 2018 was generally reduced, in part associated with unfavourable climatic conditions, and the longer-term impact requires further investigation. Nonetheless, selective limb removal is a height control strategy for the macadamia industry.

Introduction

Macadamia yields tend to increase up to very high levels of orchard PAR (photosynthetically active radiation or ‘light’) interception, approximately 94% (McFadyen et al., 2004; Olesen et al., 2007). The production benefits of tree size control generally lie in factors other than short term fruitfulness, such as with the maintenance of ground covers to reduce soil loss from orchards, which promotes sustainable farming, and with the efficient application of chemicals. However there is some suggestion of yield reductions at very high levels of orchard PAR interception (McFadyen et al., 2004), and judicious tree size control may help remediate such orchards.

The lateral growth of the canopy can be largely controlled by frequent, strategically-timed mechanical hedging (McFadyen et al., 2012, 2013) though selective limb removal will be required eventually to remove thicker branches. Such an approach results in only small yield losses relative to non-hedged controls.

Tree height control is more problematic. Mechanically hedging the entire upper canopy (‘topping’) is unacceptable. It causes large yield losses and a proliferation of water shoots, giving rise to badly structured trees (Olesen et al., 2016). Half-topping is more acceptable, with small yield losses and more controlled regrowth (Olesen et al., 2016). Selective limb removal is another possibility. McFadyen et al. (2013) found that selective limb removal near the top of the tree controlled tree height with only a small reduction in yield. However the regrowth was abundant and prohibitively complex for follow up pruning. Pruning deeper within the canopy meant that the regrowth was controlled by shade, but more of the canopy was removed and the yield penalty was higher.

McFadyen et al. (2013) worked with trees 10 m in height, and the deep pruning involved the removal of a large codominant leader. The strategy may work better with less severe pruning, and is more likely to be adopted by industry if applied to smaller trees, because the work can be done from the ground with a long-handled pole-saw instead of from an elevated platform.

Here we test the approach relative to non-pruned controls using the spreading macadamia cultivar ‘246’ and the upright cultivar ‘816’. The work is important because selective limb removal may be a height control strategy in its own right, but also because it is a necessary adjunct to any half-topping strategy, to remove woodiness and unwanted watershoots.

We assess the trees with respect to tree height, yields and nut quality. We also investigate losses caused by insect damage, because pest pressures may be lower in more open canopies (Govender, 2015), and spray penetration may be better. In this regard PAR profiles through the canopies are measured to quantify differences in canopy openness.

Materials and methods

McFadyen et al. (2016) studied the early tree training of two macadamia cultivars, '246' and '816', using an orchard of 320 trees planted at 7 x 3.9 m. The cultivars were planted in 16-tree plots, four trees down the row across four rows. On the completion of the study the ten plots of each cultivar were reallocated to two treatments, giving five plots per treatment per cultivar, with three or four fully buffered measured trees per plot. One treatment was a control where trees were not subject to selective limb removal. The other treatment was pruned to control tree height by removing the taller branches at the centres of the trees to maintain the trees close to 6 m in height. The branches were pruned 2-3 m deep within the canopy, with care not to create too large an opening in the canopy, to reduce the likelihood that the regrowth from around the pruning cut would reach the edge of the canopy. This selective limb removal occurred on four occasions: early December 2013; early December 2014; late May 2015; and late September 2016. In August 2017, selective limb removal was used to reduce the height of all trees in the trial to approximately 6 m, including the control trees. The purpose of this is to test the benefits of early versus late intervention in tree height control and harvest data is presented here for 2018.

Both treatments were side-hedged mechanically in late May 2014 and early January 2016, a standard industry practice to control the lateral growth of the canopy (McFadyen et al., 2013). The methods of McFadyen et al. (2016) were used to maintain the trees and to monitor the trial in terms of yields, nut quality and tree heights. Trees heights were measured in late June or early July. Measurements were also made in December 2016 in order to calculate changes in PAR through the canopy with respect to the top of the canopy (see below). An additional quality assessment was made for the 2017 harvest, by assessing subsamples of nuts for all harvests, as opposed to a single harvest, and using these assessments to estimate the total weights for the season of kernel, sound kernel, first grade kernel, immature kernel, insect damaged kernel, mould affected kernel and discoloured kernel. Linear mixed models were used to explain trait variability across the whole study, according to fixed effects of cultivar, pruning, season, and their interactions. Analyses of variance and t-tests were used to assess variations in nut quality for 2017.

Profiles of PAR transmission (400-700 nm) through five of the control '816' trees and five of the pruned '816' trees were measured on sunny blue sky days in December 2016, close to solar noon when the sun was 80-85° above the horizon, using a Decagon Devices, Inc. (Pullman WA, USA) ACCUPAR LP-80 ceptometer. The ceptometer has a linear arrangement of 80 sensors spaced 1 cm apart, grouped into eight non-overlapping blocks of 10 sensors. With each measurement, average PAR is calculated separately for each of the eight groups. The ceptometer was located within the canopies using graduated poles placed 0.8 – 1 m from the edge of the canopy and 1.2-1.8 m from the trunk, such that the lines from trunk to pole were at right angles to the alleyways. Measurements were made at 1 m intervals from 1-6 m above the ground. Two measurements were made at each height, horizontally, from the pole towards the trunk, and from the edge of the canopy at right angles to the alleyway to the pole. These measurements were referenced against above canopy measurements. The relationships of transmission to canopy depth were described by simple logistic curves using non-linear regression. Transmission values for the two treatments, 1.5 m below the top of the canopy, were compared using the Mann-Whitney rank sum test.

In November 2016, well before nut maturity, there was a high incidence of macadamia seed weevil (MSW; *Sigastus* sp., pers. comm. C. Maddox 2017) in the orchard, notwithstanding specific pesticide applications to limit the population. The infestation had the potential to severely compromise yields, so all the nuts on the ground beneath the measured trees in the trial were inspected for the presence of MSW egg laying sites to give an indication of the magnitude of the losses. In May 2016 shell thickness was measured for five seeds per plot, and for fifty seeds of cultivar 'A4' collected from trees immediately adjacent to the trial, to relate to MSW infestation. Shell thickness was measured perpendicular to the suture, approximately at the light-dark pigmentation boundary on the inner seed coat. The data relating to MSW and shell thickness were variously assessed by analyses of variance and multilinear regressions.

Results

Pruning weights

The mean fresh weights of prunings per tree summed across the first four pruning dates until 2016 were 23.3 kg for '246' and 31.4 kg for '816', with medians of 16.5 and 23.4 kg respectively. The cultivars did not significantly differ in the amount of canopy removed ($P > 0.05$). The heaviest pruning was in August 2017, the last pruning date, with mean and median fresh weights of prunings per tree of 26.4 and 25.0 kg for '246' and 20.9 and 15.5 kg for '816'in the pruned treatment. For the topped control treatment the mean and median fresh weights of prunings per tree were 61.8 and 51.5 kg for '246' and 66.1 and 54.5 kg for '816'. Prior to this, the heaviest pruning was in late September 2016, the last pruning date, with mean and median fresh weights of prunings per tree of 16.5 and 12.6 kg for '246' and 22.3 and 14.4 kg for '816'.

Tree height and PAR transmission

Pruning significantly reduced tree height (Fig. 1; $P < 0.05$) by 1.1 m over 4 years. The control trees were significantly taller with each succeeding measurement (Fig. 1; $P < 0.05$). The heights of the '246' and '816' control trees were not significantly different in 2014 and 2015 ($P > 0.05$) but the '816' trees were significantly taller in 2016 and 2017 ($P < 0.05$). The pruned trees were progressively taller from 2014 to 2016 because not all trees were at the height threshold of 6 m at the start of the experiment then stabilized.

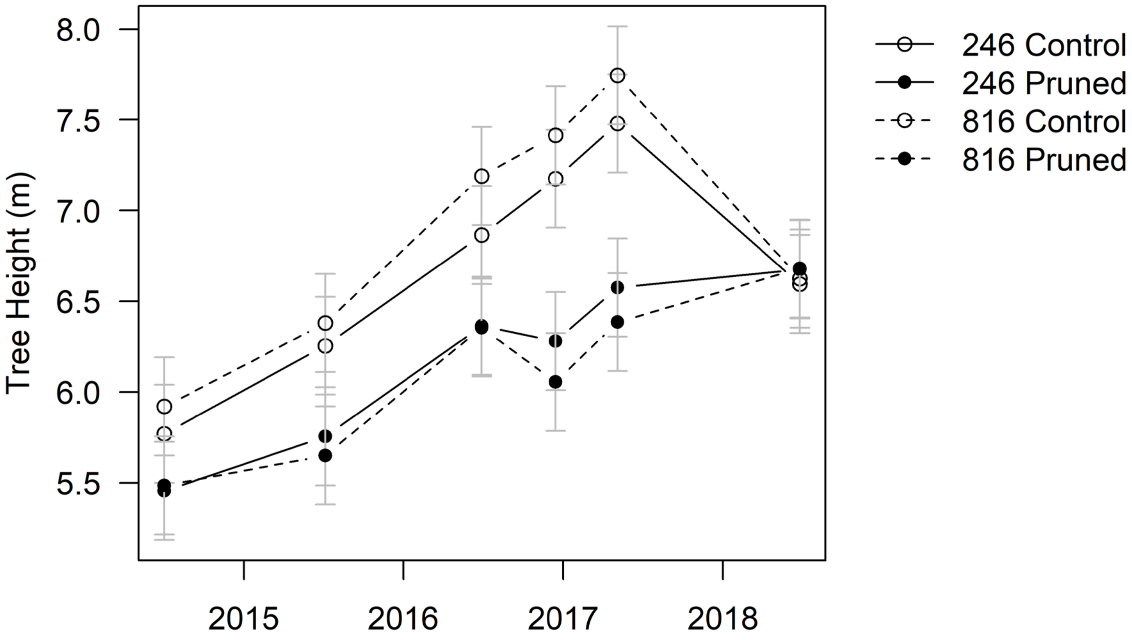


Fig. 1. Tree heights for macadamia cultivars 246 and 816, for trees that were pruned to contain tree height (December 2013 and 2014, May 2015, September 2016), or were not pruned (controls). All trees were pruned in August 2017 (height measured 2018). Each symbol is the mean of five plots, with four trees per plot (±SE). The least significant difference (LSD) at *P* = 0.05 for tree heights, estimated from a linear mixed model, is 0.21 within season and between season comparisons.

In December 2016, three months after pruning, PAR transmission through the canopy was modestly higher in the pruned trees (Fig. 2); for example, 1.5 m below the top of the canopy, transmission in the pruned trees varied from 0.055 to 0.943, with a median of 0.205, and was higher (*P* = 0.02; n = 16 for both treatments) than for the control trees which varied from 0.006 to 0.948, with a median of 0.019.

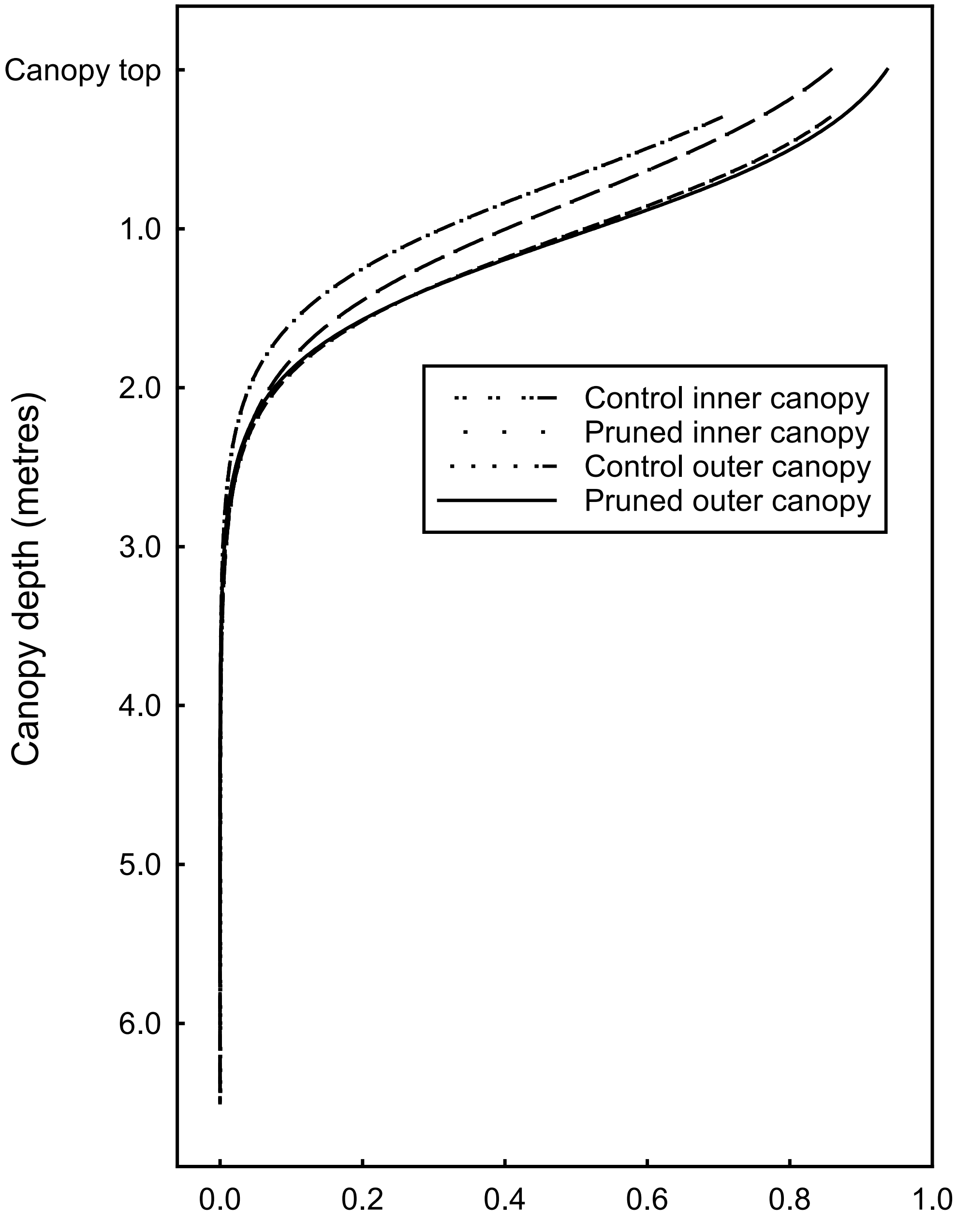


Fig. 2. Variation in PAR transmission in the outer canopy (0 – 0.8 m from the canopy edge) and inner canopy (> 0.8 m from the canopy edge, to 1.8 m) with distance from the top of the canopy for pruned and control trees of '816'. The curves are logistic equations of the form $y = 1/(1+e^{b(x-c)})$ where y is transmission (I/I_0, where I is the PAR measured within the canopy and I_0 is the PAR measured above the canopy), x is the distance from the top of the canopy, and b and c are parameters. Transmission was measured near solar noon, close to the summer solstice in December 2016. The pruned trees had been most recently pruned in late September 2016. The curves explained 0.61 – 0.75 of the variance (n = 240 for each curve), and were significant in all instances ($P < 0.05$).

Yields

The yields of the pruned trees were not significantly different (*P* < 0.05) from those of the control trees for the cumulative yields over the five years (Fig. 3). However, in 2018 the pruned '816' trees had a higher yield than the topped control '816' trees. The varieties did not differ significantly for the cumulative yields over the five years. However, the yields of the '816' trees were significantly lower (*P* < 0.05) than those of the '246' trees in 2016 and 2017 but not for 2018 when the opposite was the case.

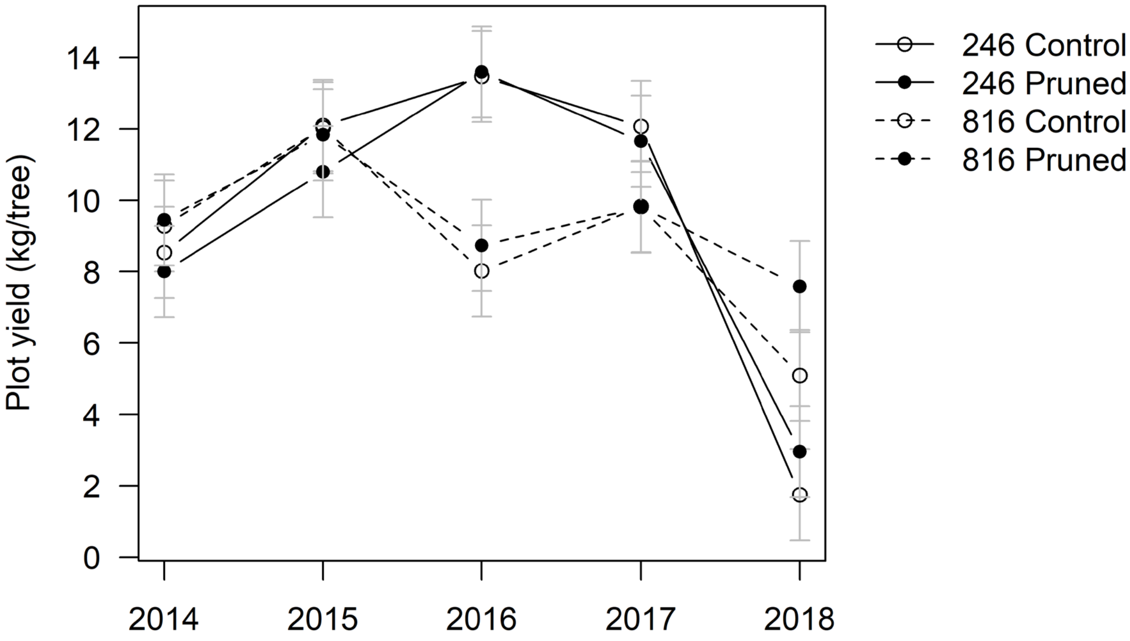


Fig. 3. Tree yields for macadamia cultivars 246 and 816, for trees that were pruned to contain tree height, or were not pruned (controls). Each symbol is the mean of five plots, with four trees per plot, except for one plot, where yields were collected from only three trees, owing to storm damage to the fourth (±SE). The least significant difference (LSD) at P = 0.05 for yield, estimated from a linear mixed model, is 1.6 for within season and between season comparisons.

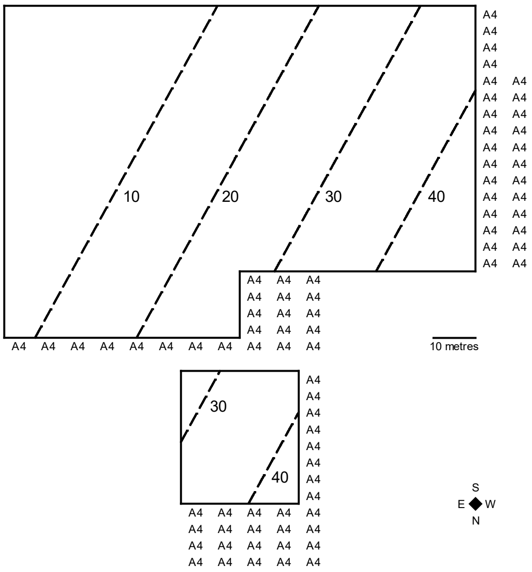
The peak harvest for '816' tended to be around April, though it was March in 2014. Similarly the peak harvest for '246' tended to be around May, but was April in 2014. The low crop load of '816' in 2016 was associated with a bimodal harvest, with a strong peak in April and a second much weaker peak in September. The second peak in September was related to out-of-season flowering.

The modest yields in 2017 were associated with dry weather conditions from spring anthesis in 2016 to mid-summer 2016/2017. The trial block was not irrigated. However, we also had issues with macadamia seed weevil in the same season.

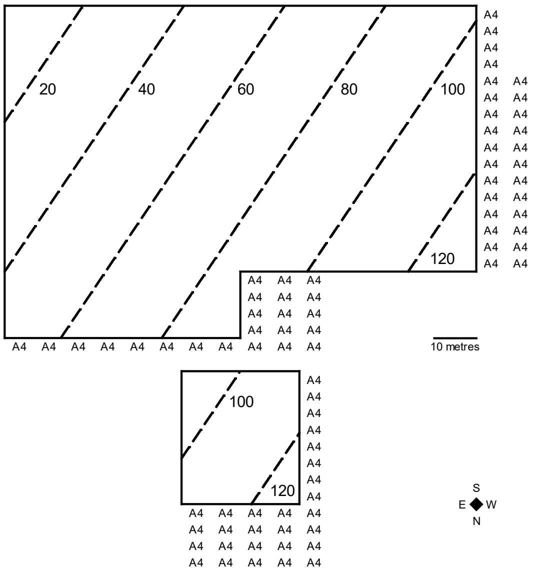
In 2018 yields were generally reduced in comparison to all other seasons. This was associated with the pruning treatment applied to all trees including the controls in August 2017. Hot windy weather coinciding with flowering of '246' may also have impacted on the yield obtained for this variety.

Macadamia seed weevil (MSW) damage in spring 2016

The control trees appeared to be no more affected than the pruned trees ($P > 0.05$), but ‘816’ appeared to be more affected by MSW damage than ‘246’ ($P < 0.05$). Pooling the control and pruned trees for each cultivar, and using the individual tree data, spatial analyses detected declining infestation from approximately the North-West to the South-East for both cultivars ($P < 0.05$; Fig. 4). Given that cultivar ‘A4’ was planted on the Northern and Western borders of the trial, MSW infestation essentially declined with increasing distance from ‘A4’. However, there was also a piece of remnant rainforest a few hundred metres to the North-West, and unsprayed macadamia trees beyond that.



A. Contours of macadamia seed weevil damage in '246'
in early November 2016 (nuts on ground/tree)



B. Contours of macadamia seed weevil damage in '816'
in early November 2016 (nuts on ground/tree)

Fig. 4. Nuts on ground with macadamia seed weevil egg laying sites in early November 2016, well before the nuts were mature: (A) cultivar 246, (B) and cultivar 816. The contours were calculated using linear models. The '246' model explained 0.34 of the variance. The '816' model explained 0.29 of the variance. Both models were significant (*P* < 0.05), with significant parameter estimates for both the North-South and East-West coordinates.

Based on nut on ground, yield losses to MSW were up to 3.5 kg/tree, but there may have been more damage in the canopies above. Against this, however, is that November is still the premature nut drop period, so not all the MSW affected fruit would necessarily have been carried through to harvest.

The shells of the control trees were thicker than those of the pruned trees ($P < 0.05$) and the shells of '246' were thicker than those of '816'. The mean thicknesses were: 2.3 mm for the '246' controls, 2.2 mm for the '246' pruned trees, 1.8 mm for the '816' controls, and 1.7 mm for the '816' pruned trees. The shell thickness of 'A4' was significantly thinner than all these treatments ($P < 0.05$) at a mean thickness of 1.4 mm.

Nut quality

There was a tendency ($P = 0.085$) in the 2017 nut quality assessment (Table 1) for the kernel from the pruned trees to have less insect damage than the kernel from the control trees, based on a two way analysis of variance. Examining the two cultivars separately using t-tests, the '246' pruned trees were found to have significantly less ($P < 0.05$) insect damage than the '246' control trees. The same was not true for the '816' comparison. There were no other pruning effects in the 2017 nut quality assessment. There were however significant ($P < 0.05$) cultivar effects with '246' having lower kernel recovery, lower first grade kernel, lower insect damage, lower incidences of mould and a higher prevalence of immaturity than '816'.

Table 1. Kernel assessed for all harvests in 2017. Kernel recovery is kernel as a percentage of nut-in-shell. All other percentages are with respect to total kernel. Numbers with different letters within the row are significantly different ($P < 0.05$).

	'246'		'816'	
	Control	Pruned	Control	Pruned
Kernel recovery (%)	34.2 a	35.2 a	37.9 b	38 b
Total kernel (kg/tree)	4.1 a	4.1 a	3.7 a	3.7 a
Sound kernel (%)	94.8 a	95.3 a	95.2 a	95.7 a
1st grade kernel (%)	91.6 a	92.9 a	94.4 b	94.8 b
Immature kernel (%)	2.3 a	1.6 a	1.2 b	1.3 b
Insect damage (%)	0.4 a	0.2 a	0.7 b	0.6 b
Mould affected (%)	0.1 a	0.2 a	0.5 b	0.3 b
Discoloured (%)	2.3 a	2.7 a	2.4 a	2.1 a

With respect to the comparison of nut quality across seasons, the were no significant effects of pruning ($P > 0.05$), but significant ($P < 0.05$) cultivar, season and cultivar x season interaction effects for nut weight, kernel recovery, unsound kernel and first grade kernel (Table 2). ‘816’ had consistently larger nuts than ‘246’. There was some evidence of crop load effects for both cultivars, with a tendency for smaller nuts in years of higher yields. ‘816’ had higher kernel recovery (except for 2018) and higher proportions of first grade kernel than ‘246’, and somewhat lower levels of unsound kernel.

Table 2. Fruit quality traits across seasons, with fruit collected close to peak harvest. Nut weight is given at 10% moisture content. The percentages for kernel recovery and unsound kernel are given with respect to nut-in-shell (NIS). The percentage of first grade kernel is given with respect to total sound kernel. Pairwise comparisons can be made within traits by reference to the letters. No common letters mean significantly different ($P < 0.05$). The least significant differences were the same for comparisons within seasons and between seasons.

	2014	2015	2016	2017	2018
Nut weight (g)					
‘246’ control	8.3 efg	7.4 b	6.8 a	7.8 cd	7.8 cd
‘246’ pruned	8.1 ef	7.6 bc	6.8 a	7.6 bc	7.6 bc
‘816’ control	8.9 h	8.2 efg	8.4 g	8.2 efg	8.1 de
‘816’ pruned	9.1 h	8.3 efg	8.3 efg	8.2 efg	8.3 fg
Kernel recovery (%)					
(100 x g kernel/g NIS)					
‘246’ control	31.5 a	36.8 def	34.5 c	34 c	40.5 i
‘246’ pruned	32.6 b	37.6 fgh	35.2 c	34.6 c	41.3 i
‘816’ control	35.9 cd	38 gh	36.9 ef	38 gh	41.1 i
‘816’ pruned	36.2 de	38 gh	37.3 fg	38.5 h	40.3 i
Unsound kernel (%)					
(100 x g kernel/g NIS)					
‘246’ control	1.8 bcde	1.9 bcde	4.6 h	1.4 abcd	3.1 fg
‘246’ pruned	2.2 cdef	0.8 ab	4.1 gh	1.2 abc	3.9 gh
‘816’ control	0.9 ab	0.6 a	2.7 ef	1.3 abc	1.5 abcde
‘816’ pruned	0.5 a	1 ab	2.5 def	1.2 abc	1.5 abcde
First grade kernel (%)					
(100 x g kernel/g kernel)					
‘246’ control	92.9 b	93.6 b	89.1 a	97.7 def	94.4 bc
‘246’ pruned	96.7 cde	94.8 bcd	87.5 a	98.5 ef	93.9 bc
‘816’ control	98.4 ef	99.4 ef	99.8 f	99.5 ef	99.3 ef
‘816’ pruned	98.9 ef	99.1 ef	99.8 f	100 f	98.8 ef

Discussion

Selective limb removal to control tree height

The selective limb removal strategy in this study succeeded in restricting tree height by 1.1 m, with only a negligible (2%) reduction in yield. The reason for the success is that limb removal had little effect on total orchard PAR interception (Olesen et al., 2007) and the trees could compensate for the loss of racemes on the pruned wood. Only a tiny proportion of macadamia flowers set fruit, and the potential for the remaining racemes at the top of the canopy to set fruit would have increased with the opening up of the canopy (Olesen et al., 2011, 2016).

The strategy can be used on trees up to 6 m, because the pruning can be done from the ground with a long-handled pole-saw. It already has some early industry adoption. For taller trees it may be used in conjunction with half-topping. The half-topping reduces half the canopy to a height that can be worked from the ground with a pole-saw strategically and with safety.

Differences in yields between cultivars

'246' had a higher cumulative yield than '816' across the first four seasons, and has had higher yields across the whole life of the orchard (McFadyen et al., 2016). In the current study, the canopy size of '246' was similar to that of '816' in the early years and possibly smaller in the latter years, given that the '246' trees were shorter. Thus superior light interception by '246' does not appear to be the explanation.

There is some evidence that '246' may have performed better than '816' in a fully self-buffered setting owing to higher self-compatibility (McFadyen et al., 2016). Differences in the phenology of flowering is another possibility, given that the first flowering of '246' is slightly earlier than that for '816' (Boyton and Hardner, 2002) or perhaps some other aspect of phenology, such as the consistently earlier nut-drop of '816' observed here. Yet another possibility is unsynchronized bienniality between the cultivars, given that, in 2016, the highest yield of '246' also corresponded with the lowest yield of '816'. And yet another is differential pest pressure, given the apparent preference of MSW for '816' over '246'. Or it may simply be an intrinsic difference in the partitioning of resources between vegetative and reproductive growth, perhaps related to the shallower location of racemes within the canopy for '246' (Salter et al., 2005), nearer to the leaves. There appeared to be a difference in resource partitioning between the cultivars in that the lower yielding '816' was taller than '246' by the end of the study, consistent with a negative correlation between branch elongation and crop load (Wilkie, 2009), but whether the difference was intrinsic or the consequence of the other factors outlined above is unknown.

In 2018, significant reduction in yield by '246' compared to '816' may have been associated with unseasonably hot winds occurring during peak flowering of '246', as anecdotally reported by growers in the district. Consequent reduction of pollination and fruit set would have resulted in the reduced yields of both control and pruned trees. However, the impact of the previous pruning in August 2017 on reducing yields in 2018 cannot be discounted.

Nut quality

There was a higher incidence of insect damage to kernel in 2017 in the control ‘246’ trees compared with the pruned ‘246’ trees. PAR transmission in the pruned trees was significantly higher in the same season. The greater openness of the pruned trees may have improved spray coverage, but open trees may also be less amenable to insect pests. Govender (2015) found a significantly higher incidence of beneficial insects in more open canopies, and a non-significantly lower incidence of insect pests.

Crop load appeared to affect nut size, with a tendency for smaller nuts with higher yields, consistent with the raceme-stripping experiments by Wilkie (2009). Wilkie (2009) also found an increase in kernel recovery with increasing crop load, but this was not obvious in our experiment. A possible reason for this is that the effect of crop load on kernel recovery is much subtler than that on nut size.

McFadyen et al. (2016) found that ‘816’ had larger nuts, higher kernel recovery and higher proportions of first grade kernel than ‘246’ and this was also found here. McFadyen et al. (2016) also reported similar incidences of unsound kernel for the two cultivars. Our results were more variable, with a tendency for a higher proportion of unsound kernel in ‘246’, largely caused by a higher incidence of immature kernel (data not shown). The detailed assessment of kernel quality for the entire 2017 harvest supported this, with‘816’ having a higher overall proportion of sound kernel than ‘246’ largely owing to a lower level of immaturity.

Macadamia seed weevil (MSW) damage

MSW is a relatively new pest of macadamia in Australia, and thought to be responsible for major crop losses. In work conducted in orchards adjacent to our experimental orchard on the same experimental farm in 2015-2016, for example, crop losses to insect pests were mostly attributed to fruit-spotting bug (*Amblypelta nitida* Stål) and MSW in a corresponding ratio of 70:30 (Maddox et al., 2016).

Virtually nothing is known about the movement, distribution and feeding preferences of MSW in either its native habitat or in macadamia orchards (Maddox et al., 2015). It can fly, so there is the possibility of rapid infestation from large distances.

Infestation was higher in ‘816’ than in ‘246’. The preference was not obviously related to crop load given that the final yield of ‘816’ was 2 kg of NIS per tree less than ‘246’, and the median losses to MSW based on nuts on ground were 0.48 and 0.12 kg of NIS per tree for ‘816’ and ‘246’ respectively. Differences in the phenology of flowering, fruit set and early fruit development may have been important. It is also possible that the thinner shell and larger kernel of ‘816’ were related to the preference for this cultivar, but how MSW might perceive these characteristics is unclear. The preference for ‘816’ may be shared by other pests given that insect damage to kernel across the 2017 harvest was higher for ‘816’ than ‘246’; and fruit-spotting bug may favour cultivars with thinner shells (pers. comm. C. Maddox 2017). However a difference in husk hardness is another possibility. Fruit with harder husks are less prone to infestation by macadamia nut borer (*Cryptophlebia ombrodelta* Lower; pers. comm. C. Maddox 2017).

The distribution of MSW within the experimental orchard was consistent with infestation from the North-West. Given that cultivar ‘A4’ flanked the experimental orchard to the North and West; is prone to out of season flowering and did throw out of season flowers over the 2016-2017 summer; and has a nut with a thin shell and a large kernel; it may be that these trees were a major refuge and source of infestation. The remnant rainforest a few hundred meters to the North-West is another possibility. Still further in this direction are unsprayed macadamia trees that provide us with a source of MSW for experimental purposes.

Conclusion and future research

Selective limb removal is an effective tree height control strategy for the macadamia industry. It has little effect on yield if undertaken judiciously, and the effects on nut quality, although small, are likely to be positive: a small reduction in kernel losses to insects.

The benefit of early versus late intervention in tree height control was not clear from this study. The yield of the harvest in 2018, following pruning in August 2017 was generally reduced, in part associated with unfavourable climatic conditions. Thus the impact of selective-limb removal, in the longer term, requires further investigation.

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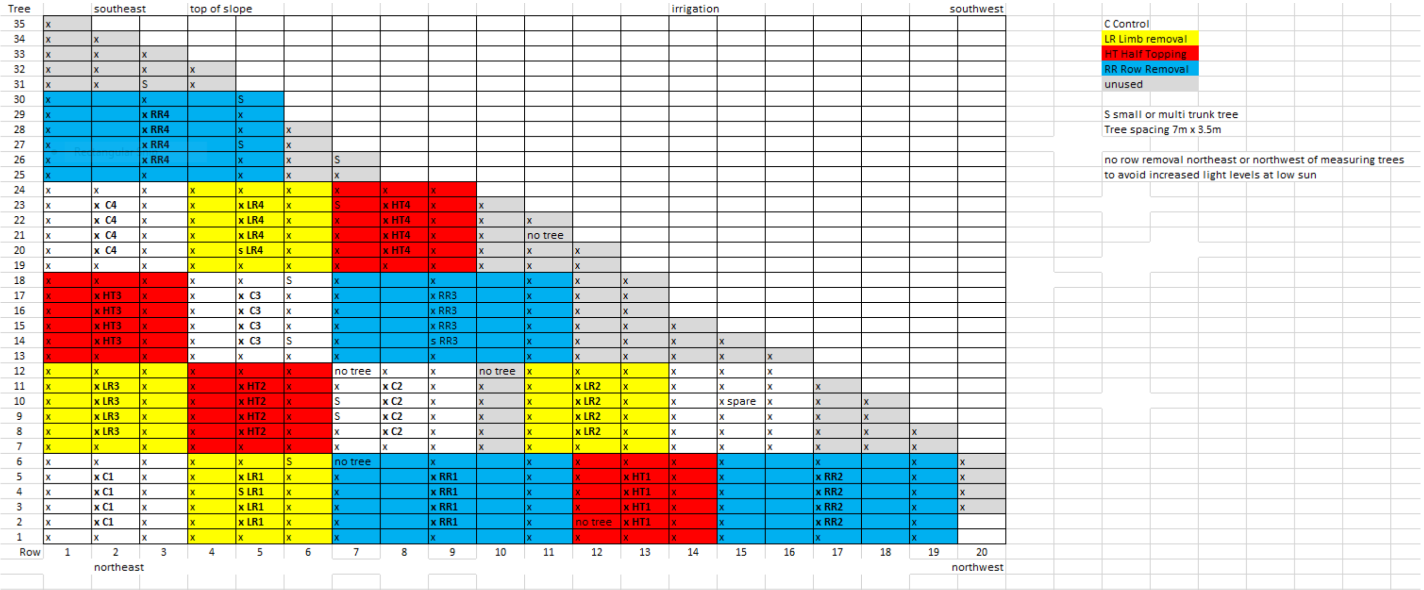
Mature orchard canopy management strategy

A comparison trial has commenced at Wollongbar Agricultural Institute field station, Alstonville, NSW. Treatments including limb removal, half tree topping and entire row removal were completed in 2019. The arrangement of treatments in the field is shown in Figure 5. Yield will be limited in 2020 due to the quantity removed as required for rejuvenation.



Tree		southeast		top of slope										irrigation						southwest
35	x																			
34	x	x																		
33	x	x	x																	
32	x	x	x	x																
31	x	x	S	x																
30	x		x		S															
29	x		x RR4		x															
28	x		x RR4		x	x														
27	x		x RR4		S	x														
26	x		x RR4		x	x	S													
25	x		x		x	x	x													
24	x	x	x	x	x	x	x	x	x											
23	x	x C4	x	x	x LR4	x	S	x HT4	x	x										
22	x	x C4	x	x	x LR4	x	x	x HT4	x	x	x									
21	x	x C4	x	x	x LR4	x	x	x HT4	x	x	no tree									
20	x	x C4	x	x	s LR4	x	x	x HT4	x	x	x	x								
19	x	x	x	x	x	x	x	x	x	x	x	x								
18	x	x	x	x	x	S	x		x		x	x	x							
17	x	x HT3	x	x	x C3	x	x		x RR3		x	x	x							
16	x	x HT3	x	x	x C3	x	x		x RR3		x	x	x							
15	x	x HT3	x	x	x C3	x	x		x RR3		x	x	x	x						
14	x	x HT3	x	x	x C3	S	x		s RR3		x	x	x	x	x					
13	x	x	x	x	x	x	x		x		x	x	x	x	x	x				
12	x	x	x	x	x	x	no tree	x	x	no tree	x	x	x	x	x	x				
11	x	x LR3	x	x	x HT2	x	x	x C2	x	x	x	x LR2	x	x	x	x	x			
10	x	x LR3	x	x	x HT2	x	S	x C2	x	x	x	x LR2	x	x	x spare	x	x	x		
9	x	x LR3	x	x	x HT2	x	S	x C2	x	x	x	x LR2	x	x	x	x	x	x		
8	x	x LR3	x	x	x HT2	x	x	x C2	x	x	x	x LR2	x	x	x	x	x	x	x	
7	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
6	x	x	x	x	x	S	no tree		x		x	x	x	x	x		x		x	x
5	x	x C1	x	x	x LR1	x	x		x RR1		x	x	x HT1	x	x		x RR2		x	x
4	x	x C1	x	x	S LR1	x	x		x RR1		x	x	x HT1	x	x		x RR2		x	x
3	x	x C1	x	x	x LR1	x	x		x RR1		x	x	x HT1	x	x		x RR2		x	x
2	x	x C1	x	x	x LR1	x	x		x RR1		x	no tree	x HT1	x	x		x RR2		x	
1	x	x	x	x	x	x	x		x		x	x	x	x	x		x		x	
Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
		northeast																		northwest

C Control
LR Limb removal
HT Half Topping
RR Row Removal
unused

S small or multi trunk tree
Tree spacing 7m x 3.5m

no row removal northeast or northwest of measuring trees
to avoid increased light levels at low sun

Fig. 5. Treatment plots for the mature orchard trial as they are arranged in the field.

Appendix 9

Mango vigour control using rootstocks

Selection of vigour controlling mango rootstocks.

Bally, I., Ibell, P., Wright, C., Mizani, A., Kolala, R., Kare, M., Scobell, Z., Maddox, C.

Photo - The effect of mango rootstocks on scion vigour. Photos are of three year old tree of the mango scion variety NMBP-4069 growing on two candidate rootstocks taken in November 2018. Left photo is the vigour-reducing rootstock 'WR181' shortlisted in this project. Right photo is one of the vigorous non selected rootstock. The low crop load on the vigorous tree was common in the experiment, demonstrating the partitioning of carbon resources between vegetative and reproductive growth.

Summary

Mango trees are large, vigorous, tropical, evergreen trees that are traditionally grown low density, extensive orchard systems. The trend towards orchard intensification in tree crops requires smaller less vigorous trees to be grown at high planting densities. Managing tree size and vigour over the life of an orchard is essential for successfully increasing productivity in intensive orchards. Dwarfing rootstocks have become one of the major tool for vigour management successful tree crop systems. In mango, only a few vigour-reducing rootstocks have been identified and none of them are used extensively in commercial orchards. A field experiment was established to evaluate the vigour-controlling performance of 97 potential rootstocks on two scion cultivars from the National Mango Breeding Program, 'NMBP-1243' and 'NMBP-4069'. This is an interim report on the progress of the rootstock selection field trial at the end of the 'Transforming Subtropical and Tropical Tree Crop Productivity' project (AI13004). The evaluation and selection of rootstocks is not yet complete, however, at this stage, a shortlist of potential vigour-reducing rootstocks with high yield efficiency has been developed for each scion cultivar. Each shortlisted rootstock has demonstrated significant scion canopy size reduction compared to 'Kensington Pride' rootstock and has high yield efficiency. Approximately 30% of shortlisted rootstocks are common between scions. Evaluation and selection will continue in the project 'National Horticultural Tree Crop Intensification' (AS18000).

Introduction

Mango trees are large, vigorous, tropical, evergreen trees, requiring a lot of space when left to grow to their natural limits of up to 40 meters high. Effective management of growth and crop (e.g., pruning, spraying and harvesting) become difficult and expensive in large mango trees. Tree size in commercial mango orchards is currently managed by annual pruning, with orchards commonly designed as low-density planting systems, either 10 m × 10 m or 6 m × 9 m (100 or 185 trees per hectare), based on the expected eventual tree size (Khan et al. 2015; Schaffer et al. 1994; Fivaz 2006; Stassen et al. 2000). Low-density tree spacing is considered to be one of the reasons for low productivity in mango orchards (Bally and Ibell 2015). Another reason for low productivity is canopy shading when trees fill their allocated orchard space. In mango, shading is eventually a problem for all planting densities, even in the low-density planting systems. Yield commonly decreases after ten years due to the tree canopies starting to shade each other. Increasing planting-density (7 × 4 m) is practised with semi-dwarf cultivars such as ‘Keitt’, ‘Calypso’ and ‘Brooks’ which generally increase yield (Bally and Ibell 2015). In India, planting the slow growing and regular bearing cultivar ‘Amrapali’ in a high-density orchard of 1600 trees per hectare was successful, however, trees required judicious pruning to maintain their productivity (Majumder and Sharma 1989).

In many temperate tree fruit crops such as apple, pear and cherry, vigour-managing rootstocks underpin intensive orchard systems and represent a significant tool for long-term vigour management. In mango, dwarfing rootstocks also have the potential to reduce scion vigour and reduce the management of tree size which is critical for the sustainable life of high density orchards (Reddy et al. 2003). Mango rootstocks have also been reported to influence scion productivity (Smith et al. 2003; Lavi et al. 1997; Negi 2000; Whiley and Saranah 1995), moderate alternate bearing (Monselise and Goldschmidt 1982), tolerance to saline and calcareous soils and poor water quality (Kadman et al. 1976; Gazit and Kadman 1980; Pandey et al. 2014), resistance to diseases and mango wilt (Rossetto et al. 1997; Arriel et al. 2016), and reducing scion vigour (Ramos et al. 2004; Reddy et al. 2003; Smith et al. 2008). Dayal et al. (2016) inferred that rootstocks alter most of the physio-chemical parameters of scion cultivars in mango, although the extent of regulation of activities was scion specific. Vigour-reducing rootstocks have the potential to lower mango canopy vigour by diverting energy from vegetative growth into productivity and contributing to sustainable high yields. But as indicated by Bally and Ibell (2015) very few rootstocks are known to reduce tree vigour in mango and those that do only work on a few scion varieties (Reddy et al. 2003). However, it has been shown that several rootstocks do have a vigour-reducing effect on scions. For example the mango rootstock ‘Vellai Kulamban’ reduced vigour of the scion ‘Alphonsio’, and increased the productivity per unit canopy volume and per land area (Reddy et al. 2003), however, when used with the scion ‘Kensington Pride’ yields were poor (Smith et al. 2008). They also found, rootstock effects varied between years and soil types, indicating the existence of strong rootstock/scion/environment interactions, making the selection of a universal dwarfing rootstock more difficult without extensive testing. Internationally, no known rootstock combines all of the above mentioned benefits and no commercially used rootstocks even combines two of the beneficial traits (Galán Saúco 2019).

Polyembryonic mango varieties are generally preferred as rootstocks because of the genetic uniformity of their multiple nucellar embryos that provide more than one rootstock per seed. In Australia ‘Kensington Pride’ is the most commonly used rootstock generally chosen for its polyembryonic nature and its availability. There is currently no evidence that ‘Kensington Pride’ when used as a rootstock imparts any vigour reducing characters to scions.

Selecting candidate mango dwarfing rootstocks is difficult as there are no known varietal traits in rootstocks that indicate their effects on scion performance. Several authors have concluded that dwarf cultivars do not necessarily become dwarfing rootstock for other scions and vigorous cultivar seedlings do not always translate their vigour to scions (Oppenheimer 1958; Swamy et al. 1972; Cedeno-Maldonado et al. 1988; Smith et al. 2008).

The aims of the experiment presented here were to identify vigour-controlling rootstocks suitable for intensive mango orchard production systems and to select rootstocks that effectively reduced canopy vigour while maintaining productivity in mango scion varieties used in the Australian mango industry.

Methodology

Aims

This experiment aimed to select vigour reducing rootstocks for mango that maintained or improved scion productivity on a yield per canopy volume basis. A field experiment was established to evaluate the specific aims of:

1. Identifying if the vigour of mango be controlled by rootstocks.
2. Screening potential vigour reducing rootstocks available in Australia from the National Mango Genepool and the Queensland Mango Breeding program using two scion varieties.
3. Identifying the production efficiency on each rootstock/scion combination as yield per canopy surface area and yield per canopy volume.

Experimental Design

A field experiment was established on Walkamin Research Station (D° MM' SS", 17° 07' 52" S and 145° 25' 33" E), to evaluate 97 genetically different, candidate vigour-reducing rootstocks on two mango scions. The experiment was designed with unequally replicated incomplete blocks, with a maximum of four replications of each rootstock-scion combination. 20 trees per row were planted 3 meters apart in North-South aligned rows, with 6 meters between rows. A total of 600 trees were planted in 20 rows.

Plant material

Candidate rootstocks were selected from a diverse range of germplasm from the Australian Mango Breeding Program and the Australian Mango Genebank. Candidate rootstocks were chosen for their dwarfing habits, their mentions in the literature as potential dwarfing rootstocks or as non *Mangifera indica* related species. Most of the candidate rootstocks were polyembryonic cultivars and varieties, which produce seedlings from nuclear embryos that are genetically uniform and genetically similar to the mother tree (true-to-type from seed). Several monoembryonic candidates were also selected. Candidate rootstocks species were mainly from *M. indicia* but several other Mangifera species and more distantly related species from the family Anacardiaceae were also included. However the non-Mangifera, Anarcadiacae species were all graft incompatible and dropped from the field experiment. The standard industry rootstock ‘Kensington Pride’ (KP) was included in the experiment as a control.

Two scion cultivars ‘NMBP-1243’ and ‘NMBP-4069’, recently released for commercial production from the National Mango Breeding Program (NMBP), were used to evaluate the rootstocks vigour reducing capacity. ‘NMBP-1243’ has high fruit quality, medium to high vigour with an open canopy and early production season. ‘NMBP-4069’ is a high yielding, high fruit quality, with a vigorous and dense canopy and mid-season production. These scions were chosen for their potential to be used in intensive production orchard systems.

Rootstock seed was collected from trees growing on the Department of Agriculture and Fisheries Southedge Research Station and other sources during the 2012/2013, 2013/2014 and 2014/2015 cropping seasons. Rootstocks were grafted from 10 months of age with one of the two scion varieties. The grafted rootstocks were field planted after the grafts had healed in either May or September of 2014, 2015 or 2016. Germination, propagation and field planting or the candidate rootstocks was staggered over 3 years because of seed and budwood availability, and propagation logistics.

All candidate rootstocks were assigned a Walkamin Rootstock number in the format of “WR###”, for their identification and to protect their intellectual property.

Tree Management

For the first three years after field-planting trees were not pruned to maximise canopy growth and accentuate vigour differences for assessments. This approach produced trees with long, thin, minimally branching limbs that became unstable in wind, causing many trees to lose one or more of the major limbs. To counteract the major limb breakage trees were pruned annually after harvest from three years old. Tree nutrition, irrigation and pest management were carried out as per commercial recommendations (Kernot et al. 2000).

Measurements and Methods

Canopy vigour was assessed biannually from planting in all trees. Measurements included the canopy dimensions of tree height (ground level to top of the canopy in cm), canopy width (diameter of the canopy across the row in cm), canopy length (diameter of the canopy along the row in cm), canopy skirt height (ground level to bottom of the canopy in cm) and canopy depth (bottom of the canopy to the top of the canopy in cm). Canopy surface area (m2) and canopy volume (m3) were calculated using the following formulas, where L, W and D are half the canopy length, canopy width and canopy depth respectively:

$$Canopy\ Surface\ Area = 4\pi \left[\frac{(LW)^{1.6}+(LD)^{1.6}+(WD)^{1.6}}{3}\right]^{1/1.6}$$

$$Canopy\ Volume = \frac{4\pi}{3} LWD$$

At each harvest, the number of fruit per tree, average fruit weight per tree measured on 25 fruit (g) and yield per tree (kg) were calculated. As mango is a terminal bearing species, annual yield data was calculated and expressed on a canopy surface area and on a canopy volume basis. Yield efficiency per surface area and yield efficiency per canopy volume were calculated as follows:

$$Yield\ efficiency\ per\ surface\ area = {Yield\ (kg)}\Big/{Canopy\ surface\ area\ (m^2)}$$

$$Yield\ efficiency\ per\ canopy\ volume = {Yield\ (kg)}\Big/{Canopy\ volume\ (m^3)}$$

Statistical Methods

Analyses of canopy surface areas, canopy volumes, yield per tree and yield efficiency per canopy surface area and yield efficiency per canopy volume have been carried out after each assessment as well as a combined repeated measures analysis across all time assessments. For the repeated measures analyses, each planting year was considered as a separate analyses and these are the results discussed in this appendix.

The repeated measures linear mixed models were fitted using factor analytic (FA) covariance models and an antedependence residual correlation model in ASReml-R package (Butler et al. 2017) within the R statistical environment (R Core Team 2019). For the yield efficiency analyses an unstructured covariance and residual model were fitted due to a maximum of only four harvests recorded. Each rootstock-scion combination was fitted as a separate treatment level in the random model. For planting years 2014 and 2015, a term representing the effect of the May and December plantings was also included in the fixed effects model (R core Team 2019).

The predicted means at each assessment are obtained from the repeated measures analysis for the rootstock-scion combinations in each planting year. The predicted means for each assessment were statistically compared to that of ‘Kensington Pride’ on the same scion. The number of times a rootstock-scion had a significantly smaller predicted mean than ‘Kensington Pride’ was calculated. Significance testing was performed at the 0.05 level.

In order to obtain a single relative value to compare the combinations across the three planting years, the area under the curve (AUC) for the predicted means with respect to time was also calculated for each combination. By expressing the calculated AUC as a percentage of the AUC for ‘Kensington Pride’ on the same scion planted in the same year, a single relative measure incorporating all assessments is obtained. For canopy measurements, a smaller tree than ‘Kensington Pride’ will have a relative AUC less than 100%, while a tree with better yield efficiency than ‘Kensington Pride’ will have a relative AUC greater than 100%.

The relative AUC, expressed as a percentage of ‘Kensington Pride’ on the same scion for canopy surface area, canopy volume and yield efficiency on both canopy surface area and canopy volume basis were calculated for each rootstock-scion combination in each planting year. The relative AUC for each category were ranked by their values and by the number of times the predicted means were significantly different to ‘Kensington Pride’. Shortlisting of rootstocks was undertaken by applying various thresholds to each of the AUC values or their comparative performance to ‘Kensington Pride’, until a suitable short list was achieved. Shortlisting was done separately for each scion cultivar.

This approach was used to accommodate variability in tree size and yield data arising from a complex mix of planting dates, seasonal environments, pruning dates, and previous crop loads.

