Final Report

Advanced production systems for temperate nut crops: Walnuts

Project leader:

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NSW Department of Primary Industries

Delivery partner:

NSW Department of Primary Industries

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Content

Summary

The Australian walnut industry has undergone a period of rapid expansion in the decade preceding this report, particularly in the Riverina region of NSW, but also in other areas where land and water are available. Most of the current, published walnut research is based on Californian production systems and climate, with minimal research completed in Australian walnut production systems. With a view to the future of Australian walnut production this project has examined a broad scope of topics. Importantly, this project has established an experimental walnut orchard for future research located in Orange, NSW, where the climate is more closely aligned to traditional walnut growing climatic conditions (particularly with cool winters and mild summers). Most of Australian walnut production occurs in the Riverina region of NSW; hence field trials investigating light and vigour management were carried out on commercial orchards in this region.

In this project we have used different platforms to:

- investigate pruning strategies to most efficiently optimise light management
- determine the effectiveness of plant growth regulators (i.e., Sunny®) for managing canopy growth
- evaluate advanced technology to model and guide high density orchard management and enable industry adoption of project outcomes
- define the nutrient and water budgets of walnut trees under Australian growing conditions and in two different climate regions
- show variable management practices of walnut orchards for different climatic conditions
- generate user friendly models to enable accurate orchard management using advanced technologies.

Keywords

Walnut, canopy, productivity, quality, climate, vigour, light, pruning

Introduction

The Australian walnut industry is the third largest temperate nut industry in Australia, preceded by the almond and macadamia industries. In the 2019-20 season, 12,800 tonnes of walnuts (in shell) were produced with a farmgate value of $55 million (Horticulture Innovation Australia Limited, 2021). Walnuts are primarily (65% of production in 2019-2020) produced in New South Wales (NSW), Tasmania (22%) and Victoria (9%), with small areas planted in Western Australia (3%) and South Australia (1%) (Horticulture Innovation Australia Limited, 2021). Walnuts are the seed of Juglans plants, including Persian and English walnuts. English walnuts have been cultivated to produce a range of edible nuts with each cultivar producing a slight variation in characteristics. Walnuts are high in Omega-3 fatty acids and are often referred to as brain foods. Globally the demand for nuts, including walnuts, is increasing.

Global walnut production is dominated by California (USA), Chile and China. China is the world's largest producer (871,850 metric tons in 2017/18, 42% of global production) and consumer of walnuts (annual consumption 0.28 kg per capita in 2016, with a total consumption of 403,719 metric tons of walnuts), and contribute 9% of walnuts to the global export market (Migiro, 2018). Increased understanding of the health benefits of walnuts, including benefits for cardiovascular health and brain function (Guasch-Ferré et al., 2018) and high Omega 3 fatty acids, has helped to push a gradual increase in demand. Australian walnut production does not yet meet local consumption demand, meaning there is potential to expand local production. Additionally, with global demand for walnuts increasing, there is potential to supply Australian produced walnuts to the export market.

Rootstocks are used for their vigour and/or soil suitability. Rootstock breeding programs, primarily at the University of California (UCLA) in the United States of America (USA), focus on rootstock development to control vigour, nematode resistance and disease resistance. Significant diseases affecting walnut rootstock varieties include crown gall and phytophthora. Approximately ninety per cent of Australia's walnuts are grown in the Riverina region of NSW near the towns of Leeton and Griffith. The climate in this region is semi-arid, with hot dry summers and cool winters but relatively little rain compared to other walnut growing regions. Other growing regions include northern Victoria (for example, near Shepparton and Mildura), Western Australia and Tasmania.

Walnut orchard management

In Australia, walnut orchards are generally planted at a relatively high density of approximately 300 trees per hectare, with 4 to 6 meters between trees and 7 to 8 meters between rows. This is relative to some spacings in California and older orchards that have 8 to 10 meters between both rows and trees. Growers can consult the work of Harold Adem (Adem and Jerie, 2004; Adem, 2010) and The Walnut Production Manual (Ramos, 1997) for information pertaining to walnut orchard management.

Often walnuts are planted in a hedgerow system, meaning that mechanical hedging (using large rotor blades) of trees is part of annual orchard maintenance. Alternatives include manual pruning by selective limb removal and tree training as a vase. To the best of our knowledge, hedging and selective limb removal are the most common practices in Australia, however there are likely as many different pruning methods as there are orchards.