Results

A shortlist of vigour-reducing rootstocks for the scion 'NMBP-1243' is presented with the relative AUC for canopy surface area, canopy volume and yield efficiency's in Table 1. The shortlist for 'NMBP-1243' was achieved by only including rootstocks that met the following criteria:

1. Predicted scion canopy surface areas that were significantly smaller than 'Kensington Pride', on 75% or more of the occasions the trees were measured.
2. Predicted scion canopy volumes that were significantly smaller than 'Kensington Pride', on 75% or more of the occasions the trees were measured.
3. Yield efficiency on a canopy surface area basis, with predicted means greater than 100% 'Kensington Pride'.
4. Yield efficiency on a canopy volume basis, with predicted means greater than 100% 'Kensington Pride'.

A short list of vigour-reducing rootstocks for the scion 'NMBP-4069' is presented with the relative AUC for canopy surface area, canopy volume and yield efficiency's in Table 2. The shortlist for 'NMBP-4069' was achieved by only including rootstocks that met the following criteria:

1. Predicted scion canopy surface areas that were significantly smaller than 'Kensington Pride', on 50% or more of the occasions the trees were measured.
2. Predicted scion canopy volumes that were significantly smaller than 'Kensington Pride', on 50% or more of the occasions the trees were measured.
3. Yield efficiency on a canopy surface area basis, with predicted means greater than 85% 'Kensington Pride'.
4. Yield efficiency on a canopy volume basis, with predicted means greater than 85% 'Kensington Pride'.

Early yields in young trees vary greatly and are not a reliable indicator of future tree performance. In this rootstock field trial, tree yields have varied considerably due to tree juvenility (tree age) and a significant first prune in their third year. This variability is not uncommon and is often referred to as irregular bearing. In many cultivars and environments, the irregular bearing becomes more regular and takes on a saw tooth biennial bearing pattern as trees mature. No link between yield efficiency and tree size is apparent in this data set, which is not unexpected as Smith et al (2008) also did not find a link between yield efficiency and tree size among 64 mango rootstocks. For the purposes of shortlisting candidate rootstocks, we have calculated the yield efficiency on both a canopy surface area and a canopy volume basis for yield collected since the trees were 3 years of age. Analyses of the yield efficiency in this way enables comparison of yield efficiency that is independent or tree size and more useful for intensive, high-density orchard systems. Yield data in the youngest trees, planted in 2016, was based on two years, while the 2014 and 2015 planted trees, was based on 4 and 3 years respectively. For this reason another year of data will be collected and analysed in the next phase of this work under the 'National Tree Crop Intensification Program' (AS18000). Additional data and re-analyses with refined repeated measures models will be used to reduce the number of rootstocks in each shortlist and to provide confidence in the estimation of rootstock-scion performance, independent of all the environmental and management variability.

Table 1. Relative AUC (%) of canopy surface area, canopy volume and yield efficiency in shortlisted vigour-reducing rootstocks for the scion cv. 'NMBP-1243', selected from 97 candidate rootstocks. Listed rootstocks have ≥ 75% of canopy volume and canopy surface area measurements significantly lower than 'Kensington Pride' rootstock and a yield efficiency ≥ 'Kensington Pride' rootstock on scion 1243. Rootstocks in bold font are common between the shortlists for scions 'NMBP-1243' and 'NMBP-4069'.

Scion cv. 'NMBP-1243'				
Rootstock	AUC Canopy Surface Area (% of KP)	AUC Canopy Volume (% of KP)	AUC Yield Efficiency (canopy volume: % of KP)	Yield Efficiency (canopy surface area: % of KP)
WR177	60.19	49.48	103.22	103.14
WR131	73.28	63.65	104.88	110.06
WR159	73.31	64.08	105.53	106.85
WR124	74.93	65.27	111.32	122.03
WR125	75.04	67.53	118.92	121.43
WR113	75.25	67.41	117.18	117.72
WR127	75.33	68.84	125.05	132.17
WR122	75.48	66.67	114.98	118.76
WR156	75.82	66.83	115.06	100.51
WR183	76.19	67.74	119.86	120.96
WR145	76.73	68.97	125.58	126.49
WR161	77.61	70.96	132.70	143.83
WR165	78.11	66.61	113.61	120.63
WR153	78.13	68.50	122.77	127.32
WR108	78.99	71.45	133.55	139.74
WR137	79.50	67.81	122.20	112.74
WR126	79.54	70.07	130.04	144.19
WR147	79.76	72.79	137.27	140.99
WR109	79.76	73.02	138.02	142.31
WR196	80.94	74.11	146.95	131.08
WR101	82.06	73.22	141.12	144.65
WR133	82.29	75.57	147.50	144.63
WR104	82.33	72.55	136.76	140.66
WR118	87.34	81.84	149.59	149.69

Table 2. Relative AUC (%) of canopy surface area, canopy volume and yield efficiency in shortlisted vigour-reducing rootstocks for the scion cv. 'NMBP-4069'. Listed rootstocks have ≥ 50% of canopy volume and canopy surface area measurements significantly lower than 'Kensington Pride' rootstock and a yield efficiency ≥ 'Kensington Pride' rootstock on scion 4069. Rootstocks in bold font are common between the shortlists for scions "NMBP-1243' and 'NMBP-4069'.

Scion cv. 'NMBP-4069'				
Rootstock	AUC Canopy Surface Area (% of KP)	AUC Canopy Volume (% of KP)	AUC Yield Efficiency (canopy volume: % of KP)	Yield Efficiency (canopy surface area: % of KP)
WR181	42.74	31.13	140.85	104.68
WR115	59.44	48.30	99.43	87.75
WR190	67.92	57.23	100.66	86.29
WR177	68.89	57.28	98.74	88.38
WR164	70.75	60.78	105.11	96.58
WR125	71.61	60.82	103.03	92.25
WR191	74.82	62.71	112.85	98.57
WR142	75.01	64.57	113.71	102.84
WR116	76.17	71.04	119.56	97.28
WR114	77.95	69.89	130.59	112.93
WR180	78.91	66.93	113.40	97.68
WR184	78.93	68.32	107.65	99.08
WR196	79.54	71.15	121.95	114.01
WR131	79.94	69.60	181.03	151.53
WR198	80.39	69.68	102.12	89.63
WR145	80.46	70.46	166.99	143.36
WR156	81.79	69.47	244.53	197.08
WR106	83.14	73.30	158.71	132.34
WR165	83.71	72.84	138.20	125.21
WR167	84.68	78.09	103.85	97.57
WR159	85.29	77.64	169.08	154.60
WR100	85.41	82.24	108.32	103.37

Discussion

Selection of vigour reducing rootstock in tree crops is complex and time consuming. This is especially so in mango, where the physiological mechanisms involved in rootstock induced scion vigour reduction are unknown. However, despite this, there is considerable need for dwarfing rootstocks in mango to enable closer tree spacing that will essentially increase the productive canopy volume per hectare, earlier in the useful life of an orchard. Smaller trees also have the advantage of being easier to manage, with operations such as spraying and harvesting simplified. Work in this project, on mango orchard intensification, has demonstrated considerable yield increases on a per hectare basis when trees are planted, trained and managed as in high-density orchards. In other crops such as apple and cherry, (Celton et al. 2006; Bondarenko 2019) reducing tree vigour has not only facilitated higher density planting but it has also tilted the balance between vegetative growth and fruit production, and has been an essential technology for the development of high productivity intensive orchard systems. Smaller, slower growing trees, reduce farm labour needed for annual pruning and management on a per-kilo of production basis. They also extend the life of the orchard. In mango, many high-density orchards have failed earlier than they could because tree vigour dominates causing excessive shading or continual vegetative growth without dormancy discouraging flowering.

Identification and selection of useful vigour-reduction rootstocks in mango is a long-term endeavour taking longer than 5 years and requiring extensive performance assessments across a range of scions, environments and management practices. At the end of the ‘Transforming Subtropical and Tropical Tree Crop Productivity’ (AI13004) project, six years progress toward these goals has been made. We have demonstrated that some mango rootstocks have the ability to reduce scion vigour (Table 1 and 2). The effect of individual rootstock cultivars varies considerably between scions, as can be seen in the differences between the shortlists for each scion (Table 1 and 2). This specific rootstock/scion effect has also been observed by others in mango (Reddy et al. 2003 and Smith et al. 2008). However, there was some overlap between the two shortlists, with seven rootstocks appearing in both lists. These may indicate rootstocks that have a wider vigour-reducing effect. As only two scions have been tested to date, it is not possible to determine if any of them can be considered as a universal rootstock for all mango cultivars. It is more likely that some rootstocks will work on more than one scion and may work for small groups of scions. Only further testing on a wider range of scions will reveal how restricted the effect of any given rootstock is. Assessment of rootstock performance, on additional popular commercial cultivars, in an intensive orchard system will determine which scion cultivars’ are suitable for growing in intensive orchard systems where tree vigour may currently be a problem. It will also help develop training, pruning and management practices for specific rootstock/scion combinations grown intensively at high-density and to document the benefits over conventional Kensington Pride rootstocks in intensive orchards.

Demonstration of rootstocks with vigour-reducing characteristics and relative strong yields is an encouraging first step towards vigour management in intensive mango orchard systems. Further evaluation of selected rootstocks’ contribution to vigour management in a high-density orchard is required to assess their effects on other vigour management operations such as training and pruning. Integration of rootstocks in to an intensive orchard system will be experimentally tested after the next round of shortlisting and selection. It is likely that rootstocks will have a significant role in canopy and crop management of future intensive mango orchards, but will not be the only management tool available. Branch training, annual pruning and the use of the growth regulator paclobutrazol, will remain as important tools. The requirement of lower vigour, smaller trees is also influencing the selection of new cultivars and Breeders are developing high yielding semi-dwarf and dwarf scion cultivars.

To date our selection of vigour reducing rootstocks has been based on the performance of two scions in a replicated selection field trial on the Department of Agricultures’ Walkamin Research Station. As the mechanisms for rootstock induced vigour reduction have not yet been elucidated, we cannot yet predict the performance of other candidate rootstocks on scion vigour. As such, it is difficult to select potential parents or progeny in a rootstock cross breeding program.

Identifying the physiological or morphological characteristics or mechanisms will be an important part of ongoing mango rootstock research that has potential to improve the efficiency of identifying candidate rootstocks in the future. Early detection of rootstocks based on their seedling performance is not yet predictable. Smith et al. (2008) also were unable to relate the morphology of different genotype seedlings to subsequent performance as a rootstock. Observation of growth at the tree and branch level and differences in canopy architecture or anatomical aspects of rootstock may identify how growth is contributing to dwarfing or reduced canopy growth. Growth analyses may also provide some information on the best training and management of low vigour canopies. Rootstock performance data from the Walkamin field trial is being used by the 'National Horticultural Tree Genomics Project' (AS17000) to develop and test phenotype prediction models from genotype. If successful, this modelling will assist in the identification of potential progeny and parents in rootstock and scion breeding programs.

1. Identification and selection of useful vigour-reduction rootstocks in mango is a long-term endeavour taking longer than 5 years and requiring extensive performance assessments across a range of scions, environments and management practices. At the end of the 'Transforming Subtropical and Tropical Tree Crop Productivity' (AI13004) project, six years progress toward these goals has been made. We have shortlisted 24 vigour-reducing rootstocks for scion 'NMBP-1243" and 22 for the scion 'NMBP-4069' (Table 1 and 2). The shortlisted rootstocks still require further assessment and analyses to identify those rootstocks worthy of selection. The evaluation and selection process is not yet complete and will continue in the 'National Tree Crop Intensification Program' (AS18000). The shortlist will be firmed-up with further evaluation and analyses of rootstock vigour and cropping performance data over time to eliminate environmental and management noise. Beyond the shortlisting, evaluation will include: continued monitoring of the short listed rootstocks in the current Walkamin Research Station field trial, testing of performance with a wider range of scion cultivars and evaluation in an intensive orchard setting.

   In addition to the selection of vigour-reducing rootstocks, there may be some benefits to be gained from:

2. Further studies to identify characteristics contributing to rootstock induced vigour reduction through continued assessment of growth and branching data to identify the relationships between vegetative growth, flowering and fruiting.
3. Investigations of rootstock and scion trunk anatomical variations between dwarfing and non-dwarfing rootstocks comparing rootstocks and scions.
4. Identifying high yielding rootstocks that do not reduce tree vigour.
5. Categorising the strength of vigour-controlling rootstocks to match vigour reduction with different scions, soil types, environments and orchard systems.
6. Breeding for, and the use of, vigour controlling scions for vigour control.
7. Further testing of seed derived rootstocks with clonally derived rootstocks through tissue culture or cuttings may help to reveal the relative contribution from root systems or stem to the dwarfing effects.

Conclusions

The search for mango rootstocks with the potential to reduce tree vigour and maintain or improve tree productivity on a canopy volume basis has made significant progress and proven to be a worthwhile endeavour. Although not yet completed the selection process is well on the way with many of the shortlisted candidates showing similar or better yield efficiency than when Kensington Pride is used as a rootstock. Plans for the next phase of evaluation will be firmed up as the current short list is confirmed. Second generation field trials are expected to start as soon as practical, within two years, to minimise any delays in this essential component of intensive high production orchard systems in mango.

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Appendix 10: Mango light relations


Light trolley with PAR sensors and data-logger

Light Relations in Intensive Mango Orchards

Kare Mahmud*[1], Paula Ibell[1], Carole Wright[1],
John Wilkie[2], and Ian Bally[1]

1 Department of Agriculture and Fisheries, Centre for Tropical Agriculture, 28 Peters St, Mareeba, Qld,
2 Department of Agriculture and Fisheries, Bundaberg Research Facility, 49 Ashfield Road, Bundaberg, Qld.


Mahmud Kare with the handheld ZEB1 LIDAR tool which has also shown potential for future light interception measures.

- Kare.Mahmud@daf.qld.gov.au or
- Paula.Ibell@daf.qld.gov.au

Introduction

The transition from large, conventional tree orchards to small tree, high productivity mango orchards has the potential to increase profits for mango growers. However, there is little understanding of how mango canopy management systems are related to light interception, light distribution and productivity in mango orchards. Hence, the objective of this research was to document relationships between light interception, canopy size and yield in 3 varieties at 3 planting densities using 3 training systems in mango orchards. Here we present early results (up to 5 years) of the light relations including light interception and light distribution. In addition, alternative planting configurations may also provide useful information for industry to optimise the productivity of high quality fruit, move to mechanical harvesting and to improve the efficiency of orchard operations in general.

Materials and methods

Mango planting systems design :

- 3 varieties:
  - Keitt, Calypso, NMBP 1243
- 3 training systems:
  - Conventional (Con)
  - Single leader (SL)
  - Single leader on trellis (Espalier)
- 3 planting densities:

Density	Spacing	Tree ha^{-1}
Low (LD)	8 m x 6 m	208
Medium (MD)	6 m x 4 m	416
High (HD)	4 m x 2 m	1250

1. Light trolley used for light interception
2. LP 80 Ceptometer used for light distribution

Light data collection

1. Light interception

2. Light distribution

Figure 1. Aerial photograph of mango planting systems trial at Walkamin research station in Far North Queensland.

Training systems

Results and Discussion

Light interception

- High density planting systems increased canopy volume / ha reaching 40% light interception in the first 5 years, this is an increase compared to a low density and medium conventionally trained systems which had <15% light interception (Figure 2a and b).
- The high density planting systems were able to utilise more light earlier than low density system, which led to increased yield per hectare, up to 5 times higher than conventional low density systems (Figure 2a and c).
- Yields at high density were increased earlier by using more precocious varieties (Keitt and Calypso).

Light distribution

- Single leader training systems (SL and espalier) increased light transmission through the canopy, which resulted in a more evenly distributed light environment within the canopy (Figure 2d);
- The SL and espalier systems had increased mean number of flowering terminals through-out the canopy compared to the conventional training system (Figure 2e).
- Conventional training at high density had a reduced number of mean number flowering terminals through-out the canopy (Figure 2e).

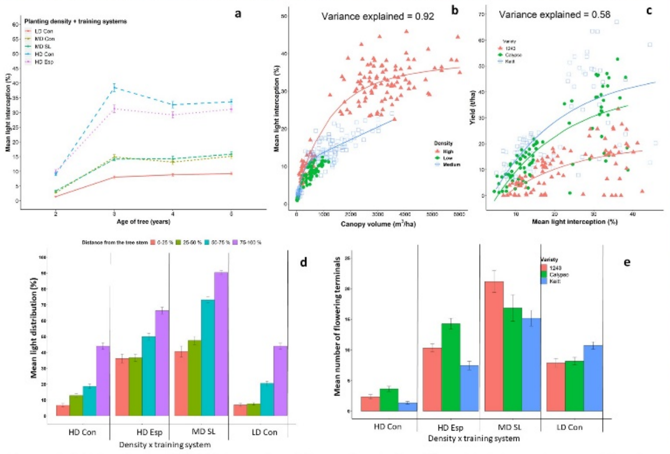

Figure 2. (a) The change in light interception (%) over time in the different training systems and density combinations; the relationship between (b) light interception (%) and canopy volume (m^3 ha^{-1}) from 2015 – 2018; and (c) light interception (%) and yield (tonnes ha^{-1}) for the Mango varieties NMBP 1243, Calypso and Keitt; (d) A comparison of the light distribution (%) within the canopy (at equal distances from the stem); and (e) the number of flowering terminals occurring, within the different training system and density combinations.

Conclusion

- Orchard intensification can increase canopy volumes to intercept more light (~ 40% at 5 years) leading to increased yields earlier than in low density, conventional training systems and yields can be increased earlier through the use of precocious varieties.
- High planting density used in combination with single leader training systems can improve how light is distributed through the canopy, where the espalier and SL training systems had a greater proportion of light distribution in the inner canopy and an increased number of flowering terminals compared to the conventionally trained trees.
- Further experiments in mango intensification are being conducted with commercial partners as a part of the CRC for Northern Australia Project, ‘Mango Futures’. For further information on the CRC project please contact Dr Geoff Dickinson, DAF Mareeba,(07) 4017 0700.
- Further work has been undertaken with University of Sydney, Australian Centre for Field Robotics using the handheld ZEB1 LIDAR tool, which has shown potential for fast, accurate and efficient light interception capture but the results are not outlined in this poster.

Queensland Government
The University of Queensland Australia
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CRCNA
manbulloo Limited
Marto's Mangoes
cirad

Appendix 11

Understanding endogenous limits to the development of crop load in mango (*Mangifera indica* L. cv. Calypso) (Part 1)

Ibell, Paula T., Wilkie, John, Bally, Ian S.E., Wright, Carole, Kolala, Ram, Mizani, Anahita, Kare, Mahmud.

Abstract

Individual tree productivity in commercial orchards depends on a range of factors such as flowering, resource allocation, and pest and diseases. The extent that flowering influences productivity and the other factors is not well understood. This study aimed to examine the relationships between inflorescence density and yield and inflorescence density and flowering in the following season. A range of flowering densities were systematically imposed in 8 year-old Calypso mango trees by removing inflorescences from terminal shoots in varying proportions of the canopy. The study found that the effect inflorescence density on crop load was less than other factors such as carbohydrate resources. Yield efficiency was optimised at a floral density of 2-3 panicles per cm^2 of trunk cross sectional area across each of the varieties. This study has shown mangoes ability to regulate crop load under different flowering densities through the allocation of carbohydrate resources and natural fruit shedding. In the future, fine-tuning of these relationships may help to manage crop load and fruit size to match market requirements.

Introduction

Commercial mango production is limited in general by low tree productivity characterised by irregular, biennial, or erratic bearing (Singh 1978, Capelli et al. 2016). Many factors have been associated with low productivity in mango, including; varieties, insufficient flower buds or terminal shoots (Stephenson 1981), pollen limitation, excessive fruit drop, vegetative growth during reproductive development (Schofield et al. 1986, Chacko 1985, Chacko et al. 1982, Normand et al. 2009, Dambreville et al. 2013), environmental conditions and the interactions among these factors (Wilkie et al. under preparation).

Erratic flowering is also a characteristic of mango, influenced by factors such as spatial variability within the tree (Lin and Chen 1981), and variable floral induction. Factors influencing floral induction include; terminal age, vegetative dormancy, and temperature. Temperatures below $14^{o}C$ have been identified as important for the floral induction in Mango, but this can vary with variety.

Mangoes bear floral inflorescences, on terminal shoots with large numbers of individual flowers. Increasing the number of flowering terminals able to bear inflorescences per unit of canopy has been proposed as a way of increasing flowering and subsequent productivity. However, if the relationship between inflorescence density and yield is likely to plateau as available carbohydrates resources become limiting. Improving the net carbon exchange from vegetative to reproduction growth (partitioning) or increasing carbohydrate production, may be beneficial to lifting productivity. Particularly if the variety is predisposed to biennial or irregular bearing.

Crop load research in mango has found a preferential translocation of carbohydrate resources from leaves to relatively few random fruitlets during the early growth stages of the fruit Chacko et. al (1995). Consequently fruitlets not receiving carbohydrates were shed from the inflorescence. The fruit drop was worse in the variety KP compared to the bunch-bearing variety Neelum. They concluded assimilates were shared more evenly in bunch-bearing varieties, indicating competition for assimilates caused immature fruit drop in mango.

Others have identified that high levels of non-structural carbohydrates are required for flowering (Sing 1960, Suryanarayana 1978, Chacko and Ananthanarayanan 1982) and young leaves are competition for the non-structural carbohydrates at flowering. Young leaves are significant sinks for photosynthates, whereas older leaves are significant sources of photosynthates (Chacko et. al 1982, Chacko and Kohli 1985).

Previous crop load work undertaken by Bally (2006) on 6 year-old Keitt trees found that removal of 70% of fruiting terminals increased fruit weight and the severity of watery pulp breakdown, and lowered tree yields slightly but not always significantly. He found that to maintain a 70% reduction (30% crop load) until harvest, required regular re-thinning of fruit, as trees naturally compensated for lower crop loads by reducing the natural fruit shedding. Bally (2006) also found that reducing fruit load in association with different nitrogen treatments increased individual fruit weight, number and quality (colour chroma and hue) as well as the occurrence and intensity of stem and side lesions and watery pulp breakdown. Bally's results however did leave some ambiguity about whether the results were due to the nitrogen, associated calcium or crop load treatments.

The overall objective in this research was to identify the relationship between inflorescence density and crop load in the mango to assist in developing regular-cropping strategies that reduce biennial or irregular bearing. Two experiments were conducted to investigate this objective. The first experiment manipulated inflorescence density in Calypso, to identify the effect on fruit set and crop load, and to determine the sustainable crop load carrying capacities for the variety Calypso, as 'baseline' for further studies. A second experiment was undertaken at the Walkamin Research Station, Planting Systems Trial in 3 to 5-year-old Calypso, Keitt and NMBP-1243 trees to determine if the baseline relationships for crop load carrying capacities varied between variety, density and training systems.

The aim of the first experiment was to develop a whole tree understanding of the relationship between inflorescence density, fruit set, yield and flowering in the following year in the commercial variety Calypso. With the initial study done in conventionally managed mango canopies, the second experiment compared three varieties, grown under different planting densities and training systems to determine if there was any variation in the relationship between available flowering and fruiting sites. The combination of the two experiments aim to provide a better understanding of the endogenous factors limiting crop load and how these factors influence yield and fruit quality in the commercial mango Calypso. The hypotheses for this experiment were:

1. That inflorescence density influences fruit set and yield;
2. That different fruit loads within canopies influence flowering in the following year.
3. That variety, planting density and training systems may influence the relationships between flowering and fruiting.

Materials and Methods

Experiment 1

This experiment aimed to understand the limits to tree yields in the variety Calypso by manipulating inflorescence density and monitoring the responses of fruit set, fruit size, carbohydrate resources and flowering in the following year.

The first experimental site was located at a commercial mango farm in Dimbulah using 32 eight-year-old Calypso trees planted at 277 trees per ha at 8 x 4.5 m. Inflorescences were removed in varying numbers from flowering trees to create trees with a range of inflorescence densities and crop loads. Fruit set, yield and fruit size were monitored, over two seasons to determine their relationship to inflorescence density.

This experiment was designed as a randomised complete block design with 4 blocks, each containing 8 trees. Each level of inflorescence removal was applied to one tree in each block. The Calypso trees are known for their consistent cropping habit which makes them an excellent candidate for floral manipulation. The experiment was spread between two rows in the orchard. The selected trees were statistically similar in size, between 2.9 – 3.1 m high, with skirts between 0.8 - 0.9 m from the ground, canopy width along the row between 2.8 - 3.1 m and a canopy depth across the row of 3.0 - 3.2 m.

Tree management

During the experiment in 2014-15 and the 2015-16 tree height was annually pruned to maintain trees at approximately 3 m. Side pruning was not conducted for the duration of the experiment. Other aspects of management included fertilisation and application of paclobutrazol following the commercial practices of the farm.

Treatments and monitoring and evaluation

Inflorescence thinning treatments were achieved by stripping flowering panicles between fingers, at full anthesis to remove all florets on the panicle. The number of terminals thinned varied between treatments to establish whole tree treatments with 0 (control), 20, 40, 60, 70, 80, 90 and 95% of terminals with inflorescences removed. A terminal shoot was defined as the shoot that occurs at the outermost periphery of the canopy. Table 1 outlines the number of inflorescences that were removed for each treatment.

Table 1: Number and ratio of flowering terminals removed for each experimental treatment in Calypso trees.

Crop Load inflorescence treatment	Percentage of inflorescences to be removed	Ratio of inflorescences removal
1 - Control	0	Nil
2	20	1 in 5
3	40	2 in 5
4	60	3 in 5
5	70	7 in 10
6	80	4 in 5
7	90	9 in 10
8	95	19 in 20

Treatment application was undertaken at full anthesis over two years (2015 and 2016). Inflorescence removal was undertaken by progressing systematically through the canopy, counting 5, 10 or 20 inflorescences at a time, and removing the proportion of inflorescences required for each treatment. For example, in the 80% removal treatment, 8 inflorescences for every 10 had all florets were removed from the inflorescences. If an inflorescence was a mixed floral and vegetative shoot then the vegetative portion was maintained. On average most terminals held only one inflorescence, however if a terminal had subsequent axillary inflorescences, they were also counted. The following information was collected on each tree at the commercial orchard site.

Tree growth

Tree canopy dimensions to calculate canopy volume and tree surface area measured at the commencement of the experiment and each year to determine inflorescence density. Trunk cross-sectional area (TCSA) of each tree (10cm above the ground) and for the assessment of yield efficiency was also calculated each year.

Flowering and fruit set

Pre-treatment and post-treatment application inflorescence counts were recorded in 2014-15, 2015-16 and 2016-17. While post-treatment inflorescence count was summarised during treatment application, regular monitoring of the canopy after treatment application identified fruit set and if any subsequent flowering occurred. To make inflorescence counts relative across the different treatments we used floral density (inflorescence count / trunk cross-sectional area (TCSA) (cm^2)) to compare pre and post treatment floral counts. Subsequent for results for yield and fruit size were then compared to floral density in a treatment to allow for a better understanding of the effects of treatments on crop load.

Yield and fruit quality

In the first year, tree yield was determined by multiplying the total fruit number at harvest by as the average fruit weight of 20 fruit. In the second year, tree yield was determined by weighing all harvested fruit. In both years, fruit quality was assessed in 20 fruit from each tree using the methods of Holmes et al. (2009).

Return flowering

Flowering in the season following treatment application was assessed by counting the number of inflorescences and comparing between seasons in 2014-15, 2015-16 and 2016-17.

Carbohydrate sampling

Non-structural carbohydrate were assessed in 2015-2016, at vegetative dormancy (April), full flowering (September, late fruit set (October), pre-harvest (November) in terminals with and without fruit at harvest (December), in four of the 0, 40, 70 and 95% inflorescence thinning treatments.

Experiment 2

The second experiment aimed to test if the baseline relationships tested in the first experiment were similar in different mango varieties grown under intensive orchard systems. Experiment 2, was conducted on the Projects Planting Systems Trial on Walkamin Research Station. The experiment has a split-split plot design with planting density at the main plot level, training system at the sub-plot level and variety at the lower sub-sub-plot level. For this dataset, each combination of density, training system and variety was replicated 3 times with the low density only having the conventional training system. The medium density has single leader and conventional training systems, and the high-density has espalier on trellis and conventional training system. Espalier trees have a much narrower depth (across the row) than the single leader at medium density. Each training system sub-plot consisted of three datum rows, with the end rows flanked by two guard rows. Each row comprised five trees of a single variety, including a guard at either end, and three datum trees in the middle.

The number of terminal growth units and inflorescences was collected on 45 trees, each year between 2015 and 2017, to understand the relationship between flowering and fruiting. Trunk cross-sectional area (TCSA cm^2) was also measured each year.

Statistical analysis

Analysis of variance (ANOVA) was used to analyse the variables in response to the treatments. Polynomial contrasts were fitted to investigate any linear, quadratic or cubic trends as the level of reduction increases. The means, p-values, standard error of the differences (SED) and 95% least significant differences (LSD) are presented. Where a significant effect was found the 95% LSD is used to make pairwise comparisons.

Results

Experiment 1

Canopy dimensions

Analysis of the variation in tree size showed there was no significant difference in tree size between the trees (height p=0.818; skirt p=0.901; width p=0.841 and depth p=0.841). Nor were canopy volume and TCSA significantly different between trees prior to, or any time during treatment application (Table 2). Despite this tree 6 in block 4 (20% reduction) was smaller than the other trees and appeared to be an outlier, as a result and for consistency the tree was excluded from the analysis.

Table 2: Canopy and stem dimensions of experimental Calypso tree for the 2014-15 and 2015-16.

Treatment Flowering terminal reduction (%)	Canopy volume (m^3)		TCSA (cm^2)	
	2014-15	2015-16	2014-15	2015-16
0	18.58	18.58	138.46	155.2
20	14.23	16.40	127.43	144.5
40	15.50	21.26	156.77	173.4
60	18.99	17.24	138.59	153.5
70	18.75	18.53	144.23	163.0
80	18.66	16.29	129.77	145.3
90	19.43	17.96	150.18	163.8
95	16.82	19.05	161.15	177.3
p-value	0.641	0.783	0.110	0.203
Average SED	3.105	3.060	12.248	13.71
Average 95% LSD	6.458	6.364	25.471	28.50

Inflorescence count

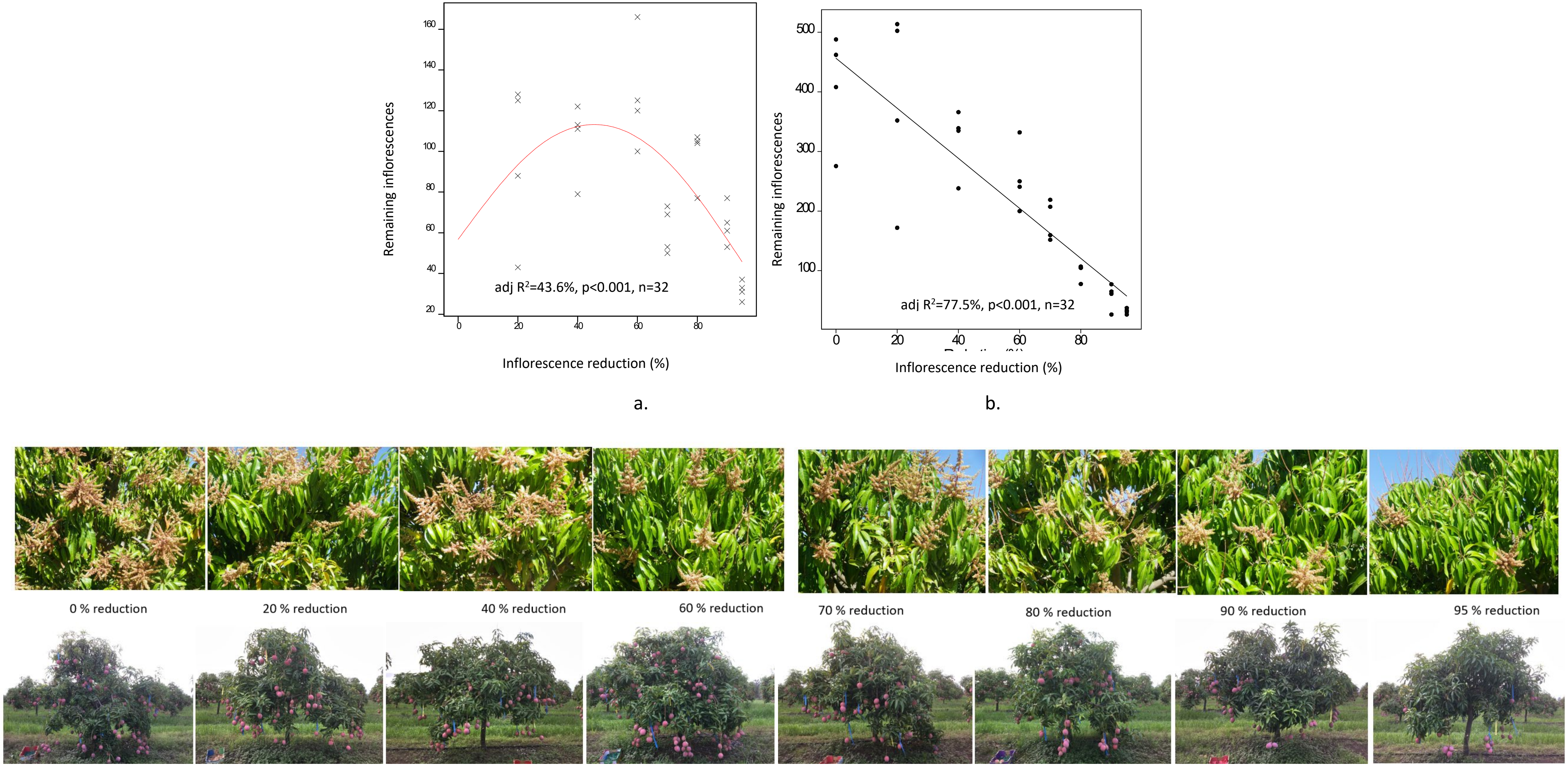
As expected, thinning inflorescences significantly decreased the number of remaining panicles in the canopy with the mean number of inflorescences decreasing as the inflorescence reduction treatment increased (Table 3). There was a quadratic relationship in the first year and a linear in the second year between the number of remaining panicles and the reduction treatment, validating that the treatment application was effective for both years (Figure 1a and b) (2014-15: adj R^2=43.6%, p<0.001, n=32; 2015-16: adj R^2=77.5%, p<0.001, n=32).

Table 3: Mean inflorescence number before and after treatments for the 2014-15, 2015-16 seasons for the Calypso crop load experiment at Dimbulah in Far North Queensland, and at the end of the experiment 2016-17.

Treatment Inflorescence reduction (%)	Inflorescence number (before treatment)			Inflorescence number (after treatment)	
	2014-15	2015-16	2016-17	2014-15	2015-16
0	408.46	385.5	374	408.4 e	385.6 e
20	481.25	500.2	342	385.0 e	400.2 e
40	532.50	604.5	337	319.5 de	362.7 de
60	639.25	563.8	383	255.7 cd	225.5 cd
70	614.75	661.2	439	184.4 bc	198.4 bc
80	439.75	535.2	413	98.8 ab	107.0 abc
90	574.50	684.8	409	57.5 a	68.5 ab
95	638.25	588.5	405	31.9 a	29.4 a
p-value	0.244	0.390	0.875	<0.001	<0.001
Average SED	98.496	127.00	77.8	52.8	66.3
Average 95% LSD	204.834	264.12	161.9	109.8	137.8
Linear	0.028	0.044	0.242	<0.001	<0.001
Quadratic	0.343	0.327	0.730	0.036	0.034
Cubic	0.780	0.703		0.785	0.408

Table 4: Mean inflorescence number / trunk cross-sectional area (TCSA) (cm2) before and after treatments for the 2014-15, 2015-16 seasons for the Calypso crop load experiment at Dimbulah in Far North Queensland, and at the end of the experiment 2016-17.

Reduction (%)	Inflorescence number /TCSA (cm^2) (before treatment)			Inflorescence number /TCSA (cm^2) (after treatment)		
	2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
0	2.95	2.81	2.42	2.95 e	2.81 e	NA
20	3.78	3.64	2.28	3.32 e	3.56 f	
40	3.50	3.88	1.94	2.10 d	2.33 e	
60	4.77	3.92	2.51	1.91 d	1.57 d	
70	4.32	4.61	2.66	1.29 c	1.38 cd	
80	3.88	4.15	2.84	0.78 bc	0.83 bc	
90	4.11	4.61	2.52	0.41 ab	0.46 ab	
95	4.33	3.65	2.29	0.22 a	0.18 a	
p-value	0.145	0.349	0.581	<0.001	<0.001	
Average SED	0.598	0.758	0.426	0.217	0.277	
Average 95% LSD	1.244	1.577	0.886	0.453	0.577	
Linear	0.019	0.049	0.368	<0.001	<0.001	
Quadratic	0.205	0.204	0.815	<0.001	0.002	
Cubic	2.95	2.81	2.42	0.090	0.011	



a.

b.

Figure 1: Relationship between inflorescence thinning (reduction %) and the count of remaining inflorescences in the canopy for (a) 2014-15 (b) 2015-16 and (c) visualisation of the inflorescence density in block 1 of the treatments and fruit set in the same trees.

Floral density

Floral density (prior to treatments)

When we compared the floral density [inflorescence count/ TCSA (cm^2)], using the untreated inflorescence count for floral density, there was no significant difference between the years (0.931). Neither was there a significant effect of treatment (p=0.145; p=0.349, for 2014-15 and 2015-16 respectively). Untreated floral densities were between 2.81 and 4.77 inflorescences / cm^2 TCSA.

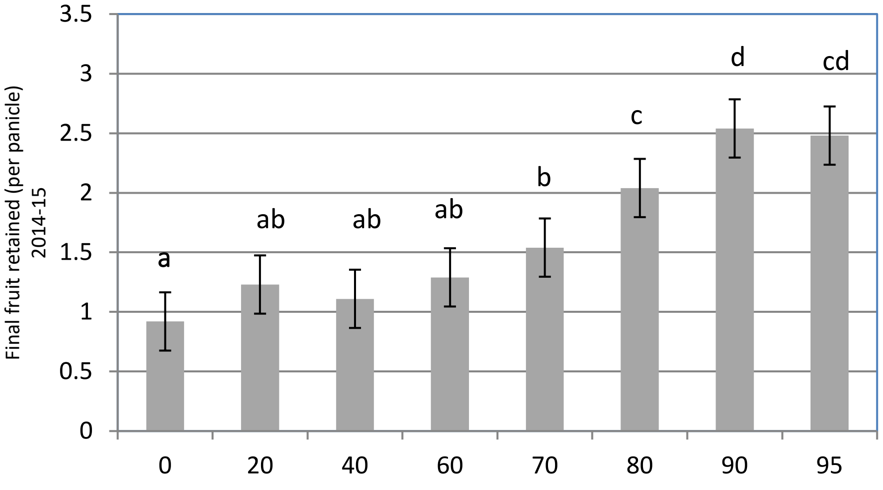
Floral density (after treatments)

When we compared the floral density [inflorescence count/ TCSA (cm^2)], using the treated inflorescence count for floral density, there were significant differences between floral densities once the reduction treatments had been imposed. The general tendency was for the mean floral density to decrease as the level of reduction increases. An ANOVA comparing the two years found a significant effect of reduction treatments (p<0.001) but no significant difference between the years (p=0.935)(Table 4). The interaction of year and reduction was not significant either. The significant effect for the treatments showed that the control had a significantly lower floral density (2.88) than that of the 60 (4.35), 70 (4.46), 80 (4.02), 90 (4.36) and 95 % (3.99) reduction treatments. There was also no significant difference between the means for the treated trees (reduction treatments between 20 - 95 %). Nor was there any difference between the control and treatment 20 (3.71) and 40 % (3.69) reduction treatments.

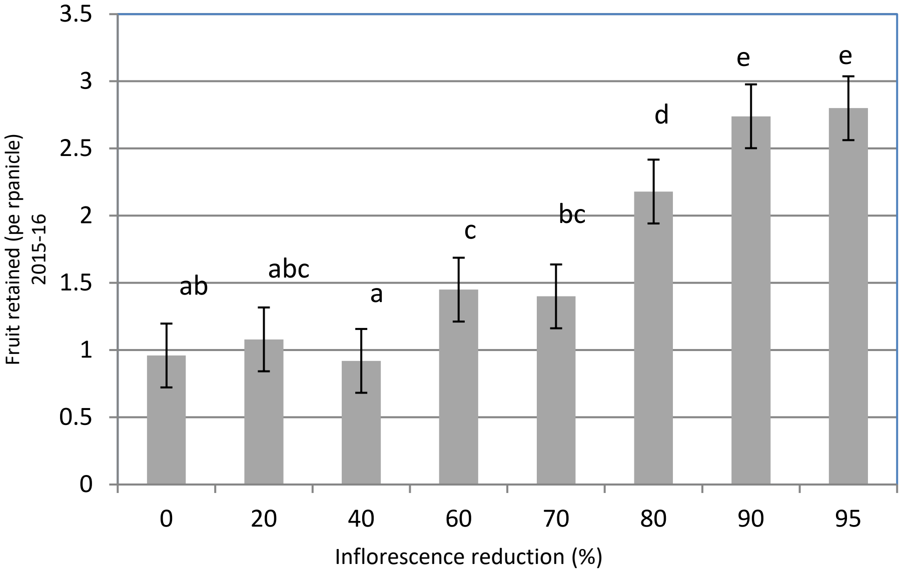
Fruit retained per inflorescence

Inflorescence thinning led to a significant increase in the number of fruit retained per inflorescence one week prior to harvest in 2014-15 and 2015-16 (Figure 2a and b). Where for both seasons, trees with ≥ 80% inflorescences thinned retained 2 fruit per inflorescence whereas inflorescences that were not thinned (control), or thinned to ≤ 70%, retained one fruit per panicle (p<0.001).

When fruit-set was monitored from fruit set to harvest, there was a non-linear relationship between time and fruit retention for both seasons (Figure 3a and 3b). The models indicate that after fruit set there was a large initial fruit drop which steadily deceased over time (Figure 4 a and b).

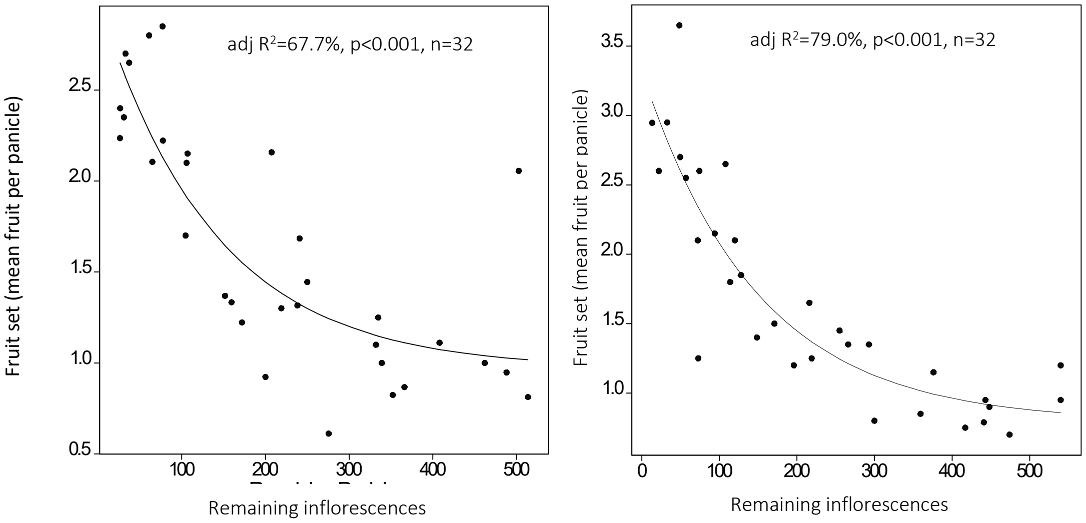


a.



b.

Figure 4: Effect of inflorescence thinning (reduction %) on fruit retention per panicle prior to harvest in the (a) 2014-15 and the (b) 2015-16. Columns with the same letter above them are not significantly different ($p<0.05$). Error bars represent +/- 0.5 95% LSD.



a. b.

Figure 3: Effect of inflorescence thinning (reduction %) on the relationship between the remaining panicles and the final fruit retention for (a) 2014-15 and (b) 2015-16 seasons.

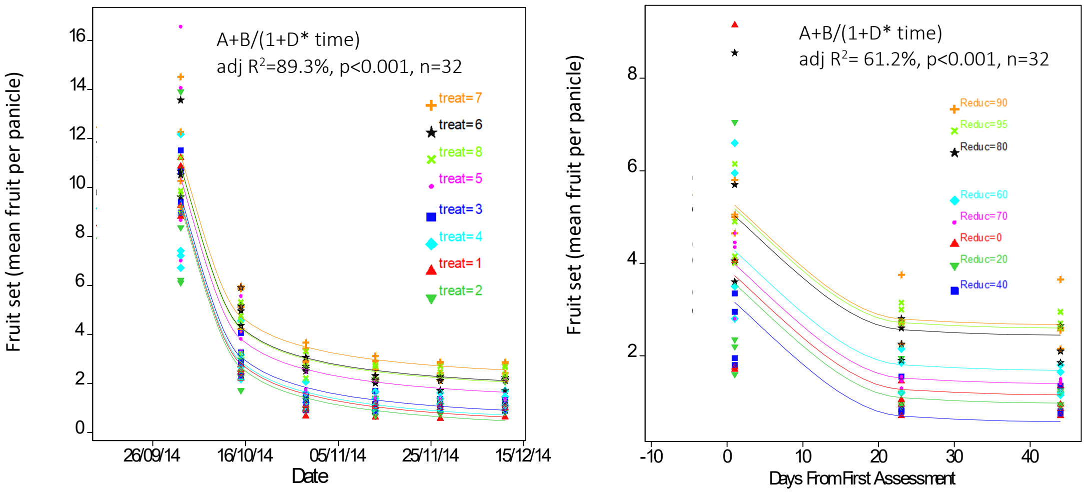


Figure 4: Fruit retention over time in Calypso trees under different inflorescence thinning treatments for (a) 2014-15 a clear non-linear relationship which was modelled by the linear-divided-by-linear model (ldl). For this data the most parsimonious form of the model is one where the treatment lines are parallel. This model explains 89.3% of the variation. The ldl curve is a rational function and can be represented as fruit set = A + B/(1 + D * time);
(b) 2015-16 an exponential model with parallel lines for each treatment explaining 61.2% of the variation (p<0.001) was fitted.

In 2015-16, The model suggests the fruit drop between assessments is constant and not proportional. The 80, 90 and 95% reduction treatments had the highest initial fruit set per panicle at the first assessment. At time 2, the number of fruit retained in these treatments dropped to similar numbers as treatments 0, 20 and 40% at time 1. Caution should be taken with the interpretation of the 2015-16 graph, as there are only three time points.

Fruit number, weight, size and quality

At harvest, in both seasons, the total number of fruit per tree was counted and fruit weight, height, length and depth were measured as a 20 fruit sample (Table 5). There was a non-significant trend of increasing fruit number until the highest inflorescence thinning treatment where in 2014-15, fruit count was significantly lower than the control and other treatments in the 95% reduction treatment only. However, in 2015-16, fruit count in the 40% reduction treatment was significantly higher than the 90 and 95% reduction treatments (Table 5).

Fruit weight at harvest, in 2014-15 was significantly higher in the 90% reduction treatment compared to all other treatments, except the 95% reduction treatment. In this year, fruit length, width and depth in the 95% reduction treatments were significantly larger than treatments with ≤ 70 % of inflorescences reduced. Fruit weight was significantly higher only in the 90 and 95% reduction treatments. In 2015-16, fruit weight was not significantly different between treatments although the 90 and 95% reduction treatments had higher mean fruit weight. The depth of fruit in the 95% reduction treatment was significantly greater than reduction treatments ≤ 80%.

At eating ripe, total soluble sugars (TSS) (Brix ^{0}) in fruit were not significantly different from any thinning treatments in 2014-15. However, in 2015-16, total soluble sugars were significantly higher in the 95% removal treatments with ≤ 80% of inflorescences removed (Table 5).

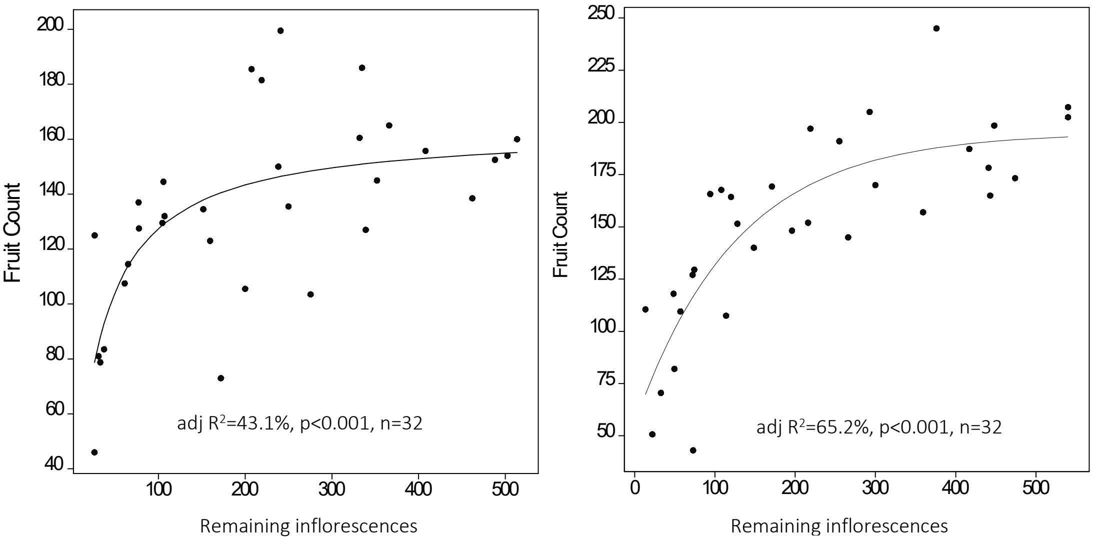
Table 5: Mean fruit count, fruit weight (g), length (cm), width (cm) and depth (cm) for each inflorescence thinning treatment in Calypso for the 2014-15 and the 2015-16 seasons.

Season	Reduction (%)	Fruit count	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Fruit depth (cm)	TSS (°)
2014-15	0	137.6 b	337.8 ab	98.80 ab	84.65 ab	75.52 a	9.50
	20	133.0 b	336.8 ab	98.95 ab	84.24 a	75.95 ab	9.87
	40	157.0 b	345.9 ab	99.77 ab	84.98 ab	76.23 ab	9.68
	60	150.2 b	340.9 ab	97.42 a	84.61 ab	75.48 a	9.32
	70	156.1 b	333.3 a	97.77 a	84.21 a	75.12 a	9.81
	80	133.4 b	376.9 bc	102.32 bc	87.00 abc	78.65 bc	9.79
	90	121.0 b	427.4 d	102.30 bc	87.38 bc	78.70 bc	9.81
	95	72.3 a	409.2 cd	104.20 c	89.35 c	80.53 c	9.87
	p-value	0.008	<0.001	0.005	0.016	0.012	>0.05
	Av. SED	19.78	20.87	1.713	1.473	1.506	-
	Av. 95% LSD	41.14	43.41	3.562	3.063	3.131	-
2015-16	0	160.1 bc	384.3	100.7	87.4	79.8 ab	10.83 ab
	20	162.8 bc	383.2	100.6	87.4	79.9 ab	11.29 abc
	40	185.2 c	384.5	101.1	87.2	80.0 ab	10.76 ab
	60	163.2 bc	368.4	98.6	86.7	78.6 a	10.48 a
	70	174.3 bc	387.6	100.7	88.1	80.5 ab	10.92 ab
	80	152.6 bc	377.9	99.9	86.9	79.5 ab	10.89 ab
	90	130.7 ab	409.7	102.0	89.0	82.2 bc	11.41 bc
	95	78.4 a	447.7	104.4	92.0	84.9 c	12.07 c
	p-value	0.016	0.052	0.256	0.063	0.023	0.018
	Av. SED	25.99	22.55	2.00	1.61	1.62	0.392
	Av. 95% LSD	54.05	46.90	4.17	3.36	3.36	0.815

Relationships between inflorescence thinning, fruit retention and fruit size

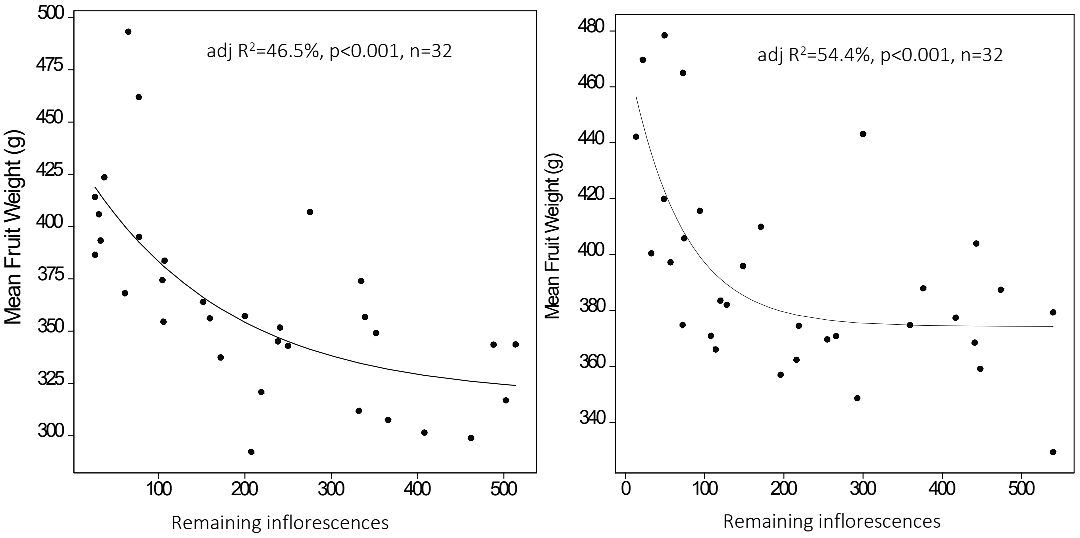
In 2014-15 and 2015-16, there were non-linear relationships between the remaining inflorescences and the mean fruit count (Figure 5a and b). If we use Figures 5a and b to identify where the rate of fruit retention flattens we can estimate what treatment was used to get attain optimum fruit set on each tree for Calypso. For example, in 2014-15 there was a flattening of the curve (Figure 5a) at approximately 140 fruit, while in 2015-16 the rate of fruit retention flattened at about 175 fruit. When we look at the number of inflorescences this fruit count relates too, we see that between 200-350 inflorescences per tree gave this level of fruit retention. Table 3 tells us that a reduction treatment of 40-60% inflorescences will leave approximately 200 - 300 inflorescences, or the thinning of 2-3 out of every 5 inflorescences.

In addition to this, a significant nonlinear relationship was found between the number of remaining panicles and fruit weight. Fruit weight increased substantially as the number of remaining inflorescence per tree decreased (Figure 6 a and b) with an increasing rate as the remaining number of inflorescences decreased. In 2015-16, the mean fruit weight reached a plateau quicker than 2014-15, with an asymptote at approximately 375 g. Fitting a broken stick model to these two sets of data suggests the break point occurs at approximately 120 remaining inflorescences. The model suggests that with 120 or more remaining inflorescences on a tree, the mean fruit weight remains constant at approximately 375 g.



a. b.

Figure 5 Relationship between remaining panicles and fruit count in the (a) 2014-15 and (b) 2015-16 seasons.



a. b.

Figure 6: Relationship between remaining panicles and fruit weight in the (a) 2014-15 and (b) 2015-16 seasons.

Exponential models were also fitted to mean fruit length, width and depth against the number of remaining inflorescences. All were found to be significant (adj R^2=20.9%, p=0.013; adj R^2=39.3%, p<0.001; adj R^2=40.1%, p<0.001 respectively) and all models suggest the mean fruit size decreases very little above approximately 200 panicles remaining per tree.

Relationships between floral density and yield efficiency

Over the two seasons (2014-15 and 2015-16), the relationships between floral density (inflorescences/ cm^2 TCSA) and mean fruit retained at harvest (adj R^2=80.4%, p<0.001, n=32) and mean fruit weight at harvest (adj R^2=47.7%; p<0.001, n=32) was represented by a parallel exponential model (Figures 7 and 8 respectively). As floral density increased, fruit retention and fruit weight decreased. Fruit retention reaches an asymptote after 3 flowers per cm^2 TCSA where fruit retention is no longer influenced by floral density. Fruit weight, reaches an asymptote at between 2 and 3 flowers per cm^2 TCSA, where fruit weight is no longer influenced by floral density. For average fruit weigh, the exponential curve explains adj R^2 46.5% ($p<0.001$) of the variation, for both years combined.

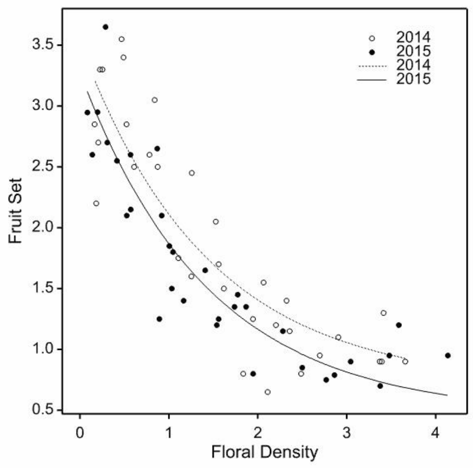


Figure 7: Relationship between floral density (inflorescences/ TCSA) and mean fruit retention over the 2014-15 and 2015-16 seasons.

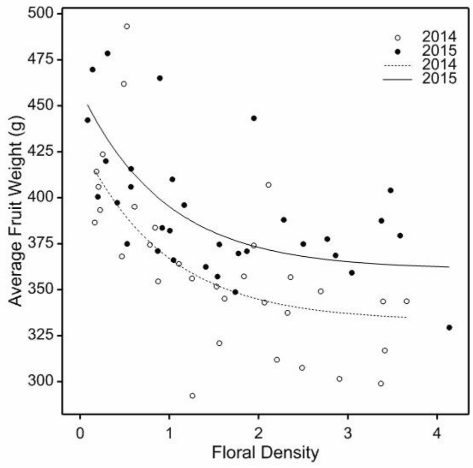


Figure 8: Relationship between floral density (inflorescences/ TCSA (cm^2)) and mean fruit weight over the 2014-15 and 2015-16 seasons.

Yield data and relationships

There was a significant main effect of treatment ($p=0.010$, combining years) and year ($p<0.001$) for tree yield and yield per ha, respectively. Reducing inflorescences, reduced the average yield per tree and per ha (Table 6) over both seasons and combined seasons, although the yield reduction was only statistically significant when 95% of inflorescences were removed. Comparing the data across years, yield in 2014-15 was 47.36 kg/ tree compared to 61.44 kg/tree in 2015-16 while yield per ha was 14.8 tonnes per ha in 2014-15 compared to 19.2 tonnes per ha in 2015-16.

Table 6: Mean yield (kg/tree and tonnes/ha) and yield efficiency (kg/TCSA) for each inflorescence thinning treatment in Calypso trees over the 2014-15 and 2015-16 seasons.

	Yield (kg/tree)	Yield (t/ha)	Yield efficiency (kg/ TCSA)	Yield (kg/tree)	Yield (t/ha)	Yield efficiency (kg/ TCSA)	Yield (kg/tree)	Yield (t/ha)	Yield efficiency (kg/ TCSA)
Reduction (%)	2014-15			2015-16			Combined years		
0	45.72 b	14.29 b	0.331 a	61.6 b	19.3 b	0.447 bc	56.77 a	17.74 a	0.412 ab
20	44.76 b	13.99 b	0.361 a	59.2 b	18.5 b	0.441 bc	52.35 a	16.36 a	0.401 ab
40	54.35 b	16.98 b	0.354 a	70.8 b	22.2 b	0.453 bc	64.24 a	20.08 a	0.413 ab
60	51.10 b	15.97 b	0.376 a	60.6 b	19.0 b	0.428 bc	58.24 a	18.20 a	0.418 ab
70	51.31 b	16.03 b	0.361 a	67.3 b	21.1 b	0.468 c	59.28 a	18.53 a	0.415 ab
80	50.18 b	15.68 b	0.394 a	57.7 b	18.1 b	0.446 bc	56.68 a	17.71 a	0.441 a
90	51.91 b	16.22 b	0.364 a	53.6 ab	16.8 ab	0.358 b	52.46 a	16.39 a	0.359 b
95	29.57 a	9.24 a	0.200 b	35.0 a	11.0 a	0.217 a	35.17 b	10.99 b	0.226 c
p-value	0.035	0.035	<0.001	0.040	0.040	<0.001	0.010	0.010	<0.001
SED	6.72	2.100	0.033	9.42	2.95	0.0480	7.014	2.192	0.057
Average 95% LSD	13.97	4.367	0.067	19.59	6.13	0.0998	14.13	4.415	0.080

A linear regression model of the relationship between fruit number per tree and yield (kg/tree) for 2015-16 (Figure 9) explained 76.9% of the variation between the parameters, and as expected, yields increased with an increase in fruit number pre tree.

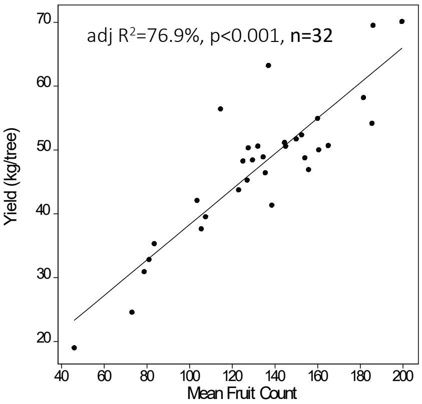


Figure 9: The relationship between mean fruit count and tree yield (kg per tree) in Calypso for the 2015-16 season, resulting from different inflorescence thinning treatments.

Yield efficiency

There was a significant main effect of treatment (p<0.010) and year (p<0.001) for yield efficiency (kg tree/ TCSA (cm^2)). Yield efficiency in 2014-15 was 0.34 compared to 0.43 in 2015-16. There was a significant relationship between floral density (number of inflorescences / TCSA (cm^2)) and yield efficiency (kg fruit / TCSA (cm^2)) (Figure 10), which varied with year (2014-15 and 2015-16) (adj R^2= 51.4, p<0.001, n=32). In the first year of the experiment yield efficiency was limited to 0.34 kg fruit / TSCA (or approximately 1 fruit) for every inflorescence, however in the second year of the experiment the relationship showed an increase in the yield efficiency with increasing floral density to a maximum of between 0.5 - 0.6 (kg tree/ TCSA (cm^2)) yeild efficeincy.

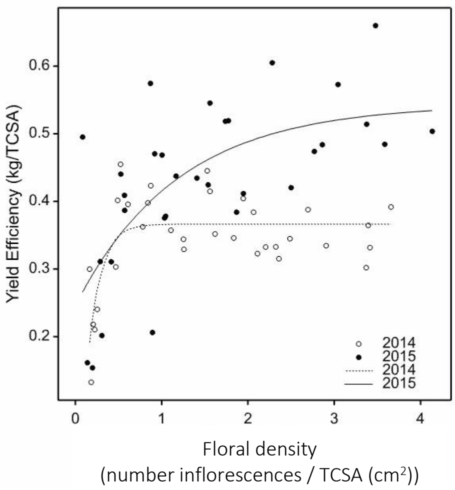


Figure 10: Relationship between the floral density (number of inflorescences / TCSA (cm^2)) and the yield efficiency (kg fruit / TCSA (cm^2))

Return flowering

To identify how inflorescence thinning treatments influenced flowering in the following year we compared the number of pre-treatment and post-treatment inflorescences in 2014-15 and 2015-16, to the number of pre-treatment inflorescences in 2016-17. Data presented in Table 7 shows that there was no relationship between the number of inflorescences (after treatment) and the number of pre-treatment inflorescences in the following year.

Table 7: The relationships (adjusted R^2 and p value) between the number of remaining inflorescences (after treatment) and the number of inflorescences in the following year for the 2014-15, 2015-16 and 2016-17 flowering events (prior to treatment). NA means the residual variance exceeds the variance of the response variable and is a very poor fitting model.

Pre-Treatment Counts (y)		
2016	NA (p=0.549)	NA (p=0.586)
2015	NA (p=0.445)	
	2014	2015
	Remaining inflorescences (x)	

There was a significant relationship between the pre-treatment inflorescences numbers in 2014 and the subsequent pre-treatment inflorescence numbers in 2015 (adj R^2=11.8%; p=0.031) as well as asignificant relationship between pre-treatment infloescence number in 2015 and inflorescence number in 2016 (adj R^2=23%; p=0.003). The pre-treatment inflorescence number in one year was weakly related to the pre-treatment number of inflorescences in the following year (Figure 11 a and b). Despite this the pre-treatment panicle count in 2014, did not have a significant relationship with the number of flowering panicles in 2016 (adj R^2=1.2%; p=0.248). Figure 11 a and b show the two fitted relationships however despite the slope being significant, the percentage of variation accounted for is very low.

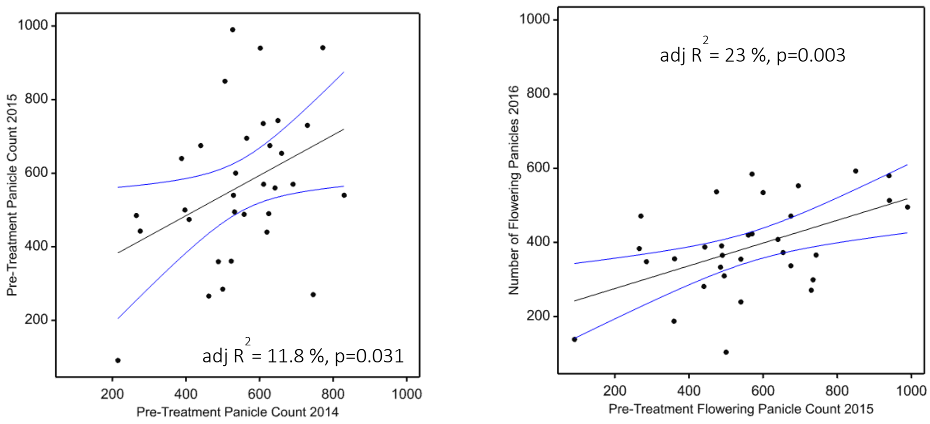


Figure 11: The relationship between the pre-treatment number of flowering panicles in (a) 2014-15 and 2015-16, and the (b) 2015-16 and 2016-17 flowering seasons.

Carbohydrate sampling

In the 2015-16 season, carbohydrate concentrations were sampled from terminal stems in the 0, 40, 70 and 95 % inflorescence reduction treatments at five different phenological stages (vegetative rest (dormancy), full flowering, late fruit set, pre-harvest and at harvest). While terminal stems were selected from the north-upper canopy, samples for pre-harvest and at-harvest carbohydrate concentrations were compared in terminals with and without fruit (crop vs no crop). At the vegetative stage, terminals were selected that had previously had fruit, while at flowering and late fruit set terminals were selected with fruit.

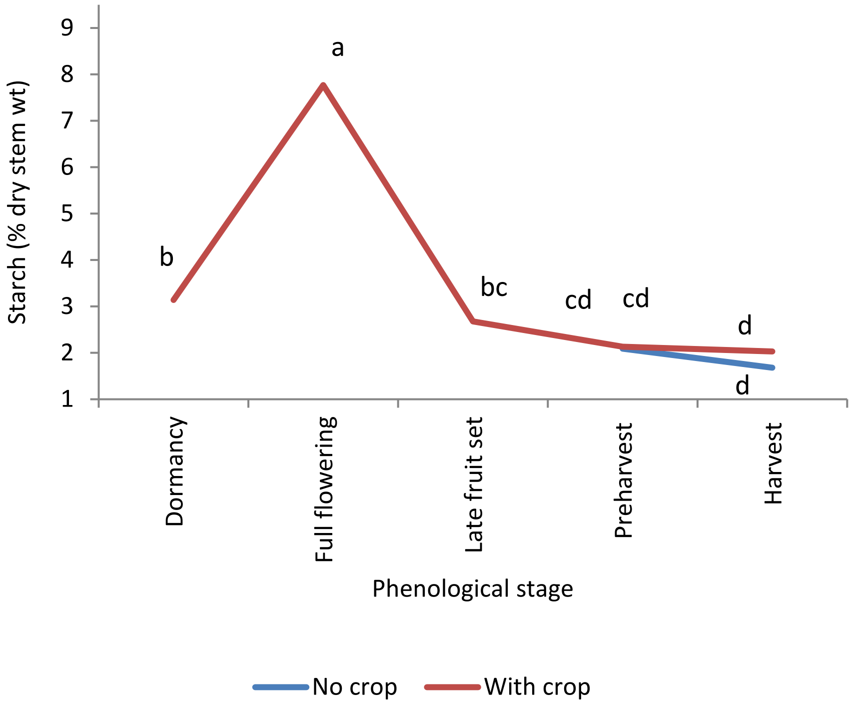


Figure 12: The water soluble starch (% dry stem weight) in terminals at different phenological stages for the 2015-16 season.

A residual maximum likelihood (REML) analysis was conducted with phase and the number of panicles per m^2 canopy area used as the fixed effects. The canopy area was only measured on one occasion in any year and therefore consistent values are used across the seven phases. A log$_{10}$ transformation was required for comparison of the starch values.

The analyses suggest a significant difference in carbohydrate concentrations between phenological stages of flowering and fruit development. ($p<0.001$) (Figure 12). There was no significant relationship between the carbohydrate concentrations and the number of panicles per m^2 canopy area ($p=0.538$). Pairwise comparisons using the 95% LSD suggest the mean starch levels at full flowering was significantly higher than all other phases. There was no significant difference in carbohydrate concentrations between cropping and non-cropping terminals from pre-harvest to harvest. Carbohydrate concentrations in dormant, pre flowering terminals were statistically similar to the late fruit set stage.

When a tensor spline was fit across starch concentrations for time and flower density (inflorescences / canopy surface area (m^2)), using a linear mixed model (with correlated residuals to account for temporal correlation between repeated measurements), there was a significant interaction between treatment and sampling time ($p<0.001$). Figure 13 shows the tensor spline for the interaction of time and flower density for water soluble starch (% dry stem weight) in terminals at different phenological stages for the 2015-16 season. The results suggest that water soluble starch was higher in terminal stems with a lower number of inflorescences and with phenology (growth stages at different times of year).

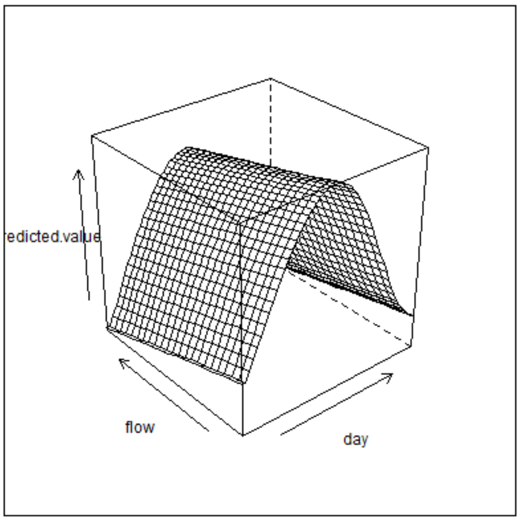


Figure 13: The interaction between phenological stage and time for concentrations of water soluble starch (% dry stem weight) in terminals with fruit, in the 0, 40, 70 and 95 % inflorescence reduction treatments for the 2015-16 season.

Experiment 2

The effect of variety on floral density and yield efficiency

Significant non-linear relationships between floral density (number of inflorescences / TCSA (cm^2)) and yield efficiency (kg tree/TCSA (cm^2)), were found in Calypso at the planting systems experiment in 2014-15 and 2015-16 (Figure 14). There was also a significant non-linear relationship between the floral density (number of inflorescences / TCSA (cm^2)) and yield efficiency (kg fruit / TCSA cm^2), for each of the varieties in the planting systems experiment

(Figure 14b). The parallel exponential model explained 82.3% of the variation between the varieties. The models associated with figure 15 are outlined below:

NMBP 1243: $y=0.246-0.2844*(0.206)$
Calypso: $y=0.5054-0.6045*(0.206^x)$
Keitt: $y=0.76-0.7763*(0.206^x)$

where x = Floral density (number of inflorescence / TCSA (cm^2)) and y = [yield efficiency = fruit weight (kg / tree) / TCSA (cm^2))]

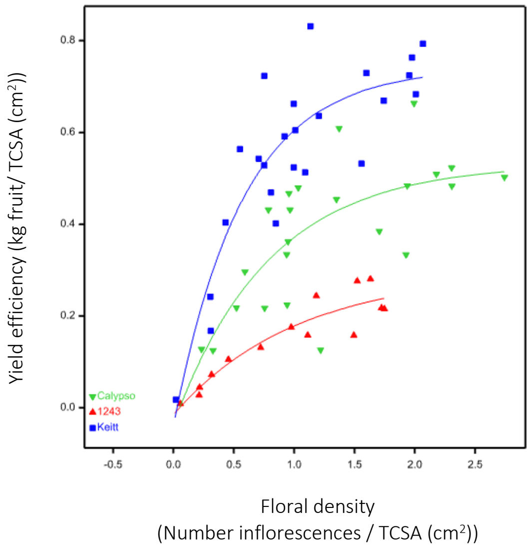


Figure 14: The relationship between yield efficiency (fruit weight (kg/tree)/ TCSA (cm^2)) and floral density (number inflorescences / TCSA) for different varieties including NMBP 1243, Calypso and Keitt at 4 and 5 years of age

We also compared the relationships between the average fruit weight and floral density and the fruiting density (number of fruit / number of inflorescences) and floral density (flower count /TCSA (cm^2)) at the PST which compared the effect of training systems and variety. There was no significant relationship training systems within each variety between floral density and average fruit weight (2016-17 and 2017-18). Although, when grouped by variety, there was a parallel relationship between varieties and resulted in significantly different intercepts between the 3 varieties, however the slope was not significanlty different to zero, suggesting floral density had no significant effect on average fruit weight (adj R^2 = 10 %, p=0.049).

When we compared the relationships between floral density and fruiting density, there was a significant effect of variety (adj R^2 = 60.1 %, p<0.001). Fitting a parallel linear regression model with variety alone as the grouping factor, showed that Keitt has higher fruiting density, followed by Calypso and then NMBP 1243 for the same floral density (Figure 15).

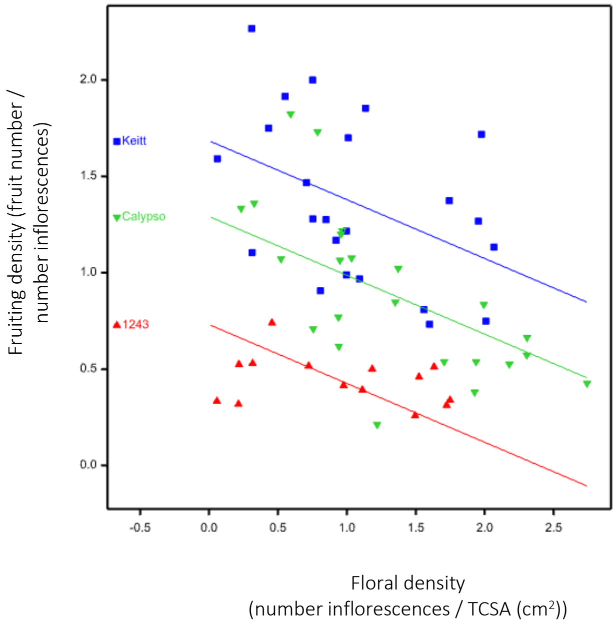


Figure 15: The relationship between floral density (number of inflorescences / TCSA (cm^2)) and fruiting density (number of fruit / number of inflorescences) for three varieties NMBP 1243, Calypso and Keitt at the planting systems experiment at Walkamin Research Station.

Discussion

This study aimed to understand the effect of inflorescence thinning on reproductive growth in the Mango variety Calypso. Thinning inflorescences at anthesis led to non-linear decreases in fruit set and the number of fruit retained at harvest. However, once the number of remaining panicles reached an upper threshold, there was little effect on fruit numbers.

Experiment 1 showed that Calypso has the ability to retain an average number of fruit (between 140 and 160 fruit per tree). As inflorescence density increased, the number of fruit retained per inflorescence also decreased. This was also shown in the relationships between floral density and fruit weight and floral density and the number of fruit retained per panicle, where both decreased with increasing floral density. In addition, low inflorescence numbers on the tree led to less fruit at harvest, and increased the fruit size and total soluble sugars (second year only).

For example, when 40 % of inflorescences per tree were thinned, yields increased slightly, although not significantly different from trees without inflorescence thinning. It seems that between 40 and 70% inflorescence thinning, resources were redirected by way of increasing the number of fruit retained per panicle. However, when 80% of inflorescences were thinned (20% retained), further resources were available to be redirected into the remaining fruit, increasing individual fruit size. Despite these results, tree yields were only significantly lower when inflorescences were thinned by 90-95%. At this point the loss of inflorescences and the increase in fruit weight could not maintain the same yield potential due to limiting inflorescences. There is also the issue of competition for resources brought about by the release of vegetative growth at high inflorescence reduction, although this aspect is not covered in this report. It is expected that any increase in vegetative growth may increase competition for resources available atfruit set because in mango they can occur at the same time. Breen (2016) found that stored carbohydrate reserves were used for canopy development in apples at the resumption of growth in spring but had little effect on fruit set, as fruit set was more reliant on newly synthesized photosynthates.

While biennial and irregular bearing in Mango can influence orchard yields in some years, the relationships between crop load and the partitioning of carbon and nitrogen resources has been demonstrated in a range of horticultural crops such as apple (Ding et al. 2017) and macadamia (Wilkie et al. under preparation). These results show that in experiment 1 yield efficiency, increased with increased when floral density was manipulated to a maximum of between 2 and 3 fruit per cm^2 TCSA or between 200 and 300 inflorescences per tree. Experiment 2 showed that while the yield efficiency varied depending on the variety, the floral density remained the same, despite variety or training system. These results suggest that while mango trees have an innate ability to grow more flowers than required, they tend to regulate the amount of fruit retained in any one year depending on available resources.

Limitations to productivity can result from either limited inflorescence density or limiting carbohydrate resources. If limited inflorescences are the problem then there maybe potential to increase yield by encourage more flowering sites in the canopy. However, if inflorescences are not limited, and carbohydrate resources are limiting, an increase in available resources during flowering and fruit development may lift productivity. This seems to be accurate to a point. In these results flower thinning in one season did not affect flowering levels in the following season, however there was a weak but significant effect of the pre-treatment inflorescence number on the subsequent pre-treatment inflorescence number the following year. In addition, while these results did indicate an increase in available carbohydrates in the terminal stems in the second year with decreasing inflorescences (per m^2 canopy surface area) and with phenological stages across the season, it is expected that any increase in carbohydrates will be shared between subsequent increases in fruit retention and competing vegetative growth. However, the effects of the treatments on vegetative growth are not covered here but will be outlined in a subsequent report (Mango Crop load part 2). If there is a sharing of resources between the two then this would help explain why there is not effect of inflorescence thinning on return flowering.

Breen (2016) showed that when artificial spur extinction (ASE) was used in apple, shoot development increased because ASE reduced the sink demand. Temperate tree crops industries employ a range of precision cropping practices including flower thinning management practices using either manual and/or chemical means. This raises the question as to whether a similar approach could be utilised in mango to regulate tree yield with more precision. The results presented here show that mango trees have a natural ability to regulate yield when flowering is not limiting. There seems to be some indication that inflorescence thinning could be used as a tool to regulate the natural variability in flowering and fruit set in the mango industry for more precision cropping to regulate peaks and troughs in production in mango from year to year at the tree level. For example, in a year with good flowering and fruit set could the number of flowering terminals be reduced to between 2-3 inflorescences per cm^2 TSCA area, to encourage more regular cropping year after year. Alternatively, if we thin inflorescences could this be used to increase fruit size to suit market requirements? However, there still remains the issue of how inflorescence thinning influences vegetative growth and how this would influences flowering and fruit retention over time.

Conclusion

The aim of this research was to develop a whole tree understanding of the relationship between inflorescence density, fruit set, yield and flowering in the following year in the variety Calypso. The second part of the experiment was to test if other tree canopies also displayed similar relationships under intensive orchard systems. Together these experiments have shown that it is resource availability that initially limits tree yield, in Calypso Mango. These results suggest that the optimal yield per tree can be attained with 2-3 inflorescences per TCSA. The second experiment showed that this result was similar across two other varieties and that training system did not influence this relationship. The application of inflorescence thinning treatments showed that Mango has an ability to regulate tree yields by redirecting saved resources (resulting from inflorescence thinning) to increasing fruit retention and/or fruit size. However, there is a point at which the increased fruit number or size did not out-way the loss of inflorescences on tree yields, and yields were reduced. In addition, the resources saved as a result of inflorescence thinning must also be shared in this regulation process between the retention of fruit, increasing fruit size and competing vegetative growth. The latter of which will be covered in a second report.

Acknowledgments

We would like to acknowledge Hort Innovation for funding this work through the Transforming subtropical/tropical tree crop productivity AS 13001; Department of Agriculture and Fisheries staff at Walkamin Research Facility and technical staff who have assisted with us on the maintenance, monitoring and evaluation of these experiments; Ontario Mangoes (David and Raymond Courtice) from Dimbulah who allowed DAF to use their Calypso orchards for some of this research and Frederic Normand from CIRAD for assistance to develop the methodology for the collection of architecture data from which the planting systems data was reported.

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Appendix 12

The influence of different training systems on yield, fruit quality and pack out in six year old, high-density mango orchard

Ibell, Paula T., Wright, Carole, Kare, Mahmud, Bally, Ian. S. E., Scobell, Z.

Abstract

Growing mango (*Mangifera indica*) at high densities has been shown to increase yield early in the life of an orchard. However, to maintain tree size and shape within their allocated orchard space, in these high-density orchard systems, trees requires intensive training and management. Currently there is little information on how planting density, tree training and management influence fruit quality. This report investigates the effects tree training and pruning system on fruit size and fruit quality including fruit Brix, dry matter, blush colour, and blemishes from sunburn, lenticel discoloration, diseases and insects. The experimental treatments included three planting densities (208 tree/ha (low density), 416 tree/ha (medium density) and 1,250 trees/ha (high density); three tree training systems, closed vase (conventional), central leader and espalier (single leader on trellis); and three mango varieties, 'Keitt', 'Calypso' and 'NMBP-1243'. Observations of fruit quality were made in 5 and 6 year-old trees. Fruit blush was more consistent and greatest in the central leader and espalier trained trees, however, there was also greater sunburn in these treatments. The results showed positive effects of variety and density on some fruit quality parameters (blush), pack-outs and yield however there were also some negative effects of the training systems which was influenced by the orientation of the trellis (west facing rows). This research also outlines potential options to consider and further research required to avoid the negative effects of row orientation and sunburn on mango fruit quality, when considering growing mango in high-density systems.

Keywords: mango, training systems, fruit quality, orchard intensification.

Introduction

With the focus on increasing productivity of mango orchards, there seems to be little benefit of increasing productivity without increases or maintenance to the quality and consistency of fruit to growers and consumers. The use of training systems to influence fruit quality and improve light distribution in the canopies has been shown in temperate crops (Musacchi and Serra 2018, Tustin et. al. 2001, Tustin et al. 1998, Wagenmakers 1994, Tustin et al. 1988, Morgan et. 1984, Robinson et al. 1983). For tropical crops like mango however this work is still lacking. While fruit quality can be defined in a range ways depending on where along the supply chain it is assessed, here were talk about quality as those accepted by the Australia Mango Industry Association (AMIA) for packing on the farm and what determines the different acceptability grades (AMIA 2016). The most important fruit quality characteristics for mango are covered by two broad categories. The first are those that identify with the consumer (determined by visual and eating quality) and the second is those that influence how fruit quality deteriorates along the supply-chain. The consumer traits refer to the visual appeal, flesh texture, flavour, healthiness and soundness of the fruit while supply chain (or marketing) traits include yield, pest and disease resistance, shelf life and robustness to harvesting and transportation systems (Bally 2006, Shewfelt 1999).

While fruit quality is affected through-out the value chain, the resulting fruit quality is strongly influenced by factors including field management (pest and disease control, training and pruning and harvest operations) (Kader 2002, Rehman et al. 2015), post-harvest environment (Hofman et al. 1997c), agronomy (timing and rate of nutrients) (Ibell et al. 2016 a & b, Smith 1989, Nguyen et al 2004; Young et al. 1962; Tarmizi et al. 1993) and post-harvest mechanical damage, disease and/or sanitation procedures (Kader 2013). In addition, several environmental factors can influence fruit quality including temperature, relative humidity, air velocity, and atmospheric compositions of oxygen, carbon dioxide, and ethylene (Kader 2013). For mango in particularly, poor fruit quality is associated poor yellow colour development when ripe (or degreening) (McKenzie 1994a, Hofman 1997b, Nguyen et al. 2004), sap-burn related skin blemishes (Bally et al 1997, Hofman 1997b), poor flavour development (MacLeod and Snyder 1985), internal disorders (or the non-pathogenic disorders affecting the fruit mesocarp including jelly seed, soft nose, internal breakdown etc.), post-harvest diseases, reduced shelf life (Paull 1994) and food safety issues that may affect the consumer (Ledger 1988). Internal fruit quality indicators including Brix and dry matter represent the total soluble sugars and maturity respectively. There are standards for mango which are identified in the Australian Mango Industry Association Fruit Quality Standards (AMIA 2016).

It is from this basis that this report investigates the effects tree training and pruning system on fruit size and fruit quality including.

Materials and methods

The aims of the experiment were to investigate the effects tree training and pruning in intensive mango orchard system on yield, fruit size internal and external fruit quality.

The experiment was carried out on the Queensland Department of Agriculture's Walkamin Research Station as part of the Planting Systems Experiment established in December 2013. The experiment was designed as a split-split-plot with planting density at the main plot level, training system at the sub-plot level and variety at the lower sub-sub-plot level. Each of three varieties, NMBP-1243, Keitt, Calypso, were planted at low, medium and high density. Within the medium density, trees are trained were either conventional or single leaders. Within the high density, trees were trained as either conventional or espalier on trellis. Within the low density, trees were trained as conventionally in a closed vase shape. There are 6 replicate blocks.

Each variety was harvested separately due to different maturity times (between two-three weeks apart). In both years NMBP-1243 was harvested first, followed by Calypso and then Keitt. Prior to harvesting, samples of four fruit from each orientation (north, south, east and west) were taken from 576 trees. A total of 384 fruit from each variety were evaluated over two seasons in 2018-19 and 2019-20, equating to a total of 1152 fruit each year. Sampled fruit were returned to the laboratory for postharvest fungal control and ripening.

Once at the laboratory, sampled fruit were dipped in a hot water solution with Schloar at 52^{o}C for 5 minutes, then ripened at 22^{o}C in controlled conditions. Prior to ripening, sampled fruit were weighed and their dimensions recorded. In 2018-19 we collected fruit weight, percent skin colour (%) with blush, blemish (cm^2) (including russet), total soluble solids (brix°), dry matter (%) and the presence and absence of sunburn and pink scale damage.

In 2019-20, we varied the parameters measured to calculate fruit grades based on 2016 Australian Mango Industry Quality Standards as outline in Table 1. In 2019-20 we rated the percentage of skin in each fruit samples covered by sunburn, damaged lenticels, and blush colour. For blemish and sap burn we measured the area of fruit (cm^2) affected by sap burn or blemishes (including russett) while scale (pink spot) was assessed by count. Once fruit was ripened to the eating ripe stage (70-100 % yellow), the cheeks off both sides of the mango were cut to reveal the internal mesocarp and the for the assessment of Total soluble solids (°Brix) and percent dry matter.

At harvest, all fruit from each experimental tree were counted and weighed to calculate tree yield. Fallen fruit were counted, multiplied by the average fruit weight and added to the tree yield for the calculation of total biological yields. Orchard yields were calculated by multiplying the total tree yield by the tree planting density per hectare.

Table 1: The ranges for parameters in the different class ratings for mango as outline in the 2016 Australian Mango Industry Quality Standards.

Grade	Sunburn	Blemish	Lenticel	Pink spot	Sapburn
1st class	Yellow bleaching to no more than 25% of the surface; no browning or dark or sunken blotches.	Less than 4cm^2 in total or 10% (cumulative). Blemish includes healed scarring, cleavage scar, and browning skin marks.	Dense pronounced spots on no more than 25% of the surface, or scattered pronounced spots no more than 50%, of the surface; not star-shaped or cracked.	Less than 6 spots or an area no more than 1 cm^2 (caused by scale)	Less than 4 cm^2 in total or 10% (cumulative).
2nd class	Yellow bleaching to no more than 50% of the surface; discoloured blotches to 12 cm^2 not sunken.	Less than 12cm^2 in total or 25% (cumulative). Blemish includes healed scarring, cleavage scar, and browning skin marks.	Dense pronounced spots on no more than 50% of the surface, or scattered pronounced spots no more than 50%, of the surface; not star-shaped or cracked	Less than 15 spots or an area no more than 4 cm^2 (caused by scale)	Less than 12 cm^2 in total or 25% (cumulative).
Reject	Anything that fails 2nd class	Anything that fails 2nd class	Anything that fails 2nd class	Anything that fails 2nd class	Anything that fails 2nd class

In 2019-20 the size of abrasions, and the presence or absence of fruit fly, internal breakdown, soft-nose, spotting bug damage and russett were also noted on each fruit.

In 2019-20, all fruit in Keitt cultivar, from three of the six replicates, were size graded as either acceptable or not acceptable to calculate harvestable yield.

Results

Tree yield

The 2018-19 was the fifth year after planning. Result showed that there was a significant main effect of variety (p<0.001) and planting density (p<0.001) for tree yields (kg). A significantly lower mean yield per tree was observed for high density trees and for variety NMBP 1243 while Keitt had a significantly higher mean tree yield than both Calypso and NMBP 1243 (Table 2). In 2019-20 there was a significant (p=0.002) three way interaction between variety, training system and density on tree yield (kg per tree) (kg/tree) (Table 2).

Table 2: The mean yield per tree for the interaction of variety, training system and density for the 2019-20 season.

Density	Training system	Cultivar (kg/tree)		
		NMBP-1243	Calypso	Keitt
low	Conventional	47.03 de	57.94 g	70.60 h
Medium	Conventional	48.53 ef	58.63 g	66.54 h
	Single Leader	44.29 de	43.30 de	49.25 ef
High	Conventional	29.13 ab	43.64 de	55.16 fg
	Espalier	28.61 a	35.32 bc	39.64 cd

Orchard yield

In 2018-19 there was a significant interaction between density and variety (p=0.006) where there was a significantly lower mean orchard yield (kg/ha) per ha in the low density treatments compared to the high density treatments. At low density there was no difference between Calypso and Kiett but at the medium density, Kiett had a higher mean yield (tonnes per ha) (t/ha) (Table 3).

In 2019-20 there was a significant three way interaction between variety, training systems and density (p=0.004) (Table 3). The mean orchard yield for conventional, high density, Keitt trees was significantly higher than all other varieties and training system combinations. High density conventional trees had the highest mean within each variety and it was significantly higher for both Keitt and Calypso compared to NMBP 1243. For NMBP 1243, there was no significant difference between the high density conventional and high density single leader training systems whereas the low density conventional trees had a significantly lower mean than all other training systems.

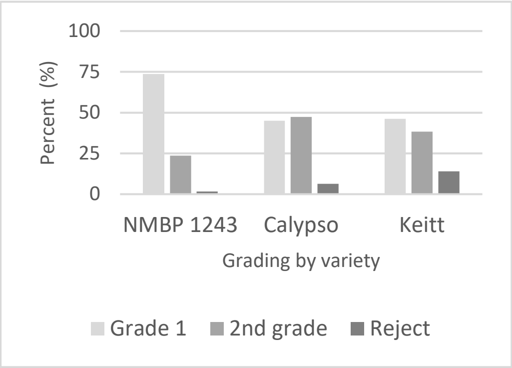
Table 3: The mean orchard yield (t/ha) for the interaction between variety and density for the 2018-19 and for the three way interaction between variety, training systems and density for the 2019-20 seasons. Comparisons of means are based on $\log_{10}$ transformations and are included in parenthesis.

Season	Density	Training system	Cultivar NMBP 1243 (t/ha)	Calypso (t/ha)	Keitt (t/ha)
2018-19	low		5.5 (a)	13.3 (b)	20.6 (bc)
	Medium		9.4 (c)	18.0 (f)	40.8 (e)
	High		11.4 (de)	21.3 (d)	47.4 (f)
2019-20	low	Conventional	9.75 (a)	11.94 (b)	14.65 (c)
	Medium	Conventional	20.02 (d)	24.06 (e)	27.35 (e)
		Single Leader	18.35(d)	17.95 (d)	20.39 (d)
	High	Conventional	35.98 (f)	54.25 (h)	68.30 (i)
		Espalier	35.62(f)	44.00 (g)	49.15 (gh)

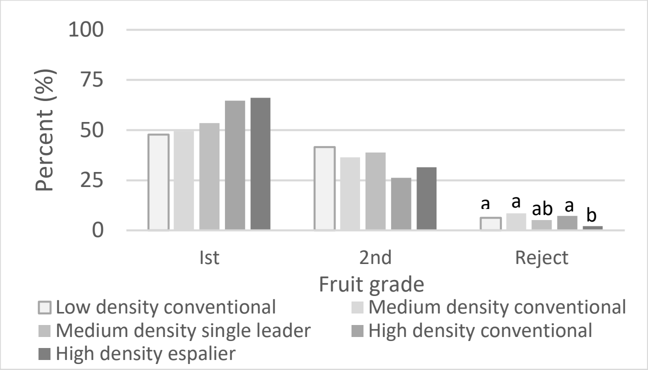
Fruit quality grading

To calculate the effect of treatments on pack out, we calculated the percentage of total fruit assessed that fell into each class, for each variable (%) in the 2019-20 season. Each fruit was graded for sunburn, blemish (including russet), lenticel, pink spot and sapburn and allocated to 1st class, 2nd class or reject class according to the accumulated grading score in line with the Australian Mango Industry Quality standards (2016). For example, the scoring took into consideration each variable and its class, if one of the variables was 2nd class, then the total fruit assessment was no higher than 2nd class. If one of the variables was reject class, then the total fruit assessment was no higher than reject class.

When we compared the proportion of fruit in each grade there was a significant difference for training system ($p=0.022$) and variety ($p<0.001$) for the proportion of fruit in the reject grade. Keitt had significantly more fruit in reject class while NMBP-1243 had significantly less fruit rejected than Calypso. The high-density espalier trained trees had a significantly lower proportion of fruit rejected than the medium and high density conventional trained trees (fig.1).



(a)



(b)

(c)

Figure 1: (a) the effect of variety on fruit grades and (b) the proportion of fruit in each training systems nested in density, from a sub-set of mango fruit in 2019-20 and (c) example of the fruit sampled from each variety from the mango planting systems experiment.

Internal fruit quality parameters

Internal fruit quality parameters do not contribute to pack-outs but they are important when it comes to maturity and flavour. The AMIA recommend minimum dry matter specifications for harvest depending on the variety.

Total dissolved sugars (Brix°)

In 2018-19 there was a significant main effect of variety (p<0.001) and canopy orientation (p=0.001) on the total dissolved sugars in the fruit mesocarp, as measured in degrees on the Brix scale. Fruit from NMBP-1243 (12.19°) was significantly lower than Keitt (13.1°) and Calypso (13.1°). Fruit in the east (12.93°) and south (12.92°) orientations had significantly higher dissolved sugars than the fruit from the north (12.62°) and west (12.75°) orientations.

In 2019-20 there was a significant three-way interaction between planting density, training system and orientation (p=0.017) and significant two-way interaction between variety by orientation (p<0.001). The results were highly variable and cannot be attributed to any one factor.

Dry matter

In 2018-19 there were significant main effects of variety (p<0.001), canopy orientation (p=0.001) and planting density by training system (p<0.001) on the percentage of dry matter in ripe fruit. The medium density, single leader trees had significantly lower dry matter (13.0%) fruit than in the high density espalier (13.5%), low (13.7%) and medium (13.7%) density conventional fruit. The fruit from the high density conventional (13.4%) was not significantly different from any other treatment. Variety NMBP-1243 (12.75%) was significantly lower than Calypso (13.9%) and Keitt (13.7%). Fruit from the north (13.2%) and west (13.4%) orientations had lower dry matter than the east (13.6%) and south (13.6%).

In 2019-20 there was a significant interaction between variety and canopy orientation (p=0.006) and training system within density (p=0.044) for dry matter. The mean dry matter was significantly lower in the medium single leader (12.072) and espalier (12.081) treatment than in the low (12.553) and medium density (12.502) conventional treatments. The low density conventional treatment also had a significantly higher mean dry matter (12.553) than high density conventional trees (12.224). Canopy orientation did not affect dry matter in Keitt, but in it was significantly higher in the north and west canopy orientation of NMBP-1243, and the south and east orientations in Calypso.

External fruit quality parameters

Fruit Blush

In 2018-19 there was a significant difference between variety and orientation (p<0.001) where Keitt and Calypso had higher percentage of skin covered by blush in the east orientation compared to the north and west (Table 4). There was also a three-way interaction for density, training system and orientation (p<0.001). For the high and medium density, single leader there was no effect of orientation however for the low and medium density, conventional trees the south had higher mean blush. For the high density conventional fruit there was lower mean blush in the north compared to the east and west sides (Table 4).

In 2019-20 there was a significant difference between variety and orientation (p<0.001) where NMBP 1243 had higher blush in the east compared to the north and west, while Keitt had significantly higher blush in the east compared to all other quarters. Mean blush in Calypso was not significantly different between any orientations (Table 4). There was also a significant three-way interaction between density, training system and fruit orientation (p=0.013) where the high density conventional fruit had lower mean blush in the north and south orientation compared to the east orientation and all other training systems in the same quarter (Table 5). Blush was significantly higher in the east for the high density conventional, espalier and the medium density single leader fruit. While the low density, conventional trees had lower fruit blush in the west compared to all other quarters.

Table 4: Mean blush (% of coloured skin/fruit) for the combination of variety and orientation for 2018-19 and 2019-20 seasons in the mango planting systems experiment.

Season	Variety	Orientation			
		North	East	South	West
2018-19	1243	53.03 cdef	51.03 cde	53.05 cdef	56.23 ef
	Calypso	43.29 ab	53.31 def	48.68 bcd	40.43 a
	Keitt	46.68 abc	57.90 f	52.77 def	47.57 bcd
2019-20	1243	58.13 bc	70.82 f	67.97 ef	65.63 de
	Calypso	52.85 ab	57.74 bc	55.97 b	52.88 ab
	Keitt	57.57 b	62.56 cd	53.92 ab	50.16 a

Table 5: Mean blush (% of coloured skin/fruit) for the combination of density, training system and orientation for the 2018-19 and 2019-20 seasons in the mango planting systems experiment.

Season	Density	Training system	Orientation			
			North	East	South	West
2018-19	low	Conventional	54.98 defgh	57.26 efgh	61.02 h	50.81 defg
	Medium	Conventional	48.85 de	53.06 defgh	56.74 fgh	45.78 cd
		Single Leader	54.58 defgh	58.43 gh	59.72 gh	56.07 efgh
	High	Conventional	24.67 a	48.65 def	31.65 ab	36.67 bc
		Espalier	55.25 efgh	53.01 defgh	48.36 def	51.05 defg
2019-20	Low	Conventional	66.39 c	68.30 b	68.43 c	59.69 a
	Medium	Conventional	62.59 c	65.28 ab	69.43 c	57.00 a
		Single Leader	58.89 bc	63.56 ab	63.15 c	56.87 a
	High	Conventional	41.02 a	61.54 ab	41.19 a	52.96 a

	Espalier	52.01 b	59.86 a	54.24 b	54.58 a

Sunburn

Sunburn was assessed at two levels, at the tree level to identify what percentage of total fruit was affected by sunburn (incidence) and at the fruit level to assess the severity of sunburn on each fruit at different orientations in the canopy.

Incidence of sunburn at the tree level

Sunburn incidence was assessed as the number of total fruit on the tree with sunburn damage on the fruit skin. At the tree level, in 2018-19 there was a three-way interaction between planting density, training system and variety (p=0.020) on the percentage of the fruit affected by sunburn. In all three varieties the mean percentage of sunburnt fruit per tree was significantly highest in the high density espalier trained trees compared to other treatments, except for Keitt, where sunburn in medium density single leader trees were as high as the high density espalier treatment (Table 6). There was no significant difference between the three conventional systems.

Table 6: The mean % of sunburnt fruit per tree in the 2018-19 season.

Density	System	Variety		
		NMBP 1243	Calypso	Keitt
low	Conventional	7.13 ab	9.01abc	11.82 cdef
Medium	Conventional	10.17 abcd	10.64 abcdef	13.35 cdef
	Single Leader	11.52 bcdef	14.31 defg	21.41 h
High	Conventional	10.66 abcde	11.71 bcdef	6.48 a
	Single Leader	15.31 fg	23.07 h	18.95 gh

In 2019-20, a similar treatment effect on sunburn was observed to 2018-19 with a significant effect of training systems within density (p<0.001). In the high density, espalier trained trees, Calypso and Keitt has significantly higher incidence of sunburn (29.12%) on fruit at the tree level than conventional trained trees (16.99%). NMBP-1243 was not assessed for sunburn in 2019-20. While the number of fruits affected by sunburn seems high it is the intensity of damage at the fruit scale that influenced pack-out classes.

Severity of sunburn at the fruit level

Sunburn severity was assessed using percent of fruit with sunburn damage. While not conducted in 2018-19, in 2019-20 we assessed the percent of sunburn damage on the fruit skin in each fruit sample. Results showed a significant interaction between variety and canopy orientation (p<0.001), and between density and training system (p=0.028). Sunburn was highest in the west quarter for all three varieties. The western quarter of NMBP-1243 had an average of 12.53 %, Calypso had 15.29 % and Keitt had 10.76 % of skin affected by sunburn, compared with 2.55 to 13.97 % at the other orientations in the tree. The proportion of sunburn on fruit was lower in the high density conventional trained trees (6.7 %) compared to the medium single leader (8.3 %), high density espalier (9 %) and low and medium density conventional treatments 8.9 - 9.7 %).

While variety, training system and orientation affected how the severity of sunburn damaged the skin, this variation was prevalent in how sunburn severity influenced the fruit grading. For example, when fruit were assessed for sunburn severity alone, according to the AMIA standards, the percent of Keitt fruit were compared, the low and medium density, conventionally trained trees had a higher percent in the Class 2 grade compared to NMBP-1243 in the same treatments. On the other hand, NMBP-1243 and Calypso from the medium density, single leader and high density espalier trees had a higher percent of fruit in Class 2 compared to the Keitt (Table 7). When all varieties were combined, the percentage of fruit in Class 1 was greatest in the high density conventional trees > high density espalier > medium density single leader > medium density conventional> low density conventional (Figure 3).

			NMBP-1243			Calypso			Keitt	
Training system	Density	Class 1	Class 2	Reject	Class 1	Class 2	Reject	Class 1	Class 2	Reject
Conventional	low	97	2.8	0	92	8	0	88	13	0
	Medium	97	2.9	0	90	10	0	93	7	0
	High	100	0.0	0	97	3	0	99	1	0
Single Leader	Medium	93	6.9	0	94	6	0	99	1	0
Espalier	High	95	5.2	0	95	5	0	97	3	0

Table 7: The severity of sunburn damage on the fruit skin in sampled fruit from the Planting Systems Trial, showing the proportion of fruit in each pack-out grade (Class 1, 2 and Reject) in the 2019-20 season.