Depending on orchard age, location and site layout, irrigation ideally involves dripper lines and a filtered fertigation system. Walnuts generally require nutritional inputs through fertigation systems (or individual farm equivalents) and foliar sprays, for example, boron.

Pest and disease management is important in Australian walnut orchards, although there are generally fewer severe issues compared with other countries. Walnut Blight (*Xanthomonas arboricola* pv. *Juglandis*, also known as Xaj), limb dieback (*Botryosphaeria vibis*) and phytophthora (*Phytophthora* spp.) are significant diseases requiring regular monitoring and management, primarily through sprays and good orchard hygiene.

Weed management is also an important consideration in a walnut orchard. There are many different approaches to weed management in the Australian walnut industry, including chemical, biological and manual control methods.

Why this project?

With a high likelihood of continuing industry growth and profitability, Australian temperate nut industries require more advanced production systems to align them with other horticultural cropping systems and future technology advancements. To address this, funding was successfully sought through the Rural Research and Development for Profit (RR&D4P) Scheme for a collaborative project “Advanced production systems for temperate nut crops”. There are three sub-projects to this collaborative work. The NSW DPI project focused on walnuts as a model crop for defining high density orchard management. The other two projects in this RR&D4P program focused on almonds as the primary model crop.

The almond and walnut industries are at different stages in their development; hence the work of each project is complimentary but differs between the two crops. For example, before this project, the almond industry already had established rootstock field trials that could be used in the almond plantings in the almond projects. However, the Australian walnut industry is in the early stages of rootstock diversification.

Higher density walnut planting requires a more definitive understanding of the crop physiology. The relationship between the amount of light absorbed by canopies and nut yields is well established on a whole tree basis, but poorly understood in relation to pruning strategies. This knowledge is essential for understanding tree establishment and canopy management in increasing yield. Understanding the relationship between canopy light management and crop productivity in high density orchards also influences the fertiliser and irrigation requirements of the crop.

Foundational research has been published on walnut production in Australia (Adem and Jerie, 2004), however more recently Australian walnut orchard management research and practices largely rely on Californian based research, broad indicators and limited understanding of the exact requirements of walnuts crops under changing Australian conditions, scientific and technological advances. To effectively manage high density orchards, we need to define some of the parameters of walnut growth. This project has used field trials (demonstration orchards) and small-scale pot and rootstock trials (shade house trials) to build on existing research and identify areas for future research that would be of benefit to the Australian walnut industry.

Methodology

Overview of experimental platforms

Walnut trees in pots in a greenhouse or planted at one of three field sites were used for the experimental work in this project. Table 1 provides a summary of the experimental platforms used in this project. Further detail is available in the detailed report (Appendix 1).

Table 1. Experimental site details. Varieties and rootstocks listed are only those used in this project and not necessarily the full range of varieties/rootstocks on each site.

Name and type of site		Street address	Manager
Shade house	Potted	1447 Forest Rd, Orange, 2800	NSW DPI
Experimental	Young	1447 Forest Rd, Orange, 2800	NSW DPI
Commercial	Mature	148 Colinroobie Road, Leeton, 2705	Commercial collaborator
Commercial	Young	2384 Tabbita Lane, Goolgowi, 2652	Commercial collaborator

Summary of experiments and methodologies

This project is a series of experiments that were conducted on the experimental platforms (Table 1) to address industry interests, as identified early in the project scoping. The experiments are summarised, including methods and measurements used in Table 2. Further details are available in the full report attached as Appendix 1.

Table 2. Summary of experiments, experimental platform used, method and measurements used and the reference to further detail in Appendix 1.