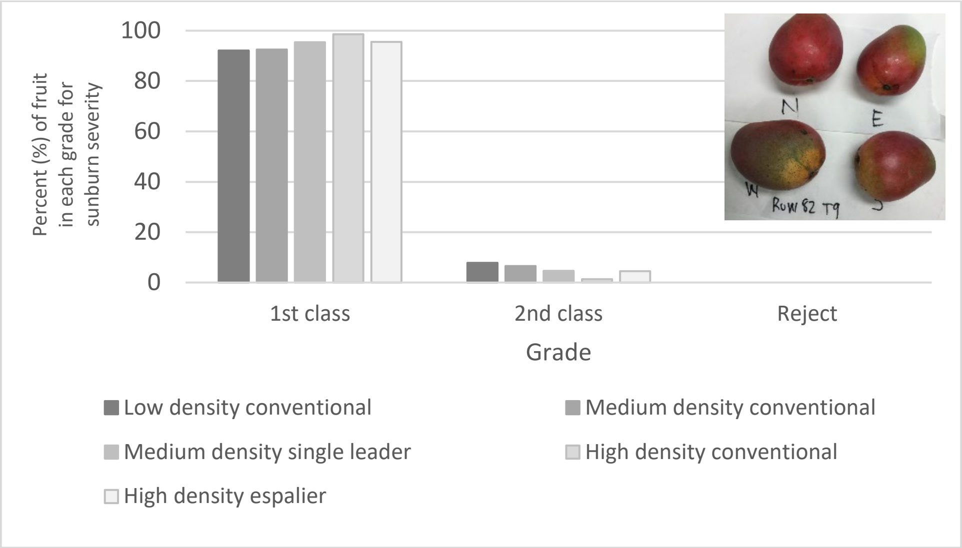


Figure 2: The percent of fruit with sunburn damage in each pack out grade (Class 1, 2 and Reject) for sampled fruit in 2019-20 from the mango Planting Systems Experiment. The insert shows an example of sunburn in each orientation, in the variety Keitt.

Blemish

Blemish severity was recorded as the area (cm^2) of fruit skin covered by blemish, in each year. In 2018-19 there was no significant effect of orientation and therefore the data is pooled and analysed with only the main effects and interactions of density, training system and variety in the fixed effects model. The only significant effect was the main effect of variety where Keitt (5.85 cm^2) had a significantly higher mean blemish on the fruit compared to NMBP-1243 (1.48 cm^2) and Calypso (1.32 cm^2).

In 2019-20 there was a three-way interaction between variety, density and training system (p=0.017) and a main effect of canopy orientation (p=0.001) on the fruit area with blemishes. Fruit blemish was significantly higher in the west (2.73 cm^2) orientation compared to all the other orientations (range from 1.93 to 1.95 cm^2).

There was no significant effect of training system on the severity (area of skin covered by blemish) in the variety NMBP-1243 (1.2-1.8 cm^2). However, in Keitt the severity of blemish was significantly higher in the high density conventional trees (6.5 cm^2) which led to more fruit being down graded into reject compared to the other conventional trees (Figure 3). In Calypso, the severity of blemish was significantly greater in the medium density single leader (2.2 cm^2) compared to the low (1.3 cm^2) or high density (1.3 cm^2) conventional trees. There was no significant difference between the medium density, single leader and high density espalier treatments within each of the varieties. Keitt had a significantly higher blemish (3.9 cm^2) than both NMBP-1243 and Calypso in all training systems except medium density, single leader where it was only significantly higher than variety NMBP-1243.

In the assessment of pack out, blemish in conventionally trained trees in 2019-20, differed between varieties with Keitt having between 16.7% of fruit in Class 2 and 11.2% of fruit in the Reject, NMBP-1243 had 6.0% in Class 2 and 1.8% in reject class, while Calypso had 5.7% in Class 2 and 1.8 % in reject (Figure 3) as a result of blemish.

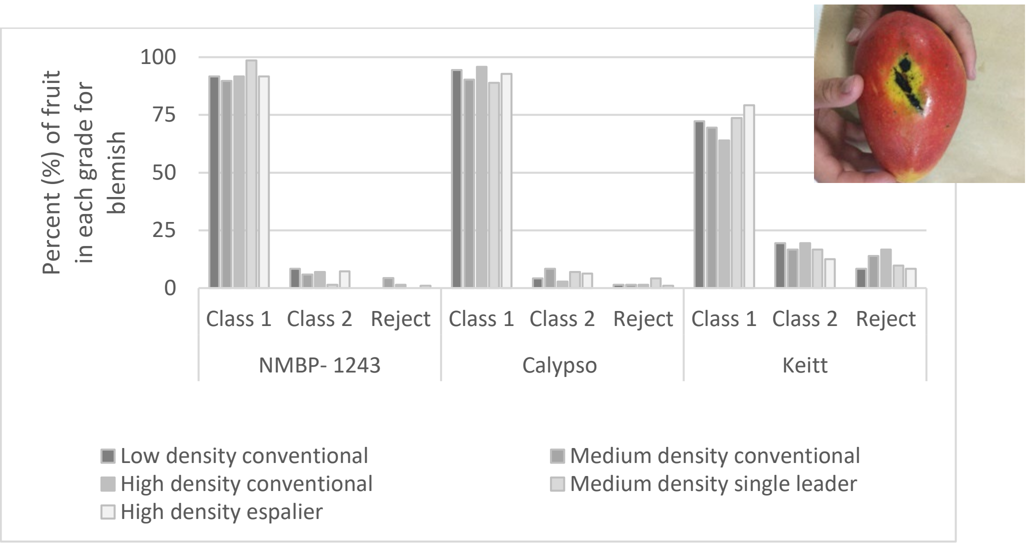


Figure 3: The effect of blemish severity (cm^2 of skin covered by blemish) on the percent of fruit in each pack-out grade (Class 1, class 2 and Reject) for sampled fruit in 2019-20 from the mango Planting Systems Experiment. The insert shows an example of blemish in the variety Keitt.

<u>Pink spot</u>

In 2018-19 analyses of the incidence of pink spot (presence or absence) was reported as the proportion of fruit with pink spot and found significant interactions between planting density and canopy orientation (p=0.033) and planting density and variety (p=0.012). The incidence of pink spot was lowest in NMBP-1243 at each density (28-51%) and highest in Keitt growing at high (97%) and medium density (95%).

In 2019-20, the severity of pink spot (measured as the number of pink spots caused by scale per fruit) on the sampled fruit is reported for Calypso and Keitt. While pink spot severity was measured, in NMBP-1243, the incidence was considered very low and could not be statistically examined. There was a significant effect of canopy orientation for both varieties (Calypso p=0.049 and Keitt p=0.003). Pairwise comparisons using the 95% least significant difference (lsd) suggest the mean number of scale was lowest on the east side (Calypso, 3.8; Keitt, 2.9) of the tree for both varieties compared to the other orientations (range from 4.45-5.04 in Calypso to 4.24-5.38 in Keitt). In Keitt and Calypso, pink spot was a major cause of downgrading. Calypso had 20.8 % of Class 2 and 4.9% of Reject fruit while Keitt had 15.6 % of Class 2 and 8.9 % of Reject fruit (Figure 4) due to pink spot. NMBP-1243 had 98.4 % of fruit in Class 1, 0 % in class 2 and 1.6 % in the reject class.

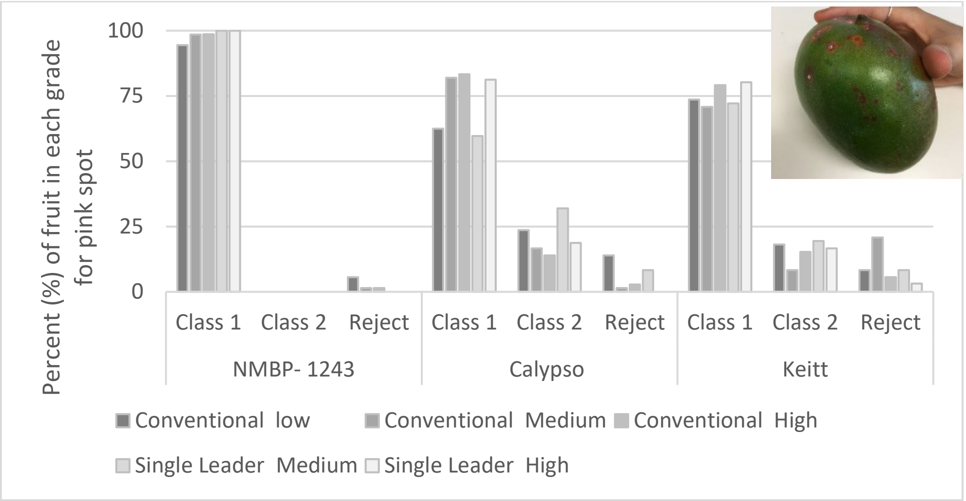


Figure 4: The effect of pink spot severity (number of spots/ fruit) on the percent of fruit in each pack-out grade (Class 1, 2 and Reject) for sampled fruit in 2019-20 from the mango Planting Systems Experiment. The insert shows an example of bad scale damage on fruit in the variety Keitt.

<u>Sap burn</u>

In 2019-20, severity of sap burn was measured as area (cm^2) of fruit skin covered sap burn on each of the sampled fruit. Orientation was not significant and as a result was dropped from the model. Sap burn contributed to the downgrading of fruit from grade 1 to grade 2 but was only significantly different for variety ($p<0.001$), where Keitt (0.6 cm^2) had smallest mean sap burn compared to NMBP-1243 (1.4 cm^2) and Calypso (1.6 cm^2).

For pack-out, the mean percent of fruit > than 4 cm ^{2} sapburn (or which downgraded fruit to Class 2) was 2.4 % in NMBP-1243, 6.8 % in Calypso and 3.6 % in Keitt (Figure 5). Calypso had a further 4.2 % of fruit downgraded to reject class (due to sap burn > 12 cm ^{2}), while NMBP-1243 and Keitt both had < 0.5 % downgraded to the reject class.

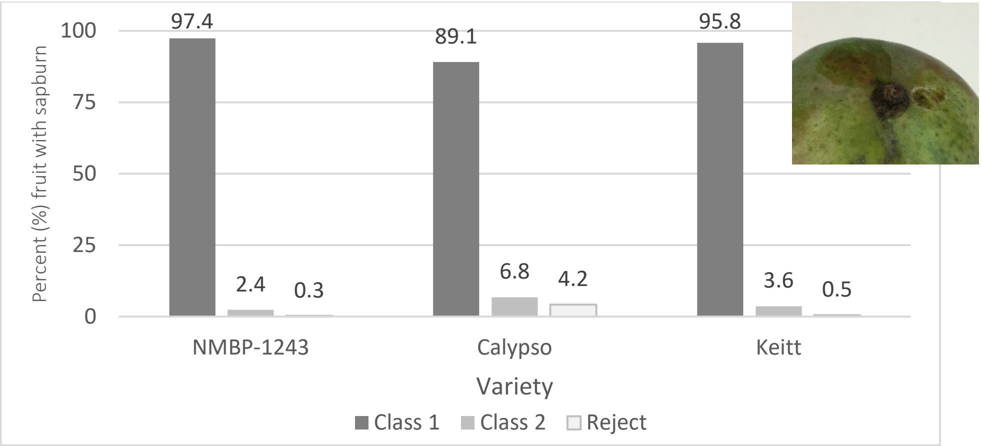


Figure 5: The effect of sap burn (cm^2 of fruit skin covered by sap burn) on the percent of fruit in each pack-out grade (Class 1, 2 and Reject) for sampled fruit in 2019-20 from the mango Planting Systems Experiment. The insert shows an example of sap burn on the variety Keitt.

Discussion

Yield parameters

This research focused on tree and orchard yield, fruit quality, and the percent of fruit in each pack out class. Increasing the planting density in the orchard has increased orchard yields in the 5th and 6th year after planting in all three varieties. In 5 year-old trees (2018-2019), yields increased in NMBP-1243 by 71% in the medium density (416 trees/ha) and by 100% at high density (1250 trees/ha) compared to the low conventional planting densities of 208 trees per ha. In Calypso, yields increased by 35% in the medium density and by 60% in the high density. Yields in Keitt, with its tendency to bunch bear, increased by 60% in the medium density and by 130% in the high density plantings. This demonstrates the importance of planting density and varietal selection in mango orchard productivity.

Training system also had a significant effect on orchard yields (in 6 year-old trees (2019-20)), where high density espalier trained trees had significantly higher yields than the other treatments, except for the high density conventional trees. The high density trees yielded higher than all other density and training system combinations. In the high density plantings, conventional Keitts, had a greater average increase in orchard yield of 366 %, NMBP-1243 had an increase of 269 % while Calypso had an increase of 354 %. While these yields are significant increases on the low conventional orchard systems, it is expected that the yields in the high density conventional trees in the 2019-20 season may not be continual or sustainable as the trees in these treatments had reached their maximum allocated orchard space. As such, trees were pruned heavily after the 2019-20 harvest. From 2020, the trees will be managed as a high density hedge to maintain their size within the allocated space. Further yield data will clarify the effect on productivity in high density training systems as a result of this management technique. Research in high density mango orchards in South Africa by Fivas et al. (1997) and Oosthuyse (1993) recommended that while high density orchards offer potential for increased yields, any real advantages of tree training and pruning can only be assessed once trees have reached their allocated space. Similarly, research in India by Ram and Sirohi (1991) found that pruning was critical to maintain yields once trees had reached their allocated space for mangoes planted at high density.

Pack outs

Fruit pack out was assessed on a sub-sample of four fruit from each tree within each plot, and as a result should be considered as indicative of a representation of fruit quality of any treatment. Fruit pack out classes differed between varieties, canopy orientation, density and sometimes training system. Overall the variety NMBP-1243 had a significantly higher proportion of class 1 fruit (73.0 %) than Calypso (45.1%) and Keitt (46.2%). The proportion of rejected fruit was higher in Keitt (13.6 %) than Calypso (6.4%) and NMBP-1243 (1.6%). This indicates the importance of variety or genetics when assessing the effects of growing systems on the marketable portion of yield assessments. The effect of canopy orientation on pack out was different in the different varieties. Again reflecting how varietal traits influence fruit quality at different canopy orientations. For example, Class 2 fruit for NMBP-1243 was predominantly from the west. For Calypso, class 2 fruit were predominantly from the south and west orientations but for Keitt, pack outs were not affected by canopy orientation, although this variety had some of the highest Class 2 and rejected fruit.

Fruit from the high density, espalier training system had a significantly lower proportion of reject fruit compared to the medium and high conventional training systems and often had the highest or second highest proportion of fruit in class 1. This indicates that the espalier and single leader tree training systems improve overall pack out of fruit adding to the value of these growing systems.

External fruit quality parameter

Fruit blush and sunburn

The experimental treatments in the Mango Planting Systems Trial had some positive and some negative influences on fruit quality and pack out grading. For example, in 2018-19 fruit blush was not influenced by canopy orientation or single leader systems (medium density single leader or high density espalier), however the conventionally trained trees across all planting densities, had less blushed fruit than the other tree training systems. In both years the high-density conventional fruit had less blush in the north and south quarters compared to the other orientations and training systems. In 2019-20, the fruit from the espalier and single leader trees had a higher percentage of skin blush in the eastern orientation. NMBP-1243 had higher blush than the other varieties in all canopy orientations. Yu et al. (2016) found that fruit blush was related to the canopy light environment in Honey Gold mango and that fruit blush could be modelled according to the light in the different positions in the canopy. Similar results have been found for apple (Awad et al 2000; Saure 1990).

In the second year, blush was greatest in the eastern canopy, however, the incidence (number of fruit with sunburn damage) and severity (% skin with sunburn damage) of sunburn was greatest in the west orientation in both years. While the single leader, espalier and high density conventional had a higher incidence of sunburnt fruit, there was no significant difference between the treatments for the severity of sunburn on the fruits.

There is some concern that the effects of sunburn resulted from the experimental design, including the orientation of training system in association with the direction of the rows. For example, the rows in in this trial were designed to run north and south, to maximise light, as a result fruit were more exposed to the east and west orientations compared in the high density espalier treatment as a result of the narrower canopies and higher light distribution near the stem in these treatments (data not included). Therefore the fruit in the high density espalier systems were less protected by foliage and more exposed to sunlight across the row, in our case on the eastern and western sides This would account for why there was twice as many fruit with sunburn in the high density espalier training system. This greater fruit exposure to sunlight is likely responsible for the increases in fruit blush as well as the increase in the incidence of sunburn in the high density espalier training sytems. This poses the question as to whether row orientation could influence the rate of sunburn in the espaliered trees. Varying the row orientations to east-west, southeast-northwest or a similar combination may lower the incidence of sunburn or the effects on fruit blush.

Palmer (1989) calculated that apple trees oriented in north-south rows intercepted more light than east to west facing rows in mid-summer (in the northern hemisphere) and the proportion of canopy intercepting light changed with row orientation. They found seasonal changes were greater in north-south facing rows compared to east-west facing rows. They attributed the variation in row orientation to the proportion of canopy that is illuminated by light. Rom (1991) identifies that while the proportion of canopy illuminated can influence on fruit morphology, development and fruit quality, it is the spectra of light that affects fruit differently. For example, ultraviolet light contributes to anthocyanin synthesis or the red colour development we refer to as blush (Haiter and Gould 2009, Sielgelman and Hendricks 1958, Walter 1967) and temperature (infrared heating) (Bergh et al. 1980). However, wavelengths within the ultraviolet zones can cause damage to fruit (Simpson et al. 1988) and hence the range of wavelengths of light and their intensity may also influence fruit quality and should be considered when designing new orchard systems. The question of row orientation, light interception and fruit quality may differ in tropical evergreen trees, such as mango, growing in high latitude tropical environments compared with deciduous trees growing in temperate climates. Observations from field experiments on how light spectra can be influenced by canopy management, row orientation and canopy positions are recommended to answer the questions hypothesized above as shown in apple trees.

Despite an increased incidence of sunburn in fruits from the high density espalier trees, the severity of sunburn was at acceptable levels for all treatments and therefore predominantly allocated to Class 1 in the pack outs. On the other hand, blemishes (which included scratches, abrasions, mechanical damage and russet) had a significant effect on pack outs. The variety with the worst blemishes was Keitt. Keitt has a bunch bearing habit which can result in the rubbing of fruit, panicles or peduncles in close proximity and which can lead to increase blemishes on the skin in this variety. There was some indication that the mixing of varieties in this experiment also led to some damage, where frequent traffic, in the high density block of Keitt (which is the last of the varieties harvested in this trial), led to an increase in the occurrence of blemishes.

Calypso had the greatest proportion of sap burn, however it is unclear that the cause of this can be attributed to the treatments, and is more likely due to postharvest handling factors. Experimental treatments are unlikely to be a factor in sap burn. Overall, pink spot was greatest in the trees with the largest, most dense canopies which is contrary to sunburn and blush which were highest in the trees with the narrowest, less dense and open canopies such as those found in the high density, espalier trained systems.

Internal fruit quality parameters

The internal fruit qualities of total soluble solids were statistically different between variety and canopy orientation however the results only varied slightly within each variety to +/- 0.5-1^0 which may not have biological or management significance. Dry matters also varied with variety and orientation although the results were not consistent between the years. The data presented in this report are a snapshot of the fruit quality based on relatively small sample sizes and may not represent the fruit populations at a whole and as a result should be treated accordingly.

Conclusion

While there was some significant increase of planting density on yield at the orchard level there were some trade-offs for fruit quality with density and training systems. This however does not out way the benefits of planting mangoes at high density on orchard yields up to six years of age. While there were some fruit quality factors that influenced pack outs, the results differed depending on variety. In addition, some of these differences maybe moreso due to factors such as management or orchard design, such as how each variety was harvested at different times to reflect their maturity with one early (NMBP 1243), mid (Calypso) and late variety (Keitt) planted evenly and randomly across the trials. As a result, some variation in relation to the parameters that impacted on the pack out assessments were not related to the treatments but more-so to other factors i.e. the management or orientation of rows in combination with training system (i.e. sap burn at harvest, blemishes in variety Keitt, sunburn to the west indicating north-south row orientation compromised quality).

External fruit quality parameters such as fruit blush were more consistent in the single leader training systems however on the down side, so was sunburn. Sunburn and blush were both influenced by orientation on the tree with the severity of sunburn greatest in the west while blush was greatest in the east. While there was a higher proportion of sunburn in the single leader training systems at the canopy scale, the overall intensity of sunburn at the fruit level did not contribute to downgrading of fruit in the pack out assessments. Internal fruit qualities did not vary with any biological significance as a result of the training systems. Overall, we can assume, that the internal fruit quality indicators assessed by this research were not negatively affected by training systems.

This research concludes that the largest canopies (low and medium density conventional trees) had the highest amount of pink scale while the thinner narrower mango canopies had both positive and negative influences on external fruit quality. Positive effects include increased and more consistent blush and reduced potential for insect damage (pink spot) while the orientation of rows, combined with narrower canopies may have inadvertently predisposed the trellis training systems to damaging light in the west, increasing the incidence of sunburn. It is recommended, as a result of this research, that further research be continued to help identify how row orientation or other protection (i.e. pruning, shade cloth cover or chemical mean) may help reduce the negative effects of sunburn, while maintaining the positive effects of mango orchard intensification such as increased yields, improved skin blush and pink spot control. Identifying the physiological mechanisms influencing fruit quality (i.e. light and temperature) in these systems could also help us manage potential future climate variability.

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Appendix 13

Hypotheses and Assumptions
for Functional Structural Plant Models

Jim Hanan, Inigo Auzmendi, Neil White, Ben Toft

Introduction

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During the course of the project, a list of hypotheses and assumptions for functional structural plant models has been maintained, created through discussion between modellers and experimental scientists. This is a living document, subject to change as our understanding of the trees' underlying physiology grows, and more to the point, as our focus on what is most important changes. Hypotheses are collected under the 4 key research areas they relate to: architecture, canopy light relations, vigour, and crop load. Superscripts refer to the number of the appendix including the models that consider each hypothesis.

Architecture (spatial distribution and connection of plant organs)

Assumptions

1) The above ground structure of a plant is determined by the activity of the buds (meristems): apical, axillary, and adventitious.[14,15,18,19]
2) An active vegetative meristem will produce metamers that make up a shoot, each composed of a segment of stem, or internode, one or more leaves, with buds in their axis.[14,15,18,19]
3) Cambium (secondary meristems) controls secondary growth (e.g. internode thickening).[14,15]
4) A reproductive meristem in a flower bud will produce an inflorescence with flowers, and possibly fruit.[14,15]
5) The plant is thus a collection of semi-autonomous above-ground organs plus roots. Similar meristematic activities happen in roots.[19]
6) A population of dormant buds is available for plant response to environmental changes.[15,18,19]
7) Management practices such as pruning and limb bending may result in release of buds to form new branches.[19]

Hypotheses

1) The indeterminate, vegetative or reproductive fate of the buds is determined by endogenous processes (apical dominance, carbon availability) affected by environmental factors (temperature, light).
2) Location of branching is determined by endogenous signals, which may be hormonal or sugar based, and/or competition for carbon allocation. This is connected to aspects of apical dominance: correlative inhibition, apical control, and epinasty, determining the branching structure (acrotonic, mesotonic, basitonic).
3) There may be different types of shoots that can be classified (long, short, water shoots, etc), which have different characteristics in terms of growth rate/duration and result in different branching and flowering / overall architecture.[19]
4) Bud release in natural growth is acrotonic, and branch bending leads to a more uniform branching pattern down the branch.
5) Shoot growth starts and ends several times during one year (flushes). The number and duration of the flushes is determined by endogenous (genetic, hormones, carbohydrates) and environmental factors (temperature, light).[14,15,19]
6) Shedding of leaves and branches is controlled by endogenous processes in response to environmental and internal conditions.[14,19]
7) Growth can be captured at different levels of detail, with metamer and/or flush (growth unit) level being our main interest.[14,15,19]
8) Meristems may be in juvenile or adult state and this may affect the characteristics of organs produced (leaf shape and colour, growth rate, absence of flowers).

Canopy light relations (amount and spatial distribution of light)

Assumptions

1) Carbohydrate assimilation is determined by photosynthetic leaf area, leaf incident light, and the leaf's photosynthetic efficiency (note there is lots of work in this area, so we may just need to find appropriate models).[14,15,18,19]
2) The amount of light absorbed by a tree canopy determines tree growth and fruit yield by producing carbohydrates through photosynthesis.[14,15,18,19]
3) Spatial distribution of light within a tree canopy plays a significant role in tree development and fruit quality by enabling production of photosynthate at different locations and the consequent allocation/transportation of photosynthate to different components, either for growth or storage.[14,15,18,19]
4) Photosynthetically active radiation intercepted by a leaf consists of both direct beam sunlight and diffuse light transmitted or reflected from the sky and surroundings.[14,15,17,19]
5) Simulation based analysis of light spatial distribution in a canopy, once validated against real world measurements for test cases, can be used to consider many more scenarios than is practically possible in the field.[16,17,19]
6) Physics-based modelling of light through a simulated canopy of fixed-position leaves, without including the supporting branches, gives a reasonable approximation of light environment found in a real canopy, at least enough for evaluation of different scenarios.[16,17,19]
7) Optical properties of reflectance and absorption measured using devices such as FieldSpec 4 spectroradiometer (ASD Inc.) on leaves that have been removed from the tree are suitable for use in light simulations, i.e. optical properties do not change significantly between picking and measuring.[16,17,19]

Hypotheses

1) Photosynthetic efficiency and light availability may interact (i.e. sun and shade leaves).[14]
2) Different tree structures result in different patterns of light spatial distribution and hence can have a significant impact on fruit production (quality: size, colour, brix, oil content) and growth (flower and bud determination).[14,18]
3) Some planting systems, combinations of tree density, tree training and pruning exist that would allow growers to optimize tree and orchard light relations and hence improve crop yield and quality.[14,16,17,19]

Vigour (the increase in internode, leaf, and shoot size over time)

Assumptions

1) The main driver of growth and development is temperature, which can be captured by thermal time (growing degree-days) with the plastochron being the amount of thermal time between leaf initiations. Expansion of organs and phyllochron (rate of leaf appearance) are proportional to growing degree-days.[14,15,18]
2) Vigour can be managed by genetics, rootstocks, plant density, pruning, crop load, training strategies and/or plant growth regulators.[174,15,18]
3) Excessive and insufficient vigour can be a problem.[18]

Hypotheses

1) Each organ has a genetically determined developmental pattern that governs its growth potential, activated (and deactivated) by endogenous or environmental signals.[14,19]
2) At any time, conditional organ growth capacity is determined relative to the organ's current biomass and growth duration, as affected by endogenous and environmental conditions.[14,15,18]
3) Actual organ growth is subject to resource availability as a consequence of inter-organ competition for available resources. In a detailed model this may be a function of location relative to sources of carbohydrates, transport resistances, and organ sink efficiency ("sink strength"), for example.[14,15,18,19]
4) There may be a positional effect on number of internodes per branch. Leading branches tend to develop more internodes than non-leading branches.[19]

Crop Load (fruit demand per supply of assimilated carbohydrates)

Assumptions

Flowering is a result of transformation of indeterminate into reproductive meristems (induction), which may then remain dormant until organ elongation commences (evocation).

Flowers may be pollinated causing conversion into fruit.

Crop load can be managed by genetics, rootstocks, pruning, fruit thinning, leaf removal and/or training strategies, as well as plant growth regulators.[14,18]

Excessive crop load can be a problem (reserve depletion), insufficient crop load can be a problem as well (reduced yield, excessive vigour and consequent shading).[14,18]

Hypotheses

1) Growth is determined by endogenous (apical dominance, carbon availability) and environmental factors (temperature, light).[14,18]
2) Reproductive (flowers and fruits) and vegetative growth (roots, stems and leaves) compete for carbohydrates.[14,18]
3) Flowers and fruit are subject to shedding controlled by endogenous (carbon availability) and environmental factors (temperature).[14,18]
4) Carbohydrate may be turned into a structural form (root, stem, leaf or fruit), or may be stored in a convertible form, subject to later recovery and reallocation. Storage should be treated as an active sink competing with other organs.
5) Fruit growth affects concurrent floral initiation and fruit set, resulting in irregular bearing.[15]

Appendix 14

Simulating growth and yield with virtual trees

Auzmendi I, Hanan JS

Introduction

Temperate trees and subtropical trees

Some temperate fruit trees have experienced an increase in orchard yield in a relatively short amount of time. This increase in productivity has been obtained mainly by orchard intensification focusing on reducing the size of the trees with new rootstocks, increasing planting density, new training systems and new cultivars. Apple orchards are one of the best examples, apple production is very important in many countries, and there has been more effort to study it and to design new systems to improve productivity than in other species.

Fruit production of trees like macadamia, avocado and mango is more important for Queensland than apple in quantitative terms, but these species have not been studied as intensively as apple. Applying the knowledge from apple intensification experiments directly to these species might not be adequate because of the following reasons:

- Species and cultivars usually have different growth habits and physiology; as a result, macadamia, mango and avocado might produce different yield results with treatments similar to those applied in apple trees. In addition, rootstocks and training systems similar to those employed in apple orchards might not be available or adequate for other species, and replicating apple experiments with other species might not be possible.
- The response to this intensification could vary during the life of the tree, initial gains when plants are young might be offset by loses when plants are mature.
- Tropical and subtropical climates differ from temperate climates. Moreover, weather can vary greatly from one growing season to another, e.g. light intensity, temperature, relative humidity and water availability, and this can have a great effect on yield.

All these considerations should be taken into account in an intensification study. One option is to follow the trial and error method for testing all the possible combinations in field experiments, i.e. planting density, training, cultivar, rootstock, age and weather. However, this would require a great amount of resources in terms of time, funding and technical effort. Another option is to gain insight based on existing scientific knowledge, but we should be aware that many plant physiological processes are not well characterised, and integrating several interacting processes might not be straightforward.

Virtual trees

An alternative approach consists on improving our understanding of the main mechanisms behind the increase of orchard productivity, e.g. light interception by tree canopies, carbon allocation among tree organs. This can be attempted by combining field experiments at small scale with models that integrate those mechanisms and their interactions to simulate the growth of virtual trees. Researchers have already used process-based models to explore alternative farming designs in several field crop systems with varying climate, soil, irrigation, fertilization, planting density and date (Keating et al., 2003). However, these models simulate biophysical processes of the plant as a whole, and they average all the vegetative and fruit organs in the canopy. In contrast, fruit tree canopies are usually more heterogeneous than field crops, due to their perennial nature and management practices. Orchard intensification requires focusing on local responses to environmental factors, e.g. light, and to physiological factors, e.g. carbon availability. Thus, we should consider, in addition to basic plant physiology, the complexity of canopy structure, i.e. branches, and individual organ variability (Grossman and DeJong, 1994), requiring in some of these aspects a higher degree of detail than process-based models.

Computational models that explicitly describe the development of the 3D architecture or structure of plants over time, governed by physiological processes are called functional-structural plant models (Vos *et al.*, 2010). In other words, dynamic mechanistic functional-structural plant models can simulate the growth of each tree organ in their spatial position every day for a growing season, mimicking mechanistic aspects of the growth of a real tree in an orchard. These models require physiological parameters with a clear meaning, estimated from measurements usually at organ scale, and they involve more complex programming and more computer resources than process-based models. The internal structure and mechanisms included in the models change as new questions arise. They should not be confused with empirical decision-support models that need to be as simple as possible, to be practically used by growers to make predictions (DeJong, 2019). Empirical models do not question the internal structure of the model or the understanding of the system. They are valid in the same scenarios in which their arbitrary parameters have been estimated, but we should not expect that they would be applicable outside those conditions (Passioura, 1996).

Currently it is not possible to simulate in full detail the complete physiology of a tree, because we still do not understand well many plant physiological processes, as opposed to other scientific fields in which the underlying principles have been well described, i.e. physical processes in disciplines like flow dynamics or electricity. Therefore, the aim of dynamic mechanistic functional-structural plant models has been to do innovative research and improve understanding in several areas of fruit tree physiology. The model provides a platform for integrating knowledge, identifying gaps and generating hypotheses (DeJong, 2019). Collaterally, it can lead to the development of simple decision support tools (Lopez *et al.*, 2011), as well as demonstrative pictures and videos for students and fruit growers (Lopez *et al.*, 2018).

Considering that no functional-structural plant model is available for our purposes, i.e. studying effects of planting density, tree size and training systems on yield in subtropical fruit trees, we use existing modelling techniques to design simple models for simulating growth and yield of virtual trees corresponding to our species. Complexity is added to the models progressively as required, comparing simulations with field results, and discussing them to enhance both models and experimental designs, as well as improving our understanding of the underlying mechanisms. Small-scale experiments are employed both to provide data for input in the model as well as to compare qualitatively outputs of our virtual tree experiments with orchard measurements.

Methodology

We used a specific modelling software platform called L-studio (Karwowski and Prusinkiewicz, 2004; Prusinkiewicz *et al.*, 2007) to simulate the growth of individual organs (fruits, leaves and stems), together with software to simulate the light environment of individual leaves (Cieslak *et al.*, 2008).

Specific measurements of individual organs and of the whole tree canopy structure were required to build virtual trees. Therefore, Auzmendi *et al.*, 2017 and Auzmendi and Hanan (2018, In press) used values already published in the scientific literature and new data collected as needed.

Mechanistic model prototype of individual organs growing in one shoot using a carbon pool model

Initially we assumed that photosynthates are accumulated on a per leaf area and light availability basis and distributed based on potential growth-demand of individual organs to drive the development and growth of a flushing shoot. This simple, generic whole-shoot carbon-pool model of local vegetative and fruit growth considered as meteorological inputs only measured solar radiation and temperature, and it was a means to integrate our key research components of vigour, architecture, light and crop load.

In keeping with the idea that we can transfer knowledge from temperate tree studies, a similar model was used to simulate growth of leaves in individual shoots. This model was employed to study how carbon availability during growth affects final leaf length variability in peach, using data from an experiment performed in 2009 at Ted DeJong's lab, University of California Davis (Auzmendi *et al.*, 2017).

Mechanistic model of individual organs growing in multiple shoots within a whole tree using a model of autonomous shoots with regard to carbon and light environment model (QuasiMC)

We added the complexity of a young tree canopy structure to simulate size at harvest of individual fruits in a whole tree. We used this virtual tree to simulate the effects of different training systems, crop loads, light availability and warmer temperature on fruit size distribution, and discuss them from a theoretical point of view (Auzmendi and Hanan, 2018).

3D representation of architectures measured in the field

We designed and implemented programs to read canopy structure data measured in the field and represent them in our modelling software, with the purpose of supporting field data verification, and also for preliminary analysis, demonstrative purposes and to start subsequent simulations of plant growth (Auzmendi and Hanan, 2019).

Mechanistic model of individual organs growing in a whole tree using autonomous units carbon allocation model (AUCAM) and light environment model (QuasiMC): Initial virtual macadamia tree

We proposed a new modelling method for simulating carbon allocation within the plant considering autonomy of branches of different ages in a tree (autonomous units carbon allocation model, AUCAM). AUCAM was combined with models of other aspects (Cieslak *et al.*, 2011), e.g. QuasiMC, to produce a simple functional-structural macadamia model to simulate tree and fruit daily growth during one growing season. These simulations were employed to explain some of the results of previous experiments, as well as to investigate the autonomic scale of a young macadamia tree measured in the field (Auzmendi and Hanan, In press).

Virtual macadamia tree growing in an orchard environment

Considering 2-year-old branches as autonomous with regard to carbon, we simulated yield and light interception in young macadamia trees growing in an orchard environment with a range of different planting densities, tree size and shapes. These results were discussed and compared with previous experiments (Auzmendi and Hanan, 2020).

Outputs

- A mechanistic model prototype of shoot growth was developed and shared with the rest of the members of the project for internal discussion in 2014 (Figure 1).

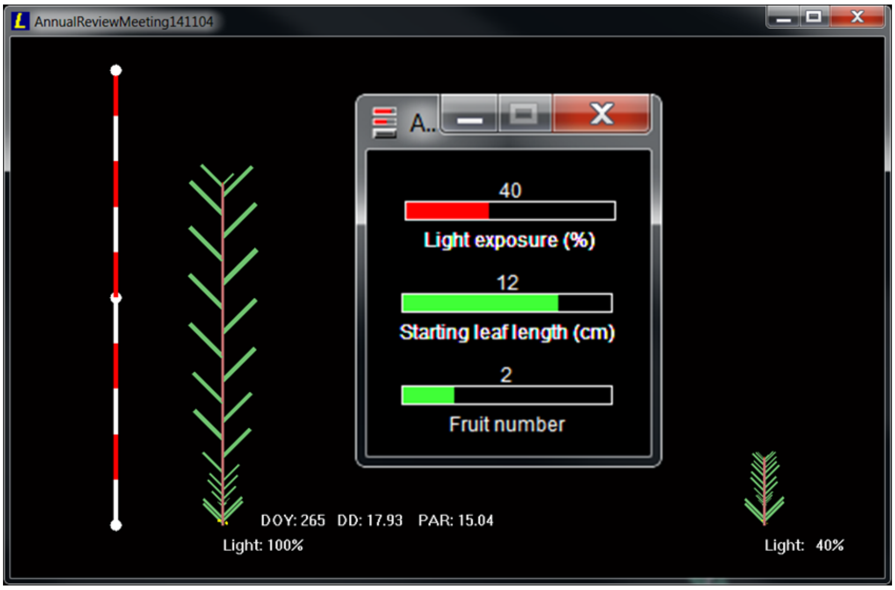

Figure 1. Screenshot of the shoot growth model prototype showing the growth with two different light intensities.

- The model of individual leaves growing in a shoot and competing for carbon was employed to investigate the most important factors that determine final length variability of individual leaves. The mechanistic model explained most part of this variability (62%, figure 2). However, it points out that more detail should be added to the model, and that there are probably other factors significantly affecting leave size that should be studied (Auzmendi *et al.*, 2017).

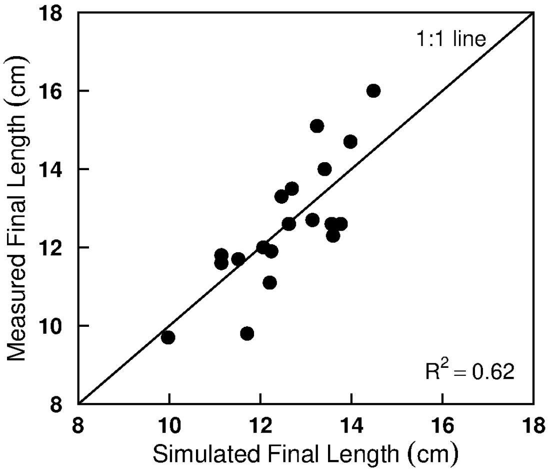

Figure 2. Relationship between simulated and measured final leaf lengths. Each observation (open circle) represents one individual leaf (Auzmendi *et al.*, 2017).

- A mechanistic model of individual organs growing in multiple shoots within a whole tree (Figure 3) using a model of autonomous shoots with regard to carbon was employed to simulate multi-factor experiments with different training systems, crop loads, light and temperature (Figure 4). These simulations were used to discuss and investigate fruit size distribution under different meteorological conditions and management, comparing them with previously published experiments (Auzmendi and Hanan, 2018).

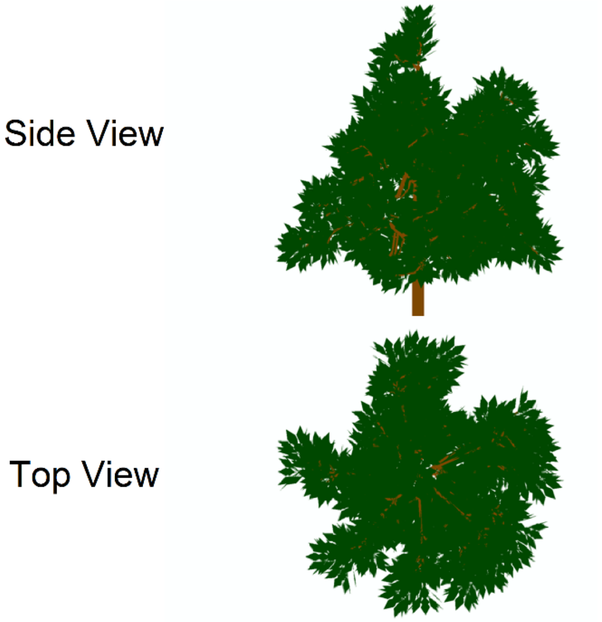

Figure 3. Initial architecture corresponding to a central leader training system used as input and visualized with the model (Auzmendi and Hanan, 2018).

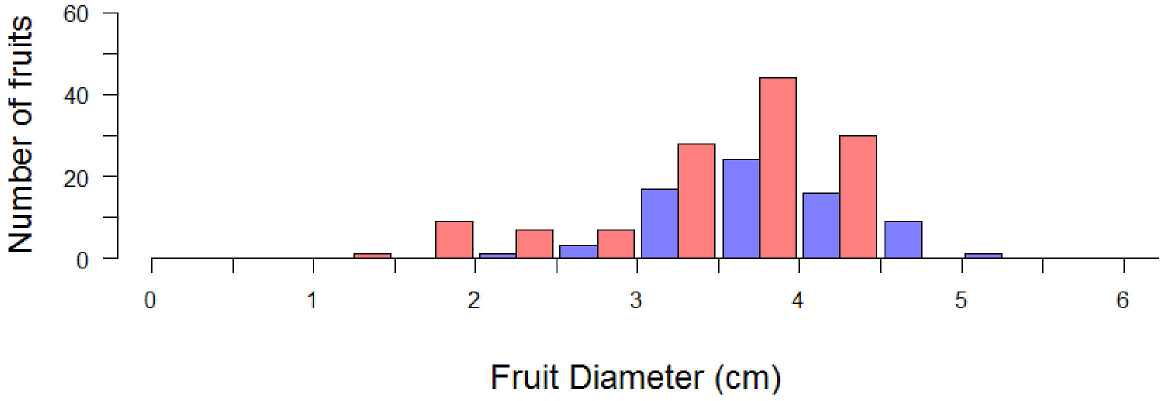

Figure 4. Simulated fruit size distribution in trees with central leader training system for one fruit per shoot (blue) and two fruits per shoot (red). Higher crop load increased the number of small fruits and reduced the number of the biggest fruits (Auzmendi and Hanan, 2018).

- Programs to read canopy structure (Auzmendi and Hanan, 2019) were used to create 3D representations of architecture for field data verification and preliminary analysis (Figure 5). They were also used for demonstrating the training systems in the planting systems trials (Figure 6, Ibell *et al.*, 2019a, 2019b), and to start subsequent simulations of plant growth with real canopies (Auzmendi and Hanan, In press) or with modified canopies, e.g. planar shapes (Auzmendi and Hanan, 2020).

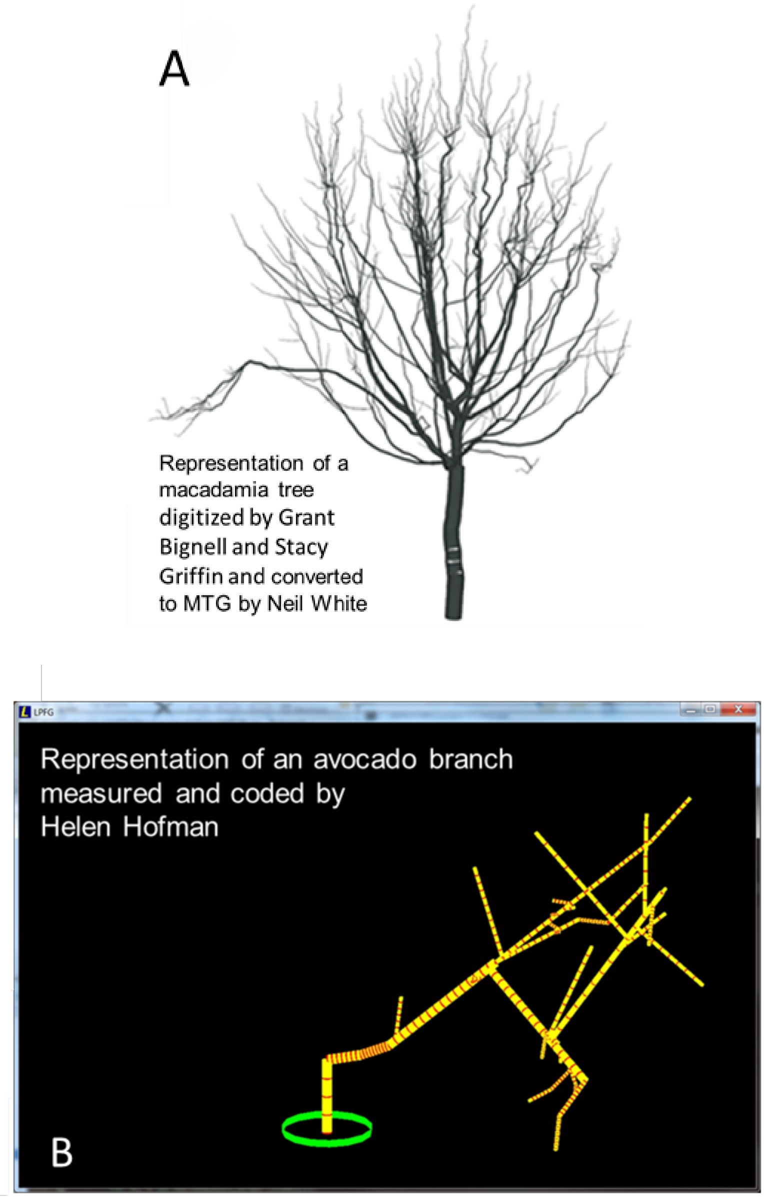

Figure 5. 3D visualization of: (A) 4-year-old *Macadamia integrifolia* tree architecture trained according to the standard local practice in Beerwah, QLD; and (B) avocado branch architecture generated from data measured in the planting systems trial of the STHPI. Length, diameter, direction and topology of each growth unit were measured in May 2016.

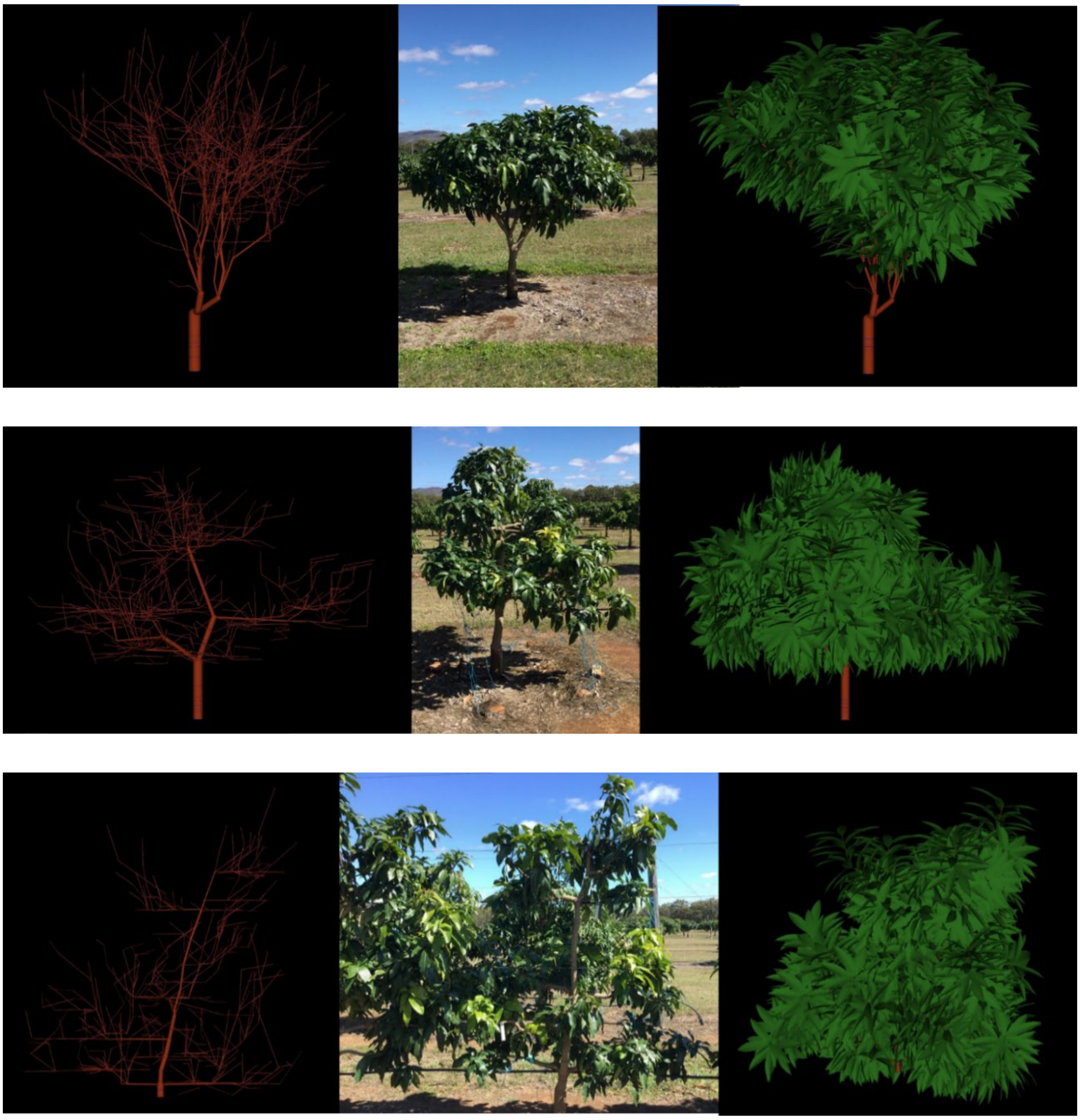

Figure 6. Mango tree architectures generated from data measured in the field and constructed directions. The mango 'Keitt' trees were planted in the Walkamin research station in December 2013 in the planting systems trial of the STHPI, directions of each growth unit were measured in November 2015, and growth unit length and topology were measured in February 2018. From top to bottom: low density (8 m x 6 m) with conventional training system; medium density (6 m x 4 m) with single leader training system; and high density (4 m x 2 m) with espalier training system. Trees were measured and coded by Paula Ibell and Ram Kolala, and converted to Multiscale Tree Graphs by Neil White.

- The autonomous units carbon allocation model (AUCAM) was described and used to implement the initial virtual macadamia tree (Figure 7). It was used to investigate and explain aspects of differences in carbon autonomy between trees, organ variability, competition between shoot and fruit growth (Figure 8 and 9), and time of autonomy, as well as to understand better the impacts that the scale of autonomy has on the results of simulations and field experiments. Compared to a measured young macadamia tree, the simulations that assumed autonomy at branch scale, i.e. two-year-old wood, showed the most realistic architectural growth (Auzmendi and Hanan, In press).

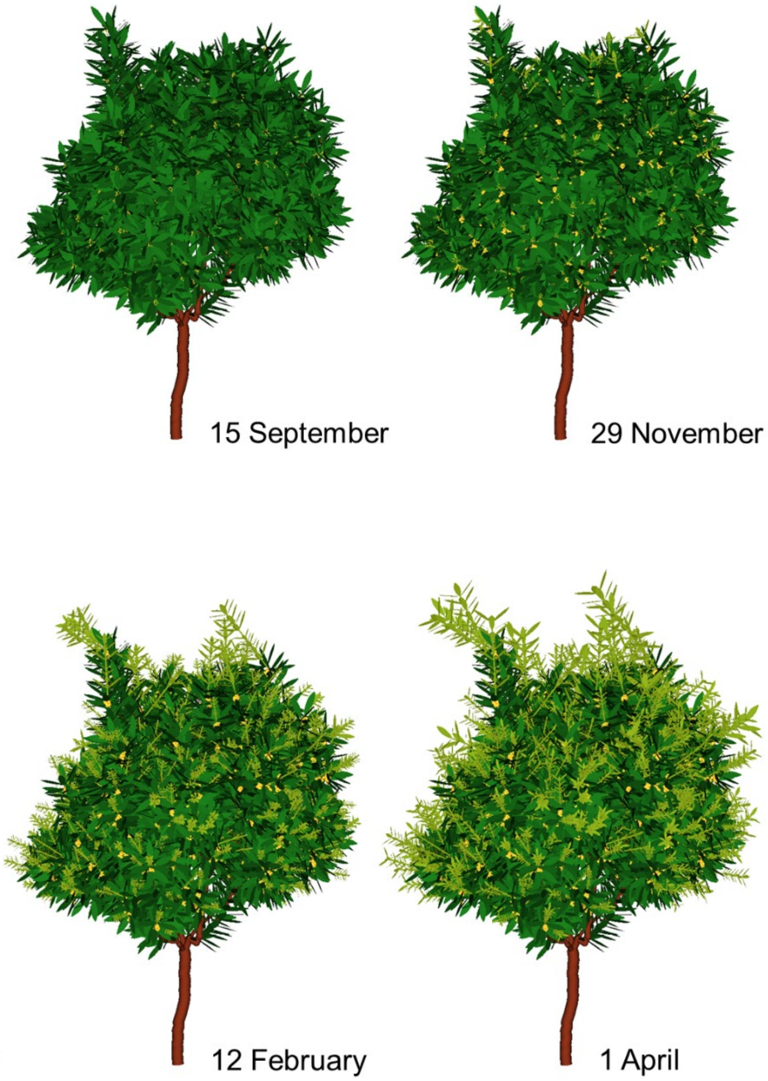

Figure 7. Visual 3D representation of the growth of a simulated young macadamia tree canopy between anthesis and harvest. The canopy includes stems (brown), leaves (green) and fruits (yellow). Leaves growing in the present season have a lighter green colour (Auzmendi and Hanan, In press).

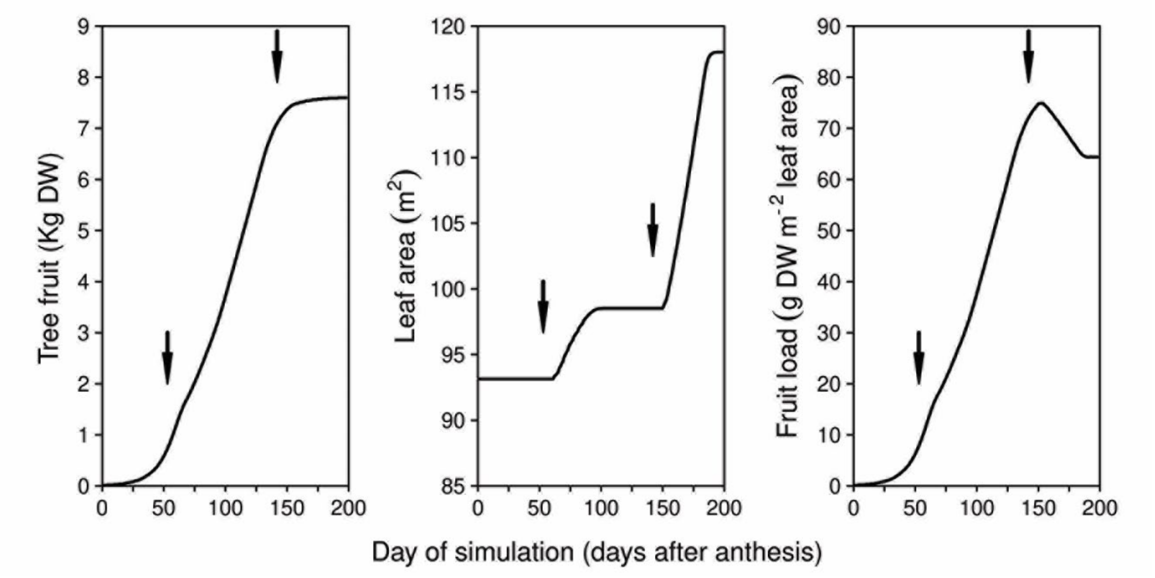

Figure 8. Values of tree fruit, leaf area and fruit load between anthesis and harvest considering 2-year-old branches to be autonomous with regard to carbon. Arrows denote dates of flush budburst (Auzmendi and Hanan, In press).

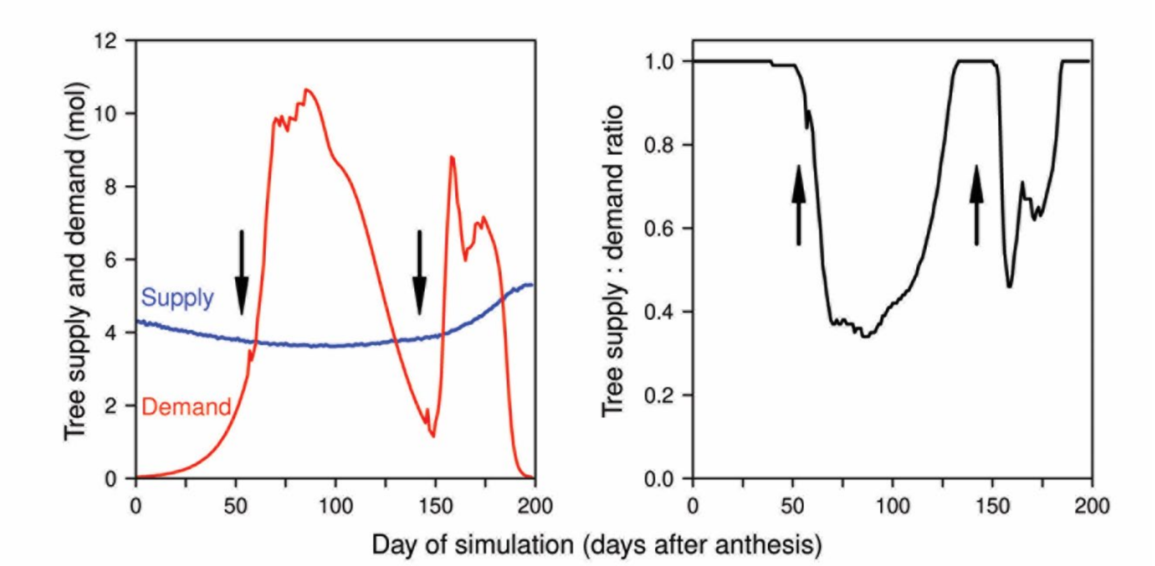

Figure 9. Values of simulated tree supply (blue), demand (red) and supply:demand ratio during the growth period considering 2-year-old branches to be autonomous with regard to carbon. Arrows denote dates of flush budburst. These values are purely conceptual and cannot be measured in the field, but they can be useful to understand carbon competition and growth (Auzmendi and Hanan, In press).

- The virtual macadamia tree was placed in an orchard environment (Figure 10) with a range of different planting densities, tree size and shapes to simulate growth and yield. We included the planting densities of our field experiments and extended the range to planting densities that have not been implemented in the field yet. Trends observed in these simulations were emergent properties of the model that matched the conclusions of field observations, e.g. yield does not increase with planting density or light interception above some level, and it can even decrease with planting density or light interception higher than that level. Our simulations generated new hypotheses, e.g. small and medium size canopies reach maximum yield at light interception values lower than large macadamia canopies (Auzmendi and Hanan, 2020).

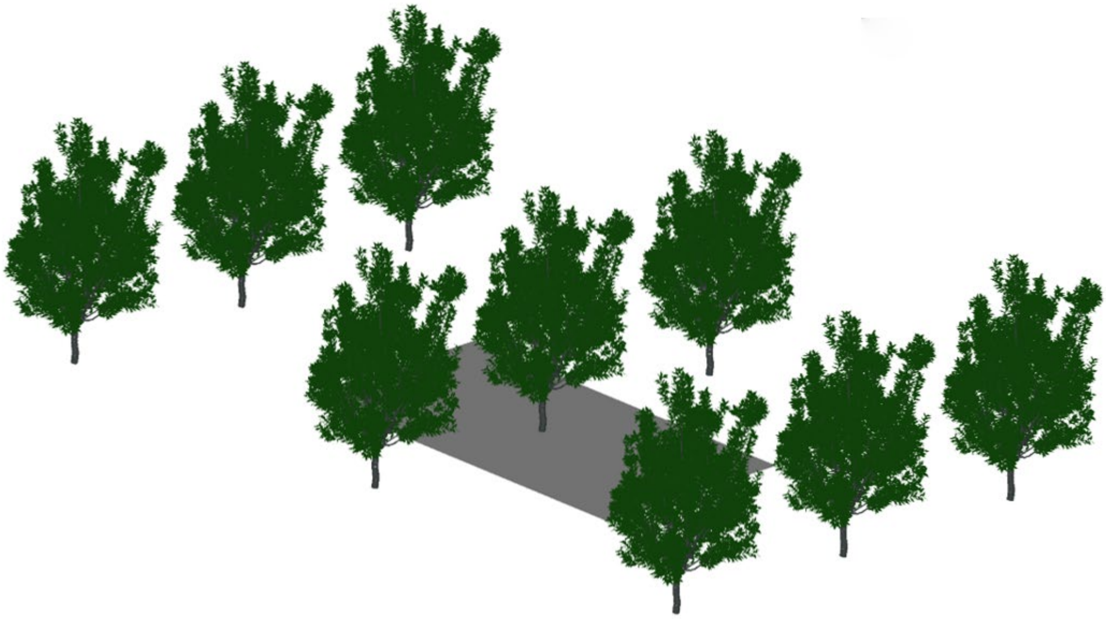

Figure 10. View of one virtual macadamia orchard at the beginning of the simulation with medium size trees, standard training and planting densities (312.5 trees ha^{-1}, corresponding to 8x4 m), showing the ground surface (grey) used to estimate light interception (Auzmendi and Hanan, 2020).

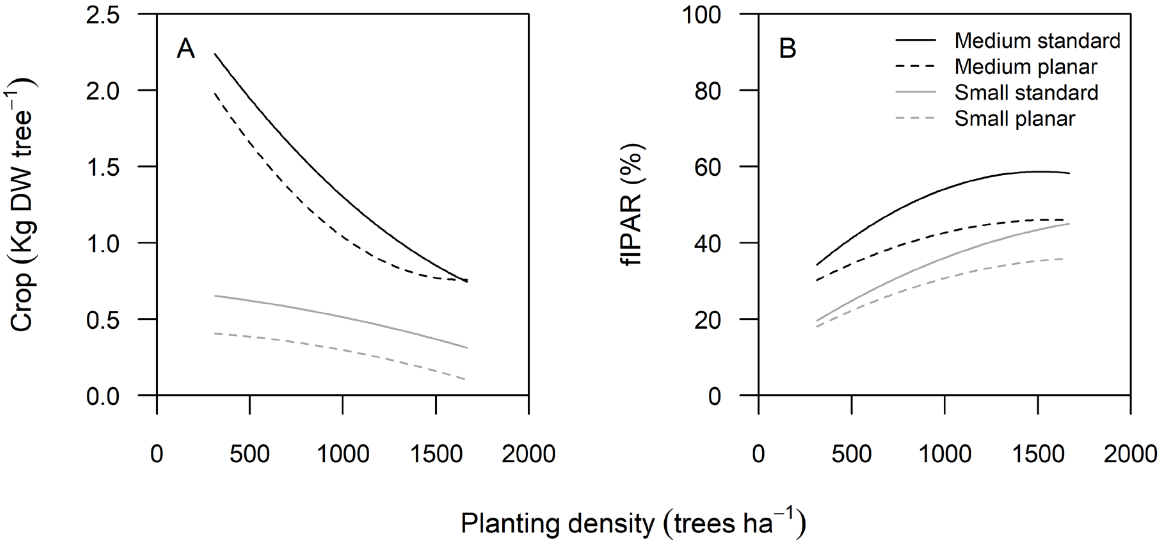

Figure 11. Simulated crop per tree and daily light interception (fraction of intercepted PAR) at harvest with different planting densities for two different sizes and shapes. Each line represents the regression curve of the results of the growth simulation of one macadamia tree from anthesis to harvest (Auzmendi and Hanan, 2020).

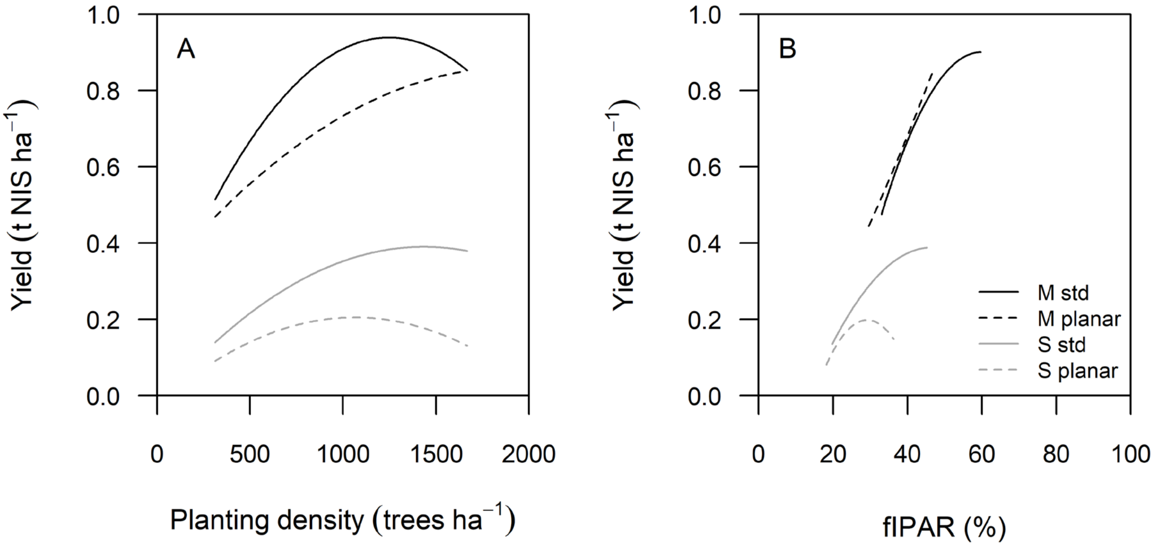

Figure 12. Simulated yield at harvest with different planting densities and daily light interception (fraction of intercepted PAR) for two different sizes and shapes. Each line represents the regression curve of the results of the growth simulation of one macadamia tree from anthesis to harvest (Auzmendi and Hanan, 2020).

Outcomes

- The iterative development and implementation of the models allowed us to increase our understanding of vegetative and fruit growth, as well as their competition for carbon, interaction between tree architecture and light interception, organ variability, duration of growth and crop load.
- The model development has helped us to detect knowledge gaps in areas like leaf and fruit drop, branching, shoot growth cessation and pruning responses. The literature reviewed for developing the models showed that the published resources on plant physiology in avocado, macadamia and mango presented more basic knowledge gaps than in temperate species like apple, peach or kiwifruit, e.g. phyllochron, individual organ growth curves, photosynthesis or temperature responses. Dedicated physiological experiments are required in these areas.
- The development of the model made clear the extensive need of data required for adapting it to specific cultivars, leading us to consider that it was more useful to develop a generic tree.
- The generic whole-shoot carbon pool model and its incorporation in a young tree canopy structure allowed us to discuss from a theoretical point of view the effects of different training systems, crop loads, light availability and warmer temperature on fruit size distribution. This facilitated debate with other project members about the results of our simulations in comparison with field observations in our Planting Systems Trials. The debate led to new hypotheses and experiment designs, e.g. organ-level growth data were collected (Auzmendi and Hanan, In press).
- The simulations with the virtual macadamia tree at different scales of carbon autonomy were employed to explain some of the results of previous experiments, as well as to investigate the autonomy scale of a field measured young macadamia tree. The new method developed represents a contribution as well to the international plant modelling community.
- The virtual trees in an orchard environment helped us to increase our understanding of and support for our hypotheses about the effects of planting distances and tree size on orchard yields. This can be useful for designing future field experiments and orchards and also helped us to generate new hypotheses.
- The rest of the members of the project have been informed of the advances of modelling regularly throughout the whole project. We had meetings to discuss the results of the simulations and which physiological aspects should be included in the model.
- The models developed have been introduced and explained to macadamia consultants and large growers in the AMS Consultants Forum, as well as to the broader scientific community in international conferences.
- One of the advantages of virtual trees is that simulations can be performed in much shorter times (hours) than field experiments (year). They also allow us to estimate values that cannot be easily measured in the field and are emergent properties of the model, like carbon supply and demand, or competition between organs; and simulations can be run in scenarios that have not been implemented in field experiments yet. For example, we used our virtual trees to simulate yield at planting densities that were not included in our planting system trial.
- Our mechanistic final leaf length model pointed out that a more detailed model was needed, so we focused on carbon allocation to investigate the role on organ growth of carbon sources outside individual shoots. This model indicated that other factors could affect leaf size as well. Work in Ted DeJong's lab (UC Davis) following the experiment and ideas used for this model have led to the discovery that phyllochron is not constant in field peaches (Davidson *et al.*,

2015a, 2015b) and it varies with carbon availability, shoot type and rank (Davidson *et al*., 2016, 2017, 2019).

- The generated tree visualizations provided support for research and extension, and were included in industry publications and conferences. For example, visualisations of mango architecture mock-ups were employed to illustrate the effects of different planting densities and training systems on tree architecture in the Planting Systems Trials of Mareeba (Ibell et al., 2019a; Ibell et al., 2019b).
- The publication of a new method to simulate carbon allocation in trees improved our understanding on modelling techniques and theory for simulation of plant growth. It also generated new insights into branch autonomy with regard to carbon in fruit trees in general, and specifically in young macadamia trees. New techniques like aspect-oriented modelling were used in the development of the models. This approach is based on organizing the code into individual aspects corresponding to physiological aspects.
- The complexity of the model and computer power required led us to investigate ways of speeding up the simulations by simplifying aspects of the model, as well as using high-performance computing.
- As tree size and complexity increased, it became very time consuming to collect all the architectural information manually, so we started investigating other alternatives to capture architectural data, such as obtaining point clouds of the canopy for its reconstruction.
- Although the main goal of our virtual trees is to improve our understanding on plant physiology related with intensification processes (planting density, tree size and training), we are finding other practical uses as we develop the models. For example, they can be used to estimate the scale of carbon autonomy in our field trees or to interpret the field experimental results of planting density and training trials. Therefore, one of the questions that arises from our modelling work is how previous theoretical models can be applied for practical uses related with our field experiments.

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Appendix 15

CTRAM: a model for carbohydrate allocation

Jim Hanan

Introduction

This appendix describes project research related to the Carbon Transport Resistance Allocation Model (Prusinkiewicz et al. 2007, Cieslak et al., 2011), which operates at organ scale within a tree. Each leaf provides photosynthate based on the light that it receives, and this is transported to competing carbon sinks such as growing leaves, internodes and fruit subject to resistance in the transport pathway. These papers resulted from the international collaboration between Alla Seleznyova from New Zealand Plant & Food Research and Jim Hanan from UQ QAAFI. These models will form the basis for similar models in future projects where this scale of modelling is appropriate to the research question and will require detailed parameterisation for the trees of interest.

Carbon transport revisited: a novel approach for solving quasi-stationary carbon transport in a system with Michaelis-Menten sources and sinks

Authors: A. Seleznyova, J.Hanan

Journal: ISHS Acta Horticulturae 1160: X International Symposium on Modelling in Fruit Research and Orchard Management

DOI: 10.17660/ActaHortic.2017.1160.39

Abstract: The Carbon Transport-Resistance Allocation Model (C-TRAM) developed for L-system plant models is based on an analogy between the equations for the osmotic-pressure generated phloem-sap flow and Ohm's law. This analogy has proved to be rather confusing because some of the model variables, for example source/sink 'electromotive force', do not have any physiological interpretation. Also, the fact that the transport equations are formulated in terms of phloem-sap flow Jp, while organ growth rates are usually expressed in mass carbohydrate flux Js, does not improve model clarity and consistency. The current paper presents a direct way of solving transport equations based on the Münch hypothesis, consistent with boundary conditions in the form of Michaelis-Menten source/sink fluxes, and accounting for the effects of carbohydrate concentration on phloem resistance. Starting from a system of equations for steady-state coupled phloem/xylem flow (Hall and Minchin, 2013), for a constant water potential in the xylem, an equation for the solute mass flow Js in a conduit element can be obtained in a closed form. The boundary problem in the context of L-system plant models was solved using analytical transformations and computational methods similar to the folding/unfolding algorithm of C-TRAM but not based on the electric circuit analogy. The performance of the transport model was tested using a simple system where exact analytical solutions were available (Hall and Minchin, 2013). To our knowledge, this is the first L-system model that combines a rigorous treatment of the carbohydrate transport in a growing system with Michaelis-Menten source/sink functions. The model will be useful in cases where the actual value of the carbohydrate concentration in phloem is important, e.g., sugar signalling. Currently the model is being extended to take into account phloem/xylem coupling.

Mechanistic modelling of coupled phloem/xylem transport for L-systems: combining analytical and computational methods

Authors: Alla N Seleznyova, Jim Hanan

Journal: *Annals of Botany*, Volume 121, Issue 5, 18 April 2018, Pages 991–1003,

DOI: https://doi.org/10.1093/aob/mcx204

Abstract

Background and Aims: Transport of carbohydrates and water are essential aspects of plant function. The aim of this study was to develop and test the methods for mechanistic modelling of quasi-stationary coupled phloem/xylem transport in the context of functional–structural plant modelling.

Methods: The novelty of this approach is in combining analytical and computational methods. The plant structure is modelled at a metamer level with the internodes represented by conduit elements and the lateral organs represented by sources and sinks. Transport equations are solved analytically for each internode and then the solutions are adjusted and 'sewn' together using an iterative computational procedure taking into account concentration-dependent sinks and sources. The model is implemented in L-studio and uses the aspect-oriented modelling approach for phloem/xylem coupling.

Key Results: To our knowledge, this is the first transport model that provides continuous distributions of the system variables in a complex developing structure. The model takes into account non-linear dependence of phloem resistance and osmotic potential on the local carbohydrate concentration. The model solutions show excellent agreement with the existing results of other analytical and numerical models. These comparisons confirm the validity of the approximations made in the model. Combining analytical and computational methods made it possible to take into account continuous sink/source distribution within internodes without much increase in the complexity of the computational procedure, because the necessary changes in the model were mostly in the analytical part. The results emphasize sensitivity of phloem flux and lateral xylem flux to the presence of distributed sinks and sources along the transport system.

Conclusions: The presented approach provides a new insight into mechanistic modelling of phloem/xylem transport in growing plants. It will be useful for both fine-scale modelling of carbohydrate dynamics and for creating simpler models at a growth unit level.

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Appendix 16
Modelling Light in Macadamia Canopies

Neil White

Introduction

Macadamia canopies are complex, large and actively managed over many years. The modelling undertaken in this part of the STHPI has been to try to understand the following:

- How the shape of the canopy changes its performance, i.e. light gathering ability
- The interaction between shape, management and orchard dimensions
- How best to model this at the scale of an orchard

The aim of the project is to develop orchards that have high productivity because the trees are smaller, which has benefits in terms of on-going management. This will need additional input from either genetics or scion-root stock interactions, and it has been assumed that these will contribute to keeping these highly vigorous trees under control.

“Light interception” has often been used as a surrogate for how well the canopy captures the available light, however, it is an ambiguous term because it does not take into account how the light is used within the canopy. For this reason the concept of Effective Leaf Area has been introduced.

Modelling the distribution of light in a canopy can be undertaken in a number of ways, from using Beers Law for totally closed canopies, to path tracing, whereby the light from a source or multiple sources is followed as it is reflected, transmitted and absorbed by representations of surfaces in the computer, and hence accounts for gaps in the canopy. The decision in this part of the work was to develop the techniques with path tracing in mind so that what was learnt at this step could be more easily used later to simulate canopy development. Rather than just tracing light from the sun direction, say at noon, a sky model can be used, whereby many million paths are traced to approximate the distribution of light from the sky for a given time period, location, and date. Figure 1 below shows the distribution of light at 10 am at a location -25.4°S and 152.8°E on 6 March. The light has a higher intensity to the northeast, but as the sky is not a single source, light also comes from other directions. The program used in this part of the study is called QuasiMC (Cieslak et al., 2008).

There are three main parameters that need to be found when using QuasiMC: Depth, R1 and R2. These are the number of times that a ray is allowed to strike a surface (Depth), the threshold energy below which it can be extinguished (R1) at a probability (R2). Finding values for these parameters was undertaken by comparing virtual measurements with field measurements. The other important parameters are the leaf spectral properties, principally reflectance and transmittance. Reflectance values for old and young leaves have been obtained, however, at this stage we do not have any direct measurements of transmittance.

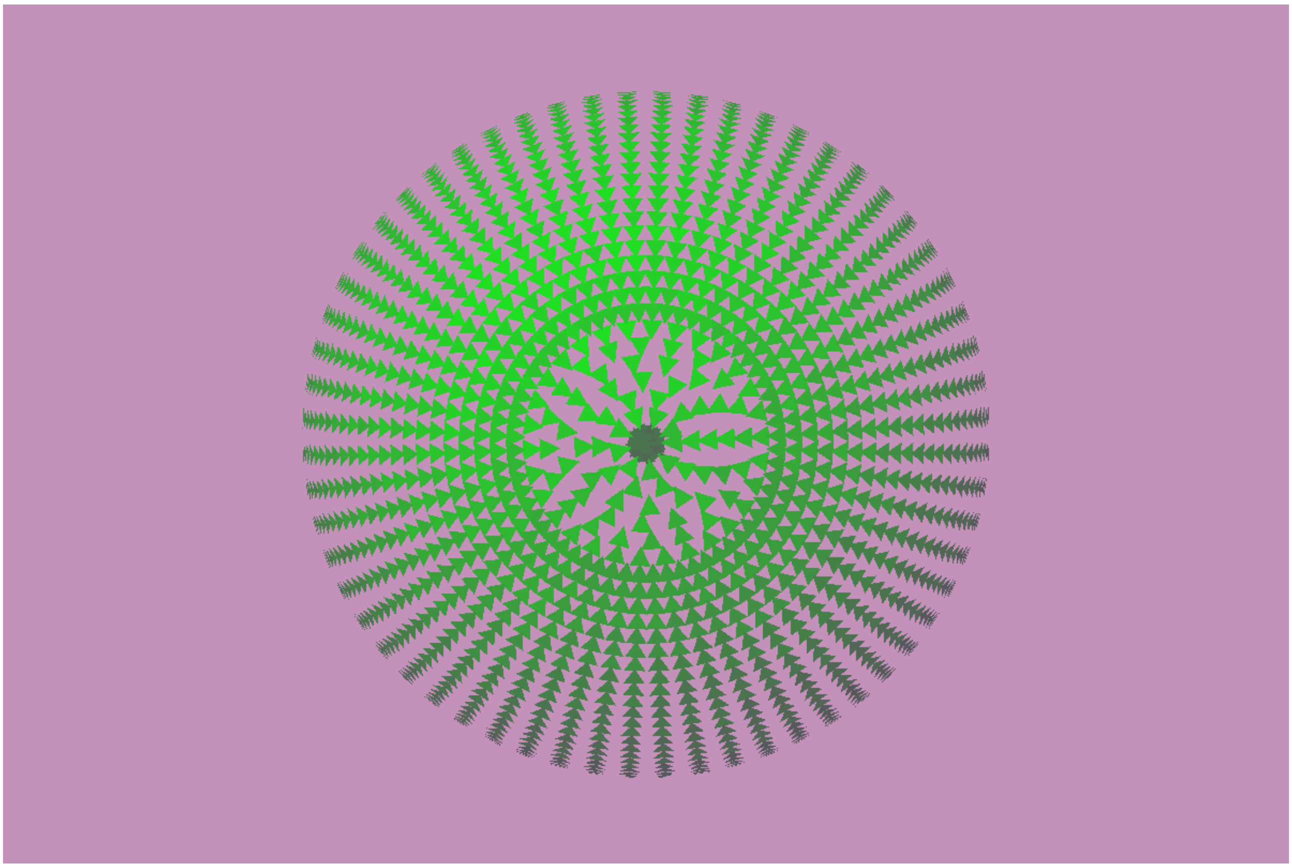

Figure 1. Hemisphere of triangle shapes placed within the QuasiMC model showing light at 10 am 6 March at 25.4°S 152.8°E.

Leaf Properties

The spectral properties of leaves affect how light is reflected, transmitted and absorbed within a canopy. The photosynthetically active region of the spectrum ranges from blue (400 nm) to red (700 nm), Figure 2. Leaves are green because they reflect highest in a region around 500 nm. As light moves through the canopy it can hit either the top side or lower side of the leaf and so we recorded reflectance for both. As leaves age their spectral characteristics change. This is very marked in trees like mangoes, but in macadamia it generally means a change from bright to dull green.

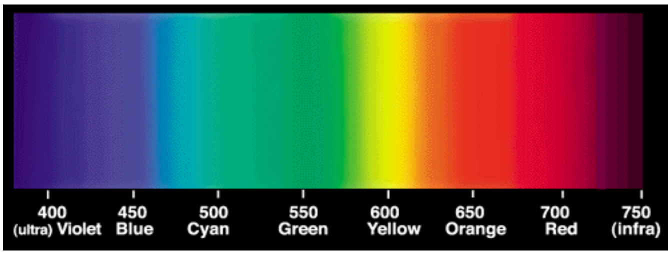

Figure 2. The visible spectrum. The Par region is from 400 to 700 nm.

Methods

The ASD FieldSpec® Spectroradiometer was used to record the reflectance from young (near the top of the branch) and old macadamia leaves (nearer the base) from their top and bottom surfaces. While the equipment was able to record from 50 to 1000nm we have limited this discussion to the photosynthetically active region (PAR) 400 nm to 700 nm.

Results and Discussion

The reflectance in the PAR is shown in Figure 3 and ranges from a low of 5% in the blue region to a peak at ~22% at 550 nm (green) for the top surface of young leaves. The reflectance and transmittance at these wavelengths are shown in Table 1. Note: the transmittance values have been approximated at this stage.

Table 1. Reflectance and transmittance values (%) used for three spectra within the PAR for modelling light within macadamia canopies.

	Old leaf (upper)		Old leaf (lower)	
Wavelength (nm)	Reflectance	Transmittance	Reflectance	Transmittance
450	3	5	3	5
550	6	10	11	10
650	3	5	6	5

	Young leaf (upper)		Young leaf (lower)	
Wavelength (nm)	Reflectance	Transmittance	Reflectance	Transmittance
450	3	5	6	5
550	13	10	21	10
650	4	5	11	5

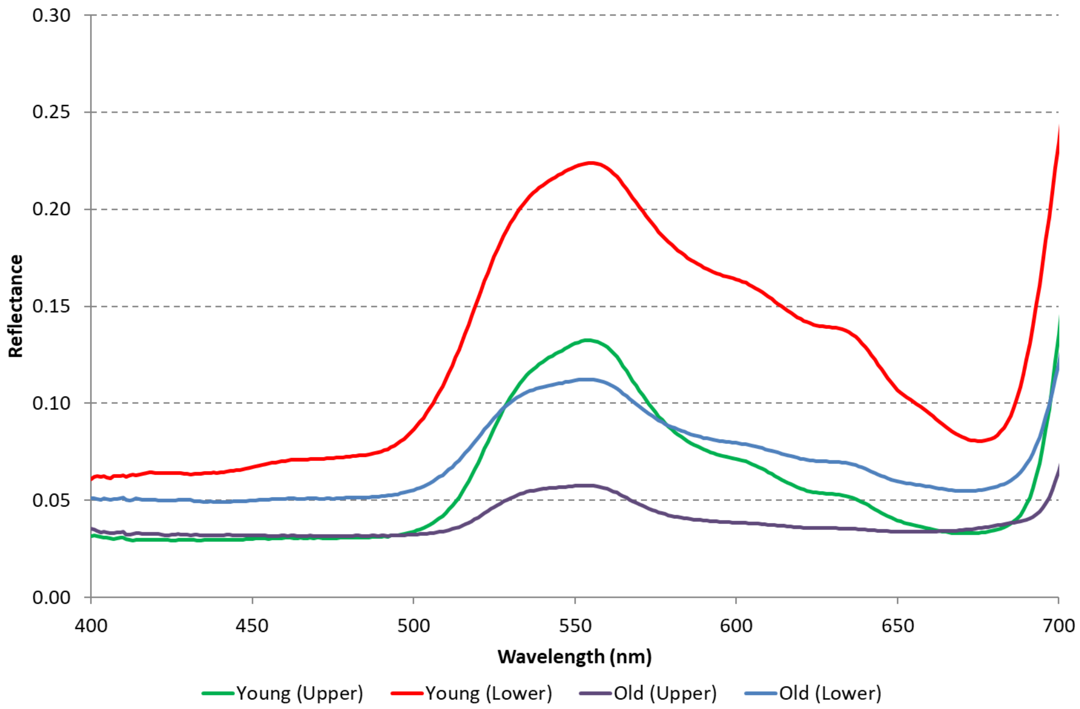

Figure 3. Reflectance of macadamia leaves across the PAR.

The Light Model - QuasiMC

How the model works

The model uses a Monte Carlo simulation (i.e. randomness) and does this in a such a way that the calculations are undertaken in a more efficient manner, the quasi part of the name. In essence, rays of light are traced from the source (in this case a probability that a light ray will originate at a given point in the sky) through a number of interactions with surfaces that represent leaves. When a ray's path intersects a leaf some of the energy will be reflected, some will pass through the leaf (transmitted), and the remainder is absorbed. The path of each of these reflected or transmitted rays is followed until it has struck a pre-set number of surfaces (the Depth) or the energy of the ray drops below a given threshold (R1). In the latter case the ray is extinguished at a set level of probability (R2).

The interaction of large numbers of rays with the surfaces representing the leaves in the canopy determines the overall proportion of light received. In this way leaves at the top of canopy might receive 100% of the available light, while leaves that are partly hidden (shaded) will receive less. This proportion of available energy can be used to drive the equations of photosynthesis to determine the amount of carbon produced. QuasiMC can be run at intervals of a day down to hours and less as required by the purpose of the model.

Calibration and Validation of the Light Model (QuasiMC)

Methodology

The leaves of two macadamia trees were digitised in 3-D and a series of ceptometer readings were undertaken at the orchard floor. The trees were recreated in VLAB with virtual sensors at the equivalent

positions. A search for the best three parameters values for Depth, R1 and R2 was undertaken using one tree (calibration) and these were tested against the second tree (validation). Depth sets the limit to the number of intersections that a ray can have with a leaf while R1 determines the light threshold at which a ray may be terminated and R2 determines the probability that it will be terminated.

Parameter Space

A total of 1,875 simulations were performed (**Table 2**) covering a range of plausible values for the three parameters. All simulations were undertaken using 32 million rays at the same latitude, longitude, time and day of the year as the field measurements. Readings from the ceptometer in the field were matched to the simulated readings of a virtual ceptometer t the same approximate position within the virtual canopy.

Table 2. Parameter space searched.

	Depth	R1	R2
Initial value	2	0.05	0.05
Increment	2	0.01	0.01
Final value	7	0.19	0.29
Number of values	6	15	25

Simulations were assessed using regression and the mean absolute error, MAE,

$$MAE = \frac{\sum(o - s)}{n}$$

where n is the number of sensors, o is the measured value in the field and s is the simulated value. A perfect score would be MAE of zero.

Leaves had the spectral characteristics that had been ascertained during earlier work.

The simulations done for the parameter space shown in **Table 2** provided the best set as being: **Depth = 5, R1 = 0.08, and R2 = 0.29**. This is interpreted by the model as - when a ray is intercepted, if it has encountered less than 5 surfaces previously, it is extinguished if its energy is less than 8% at probability of 29%.

The reflectance from the canopy floor was modelled using the three wavelengths (nominally 450, 550 and 650 nm) reflectance values were 2%, 8% and 2% for under the trees and 2%, 2% and 10% for the alleys.

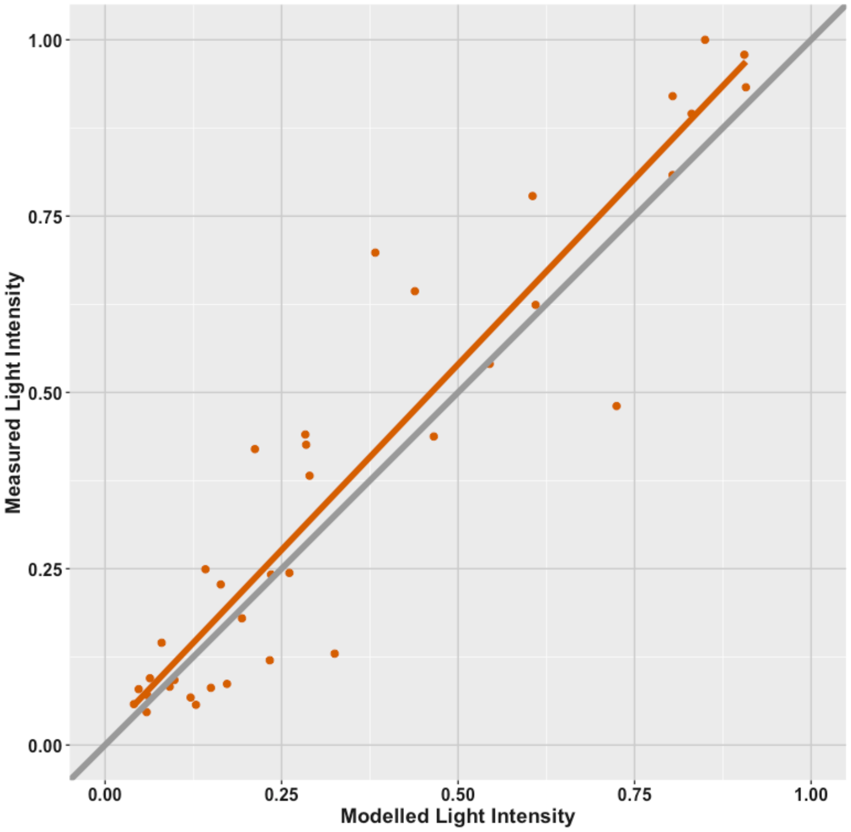

Figure 4. Validation of parameters derived from calibration of QuasiMC model. Coloured points and line show the values and line of best fit for the comparison. The grey line shows the 1:1 ratio (i.e. a perfect fit). The slope of the fitted line is 0.93.

CanopyShapes - a modelling approach for light

The approach taken by others, (e.g.Tung and Chan, 1977, Hadari, 2004, Olesen et al., 2007a), examined the performance of geometric shapes and abstracted models of the light environment. Here we continue with an abstraction of a macadamia canopy, i.e. a virtual tree is not grown, but instead of relying on surfaces and extinction coefficients the virtual canopy was created from randomly placed whorls of leaves arranged in as similar fashion to that found in the field. The CanopyShapes approach taken in this section used the QuasiMC model with the parameters that were established above. This approach allows for any geometric shape to be defined and assessed using two passes of the QuasiMC model. The first pass thins out the leaves based on a threshold and the second pass then calculates the amount of light that is intercepted by the canopy and by accounting for each leaf. The allows a greater level of detail to be examined in the analysis.

The purpose of this report is to extend the work that was undertaken and presented at *The 8th International Macadamia Symposium* in Lincang, China in 2018. It is still at an experimental stage and will be developed further during the next phase of the research. It is hoped that CanopyShapes will allow for rapid prototyping of novel orchard designs for a range of fruit and nut species to:

- provoke discussion and debate about how we manage orchards;
- aid more detailed modelling; and
- suggest fruitful field experiments.

Methodology

Once the tree shape has been defined it is randomly filled with leaves at a density of 4000 leaves /m^3. This is a high number but is thinned in the first pass of the model and tested against leaf counts taken at Alloway. A virtual orchard is created of five rows with five trees per row and subsequent observations are on the inner nine trees (Reference Trees). Additionally, a set of virtual sensors is placed across the orchard floor to calculate light interception.

Effective Leaf Area is calculated as the proportion of the light that reaches a leaf multiplied by its area. So, a leaf with an area of 20 cm^2 that receives 90% of the available light will have an effective leaf area of 18 cm^2 compared to a similar leaf that that received only 10% of the light, 2 cm^2.

Four shapes have been trialled, referred to as Tapered (industry standard), Trellis, Tatura Trellis and BigVee. The shapes (Figure 5) were compared at 8 m x 4 m (312.5 trees/ha), 5 m x 2 m (1000 trees/ha) and for Trellis only, 2.5 m x 2 m (2000 trees/ha). The alley width was set at 2 m, 1 m and 1 m respectively and the trees were skirted to 1 m. Tree height was set at 1.2, 1.0 and 0.8 times the inter-row distance, except for the Tatura shape as its geometry is not reliant on tree height. For this image the leaf thinning threshold was set at 0.001%, that is after the first pass leaves were removed that received less than this threshold of the available light. This value should not be confused with a level of light required for a macadamia to drop leaves in shaded areas. It was derived in the calibration process in against leaf counts undertaken at an orchard at Alloway, near Bundaberg (see Selection of threshold value, in the Results).

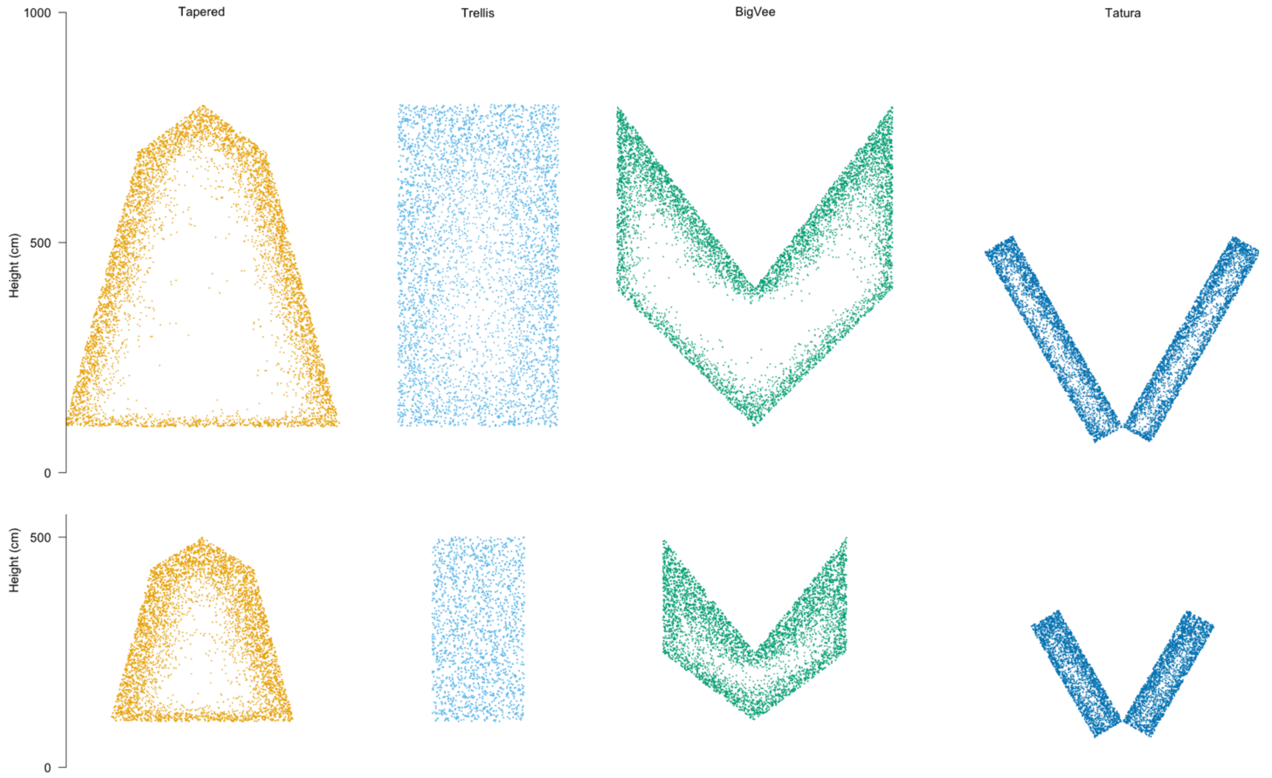

Figure 5. Relative size of shapes used in the experiments. From left to right – Trellis, Tapered, BigVee, Tatura. Shown for the 8x4 m layout (above) and 5x2 m layout (below).

Light interception at the orchard floor was calculated using virtual sensors of 50 cm x 50 cm covering 3 rows and 3 trees.

Leaf area was calculated as the sum of leaf areas remaining for each of the reference trees (N=9) and Effective leaf area was the sum of leaf areas multiplied by the proportion of light received.

All measurements were for a simulated day 355 (approximately the summer solstice) and 25°S and 152°E (nearest town Gin Gin, Queensland) and for the hours of daylight.

Validation against published data

The most useful published analysis of the relationship between canopy shape and light interception comes from Olesen et al. (2007b). In their study they utilised rectangular and tapered shapes (the Tapered shape used in CanopyShapes is modelled from their specification) and presented some data on the effect of canopy height on the rectangular shape for canopy depths up to 10 m.

These were simulated using the Trellis shape with the same layout (6 x 4 m) and a 2 m alley.

Results

Selection of threshold value

Field measurements on macadamias grown on a farm at Alloway, near Bundaberg were used to investigate which threshold value of light should be used to drop leaves from the canopy following the first phase of simulation. A transect was placed at 2m across hedged macadamias with a canopy width (across the row) of 3.5 m. The transect comprised 50 cm x 50 cm x 50 cm voxels in which the number of leaves was counted. This was undertaken for ten trees.

This was simulated using the CanopyShapes procedure with leaf light thresholds of 0 to 0.1%. A Trellis shape was used with a 3.5 cm canopy width on a 5 m x 2m orchard with a skirt of 1m. This provided a set of nine trees which could be used to compare with the field data. The void is noticeable; Figure 6 shows the transect position and the appearance of the internal void. The closest fit is a threshold whereby leaves that received more than 0.01% of the available light were retained, Figure 7. In all simulations there was a noticeable effect of reflected light from the canopy floor, see the section Calibration and Validation of the Light Model (QuasiMC), resulting in the retention of leaves that may normally have been lost. Further refinement of this approach may be required to cope with this phenomenon.

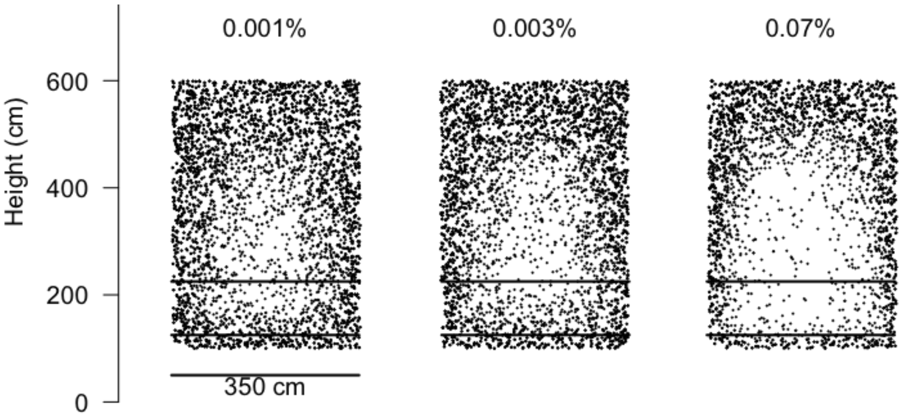

Figure 6. Looking north at 50 cm cross-section through the canopy. The two horizontal lines define the virtual transect position. Numbers above each canopy are the threshold used for thinning leaves.

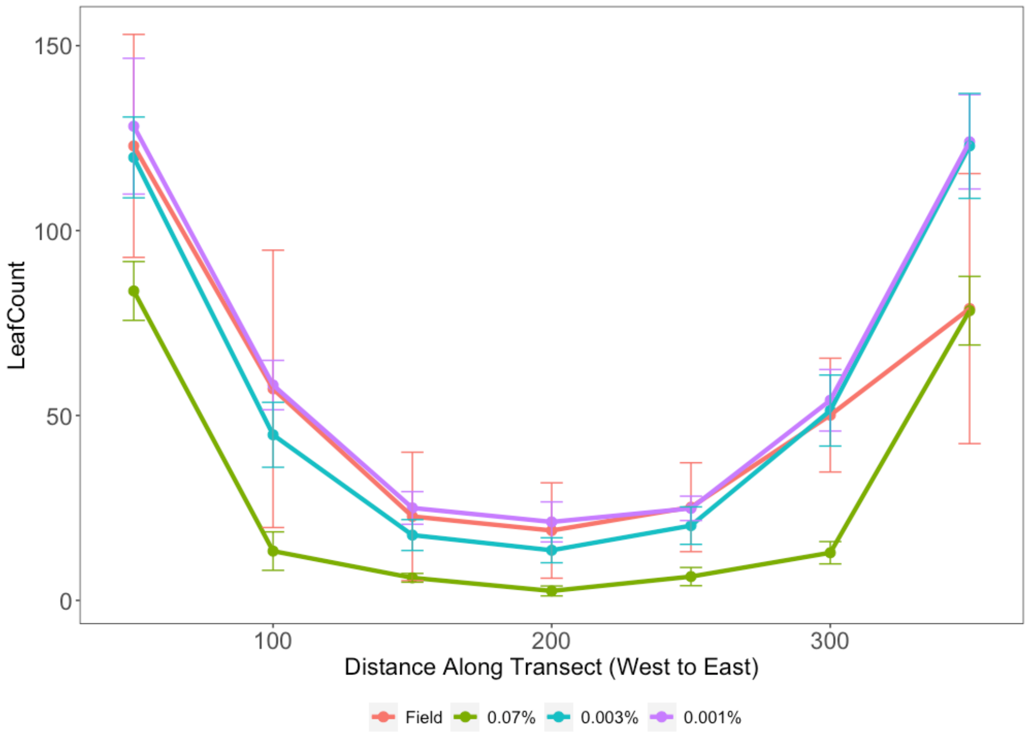

Figure 7. Mean and 95% CI of leaf counts along the transect from field data and simulation with thresholds at 0.07%, 0.003, 0.001%.

Validation against published data

Figure 4 (Olesen et al., 2007b) was digitised and is presented in against the simulations undertaken using CanopyShapes. A regression of the light interception from Olesen's data on that from CanopyShapes shows that CanopyShapes was on average ~13% lower, but the correlation was greater than 99%.

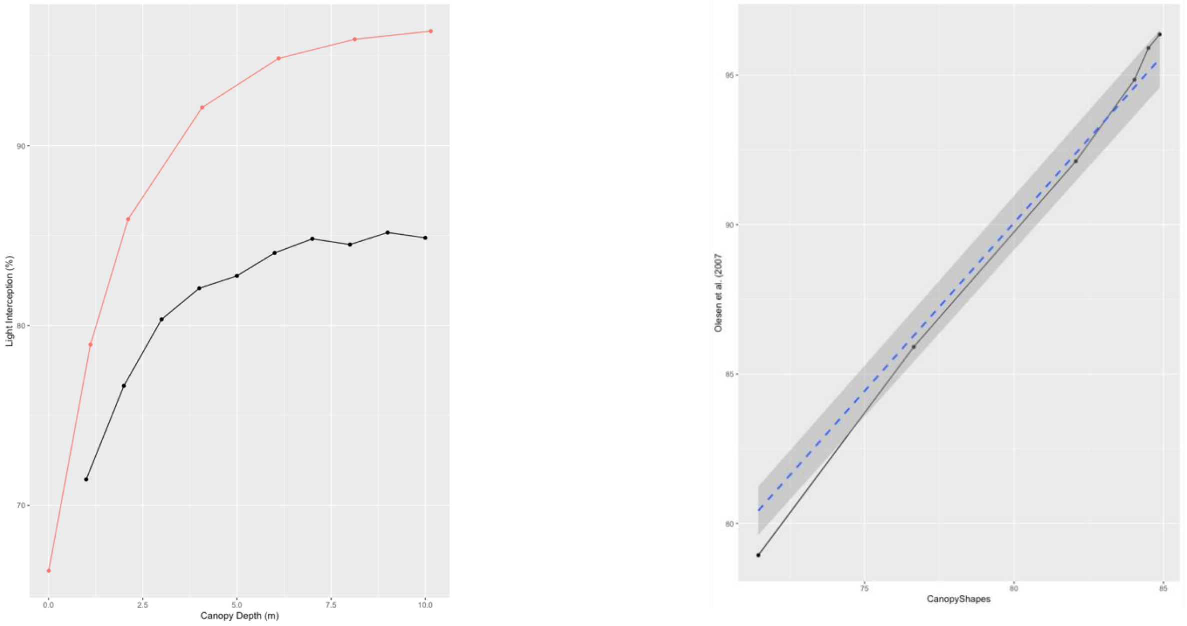

Figure 8. Comparison between data from Olesen et al. (2007b) and CanopyShapes for light interception. Left – Light interception against canopy depth (Olesen's data orange, CanopyShapes in black). Right – Olesen's data against CanopyShapes with regression line with zero intercept and 95% confidence interval. NB The figure is zoomed into to show detail which exaggerates the difference.

Light interception was highest for the Tapered shape at 5 x 2 layout for all three row-width: tree-height ratios (0.8, 1.0, 1.2) with a light interception (LI) of > 90%. The tree height had only a small effect on light interception, but had a larger effect on leaf area, in most cases increasing this without gaining much light interception, Figure 9. Tatura systems were not affected by tree height and had an (LI) of ~82% at 5 x 2 m, and a LI of ~62% at 8 x 4 m.

Leaf area in (m^2/ha) varied depending on the shape of the canopy and the density. The leaf area for the Tatura trellis was higher leaf area at the 5 x 2 m layout than at 8 x 4 m. For the other shapes leaf area generally increased with tree height, except for high density BigVee at 8 x 4 m where the trend was for decreasing light interception with height. Trellis shapes had a similar leaf area at both densities, but light interception was higher for the 5 x 2 m layout. The Trellis at the very high density of 2.5 x 2m had a much lower leaf area.

Effective leaf area (m^2/ha) shows the biggest differences between orchard density, with the larger trees at the lower density having a greater effective leaf area (LA_{eff}). The greatest effective leaf area was recorded for the BigVee style at light interceptions of ~67% at 8 x 4 m. For the 5 x 2 m layout LA_{eff} was < 10,000 m^2/ha compared to generally greater than 15,000 for the low-density layout.

Carbon Acquired (kg C/ha/day) shows a pattern similar to LA_{eff}, but in some respects provides a better understanding of what is occurring. The role of light interception is much smaller than either shape or planting density, except perhaps for the Trellis system at higher density layouts. Figure 9 demonstrates that light interception can be misleading if this is the only parameter that the suitability of an orchard design takes into account.

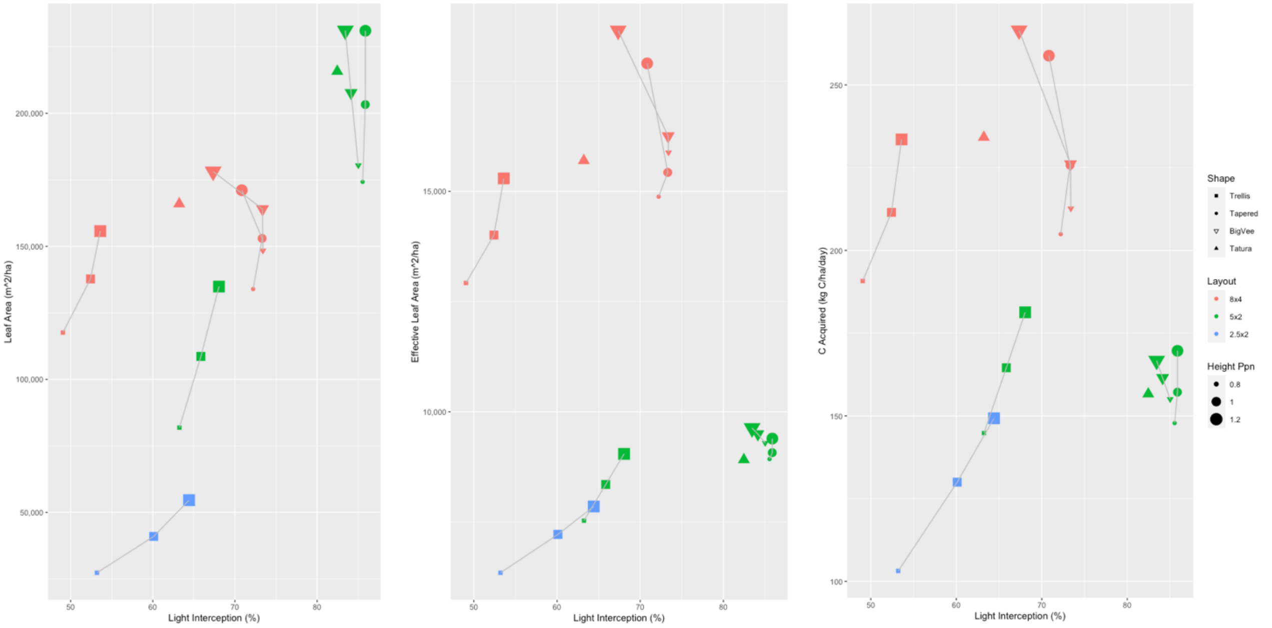

Figure 9. Light interception for tree shapes, orchard density and tree height proportion of interrow width and the relationship to leaf area, effective leaf area and carbon acquired on day 355.

Effective leaf area seems to be a more useful predictor of carbon acquisition (Figure 10) with a significant relation ($p < 0.001$) and R^2 of 0.99 for both planting densities and ignoring all other factors, shape, density etc., whereas light interception shows no relation across the set of simulations ($R^2 < 0.02$) (Figure 11). The slopes of the regression of carbon acquired on LA_{eff} at the higher density layout was 0.0179, in other words carbon acquired at the rate of ~18 g/ha/day for each 1 m^2/ha of effective leaf area. At the low density the slope is 0.0144, or ~14 g/ha/day for each 1 m^2/ha of effective leaf area. In this sense there is a bonus for using a higher density planting, all other things being equal.

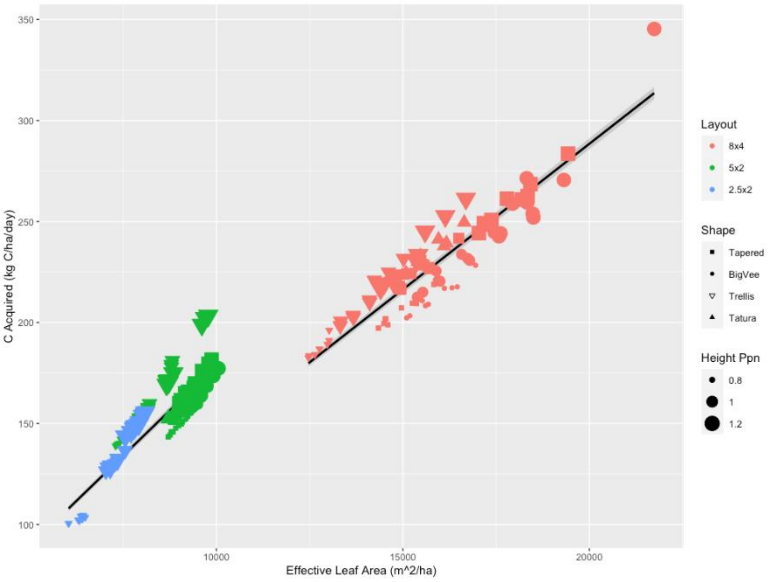

Figure 10. Carbon acquisition versus Effective Leaf Area for tree shapes, orchard density and tree height proportion of interrow width.

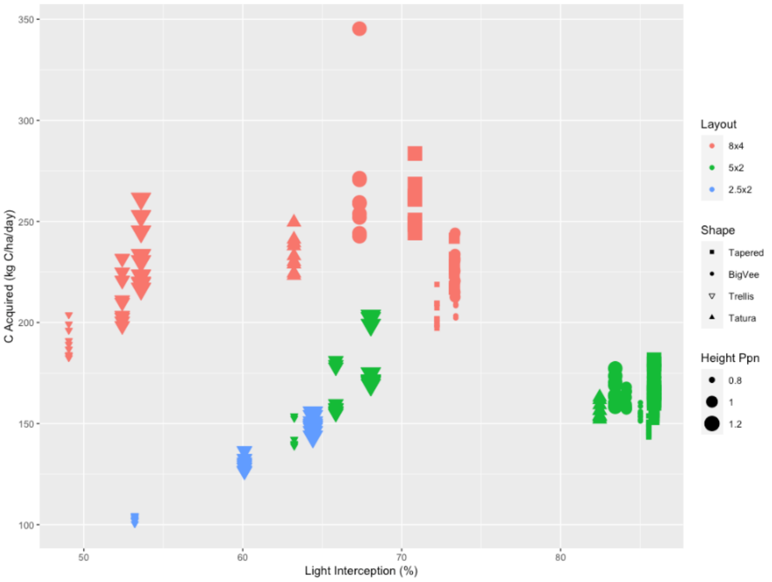

Figure 11. Relationship between Effective Leaf Area (top), light interception (below) and carbon acquired.

Discussion

The maximum light interception recorded by virtual canopies in this study were lower than that recorded in the field. McFadyen et al. (2004) recorded light interceptions up to 95%, but that was orchards up to 16 years old. Comparison between CanopyShapes and the modelling of Olesen et al. (2007b) was favourable with a strong correlation, but CanopyShapes recorded a light interception that was 13% lower. Whether this is a failing of the proposed methodology will be investigated at a later stage, but the advantage of the

CanopyShapes technique is that that is applicable to a wider range of situations as it does not rely on more complex algorithms to calculate interception of light by surfaces.

This study has been undertaken on mature virtual tree canopies and so it is difficult to compare the results with the field trials as they were less than five years old. No attempt is made here to translate the findings to yield. This will be the challenge, among others, for the new Tree Intensification Program.

A key message is that while light interception is a good surrogate for canopy volume during the development stage it becomes less useful as the canopy matures. Here I was able to calculate effective leaf area as the model allows perfect access to every leaf and the amount of light it received. This is impossible in the field. Empirical prediction of LA_{eff} would be difficult to achieve as it is an interaction between many variables. Modelling offers a route to undertake this efficiently. Further gains in understanding should come from combining this work using CanopyShapes by combining this with models of carbon allocation.

Another important finding is that the relationship carbon acquisition and effective leaf differs with planting density, although this offset by the fact that high density layouts do not achieve the same effective leaf area / ha.

To extend this work to other fruit and nut trees would require validation of the model for the light attenuation parameters and deriving/measuring the spectral properties of leaves. In the next phase of this research I intend to extend this to model by incorporating carbon allocation over a longer period.

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Appendix 17

Supercomputing and Complex Systems Modelling: the High-performance Ray Tracer and the mangoL Tree/Orchard Simulator

Liqi Han

Introduction

Light energy absorption plays a vital role in plant productivity. The quantity and quality of a plant's yield are highly dependent on the amount and distribution of light it can capture. For a single tree, techniques such as training, pruning and even selective breeding can be used to improve its architecture and the light distribution therein, and therefore to increase light interception efficiency. At the orchard scale, light interception and distribution are also influenced by planting density, row arrangement and local terrain. An optimal productivity requires a comprehensive solution to cover as many of these factors as possible. However, real-world experiments are labour intensive and time consuming. A horticultural experiment may take years and even decades.

This part of the project has developed supercomputing and virtual-plant techniques to support the investigation of different orchard designs and management practices for improvement of light interception and distribution.

Methods

Supercomputing refers to the use of computers with unconventionally large capacity and high capability for processing massively complex and data-laden problems. This high performance comes from a supercomputer or a computing cluster that is composed of many interconnected compute nodes with multiple CPU cores at each node. However, a serial program uses only one core at any time point even it is deployed to a supercomputer, making no difference from running many times on an ordinary desk computer. Most of the simulators for plant and environmental modelling are such serial programs. Based on the serially-implemented QuasiMC light simulator (Cieslak et al., 2008), we have developed a high-performance ray tracer **HP-QuasiMC** (i.e. High Performance QuasiMC) to allow parallel use of multiple computing cores for significant acceleration of virtual horticultural experiments.

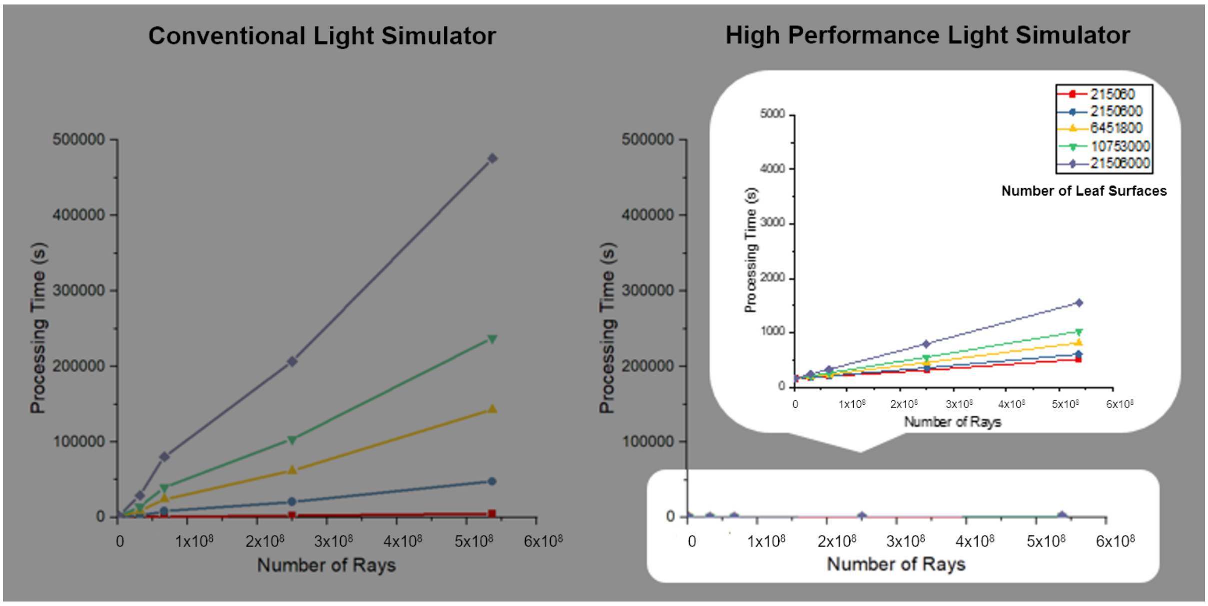
The L-system programming paradigm (Prusinkiewicz et al., 1990) has long been used to build functional-structural plant models. In this project, we have used the L-system development platform VLAB to establish a tree/orchard simulator with stochasticity defined from either empirical or conceptual data. As the first example of its use, a mango tree dataset derived from field experiments (Mizani, 2020) has been used as an instance for this **mangoL** simulator.

Results

The high-performance ray tracer is scalable to a range of multi-core systems from a desktop PC to a national-level supercomputer. Given 1000 cores for example, which is widely available in many university- or institute-owned computing clusters, the running of a virtual orchard light experiment would take the original serial light simulator 6 days while our high performance version took no more than 26 minutes (Figure 1).

The mangoL tree/orchard simulator (Figure 2) is configurable for pre-evaluation of mango orchard designs and to optimise pruning and training strategies for the maximisation of light absorption and carbon productivity.

With the high-performance ray tracer and the mangoL simulator, a number of preliminary virtual experiments have been implemented to investigate the impacts of planting density (Figure 3), row orientation (Figure 4), orchard terrain (Figure 5) and unconventional layout (Figure 6) on light energy absorption and carbon productivity[1].


Conventional Light Simulator

High Performance Light Simulator

Figure 1. The high-performance light simulator in comparison with its conventional counterpart on a 1000-core computing cluster. This experiment used different numbers of rays to evaluate light energy absorption by orchards with different leaf surface numbers. With the conventional light simulator (left), which can use only one CPU core regardless of how many cores are available, the processing time rose rapidly with the increment of leaves and rays. The high-performance light simulator (right), in contrast, made good use of all the CPU cores and thereby significantly shortened the computation time from days to minutes. The development of this high-performance light simulator was led by Liqi Han at the University of Queensland.

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[1] The calculation carbon productivity in this case is based on a photosynthesis rate model developed by Greer et al. (2004).

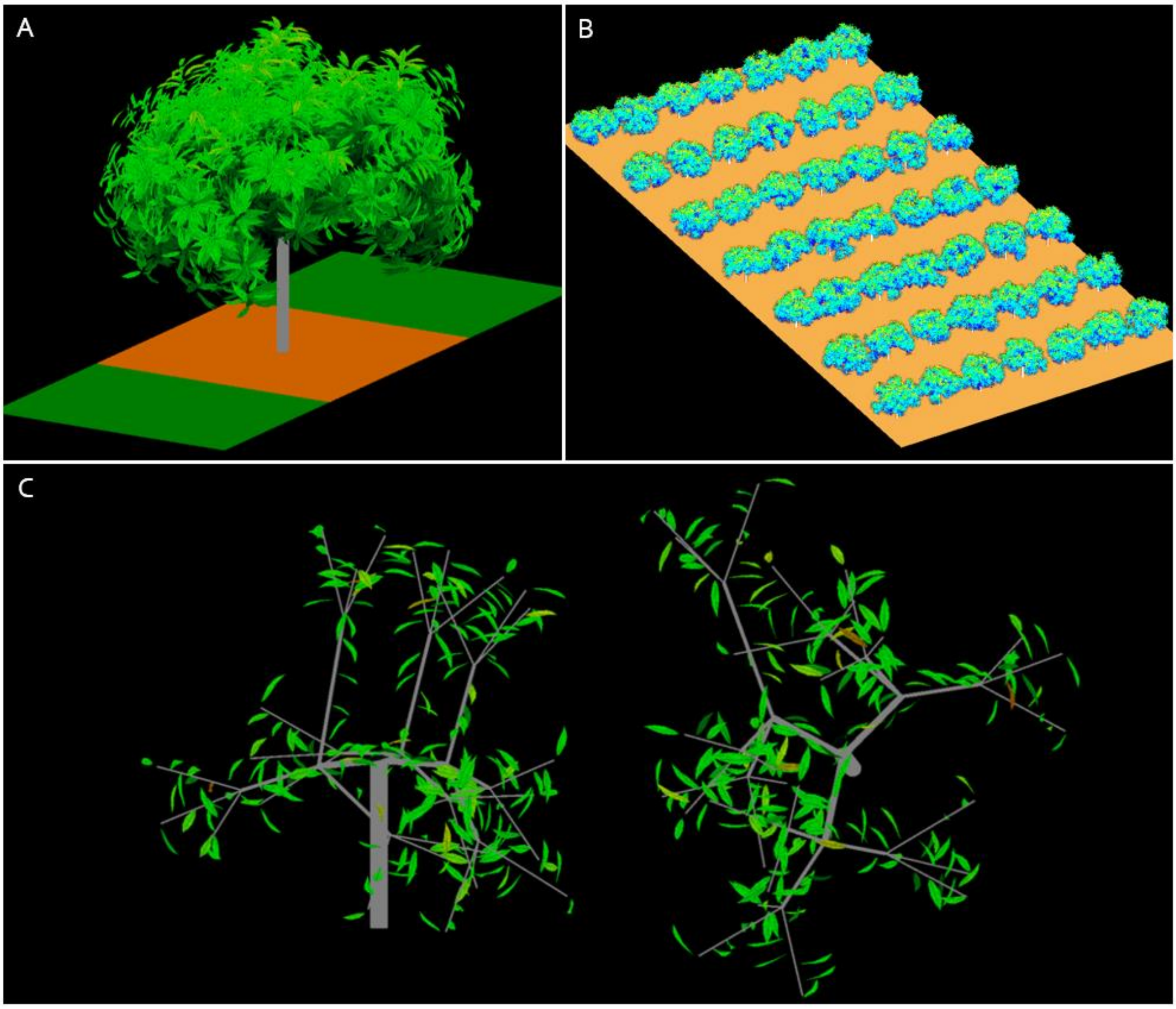

Figure 2. A tree/orchard simulator. This simulator is currently using mango tree architectural data as an example, supporting virtual experiments with either (A) one tree or (B) multiple trees in an orchard environment for investigation of light interception and distributions. Colours from blue to red (B) represents low to high flux density absorbed by individual leaves. The design and pre-evaluation of (C) canopy training systems are also supported by the integration of mangoL and HP-QuasiMC. The mangoL simulator was developed by Liqi Han at the University of Queensland based on empirical data from Anahita Mizani (Mizani, 2020).

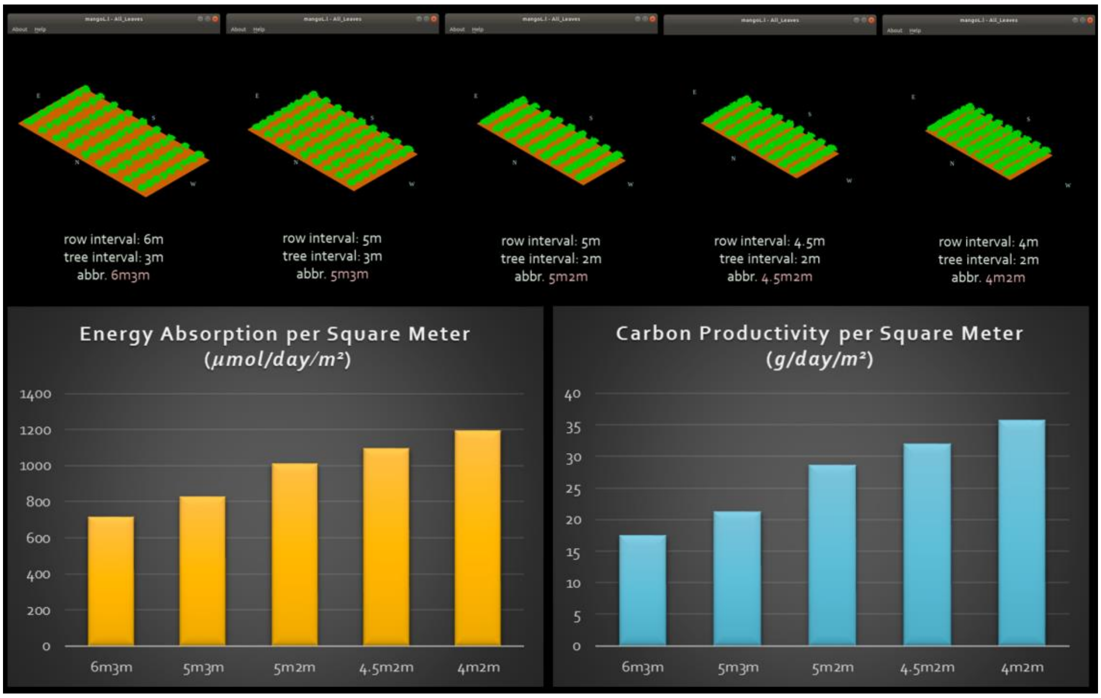

Figure 3. A preliminary virtual experiment to investigate the impact of row and tree intervals on light energy absorption and carbon productivity. This is of conventional orchard arrangement where rows are organised in straight lines. The "tree interval" here refers to the gap between any two neighbouring trees in a same row. Given 9 rows with 9 trees per row with the canopy size demonstrated in Figure 2A, using Mareeba's geographical location and Julian Day 355 to configure the light environment, this experiment indicated higher energy absorption levels and higher carbon productivities from higher planting densities within the scope of investigation.

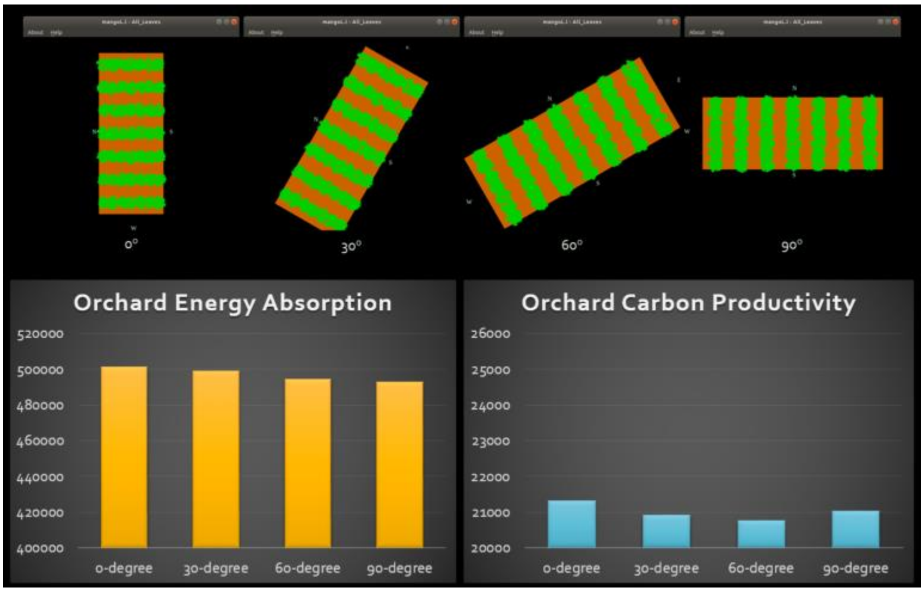

Figure 4. A preliminary virtual experiment to investigate the impact of row orientation on light energy absorption and carbon productivity. Using the same local light environment as was configured for Figure 3, an orchard setting (row interval 5 m, tree interval 2 m) was rotated clockwise by 30°, 60° and 90° respectively. The orchard-level energy absorption decreased slightly during the rotation, while the carbon productivity increased when the rows were turned to 90°.

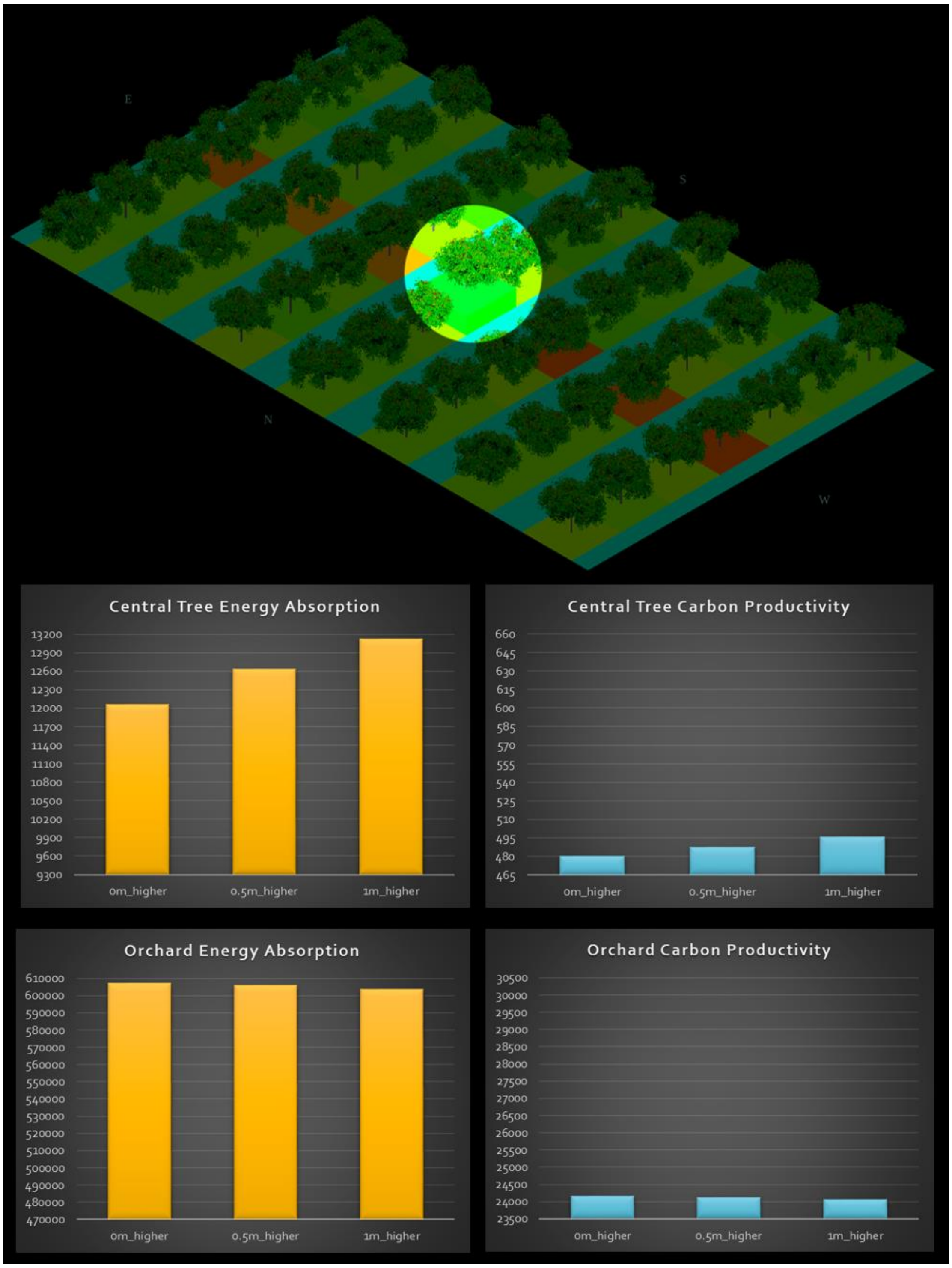

Figure 5. A preliminary virtual experiment to investigate the terrain effect on light energy absorption and carbon productivity. In this investigation of terrain effects, the central tree's location was adjusted vertically from 0 m to 0.5 m and then to 1 m. This did improve the tree's exposure to light and increase its carbon productivity, but did not create a significant impact to the overall orchard result. However, more complex terrains and combinations of different tree heights are allowed by mangoL and deserve future experimentations to help optimise orchard designs.

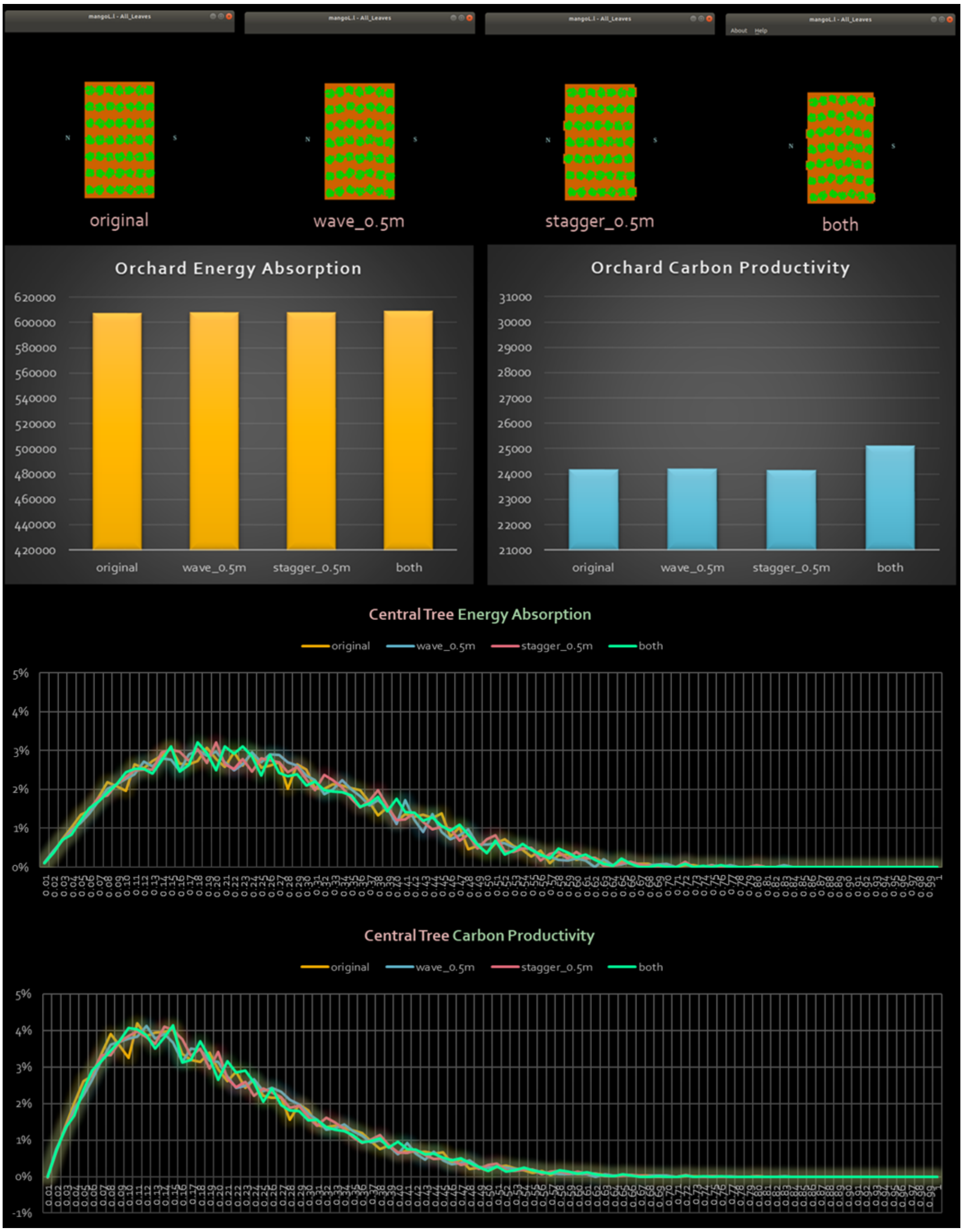

Figure 6. A preliminary virtual experiment to investigate the impact of unconventional orchard layout on light energy absorption and carbon productivity. Based on a conventional orchard setting (with the canopy size demonstrated in Figure 2A, in north-south rows, where row and tree intervals were set as 5 m and 3 m respectively), "wave_0.5m" applies a fluctuation of half a meter to every other tree east or west from the original row line, "stagger_0.5m" represents a half-meter shift of an entire row north or south, and "both" combines these configurations. The in-canopy light distribution as well as the carbon productivity can also be evaluated through the percentage of leaves with different energy or productivity levels (where the flux density level is normalised to a value between 0 and 1). This experiment did not find significant differences in light absorption and carbon productivity among the four different orchard settings at this tree age.

Discussion

The high-performance light simulator as well as the mangoL simulator have been applied in this project to support the investigation of light environment in relation to orchard design and management. Together they are capable of calculating energy absorption and carbon productivity (as a result of photosynthesis) of every single leaf and thereby evaluating the distribution and assimilation of light at different scales from individual leaf to the entire orchard, which can help our understanding of how conceptual planting systems and potential management could affect light interception and distribution. In addition to mango, these simulators could be easily configured for virtual experiments with macadamia and avocado crops.

Following the preliminary virtual experiments that have been enabled by HP-QuasiMC and mangoL to test their technological capability, more complex experiments should be designed and implemented with these tools to further investigate the impacts of planting density, row orientation, unconventional layout, terrain, pruning and training systems as well as their joint effects on orchard light environment and yield.

Also, the mangoL simulator so far has been driven by parameters derived from field measurements (Mizani, 2020). Although the *in silico* trees created this way represent many features of the trees *in situ*, they do not have direct counterparts in the real world. In future we see LiDAR scanning as a promising approach to creating digital orchard twins that more closely match reality.

Appendix 18

Model Development for Avocado, Mango and Macadamia

Ming Wang, Anahita Mizani, Ben Toft, Inigo Auzmendi and Jim Hanan

Introduction

This appendix provides a summary and guide to project literature for avocado models developed by Ming Wang, mango models developed by Anahita Mizani and macadamia models by Ben Toft during the course of their PhD work undertaken in the project. The models described in Appendices 17-20 and 22 are also able to be applied to avocado and mango but require species specific parameterisation, some of which has been undertaken.

The models were developed using L-studio (algorithmicbotany.org), which provides the set of tools required to develop models as an L-System (Lindenmayer, 1968a, Lindenmayer, 1968b), and PlantGL/OpenAlea (Pradal et al., 2009).

Avocado Modelling in Ming Wang's PhD Thesis (Wang 2018)

An annual growth module (AGM) of avocado is the development of the unit of tree branching architecture over a year, which is made up of a mix of proleptic and sylleptic shoot types. An avocado tree can be viewed as an aggregation of multiple AGMs, occurring over many successive years of growth. Cultivar differences can be clearly seen in the relative frequency and dimensions of these shoot types, which ultimately determine the structure and shape of the mature tree. Understanding these relationships and the natural growth habit of trees, i.e. their architecture, is fundamental to the development of more intensive growing systems for avocado. Additional insight into architecture and growth patterns could translate into applied outcomes, so that better canopy management strategies can be conducted more effectively in avocado orchards. Thus, development of such an avocado architecture model is timely.

Architecture model of an AGM (Thesis Chapter 2, Wang et al., 2016)

The architecture model was constructed to simulate the relationships between proleptic and sylleptic shoot types and the natural growth habit of trees, based on the Pattern Oriented Modelling (POM) approach. The model was able to reproduce multiple observed patterns of architecture and shoot growth simultaneously, and to make independent predictions providing insights into branching architecture, which were consistent with independently generated findings of other studies. It generated insights into the development of branching architecture of an AGM. The model showed that the probability of shoot extension and the probability of an axillary bud turning into a sylleptic/proleptic shoot played an important role in determining the whole structure of the annual growth module.

Functional-structural plant model of an AGM (Thesis Chapter 3, Wang et al., 2018)

The architecture model was further developed into a functional–structural plant model of an AGM, taking the aspects of photosynthesis and adaptive carbon allocation at the organ level into account. After model calibration, the model simultaneously reproduced multiple observed architectural patterns. The model then successfully predicted, without further calibration, the validation patterns, such as the mean length of each growth flush, the mean leaf area per shoot and the mean number of nodes (leaf nodes only) per growth unit. It also predicted the observed developmental timing of the leaf sink–source transition stage as well as the leaf expansion duration and the peak vegetative flush growth duration. Such accurate predictions demonstrated that POM can help to improve the 'structural realism' of functional-structural plant model models, i.e. the likelihood that a model reproduces observed patterns for the right reasons. Structural realism increases predictive power so that the response of an AGM to changing environmental conditions can be predicted. The model supports the hypothesis that carbon allocation can be modelled as being dependent on current organ biomass and sink strength of each organ type. Therefore, the constructed model is ready for potential applications. For example, it is known that competition for carbon between developing fruitlets and developing leaves on indeterminate floral shoots in avocado trees is a key determinant of final fruit yields. Using such a realistic model, an accurately predicted developmental timing of the leaf sink-source transition stage during a growing season allows the exploration of methods for identifying horticultural practices that maximise fruit yield. Orchardists can remove leaves still acting as sinks and drawing resources away from fruit at some time during the period of early fruit set, then final fruit yield should increase. Also, the fruit component can also be further incorporated into the model to investigate other factors that influence interactions between fruit and vegetative growth, such as early summer fruit drop.

Mango Modelling in Anahita Mizani's PhD Thesis (Mizani 2019)

In order to use modelling to help gain a better understanding of cyclic vegetative growth patterns in mango, and thereby aid in intensifying mango production, Anahita Mizani started with a baseline study monitoring five mango varieties' vegetative growth. The number of leaves, leaf colour, leaf length and width, petiole length, internode number, internode length and diameter of the growing growth in the western and eastern sides, and the middle of the tree canopy were measured (Thesis Section 3.4). An L-system model of mango flushing was developed capturing number and size of leaves in seasonal flushes in different varieties and climates as governed by interaction of plant physiology with light and temperature. This formed the basis of a model taking into account growth-unit location in the canopy (Figure 1) via variation in temperature and light distribution.

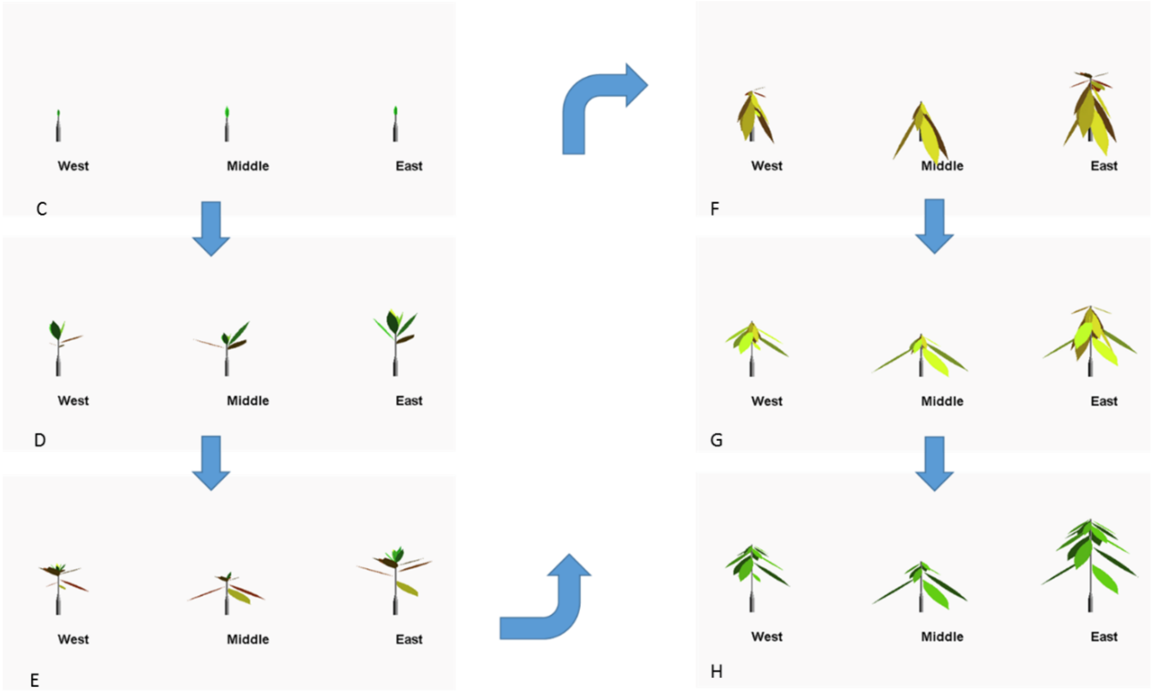

Figure 1. Vegetative growth model based on local temperature in west, middle and east part of a simulated mango canopy. Labels C to H represent stages of vegetative growth development, which define leaf angle and colour. Changes in size are determined by a degree-day relationship (Thesis Figure 3.21).

To verify the light component of this model, data captured in light relations studies was compared to model predications of canopy light-distribution resulting from different pruning methods. The quasiMC light environment modelling package was used to determine light levels at simulated light sensors for comparison to field data, and at each leaf for more detailed analysis of light distribution (Figure 2, further details in Thesis Section 3.4).

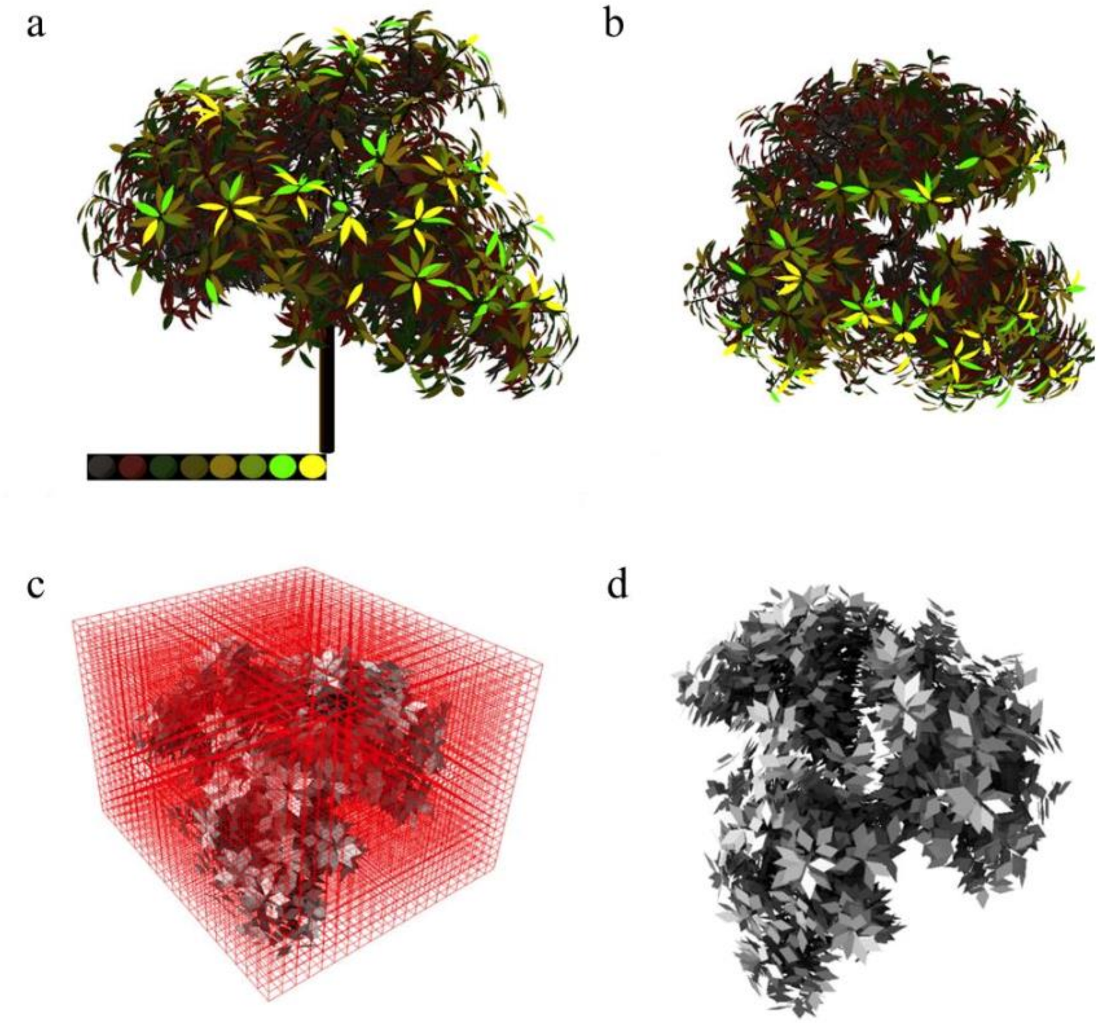

Figure 2 Mean light transmission percentage, at leaf level in window pruned tree from lowest, brown, to the highest, yellow. Landscape (a) and overhead view (b). Leaf locations in the quasiMC voxel matrix (c). Light transmission percentage for each leaf without branching structure. The higher the mean, the lighter the colour (d) (Thesis Figure 3.14).

Future prospects

Light has an important role to play in final fruit quality, particularly blush. By incorporating fruit location and development into the model, the light environment of each fruit can be characterised. The combined flush and fruit growth and light model can predict the light received by each fruit over the course of its development. This can make the estimation of light distribution easier and more economical by reducing the number of field light measurements needed. Outputs of the light model could be used to test various pruning and training options to improve light transmission inside the canopy and to help understand how it affects fruit quality. For example, virtual thinning could be undertaken and resultant simulated light distribution analysed to see where in the canopy fruit are more likely to receive the optimum light during development. Similarly, the effect of experimental pruning methods on light distribution can be analysed before trialling in the field, to help optimise experimental work.

Macadamia Modelling in Ben Toft's PhD Thesis (Toft 2019)

The elongation of internodes and growth units was monitored to determine physiological parameters in macadamia by fitting a logistic model to the length data as a function of thermal time (Figure 3, thesis Chapter 3 and Toft et al., 2018). These parameters could be used subsequently in functional-structural plant models.

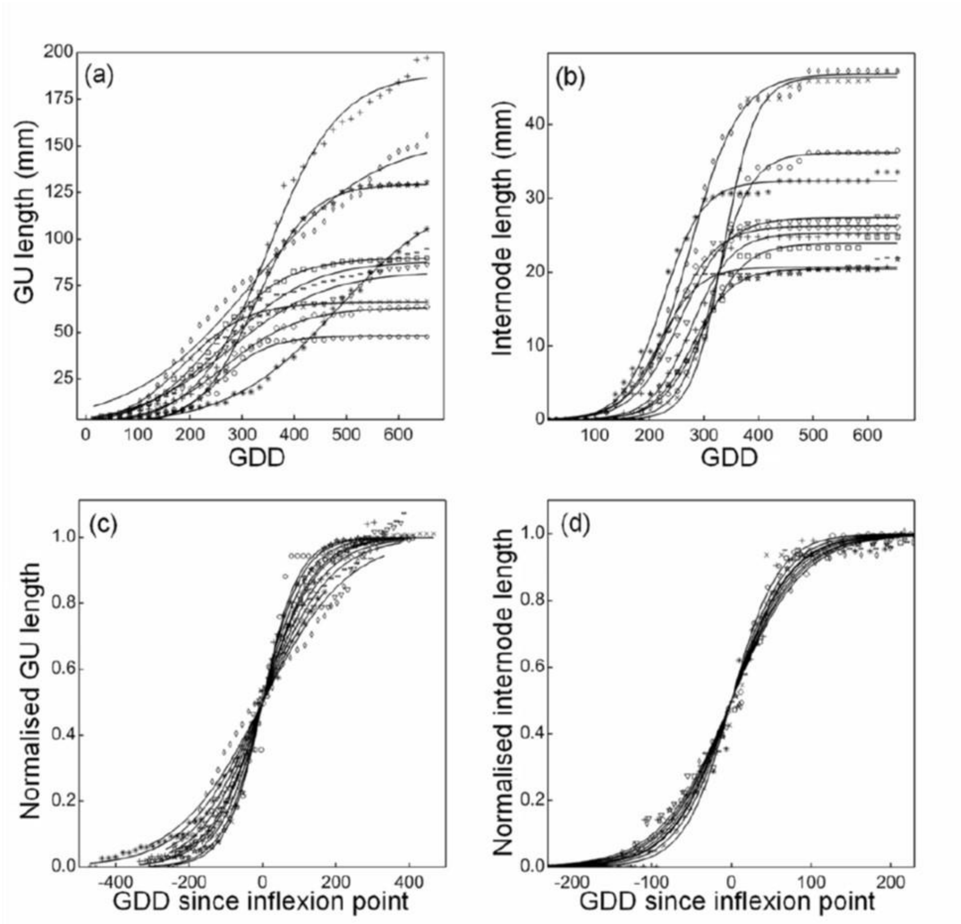

Figure 3 The length of 10 whole growth units (GU) and of the third internode of each GU are shown as related to thermal time (GDD). Logistic models fitted to length of (a) GU and (b) internode of GU expressed over GDD. Logistic models of normalised length at (c) GU scale and (d) internode scale. In (c) and (d), the inflexion points are constrained to 0 GDD and all lengths are normalised to maximum length. Cultivar '741' scion on 'H2' seedling rootstock, one year since planting. (Thesis Figure 3.1).

Ben Toft also measured and coded tree architecture, i.e. topology, internode length, inclination and orientation at different times of the year in two cultivars with bent branches and unbent branches. These data were used to simulate 3D tree architecture using PlantGL, and visualize the time of growth of the units, as well as the effect that bending had on tree architecture and growth. These visualizations were employed for data verification and preliminary analysis (Figure 4, thesis Chapter 6 and Toft et al., 2018).

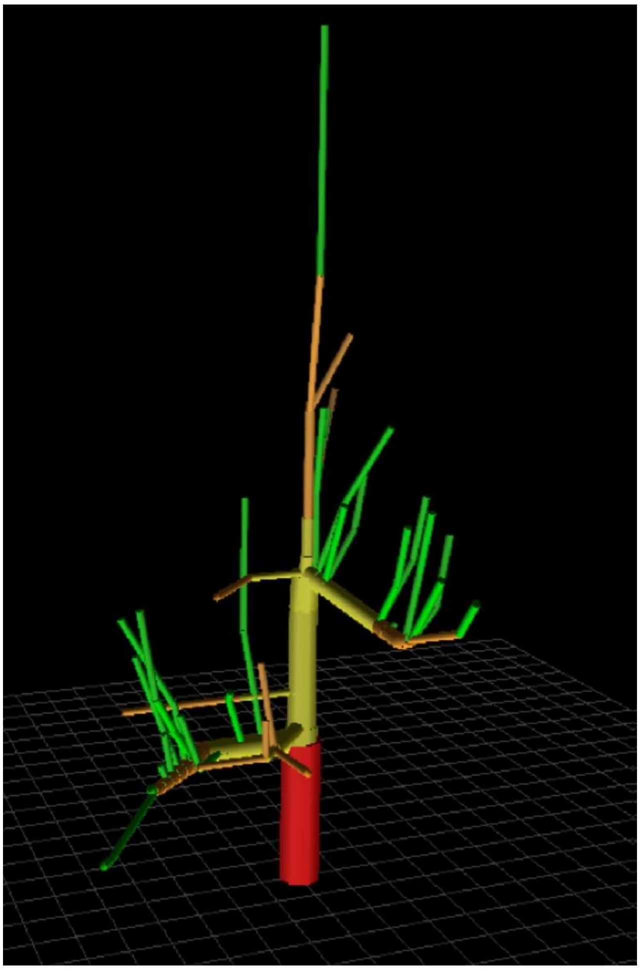

Figure 4 Graphical representation of an 'A203' tree in trellis with bending treatment, created using data collected in multi-scale tree graph (MTG) format. Colour represents time of growth of GUs. Red, rootstock; yellow, growth in time A (whole tree); orange, growth during time B (two subsampled BUs); green; growth during time C (two subsampled BUs). Time period D not included. Multiple second-order shoot growth has occurred along the first-order axes in the time period since bending. (Thesis Figure 6.2).

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Appendix 19

A Model of Macadamia with Application to Pruning in Orchards

Neil White and Jim Hanan

Summary

A self-organising model of macadamia, expressed using L-Systems, was used to explore aspects of canopy management. A small set of parameters control the basic architecture of the model, with a high degree of self-organisation occurring to determine the fate and growth of buds. Light was sensed at the leaf level and used to represent vigour and accumulated basipetally. Buds also sensed light to provide demand in the subsequent redistribution of the vigour. Empirical relationships were derived from a set of 24 completely digitised trees after conversion to multiscale tree graphs (MTG) and analysis with the OpenAlea software library. The ability to write MTG files was embedded within the model so that various tree statistics could be exported for each run of the model. To explore the parameter space a series of runs was completed using a high-throughput computing platform. When combined with MTG generation and analysis with OpenAlea it provided a convenient way in which thousands of simulations could be explored. We allowed the model trees to develop using self-organisation and simulated cultural practices such as hedging, topping, removal of the leader and limb removal within a small representation of an orchard. The model provides insight into the impact of these practices on potential for growth and the light distribution within the canopy and to the orchard floor by coupling the model with a path-tracing program to simulate the light environment. The lessons learnt from this will be applied to other evergreen, tropical fruit and nut trees.

Introduction

Macadamia are vigorous and tend to need a high level of management. Management decisions made in the first few years can have long-term consequences when combined with the longevity of a tree. Decisions include row and tree spacing, cultivar selection, orientation, training, pruning, hedging and topping. In this section of the work for AL13004 we have developed a model of macadamia growth that will allow for virtual experiments to be done. These might include novel training schemes, planting densities and canopy management practices by providing a framework on which to test ideas over the life of a tree under controlled conditions.

The early stages of this development were to understand the experimental and computational requirements needed to build a functional model and be aware of the limitations and sensitivities and hence help to direct further experimental work. The initial model utilised the data gathered during and earlier DAF project in which trees within an orchard were digitised. These trees ranged from 1.4 to 4 m in height. We assume that the light and space environment of buds contributes strongly to the fate of vegetative buds and that this largely determines the resulting structure. This approach is referred to as the self-organisational model (Palubicki et al.,

2009) and collects the light from the leaves and redistributes this as “vigour”. The vigour represents the number of internodes that can be grown. In our model we have separated the sensing of light from the demand so that light is sensed by leaves rather than buds, but it is the light and space environment of the bud that determines if it can grow out. Macadamia have leaves that are distributed as whorls of three or four leaves and that each leaf can have as many as five axillary buds (Bennell, 1984), however, at this stage we have only included one axillary bud per leaf. The disadvantage of the self-organising model is that there are many parameters that control tree shape. Some of these relate to the accumulation of vigour and light, but others control the way that light is reduced within the canopy, the sphere of influence that one plant part has on another and the way in which decisions are made in terms of the fate of buds.

Materials and Methods

Five cultivars (H741 H816, H842, A268 and Daddow) comprising heights from 1m to 4m were digitised using a Fastrak 3D magnetic digitiser (Figure 1) with the LongRange Transmitter (Polhemus Inc. www.polhemus.com). Some trees were redigitised on a number of occasions. Digitisation included the position of each node, whether leaves were present or not and the width of main structural branches. The study orchard was at Beerwah in southeast Queensland. The Floradig software (Hanan and Room, 1992) was used to record the topology of the tree and also provide information on the orientation of successive nodes. The data created in Floradig were converted to multiscale tree graphs (Godin et al., 1999) and analysed with Openalea (Pradal et al., 2008).

The models were developed using the Virtual Laboratory, VLAB (Linux and OS X), (Prusinkiewicz et al., 2000) which provides the set of tools required to develop models as an L-System (Lindenmayer, 1968a, Lindenmayer, 1968b). A Windows-based version of VLAB, called LStudio, is also available (algorithmicbotany.org).

The set of fully digitised trees was used to parameterise the self-organising model by employing the high throughput computing techniques in HTCondor (Thain et al., 2005) to explore values for the parameters. The limitation was that we only have data for trees up to 4 m tall and that these had been subjected to orchard management, so there is a risk that we have fitted our model to managed trees (Figure 2).

Simulations were run that averaged the light intensity from the blue, green and red parts of the photosynthetically active radiation (PAR) spectrum (400 to 700 nm). A series of 85 simulations were undertaken to examine the goodness of fit between the virtual sensors and the data collected from the ceptometer using parameters for light extinction within QuasiMC.

In a further set of experiments we measured the light interception at multiple levels through two different canopies using a ceptometer (LP80 Accupar, Decagon Inc) in order to parameterise the light model, QuasiMC (Cieslak et al., 2008). The reflectance spectra of leaves were obtained using a FieldSpec 4 spectroradiometer (ASD Inc.) for the upper and lower leaf surfaces for mature leaves (see Appendix 16).

Figure 1. Digitising a macadamia using the Polhemus Fastrak

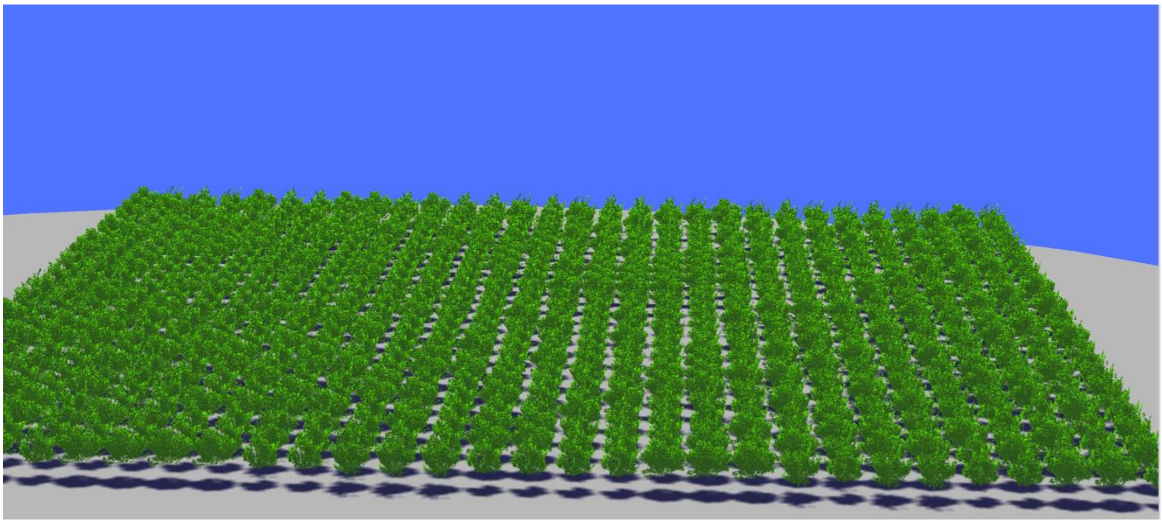

Figure 2. Virtual orchard created using some of the digitised trees.

Table 1. Details of digitised trees used for model development.

Variety	Nodes	Branches	Height(cm)	Width(cm)	Planted	Digitised	Volume (m^3)
816	264	45	166	51	Feb 06	Aug 08	0.05
816	2147	379	232	161	Feb 06	Mar 10	1.25
816	504	89	156	65	Feb 06	Aug 08	0.15
816	295	61	157	51	Feb 06	Aug 08	0.04
816	262	47	157	55	Feb 06	Aug 08	0.07
842	722	87	193	101	Feb 06	Aug 08	0.39
842	2917	341	334	280	Feb 06	May 10	3.54
842	1007	205	201	119	Sep 04	Sep 08	0.69
842	1626	205	263	160	Sep 04	Oct 08	1.53
842	8858	1021	381	323	Sep 04	Apr 10	11.1
842	9583	1116	401	339	Sep 04	Sep 10	12.18
842	7146	757	360	349	Sep 03	Jul 09	12.23
842	8189	903	360	344	Sep 03	Nov 09	12.46
842	9390	1064	401	374	Sep 03	Nov 09	18.25
741	1615	217	200	141	Sep 04	Nov 08	1.04
741	2763	364	249	204	Sep 04	Dec 08	2.94
741	1814	204	233	154	Sep 04	Dec 08	1.59
741	1981	297	241	143	Sep 04	Dec 08	1.42
741	4328	717	301	219	Sep 04	Jan 09	4.21
741	5932	842	391	282	Sep 03	Jan 09	9.33
741	6275	682	415	265	Sep 03	Mar 09	9.72
741	8772	1014	459	326	Sep 03	Apr 09	14.79
A268	513	61	193	100	Sep 07	Mar 10	0.28
A268	281	28	160	81	Sep 07	Mar 10	0.17
Daddow	425	69	232	103	Sep 07	Jun 10	0.37
Daddow	404	65	176	75	Sep 07	Jul 10	0.11
Daddow	645	90	232	103	Sep 07	Dec 10	0.37
Daddow	1079	166	213	102	Sep 07	Mar 11	0.43

Results and Discussion

Development of the Model

The data for the various cultivars have been combined to create a set of relationships on which to base model validation. Primary among these are height, canopy volume, the number of nodes and branches produced and overall form of the tree. A few parameters are hard coded into the model, i.e. they are not an emergent property of the self-organising model but were derived from the database of digitised trees. These included distributions for branch angle, leaf length, leaf angle, and length of nodes, maximum and minimum number of nodes added during a flush, and the number of nodes in the main stem below the first branching point.

The time step of the model is the flush, of which there are two – the summer flush and smaller early spring flush (Stephenson et al., 1986) with a factor to limit production of new growth during the early spring. There were no other temperature effects on growth in this model. When light is converted to “vigour” it influences the number of nodes that are added during a flush and without the differential limiting of early spring growth the trees grew too quickly. The steps that determine the growth of the orchard and light interception are shown in Figure 3. The steps contained within the box rely on (Palubicki et al., 2009) with further refinements by Brendan Lane (University of Calgary) pers. comm. 2010.

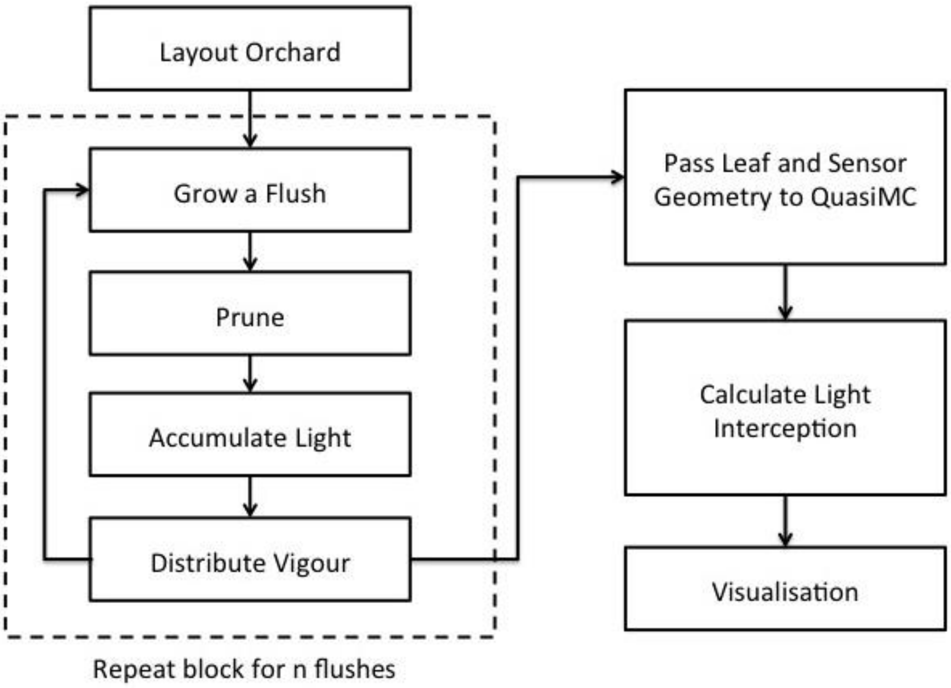

Figure 3. Flowchart of macadamia model and integration with QuasiMC.

Validation of the Model

Height of the tree was largely determined by lambda, i.e. the parameter that allocates resources preferentially either to main or lateral branches, the conversion of light into vigour, node length and to some extent factors that control gravitropism and phototropism. The production of nodes is a result of the amount of light gathered by leaves (using the shadow model) and redistributed after converting light to vigour. This is a simple factor and was tuned during the parameter search. The relationship between the flushes and the number of nodes produced is shown in Figure 4 (r^2=0.94). This relationship (Figure 5) and those for tree height, canopy volume and number of branches (not shown) were used to assess the validity of the modelled output over time. The number of internodes produced fits the observations quite well up to 14 flushes. After that it tends to deviate and produce fewer nodes than expected. At present we do not have data on trees larger than 4 m, but this will be required to validate the model further. Tree height also deviates, being slightly shorter than projected. These departures seem to be largely due to the lower light interception received by leaves once the canopy closes. The projection of the relationship from before the canopy closes is unlikely to be valid after the canopy closes. This needs further measurement and will probably require a technique other than 3D digitisation, such as LIDAR.

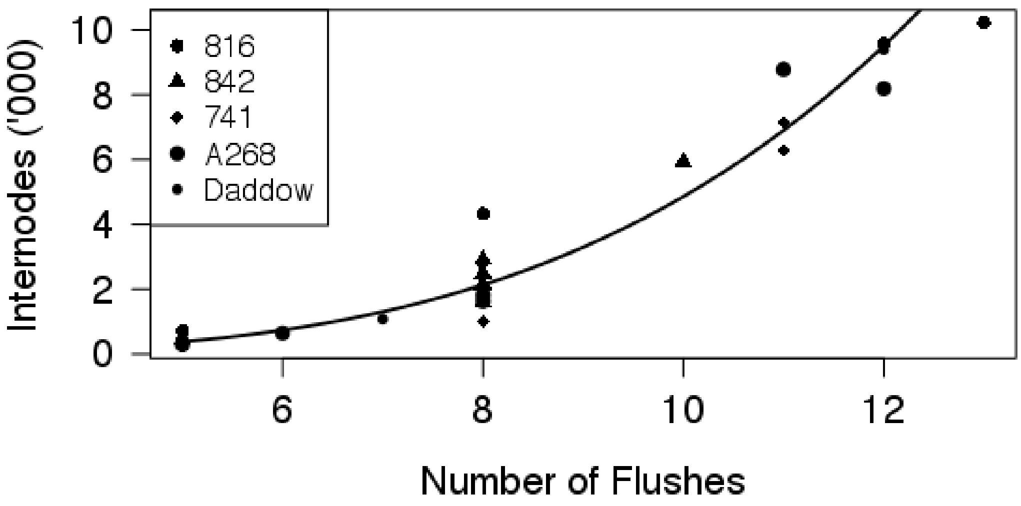

Figure 4. Relationship between number of flushes and the number of nodes on five cultivars of macadamia.

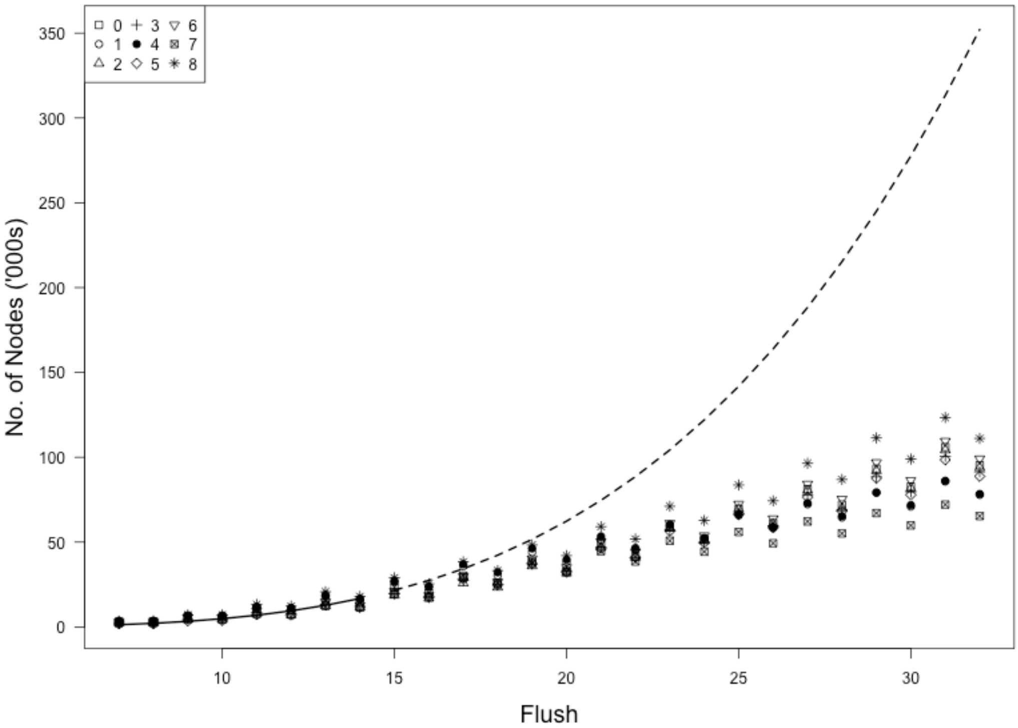

Figure 5. Modelled relationship between number of internodes. Tree 4 is at the centre of the guard trees. The black line shows the relationship derived from the five cultivars that were digitised (Fig. 2). The dashed line is a continuation of that relationship.

Light Interception

Yield is largely the result of light interception and resource allocation within the plant. Designing the high productivity crops of the future will depend on **efficient** light interception and optimising vegetative growth to ensure resilient canopies that also allow resource allocation to the crop. The QuasiMC model allows the trees to be placed within a realistic light environment and to compare the levels of light interception for various orchard layouts and management strategies. Comparisons between measured light (PAR) and modelled light were done to ensure that the light intercepted by leaves and virtual sensors was reliable. QuasiMC uses a set of parameters to inform its sky model (see Appendix 16). These include the location and time of day and information on leaf reflectance and transmission. It can also operate at multiple wavelengths and 450 nm (blue), 550 nm (green) and 650 nm (red) were chosen to represent PAR. The mean of the resultant intensity at the three wavelengths was used to compare with the PAR reading from the ceptometer. At this stage we only know leaf reflectance from macadamia leaves with transmittance being inferred from other plants (Table 2).

Another important part of the path tracing involves what is referred to as Russian Roulette and also the number of intersections that a ray can have. After reflection or transmission if the intensity of the resultant light ray is below some threshold it is terminated with some probability. Subsequently, if the light ray has now intersected with the maximum number of leaves or sensors it is also terminated. The set of 85 simulations that examined the goodness of fit of the QuasiMC model to reliably model light interception was a factorial with thresholds of 1%, 2%, 3% and 4%, with probabilities of 30%, 40%, 50% and 60% and number of intersections 1, 2, 3 , 4, and 5. The goodness of fit was tested by ranking the root mean squared error and the regression coefficient. There were inconsistent results between the horizontal sensors and the vertical sensors and to some extent between the two tree canopies. However, the best compromise was to use a threshold of 2% with a probability of 60% and to allow a maximum of three intersections.

Table 2. Reflectance and transmission values used in modelling of the light environment of the canopy within QuasiMC.

	Reflectance (%)			Transmission (%)		
Leaf Surface	Blue	Green	Red	Blue	Green	Red
Upper	3	6	3	5	10	5
Lower	3	11	6	5	10	5

The application of this procedure can be demonstrated by comparing the orchard floor light interception in a similar way to the field experiments undertaken by (McFadyen et al., 2004). In our virtual experiments the transects were extended from the centre of the adjacent rows and placed midway between the adjacent trees and 20 cm south of the tree at the centre. These simulations were undertaken for the full path of the sun and at various times during the year. Light interception at summer solstice was 94% for the unhedged canopies (
Figure 6) and 77.4% for the hedged canopies (Figure 7). Hedging was undertaken during the spring flush to create a 2 m alleyway. The orchard was planted with 7 m between rows and 4 m between trees. These results were similar to the values given in (McFadyen et al., 2004). At this stage QuasiMC does not include structural components in any meaningful way. Inclusion of main branches may help improve the fit between the ceptometer readings and provide better estimates for modelling within canopy light distribution.

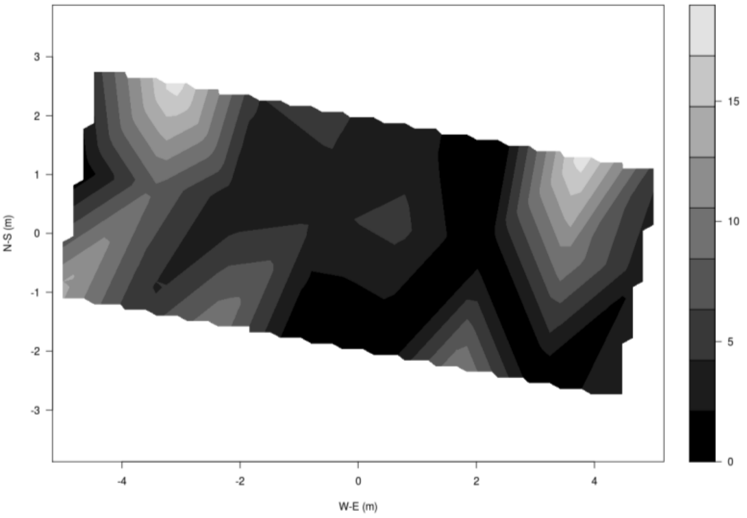

Figure 6. Light interception at orchard floor for unhedged orchard. Light intensity expressed as percentage of above canopy light.

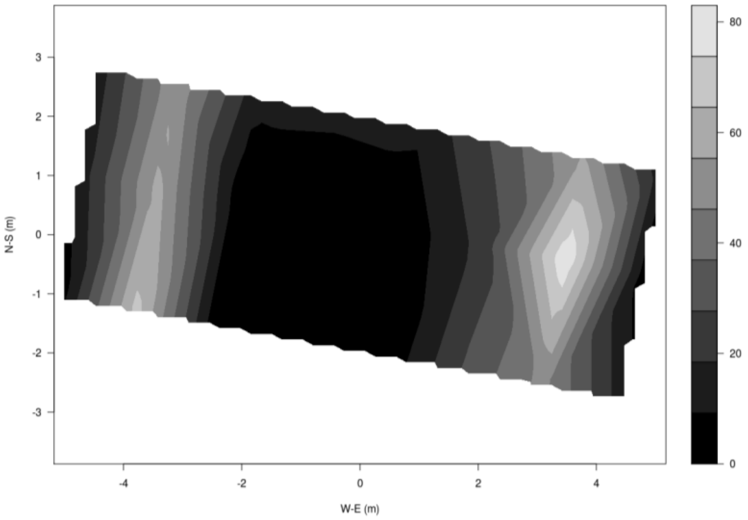

Figure 7. Light interception at orchard floor for hedged orchard. Light intensity expressed as percentage of above canopy light. Note that the scale extends to 80%.

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Appendix 20

INSIGHT INTO THE FLOWERING REGULATION AND THE ROLE OF FLOWERING LOCUS T (FT) IN MACADAMIA

Ye Gong, Francois Barbier, Craig Hardner, and Christine Beveridge

Summary

Macadamia is one of the important commercial evergreen fruit crops in Australia. Flowering is a vital stage for fruit production, and Flowering Locus T (FT) is recognised as a pivotal floral inducer in the arabidopsis flowering network system, as well as in other species including crop trees. The aim of the study was to understand better the flowering regulation mechanism in macadamia for improving crop productivity. MacFTa/b was identified in macadamia and characterised to show they may induce flowering in arabidopsis. In-silico analysis of the MacFT genes predicted the distribution of CONSTANS (CO), SHORT VEGETATIVE PHASE (SVP) and APETALA2 (AP2) within the promoter and provided that MacFTa and MacFTb may not only induce photoperiod. To clarify the molecular network of two macadamia FT-homologous genes, MacFTa/b gene expression pattern in buds and leaves were analysed in mature macadamia prior to induction of flowering within a season. We determined that MacFTa/b gene expression located in buds may be most important for flowering. The expression pattern of MacFTa/b Dormancy Associated MADS-box (DAM), and BRANCHED1 (BRC1) in 2018 and 2019 indicated that BRC1 might inhibit bud break, and MacFTa may induce flowering. A defoliation experiment confirmed that buds might serve a vital role for independent flowering induction. To further understand the role played by the FT gene in Macadamia (MacFT) in flowering regulation, MacFTb was fused to GFP protein and then introduced into *Nicotiana benthamiana* leaves by agroinfiltration to identify the subcellular localisation in the nuclear and cytoplasm and further support their transcriptional regulatory role. Altogether, this study suggested that MacFTa and MacFTb may promote floral induction in buds, and BRC1 may inhibit bud break in buds.

Introduction

Flowering is a fundamental process in plant reproduction

Flowering is an important process in a plant's life and plays a crucial role in fruit production. Thus, developing a greater understanding of flowering regulation is fundamental to develop new varieties and management approaches for improved productivity (O'Connor et al., 2018). Flower induction is defined as the transition from vegetative growth to reproductive growth, and this is the most critical part of flowering in higher plants because it may have strong impact on fitness (Huijser and Schmid, 2011). Flowering induction is the result of flowering genes adaptation to the environment, with the temperature and the day length considering of the two main factors (Alter et al., 2016).

Flowering plants are only able to flower once they undergo a developmental switch from a phase of vegetative growth to the reproductive phase (Roux et al., 2006). From direct observation, the plant floweringis a complex process. In macroscopic perspective, once the flowering period is under control, all aspects of the floral transition can be manipulated including floral primordia initiation, dormancy, and formation of inflorescences and floral bud (Conway, 2016). In annual plants, like arabidopsis, the switch from the vegetative phase to the flowering phase only takes a few weeks when conditions are appropriate (Jung and Müller, 2009). However, unlike annual plants, perennial trees face a long juvenile phase prior to flowering. Studying the induction of flowering at the molecular level can provide more accurate clues to determine the regulation of flowering and the time of flowering, and this also offers a novel strategy to overcome the study limitations of a long juvenile period of perennial trees. Under proper conditions, the trees can cycle between the juvenile phase and flowering year after year (Jung and Müller, 2009). Many studies show that the induction of flowering in perennial plants is carried out by observing the relationship between macroscopic flowering (such as flowering phenotype and flowering time) and physiological factors or environmental factors, and there may need several months or years between flower induction and macroscopic flowering. In some species, there is a lag between the floral induction and the formation of the flower primordium (Sedgley and Griffin, 2013). Like in *Banksia coccinea*, the same sub-family of Proteaceae as macadamia, the transition between floral primordia initiation and macroscopic appearance of the inflorescence took 1 to 9 months (Fuss and Sedgley, 1990). And in olive (*Olea europaea*), the timing of floral induction is more than half a year which is earlier than its actual flowering time (Rallo and Martin, 1991). As for in mango (*Mangifera indica*), the floral induction is controlled by low temperatures. However, low temperature is not encountered in tropical regions, which cause challenges for the flowering process exploration in mango (Vyavahare et al., 2015).

FT in flowering regulation

Homologs of FT

From the microscope perspective, previous studies have shown that the plants have developed a mature regulated network for controlling the reproductive transition through the function of activators and repressors in molecular level (Yant et al., 2010). FLOWERING LOCUS T (FT) is a florigen for flowering and seems to be essential for the acceleration of the floral transition among phosphatidylethanolamine-binding protein (PEBP) family genes. The members of the PEBP family are determined to have great importance for changing plant architecture and regulation of flowering time in plants, including FT, TWIN SISTER OF FT (TSF), CENTRORADIALIS (CEN), BROTHER OF FT (BFT) and TERMINAL FLOWER 1 (TEL1). Reproductive developmental phase changes are marked by floral induction which is highly sensitive to several internal and external factors, including gibberellic acid (GA) (Wang et al., 2016); plant age (Bäurle and Dean, 2006); temperature (Bi et al., 2019) and photoperiod (Song et al., 2013). These factors, directly and indirectly, affect the gene expression to regulate floral induction. The regulation of FT expression is involved in several flowering pathways, such as vernalisation, the long-day, temperature and autonomous promotion. Loss-of-function FT mutants are considerably late in flowering (Koornneef, 1991), and overexpression of FT in arabidopsis accelerates floral induction and causes early flowering (Kobayashi et al., 1999). So far, FT-homologous genes have been discovered in a few flowering plants, while its functional diversity has been confirmed in different species. In apple (Malus Domestica), pea (Pisum sativum) (Hecht et al., 2011), poplar (Populus spp) (Böhlenius et al., 2006), rice (Oryza sativa) (Cai et al., 2014), tomato (Solanum Lycopersicum) (Lifschitz et al., 2006), and wheat (Triticum aestivum) (Lv et al., 2014), FT-homologous genes act as flowering inducers (Kotoda et al., 2010); FT in Kiwifruit (Actinidia spp) is an inducer not only for flowering but also for dormancy release (Varkonyi-Gasic et al., 2013); In Sugar beet (Beta vulgaris) (Pin et al., 2010) and tobacco (Nicotiana tabacum) (Harig et al., 2012), some FT- homologous genes are a represser for flowering. Thus, FT-homologous genes are involved in the control of floral transition across some species. However, the role of FT-homologous genes in macadamia is still poorly understood.

Transcriptional Regulation of FT expression

In arabidopsis, expression of FT is highly regulated on the transcriptional level by several interdependent or independent inputs. The complex integration of many regulatory networks at FT is structured so that the plants flower at the optimal moment when the plants adapt to the environmental conditions and internal cues. Thus, transcriptional regulation plays a crucial role to maintain FT expression (Qin et al., 2017; Shim et al., 2017; Wickland and Hanzawa, 2015). Transcription factors can directly bind to FT promoter and regulate the first step of its expression, such as MADS-box, AP2, NF-Y, and bHLH and MYB (Liu, 2013; Qin et al., 2017). Intensive studies for analysis of TFs have shown that different TF complexes bind to FT promoter at the distal and proximal cis-elements that seems to cause initiation of reproductive transitions (Shim et al., 2017). In addition, TFs also can interact with the 5'untranslated regions (5'UTR) of FT (Qin et al., 2017).

Limitations of FT transcription was determined by in situ hybridisation, and this was required the application of β – glucuronidase (GUS) gene and driven by 8.9 kb FT promoter (Takada and Goto, 2003). Promoter truncation experiments in arabidopsis demonstrate that the 5.7 kb sequence upstream of the FT translation start site contains sufficient regulatory elements which can mediate spatial and temporal expression of FT in long days (Adrian et al., 2010). In Arabidopsis thaliana, 5.7 kb FT promoter has three phylogenetically putative regulatory regions, which are Block A, Block B and Block C and located on the proximal, middle and distal (Figure 1). In 5'UTR, AP2 repress FT by Figure 1 The transcription factors in arabidopsis FT promoter and 5'UTR.binding to AtFT at -64 and -23 regions via TTGTT or AACAAA consensus sequences (Dinh et al., 2012). Block A (1-400bp) containing 5'UTR conserved binding sites for several known transcriptional factors. In Block A, CO acts in the long day flowering pathway to up-regulate FT mRNA, and it was found to bind DNA via TGTG(N2- 3)ATG motifs which is present in -161 and -222 positions in vitro (Tiwari et al., 2010). TEMPRANILLO1(TEM1) and RTEMPRANILIO2 (TEM2) belong to transcription factors of the RAV (RELATED TO ABI3 AND VP1) family act as novel direct FT repressors via direct binding to its 5'UTR region (-43bp) (Castillejo and Pelaz, 2008). CYTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX (CIB1) interacts with various E-box element (CANNTG) in FT 5'UTR to stimulate the FT mRNA expression (Liu et al., 2008).

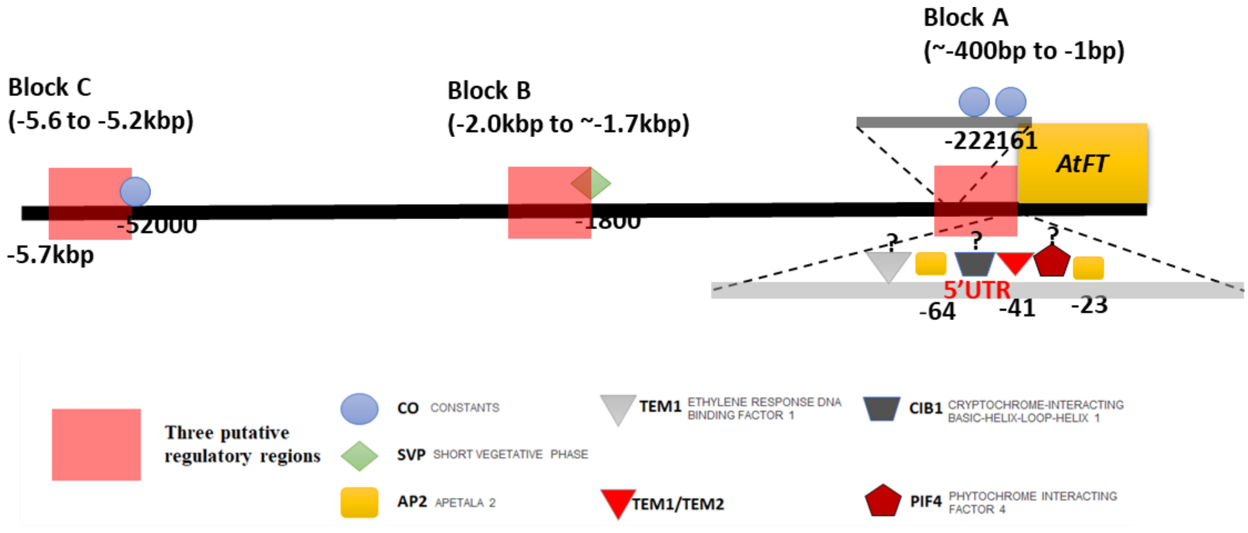

Figure 1 The transcription factors in arabidopsis FT promoter and 5'UTR.

In Figure 1, in addition to these transcription factors in FT 5’UTR, FT mRNA expression is also activated by PHYTOCHROME INTERACTING FACTORS 4 (PIF4) protein which is a bHLH transcription factor that regulates architectural responses to high temperature for the induction of flowering (Kumar et al., 2012). Block B (1.7kbp-2.0kbp) and Block C (5.2kbp-5.6kbp) includes recognised two putative cis-regulatory elements by transcription factors to reflect the FT regulation (reference), but this is not largely known. In Block B, SVP represses FT by binding to a CArG motif (CC(A/T)6GG) in the 1.8kb region upstream of the FT promoter (Lee et al., 2007). In Block C, conserved sequences are essential for FT promoter function. CO not only bind to FT in Black A, but CO also interacts with Nuclear Factor YB and YC (NF-YB and NF-YC) in the 5.2kb upstream of FT promoter region to assemble a functionally trimetric complex and activates FT expression in the transcription level (Wenkel et al., 2006).

FT in floral induction

In model plants, FT encodes a phloem mobile protein that is produced in leaves and moves to the shoot apical meristem (SAM) to promote floral induction (Figure 2, Alter et al., 2016). FT is a floral inducer under inductive day length conditions, which is directly regulated by the CO in leaves. The transcription factor CO, whose expression is tightly controlled by photoperiod, binds to FT promoter to induce its transcription (An et al., 2004). Accumulation of CO messenger RNA (mRNA) in phloem companion cells shows a circadian controlled pattern, and CO protein is stabilised by a light. Once that happens, CO binds to the cis-regulatory element (CREs) in the FT promoter to up-regulate FT expression. In short, CO protein accumulation only occurs when daylength is sufficient, and it is then able to induce FT, which is then transported from leaves to the shoot apex.

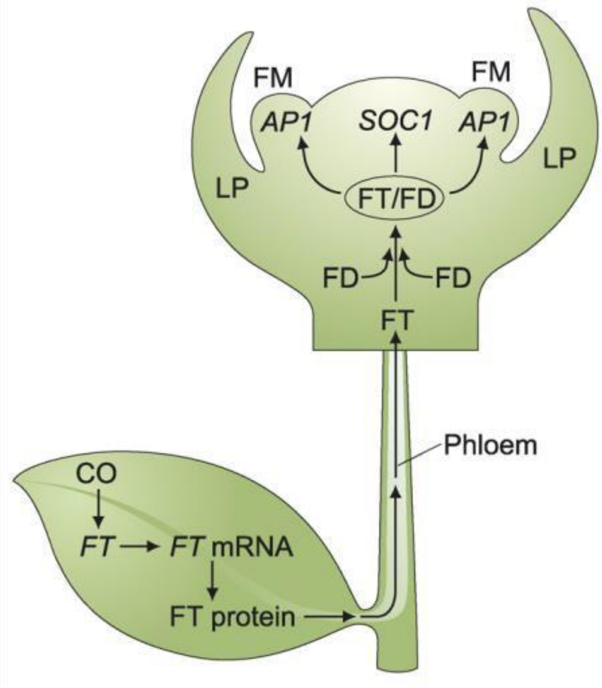

Figure 2: Flowering genes regulation in Arabidopsis. Under log days condition, FT is upregulated by CO, and its protein travel from leaves to SAM. In SAM, FT interacts with FD to trigger AP1 and SOC1 expressions, leading to flowering (Zeevaart, 2008).

Once FT protein enters into the shoot apical meristem (SAM), it interacts with the bZIP transcription factor Flowering Locus D (FD) as well as 14-3-3 proteins to form a “Florigen Activation Complex” (FAC) and then triggers the expression of meristem identity genes, such as the APETALA (AP1) gene, SUPPRESSOR OF

OVEREXPRESSION OF CONSTANS (SOC1), leading to SAM transition from vegetative to early floral development (Jaeger and Wigge, 2007; Taoka et al., 2011)

FT in Bud break

Unlike the model plant, the flowering of perennial plants is a combination of floral induction and bud break in that bud- dormancy release and reset are unique for the perennials (Castede et al., 2015). There exists limitation, as there lack of the model in the subtropical area, the study has applied the tropical instead of subtropical trees. In temperate horticultural trees, adequate timing of the onset and duration of winter dormancy are essential to avoid unfavourable winter conditions and ensure flowering in the following season (Rohde and Bhalerao, 2007). Bud dormancy states can be separated into paradormancy, endodormancy and ecodormancy (Champagnat, 1989; Crabbé and Barnola, 1996). Paradormancy is characterized as inhibition by correlation in that competition among organs, auxin and other signals regulate the inhibition of growth (Horvath, 2009). At the end of the autumn, the exposure to low temperature and reduction of the photoperiod induce the formation of winter buds, and the buds will become endodormant (Anderson, 2015). Within the period of endormancy, inhibition of bud growth is regulated by internal signals. In general, a period of chilling temperatures could overcome inhibition (Horvath, 2009). Once endodormancy is released, buds may also be repressed from bursting/breaking and enter into ecodormancy. However, if the conditions for favourable growth resume, the ecodormant buds grow immediately (Anderson, 2015). Temperature and day length are major environmental cues that regulate growth and dormancy cycle (Rohde and Bhalerao, 2007). These regulation network systems at the cellular level have been studied in different species. So far, the DAM gene is one of the most important of genes in response to control dormancy, which is homologous to SVP gene in arabidopsis (Abbott et al., 2015). SVP acts as a transcriptional repressor in the flowering regulation system, and it directly represses the FT mRNA to delay flowering (Gregis et al., 2013; Jang et al., 2009). Thus, the DAM could act as an inhibitor of FT-homologous gene in the perennial tree (da Silveira Falavigna et al., 2018). Additionally, DAM also can regulate dormancy by regulating BRC1. BRC1 is expressed throughout the development of axillary bud and plays a critical role in shoot branching. Under low temperature, the overexpression of DAM inhibits bud outgrowth by inducing BRC1 expression (Niwa et al., 2013). To sum up, the DAM could act as an inhibitor of FT- homologues while acting as a promoter of BRC1 to regulate the dormancy of the perennial tree.

The flowering challenge in macadamia

Flowering is well known in model crops, but the information is missing for non-model crops so we need to examine if results in model crops can be translated to specialty crops. Thus, this study aims to better understand the flowering regulation and the role of FT in macadamia. Macadamia (*Macadamia*, F. Muell) as an international commercial crop is the only domesticated on a scale in Australia with 50,000 tonnes of production per year (ausmacadamias, 2019). Commercial Macadamia breeding programs aim to improve yield. However, due to the relatively long juvenile period and growth complex nature, irregular flowering of most of the commercial cultivars causes the yield to be hard to control (O'Connor et al., 2018). Additionally, limited genetic resources are a hindrance to the efficient and fast development of new cultivars. In Australia, the growth cycle of macadamia fruits in winter, and floral induction occurs from May to June. Bud initiation starts in May, followed by 50-96 days of bud dormancy and then raceme and floret elongation (Hardner et al., 2009). In nature, the peak of flowering is usually during the late winter and early spring (Heard and Exley, 1994). The change in flowering time has an impact on flowering obvious (Howlett et al., 2015). Therefore, understanding the physiological mechanisms of flowering regulation at the molecular level in macadamia is important for to allow a better understanding of how to shorten the juvenile and dormancy periods, and it also has certain propelling significance for the improvement of yield.

The aim of this study was to better understand the flowering regulation and the role of FT in macadamia. FT homologs were identified, and their expression was monitored in various macadamia tissues. Potential roles in the regulation of flowering were studied on buds from defoliated branches and non-defoliated branches. To functionally characterize MacFT genes, a full-length macadamia FT codon region system (MacFT) was characterized using ectopic overexpression in pea (*Pisum sativum* L.) because pea has a relatively short flowering period. MacFT gene promoters were analysed in to predict the potential transcription factors that may regulate FT expression in macadamia.

Materials and Methods

Plant materials and growth conditions

The study is designed to explore the regulation of flowering genes in macadamia. The samples in the study were collected from the experimental field at the Bundaberg research station, Australia. Eight qualified trees from Macadamia (Macadamia F.Mull) cultivar "741 "were selected from the middle direction conventional guard trees in a macadamia trial. Axillary buds were collected from the trees which were located on centre-west every three weeks, specifically start from the 19 March 2019 and lasts for six times (19/03/19, 9/04/19, 30/04/19, 21/05/19, 11/06/19, 2/07/19) as same as last year. For the defoliation experiment, an additional tree was chosen in each repeat, resulting in four trees from cv741. Experimental trees were marked in the trial plan, and Four axillary defoliation buds were collected on the 11 June 2019. The buds were placed in an Eppendorf tube on the dry ice and shipped to the laboratory for analysis. Furthermore, other axillary buds and leaf discs were collected from a macadamia tree located on-site at The University of Queensland, St Lucia campus on 28th July. Immediately after collection these samples, all the samples were rapidly transported on dry ice and then stored in at -80 °C for laboratory use.

For transgenic experiments, Arabidopsis thaliana ecotype Columbia (Col-0) and Nicotiana benthamiana wild-type plants were grown on soil using a standard potting mix (UQ23 soil Mix + Osmocote and Dolomite) and in a growth room (LD, 21°C, 16h light/8h dark) regime. For observing the flowering phenotype, pea L107 and transgenic line pea L107 were grown in UQ23 mix with osmocote in 2L pots, grown in PC2 glasshouse 24'C day/18'C night in an 18-hour photoperiod (natural photoperiod extended to 18 h by fluorescent lighting from 4 am-10 am and 4 pm-10 pm).

Defoliation experiment

Based on the expression pattern in MacFT genes, the expression in buds is much high during May and June. To determine whether bud is most important for flowering, the branches from macadamia cv741 were defoliated on 1 May 2019, and then the defoliated branching back to the trunk. All the leaves on the branch were snipped off using fruit snipping secateurs. Branches of about 1.5 to 2cm in diameter were tagged on the west side. Axillary buds were taken from the first node after the bracts (i.e. the second node on the GU) from a GU most likely to flower. Two terminal growth units on each sample and control branch were tagged and monitored weekly for raceme or vegetative growth emergence until the beginning of July. In August a total count of racemes and new vegetative shoots on the branches was made.

Polypeptide sequence analyses

The possible similarity sequence of MacFTa/b for homologous alignment was identified using Geneious primer software and online ExPASy–Translate tool (https://web.expasy.org/translate/). The BLAST tool (https://blast.ncbi.nlm.nih.gov/) was also applied to obtain higher similarity sequences, and after this, the neighbour-Joining method was launched for constructing a phylogenetic tree by using MEGA6 software.

Molecular cloning and vectors construction

To further characterise the function of different form of FT in macadamia, we constructed the vectors for subcellular localisation and over-expressions of MacFTa/b proteins. Specific primers (MacFTa and MacFTb) were designed based on the FT gene CDS (coding sequence) of Macadamia (using the SnapGene). The cDNA was diluted to 30ng/µl. After this, MacFTa gene was amplified by using PCR and followed the thermal cycler programmes: 98°C for 30sec followed by 35 cycles of 98°C for 10sec, 50- 60°C for 30sec, 72°C for 15sec and 72°C for 5min. Amplification of MacFTb is different from amplification of MacFTa, and the following program: 98°C for 30sec followed by 35 cycles of 98°C for 10sec; 58-63°C for 30sec; 72°C for 20sec and 72°C for 5min. All PCR products were run on a 1.5 % agarose gel, and the target fragment was extracted by the QlAquick Gel Extraction kit based on the size of the band.

To construct the vector for subcellular localisation of MacFTa/b proteins, the Gateway cloning method was used. The target fragments were ligated into the pDONR221 vector by using Gateway® BP Clonase® II enzyme and transformed into Escherichia coli (DH5α). After incubation at 37°C, the single colony was picked from a selective culture on Lysogeny broth (LB)-agar plate (Kanamycin 50µg/ml) and incubate this on LB liquid medium containing 50µg/ml, then shook at 230rpm in 37°C chamber. The target plasmid was extracted by QlAprep Spin Miniprep Kit and ligated into the pGWSB452 vector (N-terminal GFP tag) using Gateway® LR Clonase® II, following transformed into Escherichia coli (DH5α). The single colony was cultured in 50µg/ml of spectinomycin LB-agar (Appendix B) plates in 37°C and then incubated in LB liquid medium with the same concentration of antibiotic. After this, the target DNA was extracted by QlAprep Spin Miniprep Kit and transformed into Agrobacterium tumefaciens GV3101. Finally, the agrobacterium was cultured on a selected LB-agar plate containing 50µg/ml of spectinomycin, 50µg/ml of gentamicin and 20µg/ml of rifamycin and then screened positive clones.

To create the over-expressing constructs of MacFT protein, the entry colony pDONR221::MacFTa/b was ligated into pMDC32, following transformed into Escherichia coli (DH5α). The single colony was picked up from selective cultured on LB-agar plates with 50µg/ml of kanamycin and then incubated in LB liquid medium with the same concentration of antibiotic. The extracted target DNA was transformed into Agrobacterium tumefaciens GV3101 by electroporation using the transformation apparatus (Bio-Rad). The agrobacterium was cultured and selected on LB-agar (Appendix B) plates containing 50µg/ml of kanamycin, 50µg/ml of gentamicin and 20µg/ml of rifamycin, and then positive clones were incubated in LB liquid medium. The target plasmid was extracted by the QlAquick Gel Extraction kit. Based on the size of the band, plasmids were confirmed and sent to a company (AGRF) for sequencing for further verity whether the construction of the vector is correct.

Leaf agroinfiltration of GFP-MacFT fusion protein in tobacco and pea transformation

Agrobacterium tumefaciens GV3101 harbouring the constructs 35S::GFP::MacFTa/b or 35S::GFP (used a control) were cultured over-night in LB medium with 50ng/µl Spectinomycin, 50ng/µl kanamycin, 10mmol/L MgCl2 10mmol/L MES-KOH(pH=5.6), 100µmol/L acetosyringone to the stationary phase

(OD=0.5-0.6) at 28°C. In addition, each expression constructs transformed into p19 (which is a suppressor of gene silencing from the tomato bushy stunt virus) and incubated the same culture medium as earlier to OD600=0.3. Agrobacterium tumefaciens and p19 were mixed and adjusted to the appropriate and centrifuged at 3000g for 15 min at room temperature. After centrifugation, the mixture was re-suspended in the infiltration buffer (Appendix B) to OD600= ~1. The agrobacteria cells were infiltrated with a 1-mL syringe without a needle into the 4-week-old leaves of N. benthamiana. After 3-5 days the infiltration, leaves were selected to detect GFP fluorescence by using a Laser Scanning Confocal Microscope at an excitation wavelength of 488nm and a 505-530nm band-pass emission filter. For pea transformation, we constructed 35S::GFP empty vector as control and 35S::GFP-MacFTa/b, and then we transiently expressed the 35S::GFP-MacFTa/b in pea L107 leaves instead of 35S::MacFTa/b to observe the phenotype. This process was used in the same agroinfiltration method and treated with a first open leaf from 1-week pea L107 seedlings. This treatment was repeated weekly until flowering. After 1.5 months, the phenotypes (such as flowering date and number of leaves) were observed to determine the potential role of MacFT in flowering.

Arabidopsis transformation by using floral dipping methods

To get transgenic Arabidopsis line, wild-type Arabidopsis was infected using the floral dipping method with the transformed agrobacterium (Zhang et al., 2006). Firstly, the agrobacterium harbouring 35::MacFTa/b was incubated into a 500ml LB liquid medium (Appendix B) with 50ug/ml of Kanamycin, 50ug/ml of gentamicin and 20ug/ml of Rifamycin at 28°C for two days and the OD is around 0.5. The agrobacterium culture was then centrifuged at 40,000g for 10 min at room temperature. After centrifugation, the cells were resuspended in a fresh 5% sucrose solution, and 0.02% of Brush wet was used to dip aerial parts of WT arabidopsis for 10 s. With this procedure, the dipped plants were covered with a plastic cover. Then we placed the dipped plants under high humidity and treated for 16-24h. After treatment, dipped plants have removed the cover and letting grow under the normal condition for one month.

Primer design

MacFTa/b primers were designed from macadamia sequence when available. Primers were designed using the Snapgene software and online Primer3 (http://primer3.wi.mit.edu/), optimised for produce length, primer length, annealing temperature, G/C content. For this research, details for all primers were presented in Appendix A, Table S2.

Expression analysis of relative flowering gene in macadamia

Preparation of RNA Extract

Dormant axillary buds and mature leaves samples from macadamia were ground into powder with a ball mill grinder for RNA extraction. Total RNA was extracted using Cetyltrimethyl-ammonium Bromide (CTAB)/SDS – based RNA extraction method (Barbier et al., 2019). Each Sample was added with 625µl of CTAB (Appendix B) and 25µl of Dithiothreitol (DTT) 0.5M and then incubated at 56°C for 5 minutes. After incubation, 65µl of 10% SDS was added into each sample. After centrifuging at top speed for 15 minutes at normal temperature, 450µl of clarified supernatant was transferred into a new 2ml 96 well plate, and the same volume of pre-cooled isopropanol as the supernatant was also inserted to each well. The plates were left at -20 degrees for 15 minutes and then centrifuged at 4°C for 50 minutes at top speed. The supernatant was discarded, and samples were washed in 70% ethanol and centrifuged at 4°C for 15 minutes at top speed. After drying, the pellets were resuspended in 60µl of RNase-free water and transferred to a slim profile plate for storage at -80°C. Besides this, 16µl of the RNA samples was taken for cDNA synthesis, regardless of the concentration.

RNA Quality Control

Eight RNA samples were randomly selected from the plate. The RNA concentration was verified with Nanodrop measuring the absorbance at 260nm. For determining RNA purity, 5µl of the samples and 1µl of loading dye mix were added in the 1.5% gel (0.7 g of agarose, 2.5µl of Red Safe gel stain, and 50 ml of 1 x Tris-acetate-EDTA (TAE), pH 7.5), and the gel was run for 20 minutes at 150V. The gel result was visualised using UV light.

cDNA Synthesis and Quantitative Real-Time PCR (qRT-PCR) Analysis

Following the manufacturer's instructions, the first-strand cDNA was generated by reverse transcription using 2ul of 5 x iScript Supermix (Bio-Rad) and 8µl of RNA. Then, cDNA as a working template was diluted to 1ng equivalent RNA in the RNase free water per µl. The analysis of the expression level using qRT-PCR was described by Udvardi et al. (2008). qRT- PCR was performed using 5µl of 1ng/µl cDNA, 1.5µl of 1mM primer (Appendix A, Table S1) mix and 3.5µl SensiFAST™ SYBR® No-ROX Kit (Bioline). PCR programs were as follows: 95°C for 2 minutes; 45 cycles of 95°C for 5s; 60°C for 10s; 72°C for 10s. After that, threshold cycle values and were calculated using CFX Manager software (version 3.1; Bio-Rad), and the analysis of qualification amplification was conducted by using the LinRegPCR program (http://linregpcr:nl/).

MacFT promoter analysis

The aim of this analysis is to identify putative transcription factor binding FT promoter in macadamia compared to arabidopsis. In order to achieve this, we used the FT promoter (5.7kbp) sequence from Arabidopsis. We searched the database in Tair and PlantPlan 3.0 to identify all possible Arabidopsis transcription factors that could bind to motifs in the Arabidopsis FT promoter sequence (Arabidopsis FT promotor database). After this, we then restricted our analysis to a subset of transcription factors relevant to floral initiation. By analysing MacFTa and MacFTb promoters, we identify binding sites corresponding to transcription factors which have been demonstrated to be involved in flowering in other plant models such as arabidopsis. Finally, we then compared the result to arabidopsis FT promoter to identify the differences which may be responsible for differential regulation of FT in arabidopsis and macadamia.

Results

The functional characteristic of FTa/b in macadamia

Identification of FT-homologous gene in macadamia

We isolated multiple sequences from the cDNA library prepared from macadamia flower bud, young leaf and shoots by using Geneious ver 11. To identify FT-homologous genes in macadamia, all the PEBP proteins from arabidopsis and these sequences corresponding proteins from macadamia were used to construct the phylogenetic tree in MEGA 7 following Maximum Likelihood phylogenetic analysed parameters with defaults setting.

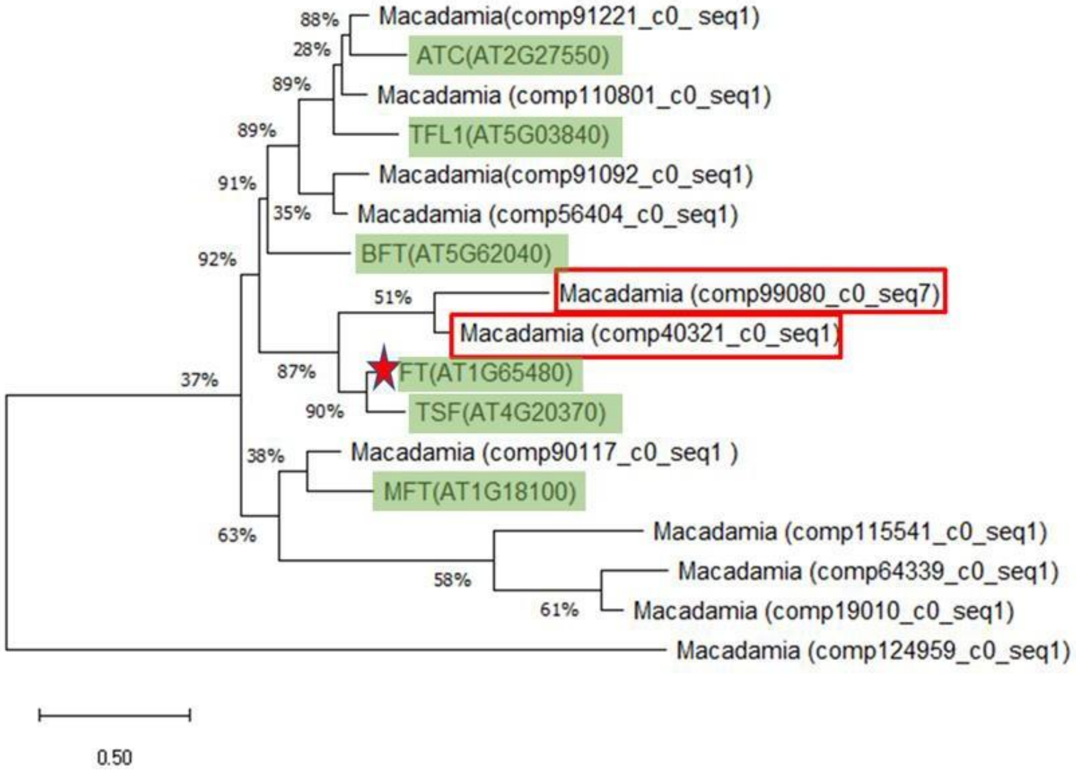

Figure 3. Phylogenetic analysis of macadamia FT-homologous protein by using the Maximum Likelihood method and JTT matrix-based model. The phylogenetic tree was constructed based on the amino acid sequence similarities in a PEBP family proteins from arabidposis and macadamia. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 6 amino acid sequences. There was a total of 405 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. FT family proteins from arabidopsis were marked in green solid box, and two possible FT proteins from macadamia were marked in red box. The FT protein in arabidopsis is marked in red star.

Macadamia (component 99080_c0_sequent7) and component 40321_c0_sequent1) were phylogenetically closely related with arabidopsis FT(AT1G6480) (Figure 3), suggesting that two FT- homologous proteins exist in macadamia.

Isolation of sequence analysis of two FT-homologous genes from macadamia

To gain insight into the possible function of the unidentified macadamia FT proteins, the sequences of the FT were used to search the Protein Families database of alignments and public database from NCIB (https://www.ncbi.nlm.nih.gov/). The CDS fragments of MacFTa/b were cloned by specific primers pair of these genes by PCR using cDNA library derived from macadamia leaves. We designated them as MacFTa and MacFTb for keeping then differentiated from both in the same gene family. FT homologues from rice (Oryza sativa), potato, maize (Zea mays), tobacco, jujube, Chinese white pear, peach, apple, Japanese apricot and arabidopsis, and 13 sequences from these plant species were used to construct a phylogenetic tree.

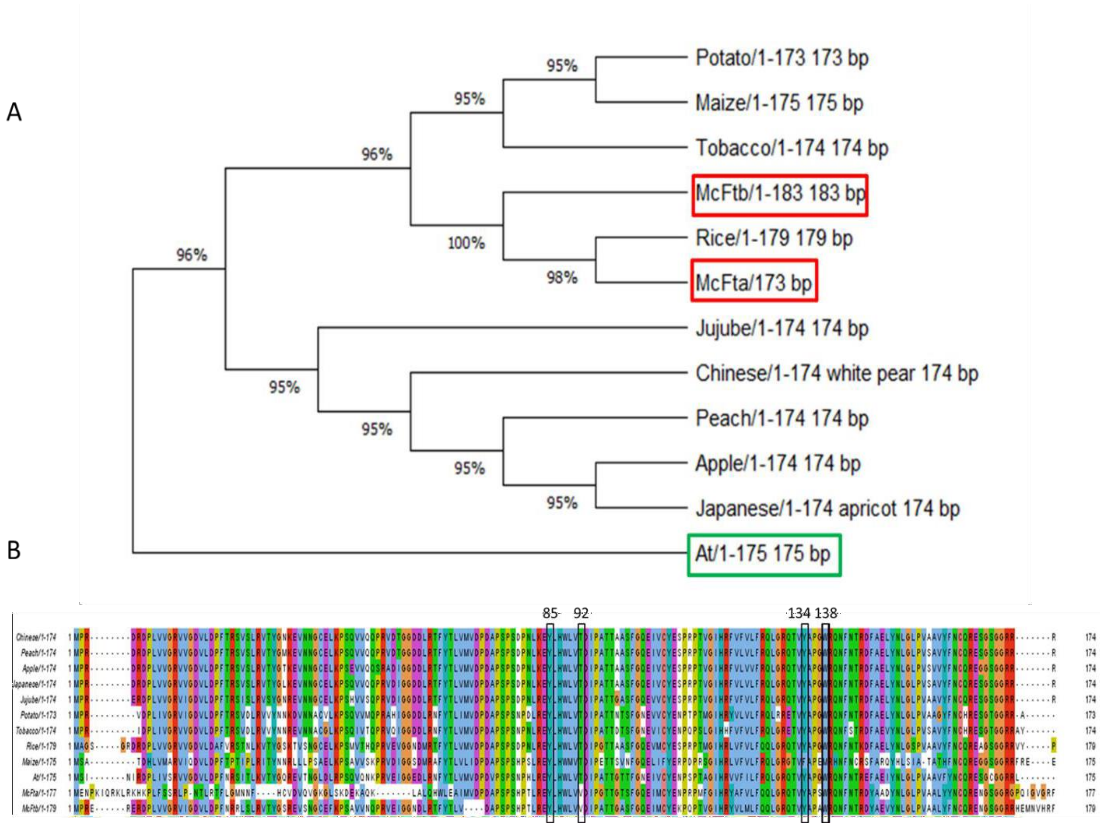

Figure 4. Phylogenetic analysis of FT-homologous genes in some species. A. FT-homologous proteins phylogenetic tree constructed with Maximum likelihood analysis of already published sequences from other species with identified FT related transcripts from macadamia. B. Sequence alignments of the FT-homologous transcripts with macadamia identified transcripts. Neigbor-joining tree for amino acid sequences aligned with ClustalX and rooted on AtFT175 is shown. The bootstrap values are indicated as a percentage above each branch. MacFTa/b were marked in red squares, and FT gene in Arabidopsis was marked in green squares. FT gene in rice was marked in red star. The residues were marked in black box.

The coding protein of MacFTa/b from Macadamia was 177bp and 179bp, respectively. Homologous alignment results showed that MacFTa/b sequence had extremely high homology with the sequences of FT protein from other species (Figure 4A). MacFTa/b shared 98% identity in amino acid sequence to its homologous in rice that is the highest among all species. The multiple sequence alignment revealed some macadamia specific amino acids (Figure 4B). At site 84, 134 and 138, tyrosine (Y), all species have tyrosine (Y) and tryptophan (W), respectively.

However, At site 92, valine (V) were MacFTa and MacFTb specific. Altogether, these results suggest that MacFTa and MacFTb are homologous to characterised FT from other species.

MacFTa/b promoter analysis

To further explore a role for both the MacFT genes in flowering regulation, a computational approach was used to identify transcription factors binding sites in 5.7kb upstream of the FTa/b in macadamia based on the FT gene transcription regulation in arabidopsis.

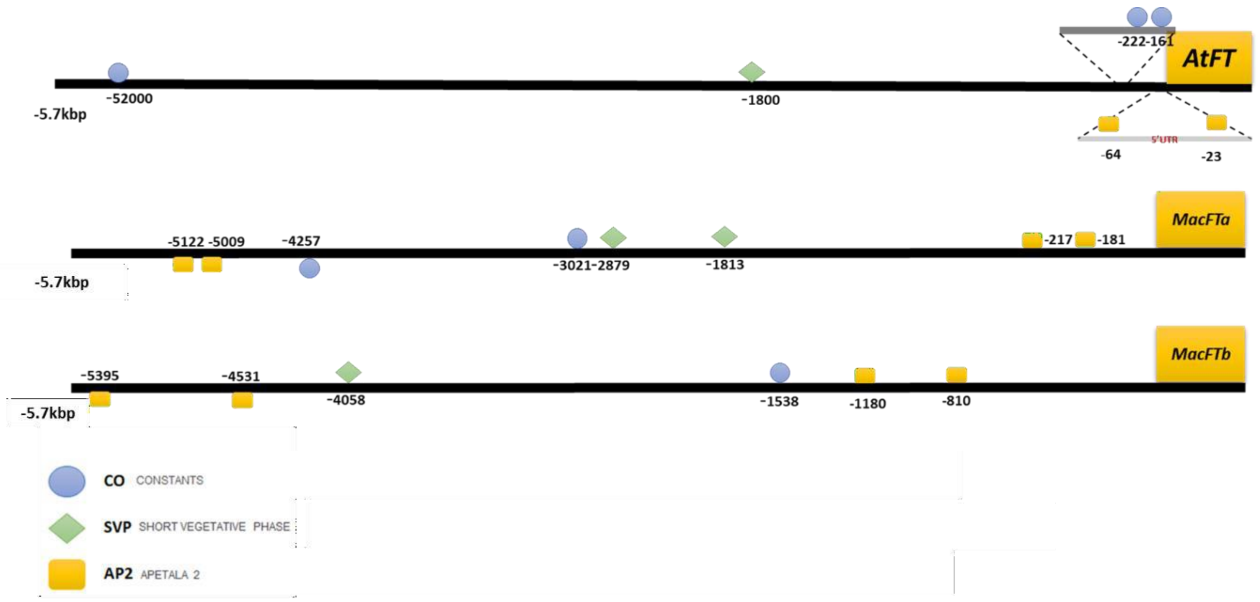

Figure 5.: In-silico 5.7kb upstream analysis of FTa/b in macadamia. A. The crucial transcription factors that can bind to the 5.7-kb region upstream of AtFT. B. Analysis of Macadamia FTa. C. Analysis of macafamica FTb. The analysis was performed by PlantPAN 3.0, the transcription factor binding motifs from arabidopsis were used to screen the promoter region of MacFTa and MacFTb. Binding sites are represented by the following symbols. Blue circle: CO; Green diamond: SVP; Yellow square: AP2.

In Figure 5, CO, SVP and AP2 motif were identified and localised in AtFT promoter and 5'UTR regions. Based on these TFs' motifs, the corresponding binding sites of TFs were identified in the 5.7Kb upstream of MacFTa/b. In comparison to AtFT promoter, MacFTa promoter showed more similar than MacFTb in the corresponding motif sites at a distance. In MacFTa promotor region, there are two AP2 motifs which were localised close to the transcription start site. Around 1800 base pair from the start site, the promoter of AtFT and MacFTa contained an SVP motif. Additionally, using the CO binding motif also found CO binding motif in MacFTa/b promoter regions, but the binding motif was located far from the transcription start site were observed among AtFT and MacFTa promoter. In addition, the motif distribution on MacFTb was a little bit different than a binding site of CO and SVP were localised at 1538bps and 4058bps, respectively. Altogether, CO, SVP and AP2 are important regulators of MacFTa/b expression that seems to be consistent with AtFT regulation.

Regulation of flowering in macadamia

Tissue-Specific Expression of FT-like Genes in Macadamia Tree

To analysis the expression pattern of MacFTa/b in different organs in macadamia, the buds and leaf discs of macadamia were selected for qRT-PCR analysis. MacFTa/b were constitutively expressed in different tissues of macadamia. We monitored the expression of FT preceding flowering in macadamia leaves (parenchyma and vascular) and buds. The expression of MacFT genes by qRT-PCR was performed in order to determine whether FT is produced on buds and how does FTa/b transform in macadamia.

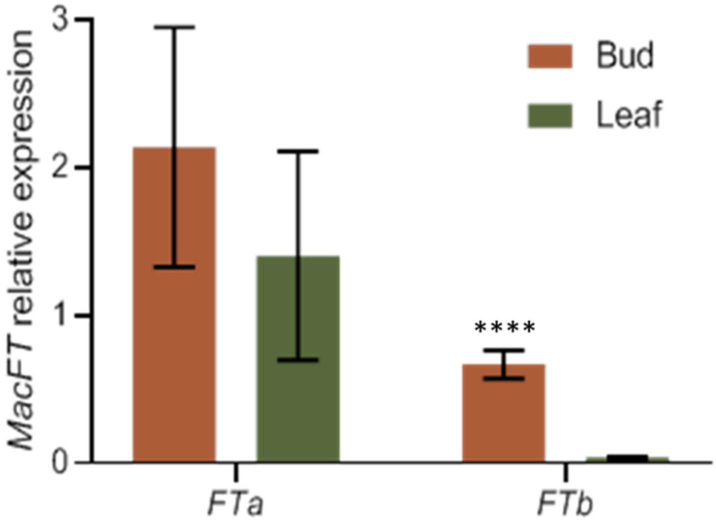

Figure 6. Expression patterns of MacFTa/b in different tissues of Macadamia with qRT-PCR. Brown = bud; dark green = leaf dices. Samples were harvested at 10-11 am at the end of July. Date are means ± SE from the results of 4 biological replicates. A two-tailed Student's t-test was performed to compare the significance between buds and leaf dices, and $p<0.0001$.

According to Figure 6. MacFTb was mostly expressed in axillary buds and a faint expression in the leaf dices, whereas MacFTa showed the main expression in leaves dices and buds with the higher expression in buds. Comparing MacFTa and MacFTb from different organs, the expression of MacFTa was obviously higher than that of MacFTb. In general, MacFTb in buds is mostly expressed.

Regulation of flowering gene expression

Following the identification of critical flowering related genes, their temporal expression pattern was examined using macadamia bud samples from cv741. We qualified these relative gene expressions using qRT-PCR and then compared these expressions from 2018 with 2019.

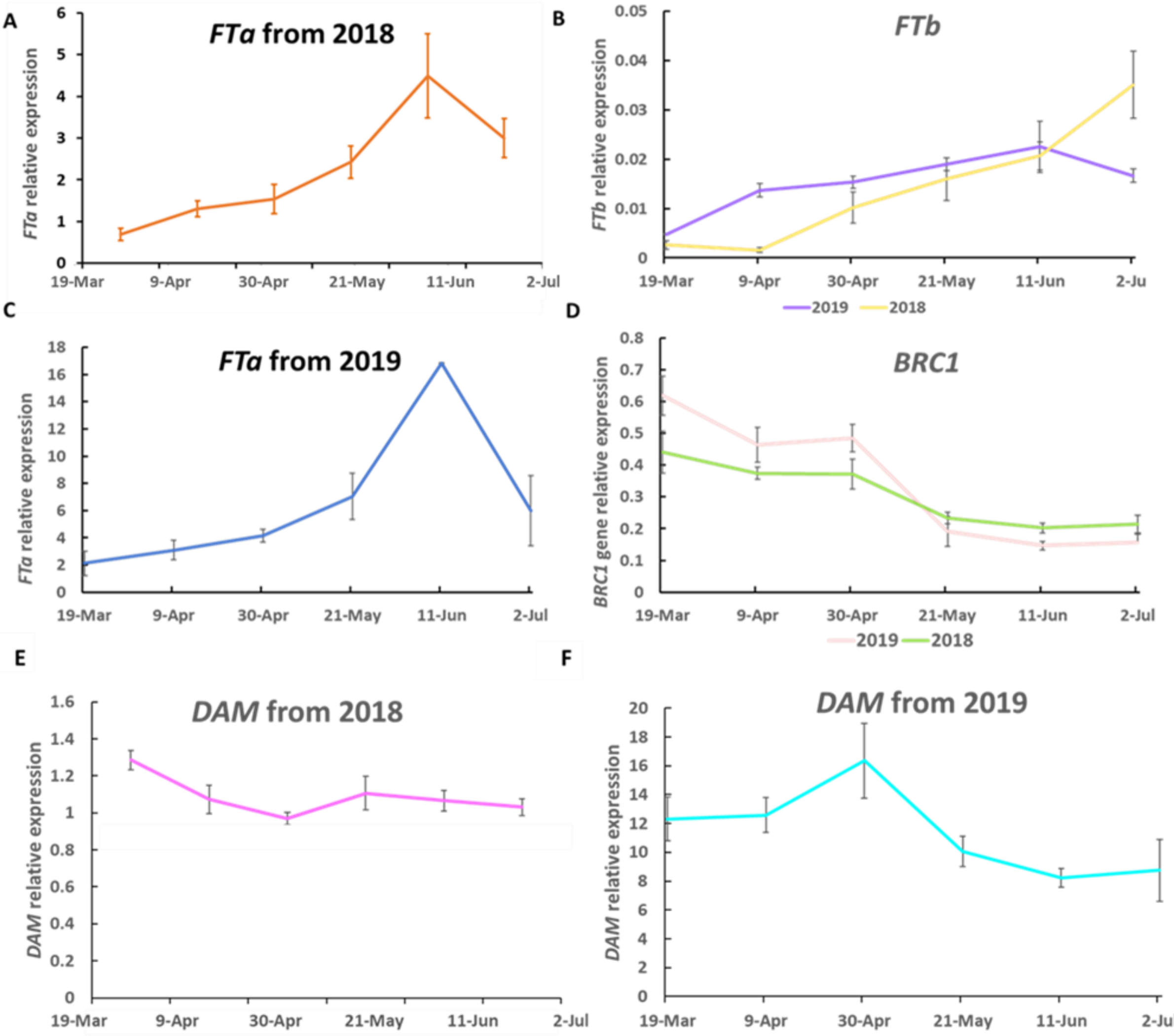

Figure 7. Expression of MacFTa, MacFTb, BRC1 and DAM genes by samples data (19/3, 9/4, 30/4, 21/5, 11/6 and 2/7) from macadamia cv741. A. MacFTa relative expression from 2018. B. MacFTb relative expression from 2018 and 2019. C. MacFTa relative expression from 2019. D. BRC1 relative expression from 2018 and 2019. E and F, DAM relative expression from 2018 and 2019, respectively. Measurements of 8 trees were averaged. Bars denote standard error of the mean.

The FTa expression in buds showed an overall upward trend with increase time in 2018 and 2019 (Figure 7A and Figure 7B). Additionally, from May to June, FTa expression in 2018 and 2019 has highly increased and then dramatically dropped in July. The difference was that FTa expression in 2019 is much higher than that in 2018. Basically, here FTb expression increased from May to June, and the trend was quite similar for 2018 and 2019. In general, the trend of expression of FTa in buds was relatively stable than that of FTb after two years of study.

Overall, there was a slight difference between 2018 and 2019 in the BRC1 expression pattern in buds (Figure 7D). 30th April was as the turning point. BRC1 expression in 2019 reduced more obviously than that in 2018 so that BRC1 expression in May 2019 was lower than that on May 2018. In addition, BRC1 expression has represented a general decline to June at which time the difference in the BRC1 expression was relatively consistent in buds (Figure 7D). However, in July, BRC1 expression in 2018 had the same increase as 2019.

The DAM expression was higher in 2019 than in 2018, and the trend of DAM expression in 2019 was completely opposite to that in 2018. Nevertheless, the trend of FT expression remained similar over the two years (Figure 7E-F). Thus, the expression of FTa gene was less affected DAM expression.

The effects of defoliation on flowering and expression MacFTa/b

To determine the effects of defoliation on flowering and FTa/b expression in macadamia buds, the defoliated experiment was conducted on the tree of cv741, during early May of 2019. We defoliated the branches back to the trunk. Axillary bud samples in control and defoliated branches were taken one month later from the first node after the bracts. A total count of racemes and new vegetative shoots were recorded observations on the location and marking in August. At the same time, the number of racemes in the normal branches also were recorded.

Following defoliation, we observed the racemes count of the branches were especially different. The racemes percentage of defoliation group was lower than those of the intact group (Figure 8A), but the racemes formation was no significant effect of defoliation.

As is shown in Figure 8B, analysis of changes in MacFTa and MacFTb expression revealed very significant treatment effects ($p<0.01$) that these gene expressions much increased after defoliation. Strikingly, DAM and AP1 expression weren't influenced under defoliation, but higher BRC1 expression was observed in the defoliated group. In general, defoliation may not affect flowering.

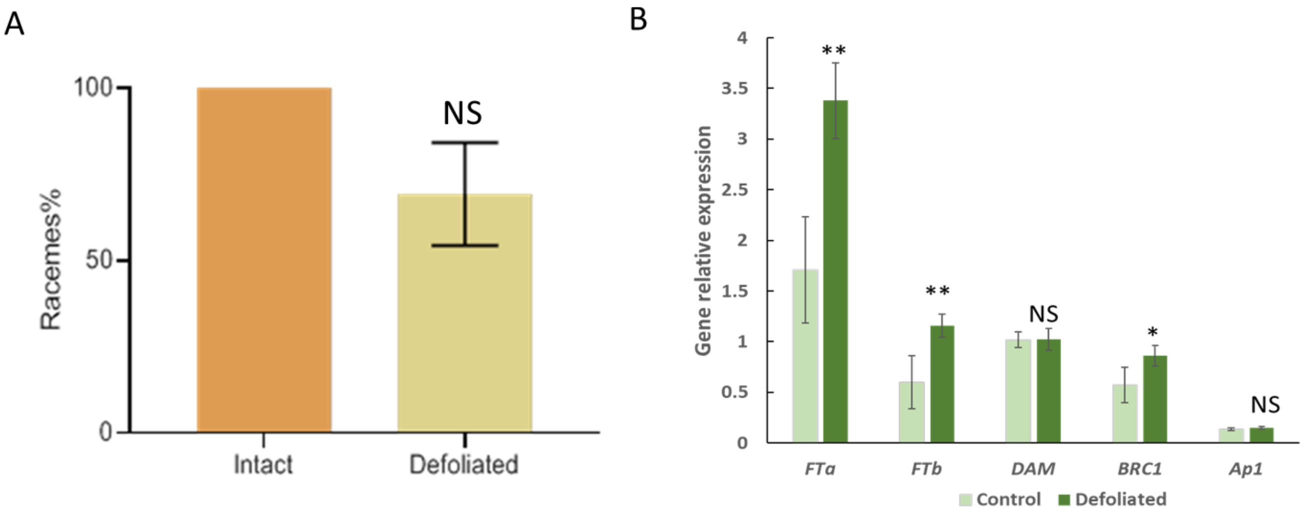

Figure 8. The influence of defoliation on the flowering of macadamia. A. Mean (±SE) percentage of racemes between normal and defoliated branches; B. Respective qRT-PCR analysis of the expression of FTa, FTb, DAM, BRC1 and AP1 in macadamia buds from normal and defoliated branches (n=4 replicate branches). A two-tailed Student's t-test was performed to compare the significance between defoliated and no-defoliated branches. The start signals indicate significant difference between treatments at $p<0.05$, and two stars mean $p<0.01$. NS= no significant different. Turmeric= the percentage of racemes from normal branches (as intact); Pastel yellow= the percentage of racemes from defoliated branches; light blue =gene expression in normal branches (control); green = gene expression in defoliated branches.

The role of FT in macadamia

Subcellular localisation of MacFTa/b Proteins

Since the construction of MacFTa vector didn’t work, and the study doesn’t have enough time, we focused on study of MacFTb. To determine the localisation of MacFTb at the sub-cellular level, we analysed the subcellular distribution of MacFTb protein fused to the GFP protein. Full-length MacFTb coding sequence was fused in-frame to the 3’ end the GFP gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The construct and empty vector pGWB452-GFP were introduced into N. benthamiana epidermal leaves stably via agroinfiltration, respectively. The plasmid map showed in Supplementary Material C, Figure S1. The signal of GFP-MacFTb fusion protein was detected using a confocal laser scanning microscope.

In Figure 9, GFP fluorescence of the control vector was evenly distributed throughout the observed cytoplasm, nucleus, plasma membrane and microtubules of N. benthamiana epidermal cells. Leaf cells expressing MacFTb-GFP fusion protein displayed a more localised GFP signal, limited to the nucleus and cytoplasm. Altogether these results demonstrate that MacFT is localised in the cytoplasm and nucleus as previously reported (Li et al., 2009a; Li et al., 2015), suggesting that MacFTb may play a role similar to FT homologues from other species.

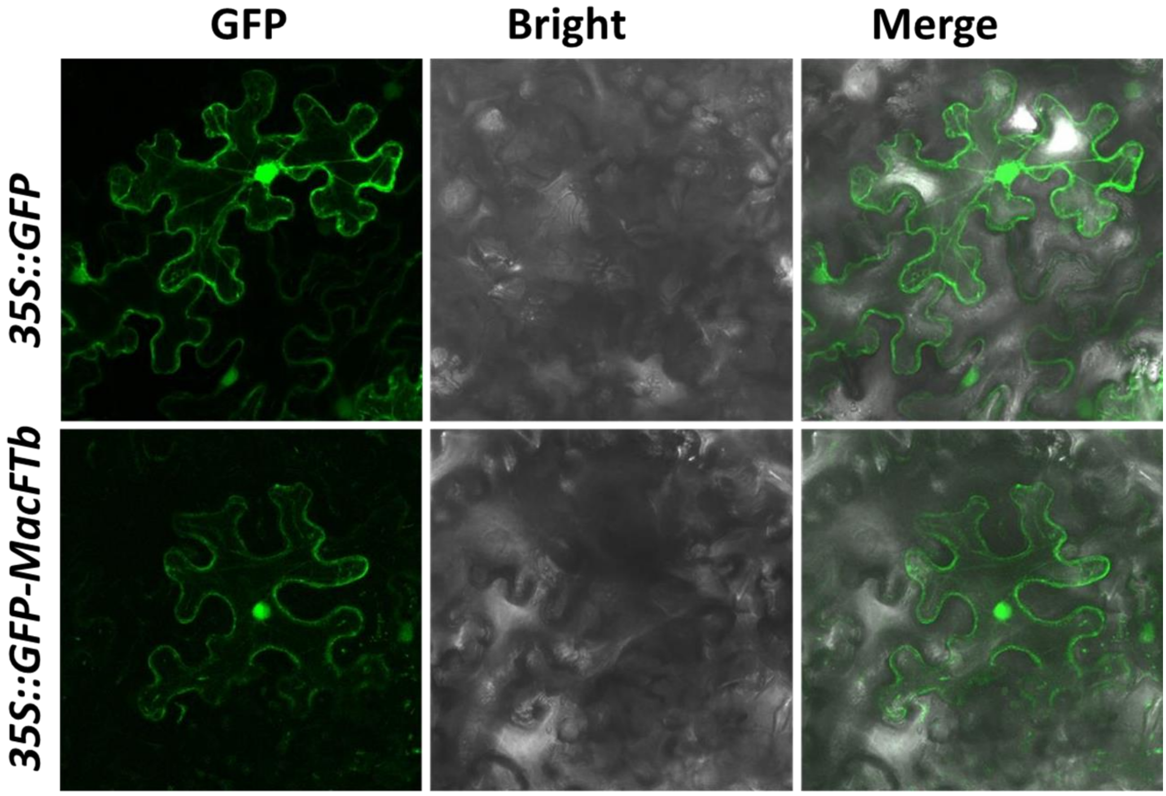

Figure 9. Subcellular localization of MacFTb. GFP alone and GFP-MacFTb fusion protein were transiently expressed in N. benthamiana epidermal cells, respectively. Flulrescence patterns of GFP-MacFTb fusion protein (bottom lane) were compared to those of the diffuse GFP control (upper lane) in N. benthamiana epidermal cells. GFP, GFP fluorescence; Bright, bright field; Merge, the merged image of GFP and Bright.

Transient overexpression of MacFTb in pea

To elucidate whether the overexpression of MacFTb affects in flowering regulation, MacFTb was transiently overexpressed in pea under the control of the strong and constitutive CaMV 35S promoter by agroinfiltration using 35S::GFP-MacFTb construct. The first open leaf of pea L107 seedlings was infected with Agrobacterium tumefaciens GV3101 carrying 35S::GFP-MacFTb and 35S::GFP respectively, and this infection was repeated weekly. We constructed up to 18 independent pea plants expressing MacFTb, 18 independent WT pea plants with GFP fluorescence of the control vector, and four independent WT pea plants (untreated plants). 1.5 months after last agroinfiltration we recorded the date of the first flower to open and counted the number of leaves from these plants. In addition, we could detect the GFP signal in the leaves, showing that the construct was expressed.

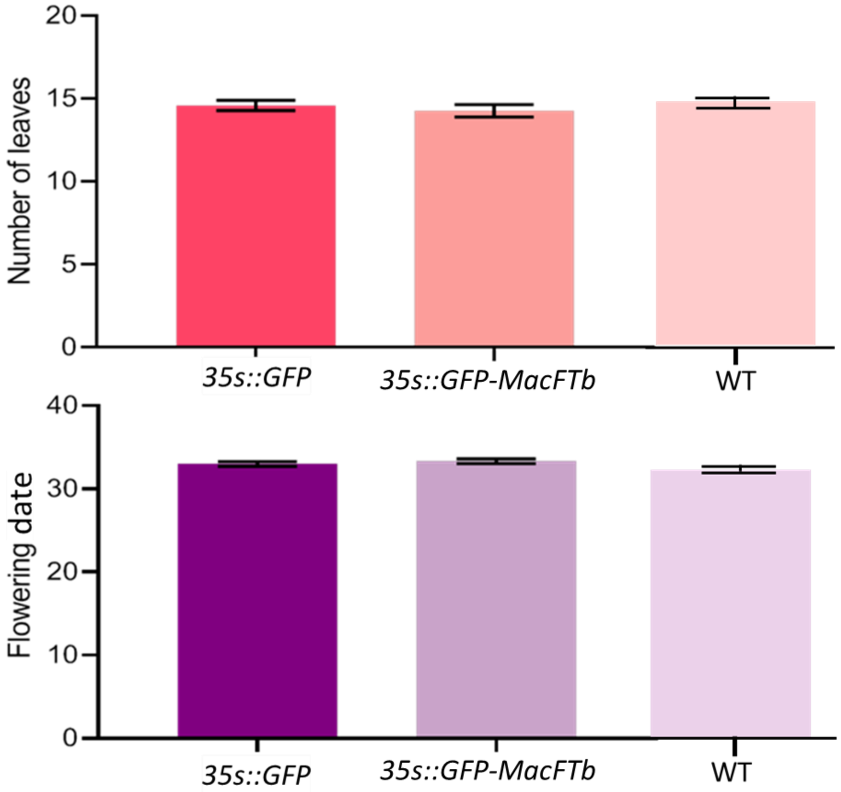

Figure 10. Impact of MacFTb gene transient expression in WT pea L107. MacFTb -overexpression plants containing 35S::GFP- MacFTb grew under LD condition (16h of light/8h of dark) using 35S::GFP and WT lines (untreated plants) as representatives (A) Comparison of the number of leaves (B) Comparison of flowering date. Measurements of 18 plants were averaged. Bars denote standard error of the mean.

When WT-type plants and 35S::GFP plants grew the opening of the first flower, it can be seen that the average number of leaves and flowering time were 14 and 33 days, respectively (Figure 10 A-B). This phenotype in WT plants was similar to that in 35::GFP-MacFTb transformed plants. Thus, overexpression of MacFTb in pea leaves didn't affect the flowering phenotype, and the GFP may have stopped the FT to be mobile.

Discussion

Characterisation of the macadamia FT-homologous gene family

Flowering time is regulated by a network of flowering genes. Several studies have demonstrated FT homologous genes are key inducers to promote the transition to flowering (Zeevaart et al., 2007). The current study has identified two homologues of FT genes in macadamia and compared them to homologs in arabidopsis and other close species (Figure 3 and Figure 4). The aim of the study was to identify the likely macadamia FT(s) and in order to better understand the role(s) in flowering regulation system. According to AtFT amino acid sequence at position 85, macadamia FTs had a tyrosine (Y) that was consistent with most other species (Figure 4B). The Y residue in the FT protein was identified that it actives flowering and control flowering time by gain-of-function studies (Hanzawa et al., 2005). Another defining position, at position 134 and 138 (numbered according to AtFT), tyrosine (Y) and tryptophan (W) in macadamia FTs sequences have also respectively been shown putative flower inductive function of these proteins. Tyrosine in all inducer FTs is localised at 134, while there is not in most repressor FTs (Ho and Weigel, 2014). In addition, the conserved tryptophan residue at position 138 is in all inducer FTs and not in most repressor FTs (Ho and Weigel, 2014; Wickland and Hanzawa, 2015). Taken together, it suggests that macadamia FTs are likely to be possible inducer of flowering initiation.

Promoter analysis of MacFTa and MacFTb

Transcriptional regulation plays a key role in gene expression, which is mostly regulated via the interactions between TFs and gene promoters. TFs binds to specific short promoter sequence (motifs) to demonstrate the features of gene expression (Liu and Stewart Jr, 2016). In arabidopsis, CO motifs bind to FT promoter at the proximal promoter to induce FT expression (Adrian et al., 2010). In addition, in response to long days, CO and CO-like protein physically interact with NF-YB and NF-YC in the 5.3 Kb upstream region of FT promoter (Ben-Naim et al., 2006; Wenkel et al., 2006). Interestingly, CO could associate with a CO/NF-Y complex, and proximal promoter could link the proximal promoter and distal promoter to form a chromatin loop structure (Cao et al., 2014). However, in the MacFTa and MacFTb promoter, CO binding sites were not located at the proximal promoter, suggesting that MacFTa and MacFTb expression may not be necessary to be induced by photoperiod in long days. Namely, MacFTa and MacFTb may be directly induced in buds to trigger flowering. In addition, there were SVP and AP2 binding sites at the MacFTa and MacFTb promoter regions (Figure 5), indicating that MacFTa and MacFTb may also be regulated by SVP and AP2. Thus, CO, SPV and AP2 regulate FT- homologous genes in macadamia may be similar to that in arabidopsis.

Expression pattern of FT-homologous genes in macadamia

Florigen is a long-distance signal hormone that can travel through the phloem vasculature from leaves to the SAM (Zeevaart et al., 2007). In arabidopsis, the transcripts of FT are expressed in leaves, in response to photoperiodic perception occurring in this organ, but FT encodes a phloem mobile protein that its protein moves to the SAM and acts as a florigen signal to promote floral induction (Corbesier et al., 2007). A number of studies in sunflower (Helianthus annuus) (Blackman et al., 2010), maize (Lazakis et al., 2011), tomato (Lifschitz et al., 2006), tobacco (Harig et al., 2012) and soybean (Kong et al., 2010) have found that the FT-homologous genes are highly expressed in leaves as well. However, in some species, FT-homologous genes were not restricted to leaves, and these genes were mostly expressed in buds. For example, an FT-homologous gene (MdFT1) in apple (Malus Domestica Borkh) was expressed mainly in the apical buds (Kotoda et al., 2010). In citrus, a flowering gene, FT was also expressed mainly in buds (Goldberg-Moeller et al., 2013). Additionally, the rose FT (RoFT) was specifically expressed in flower buds (Remay et al., 2009). In macadamia, the expression pattern of two FT-homologous genes (MacFTa and MacFTb) was mainly detected in buds and follow lower in leaves. In addition, MacFTb expression in buds is far exceeding that in the leaves (Figure 6). The transcripts of MacFTa in leaves and buds are higher than that of in MacFTb. This suggests that the variation in the expression pattern of the two FT-homologous genes in macadamia may lead to different roles of the two FT-homologous genes in flowering regulation system.

MacFTa/b, BRC1 and DAM expression in buds may affect different regulatory networks

The florigen FT promotes floral induction in annual plants as well as in perennial trees. Thus, FT can be considered as a marker for floral induction (Böhlenius et al., 2006; Yoo et al., 2010). Perennial trees are distinct from annual plants. That perennial trees have an annual flowering pattern for many decades once they enter a yearly flowering cycle. Recently, some studies about FT regulation in trees have been established. In poplar (Hsu et al., 2011), citrus (Pajon et al., 2017), and avocado (Ziv et al., 2014), FT-homologous gene expression is upregulated a few weeks before floral induction. FT- homologous gene expression patterns in these species are consistent with that in macadamia. In 2018 and 2019, the transcript of MacFTa in buds was much increased during floral induction which is from May to June (Figure 7A and C). Thus, MacFTa expression in the bud may be important for floral induction in macadamia.

BRC1 is an integrator, and it acts as a negative regulator of axillary bud outgrowth in arabidopsis (Aguilar-Martínez et al., 2007; Niwa et al., 2013). Moreover, modulation of BRC transcription can be affected by environmental and developmental stimuli, and the function of BRC1 also is necessary for response to these stimuli (Aguilar-Martínez et al., 2007). In this study, the expression of BRC1-homologous gene in buds was dramatically reduced during dormancy release which is from April to May for macadamia (Figure 7D), suggesting that BRC1 in macadamia may play a negative role during bud break.

DAM gene is identified in some woody species and involved in bud dormancy induction and flowering time regulation. In peach (Li et al., 2009b), Japanese apricot (Prunus mume) (Yamane et al., 2008), raspberry (Rubus idaeus) (Mazzitelli et al., 2007) and Japanese pear (Pyrus pyrifolia) (Ubi et al., 2010), DAM homologous gene integrates environmental cues to regulate the endodormancy release. DAM as an SVP-like gene is demonstrated to inhibit FT to induce dormancy. As illustrated in the results (Figure 7E-F), in 2018 and 2019, although macadamia DAM expression in buds didn’t show a clear expression pattern. This is contrasting with other reports in perennial plats (Horvath et al., 2010). This suggests that the role of DAM during bud break is not as important as for at perennial plants. However, this result is contrasting with the presence of DAM binding element on MacFTa/b promoter. Since the expression of these two genes increases quite early in our time-course, it is not unlikely that the decrease in DAM expression may have occurred prior to the first sampling. More experimentations need to be carried out to reveal the role played by DAM during bud break.

Defoliation may not affect flowering in macadamia

Defoliation experiment in plants remove not only the source of the inductive signal but also the source of photosynthate (McCormick, 2011). In model plants, the leaf is a main organ to receive a photosynthetic signal organ. Under long day, the photosynthetic signals induce CO accumulation in the leaves results in the activation of FT, and FT protein is transported in the sieve tubes to the buds to induce flowering (Zeevaart, 2008). In response to defoliation, recently fixed carbon from discrete storage organelles can be mobilised (Thornton et al., 2000). In early studies, defoliation affects the leaf growth rates but this only keeps on a few weeks (McCormick, 2011; McNaughton et al., 1983). These results suggest that the fixed carbon seems to be mobilised and utilised in other organ for plant growth. In this study, MacFTa and MacFTb expression in buds increased after defoliation few weeks, and defoliation didn't significantly affect the relative flowering intensity (Figure 8A). These results suggest that the two-homologous gene is mainly triggered in buds, and MacFT and MacFTb may also be short term regulated by independent floral signals, such as sugar. In addition, BRC1 expression in macadamia buds of the defoliated branch was reduced (Figure 8B), suggesting that the presence of decreased expression of BRC1 is consistent with that in Arabidopsis may be inhibited by sugar in buds. Furthermore, the expressions of DAM and AP1 gene in macadamia buds of undefoliated branches were same as expression in buds of defoliated branches, suggesting that the two genes during floral induction time may not be affect defoliation and mainly express in buds. Thus, MacFTa and MacFTb expression in buds may play an important for flowering in macadamia.

The localisation and overexpression of MacFTb

FT mRNA and its protein are of low abundance in plant tissues (Stadler et al., 2005; Zeevaart, 2008) In most studies, localisation and movement of FT have been demonstrated by using GFP or YEP fusion protein. In arabidopsis (Abe et al., 2005), rice (Tamaki et al., 2007), and tomato (Shalit et al., 2009), FT-GFP were observed in nucleus and cytoplasm. In addition, FT-GFP fusion protein in these plants has been detected to move through the phloem from leaves to the SAM as the position of flower formation. In this study, the 35S::GFP-MacFTb construction by using CaMV35S promoter fused to the MacFTb CDS was successful to be infiltrated in tobacco, and GFP-MacFTb was observed in nucleus and cytoplasm that is consistent with in these plants (Figure 9). The 35S::GFP-MacFTb didn't affect flowering phenotypes in pea leaves (Figure 10), indicating that MacFTb protein may not a mobile signal so that MacFT may not move from leaves to buds to trigger flowering. Or, agroinfiltration using the vector may not work in pea. For determining these assumptions, we have harvested the pea leaves samples and quantified the MacFTb gene expression. However, time is not enough to do this.

Conclusions and future directions

Molecular mechanisms controlling flowering regulation has been important elaborated in model plants. A thorough understanding of the mechanism of flower development process and regulation of flowering genes in horticulture crop is useful for improving crop yield. MacFTa and MacFTb, two FT homologous genes from *Macadamia* F. Muell, were identified and characterised. The deduced MacFTa and MacFTb proteins may be floral inducer and have highly conserved residues which were consistent with inducer FTs in other species. By analysing MacFTa and MacFTb promoters and 5'UTR, MacFTa and MacFTb may not be induced by photoperiod in leaves. In addition, CO, SVP and AP2 regulate the two genes in macadamia was similar to that in arabidopsis. The expression of MacFTa and MacFTb in buds may play an important role for floral induction. MacFTa and MacFTb expressions were few effects on the expression DAM and AP1 in buds, and BRC1 may inhibit dormancy release in macadamia. MacFTb is a transcription regulator, and the subcellular localisation is in the nuclear and cytoplasm. Transient overexpression of MacFTb in pea didn't cause the change in flowering date and number of leaves. To confirm the role of MacFTb, MacFTb expression will be quantified in treated pea leaves, and the experiment will be retest by creating fusions at the N-and C-terminal of MacFTb. When both orientations can work, a direct comparison of the two fusion proteins may reveal which of the two better determine the function of MacFTb. For determine the role of MacFTa, the study of MacFTa will be same as of MacFTb that GFP-MacFTa will be constructed to determine the subcellular localisation and overexpress in tobacco or pea under short days. To further understand the flowering regulation mechanism, the expression pattern experiment will be repeated next year. In addition, more samples will be harvested to use in defoliation experiment to improve the experimental accuracy.

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Supplementary Material A

Table S1. Primers used for qRT-PCR

Name	Sequence
MacBRC1-R	CCTTGCACTGGTTGAGCAAC
MacBRC1-F	GTAAGAGACCGCCGGATGAG
MacDRM1-R	AGCCAGTCGTAAACAGTGGG
MacDRM1-F	TGACGCCACCTTCTGCTAAG
MacAP1-F	GCCAGAACTCACCTCCCTTC
MacAP1-R	ACATCCTGTGCCTCTCTCCT
MacFTa-F1	TATGCTCCTGCTTGGCGTCA
MacFTa-R1	GTCCGCCGGATCCATTCTCT
MacFTb-F1	CAGCTTGGGAGGCAGACTGT
MacFTb-R1	GGTCCTCGTCCACCTGAACC
MacDAM-F	GGAGAAGAGCTGCAAGGGTT
MacDAM-R	TCTTTGGAGCGCGCTAATCT
MacEF1a-F	AAGCGTGGTTTCGTTGCTTC
MacEF1a-R	GCCTGGGTGGTTCATGATGA
MacMON1-F	CGGCATGTCGATGAGGATGA
MacMON1-R	CTTGCCAGAGTGACTCAGCA

Table S2. Primers used for cloning

Name	Sequence
attB1 - McFTb	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGCCAAGGGAAAGAGAAAGGGATCC
attB2 - McFTb	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGAATCTATGAACATTCATTTCATGTCTTCGTCCG
attB1 - McFTa	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGAAAACCCAAAAATACAAAGAAAGCTCAG
attB2 - McFTa	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAAAATCTACCAACACCTATTTGTGGTCC

Supplementary Material B

CTAB buffer

Chemical	Concentration (in solution)	500mL buffer
CTAB	2%	10g
NaCl	1.4M	40.6g
0.5M EDTA, pH 8.0	20mM	20mL
1M TRIS, pH 8.0	100mM	50mL
PVP40 (mw 40,000)	2%	10g
$MgCl_2$	5mM	0.24g
Water	-	Make up to 500mL

LB (PH 7.0)

-NaCl (10 g/L)
-Tryptone (10 g/L)
-Yeast extract (5 g/L)

LB agar (PH 7.0

- Agar (20 g/L)
-NaCl (10 g/L)

-Tryptone (10 g/L)
-Yeast extract (5 g/L

SOC medium (90ml)

-2g Bacto Tryptone
-0.5g Bacto Yeast Extract
-0.2ml of 5M NaCl.
-0.25ml of 1M KCl.

-1ml of 1M MgCl2

-1ml of 1M $MgSO_4$
-2ml of 1M glucose

-Adjust to 100mL with distilled H_2O
-Sterilize by autoclaving

Infiltration buffer

-10 mM $MgCl_2$
-10 mM MES (pH5.6)
-100 µM acetosyringone

Supplementary Material C

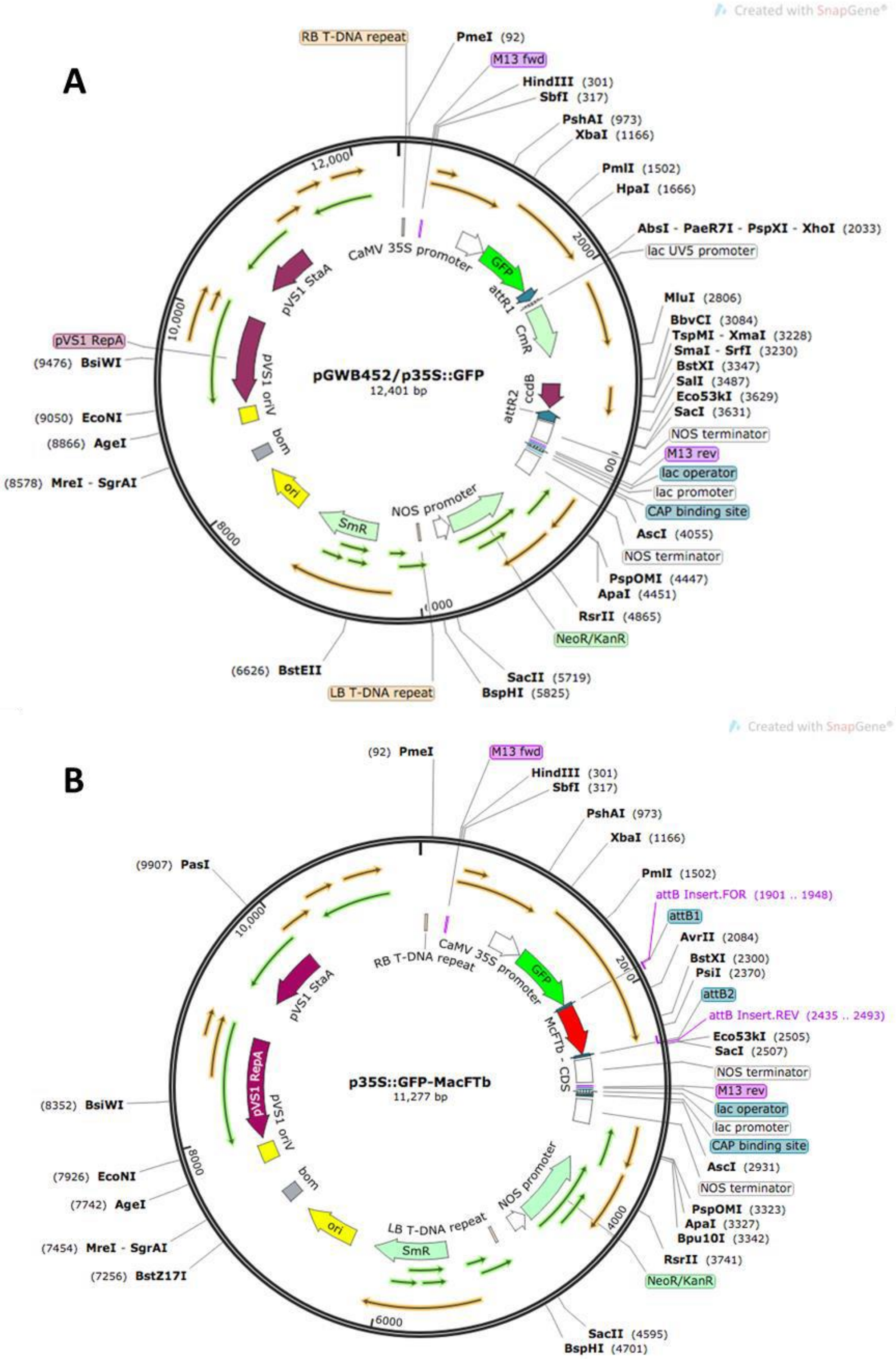

Figure S1. The plasmid map of *p35S::GFP* and *p35S::GFP-MacFTb* (backbone, pGWB452).(A) Plasmid map of pGWB452 (B) Vector containing full-length cDNA of *MacFTb* (in red colour) with a GFP (in bright green colour).In bacterial, Spectinomycin and Chloramphenicol are the bacterial resistances. In plant, Kanamycin is the selectable marker.