Experiment	Platform	Methods	Measurements used	Reference
Rootstock, density and regional tree growth comparison	Experimental and young commercial sites	Established experimental orchard with a variety of rootstock and scion combinations. Selected trees on young commercial orchard to measure annually.	Tree height Tree diameter near graft union Soil analysis Leaf nutrition analysis	Chapter 2
Using plant growth regulators for chemical management of walnut trees in Australia before planting, during early growth and at maturity	Young and mature commercial orchard	Application of Sunny ® plant growth regulator to mature and young walnut trees. The product was applied at three different rates at irrigation dripper points on two commercial orchards.	Canopy volume Stem and internode length Bud formation and catkin formation Leaf/leaflet size Yield and quality assessment	Chapter 3
Understanding the impact of selective limb removal on the penetration of light into walnut tree canopy using simple and advanced technologies.	Mature commercial orchard	Commercial implementation of selective limb removal and hedging pruning strategies.	Canopy light interception (photosynthetically active radiation under tree canopy) Yield and quality assessment	Chapter 4
Review of technology options for guiding orchard management	Desktop review	Identified and investigated possible technologies to guide orchard management.	Literature review Consultation with Agriculture service providers and data validation	Section 4.2.5
Improving soil health to maximise growth of walnut trees through soil ameliorants	Experimental orchard	Consulted industry representatives and literature to identify soil management options used by the Australian walnut industry. Conducted small scale experiment using key soil ameliorants identified through consultation process.	Literature review Interviews Soil analysis (chemical and microbial) Tree height Tree diameter at graft union	Chapter 5
An investigation into walnut nitrogen requirements for early biomass production	Shade house – pregerminated seeds	Supplied nutrient solutions with varying ratios of ammonium and nitrate to four rootstock varieties of pregerminated walnut seeds	Seedling germination time and height Biomass of stems, leaves, roots Leaf area Nutrient analysis	Chapter 6
Developed a method for non-destructive measurement of leaf area in walnut leaves	Mature commercial orchard	Measured the area a large sample of leaves and terminal leaflets collected from three different walnut varieties (n=500, 1250 and 2500 leaves for Vina, Chandler and Serr cultivars respectively). Determined strong correlation ($r=0.91$-0.95, $p<0.05$) between leaf and terminal leaflet area.	Leaf area	Section 3.3.4

Outputs

Detail is provided in full project report (Appendix 1), however the outputs from this project, briefly, are:

- Established demonstration site for future research. The NSW DPIE Research Services branch is compiling an information package and business case to attract further research investment and maximise the use of the demonstration site. In the immediate future the NSW DPIE Research Services Branch are developing resources to use the demonstration site as a "testbed" for future research projects relevant to the walnut industry and plan to replicate the site in the Riverina using potted walnut trees from this project. This/these demonstration sites are long term assets for the walnut industry and for ongoing walnut production research.
- APVMA Permit granted for the use of Sunny® in walnuts to help with vigour management. Vigour management is a considerable cost to the industry; hence management options were identified as a priority for investigation
- Report on the effectiveness of Sunny® on mature walnut trees, the impact of this on walnut yield and quality and a comparison with paclobutrazol in potted trees. This report includes an investigation into some of the observed impacts of chemical management practices on walnut trees.
- Assessment of pruning strategies in walnut orchards. Like vigour management, pruning is a significant cost to the industry. This report has addressed the impact of pruning on light interception, yield and nut quality. This work includes a short review of advanced technology options for walnut orchard management including a comparison of data collected manually and from an unmanned aerial vehicle.
- Review of soil management practices and small-scale demonstration of the impact of soil management options on soil health. This was completed at the demonstration site in consultation with industry collaborators for the purpose of improving sustainable management practices.
- Investigation of nitrogen source preferences of walnut seedlings as an indicator of the different uptake mechanisms and nutritional requirements. This was another small-scale experiment to guide future experiments.

Recommendations

This project aimed to investigate methods to improve advanced production systems for the future development of the Australian walnut industry. Extensive trials on experimental and commercial orchards and controlled pot trials have resulted in a greater understanding of tools and strategies for vigour and light management to optimise current and future orchard productivity, soil ameliorant strategies to increase orchard sustainability and improved our understanding of walnut nutritional requirements. Growers now have a chemical option for vigour management through the approval of an APVMA permit for the plant growth regulator Sunny® (active constituent uniconazole-p).

An experimental orchard was established at the Orange Agricultural Institute, which will be a valuable resource for the Australian Walnut Industry to support future research and provide objective, reliable outcomes without relying on commercial partners interrupting production

Vigour management

Multi-year experiments on non-bearing and bearing walnut trees of three different cultivars found the plant growth regulator, Sunny®, to be an effective vigour management tool. This will be advantageous for growers interested in chemical management options. Sunny® application significantly reduced the crown volume, stem length, internode length and leaf area of Chandler, Serr and Vina walnut cultivars. The reduction was greatest at higher application rates, including the manufacturer suggested rate of 1.5 L/ha. The reduction in tree vigour was not consistently related to the timing (autumn or spring) of Sunny® application. There were also no consistent differences in nut quality (nut size, kernel colour, weight, etc.) that were conclusively related to the application of Sunny®.

Walnut trees are naturally large vigorous trees, therefore optimising vigour management for commercial walnut production is a considerable area for further work. Further research opportunities would be:

- comparing Sunny® with other plant growth regulators, particularly products with paclobutrazol as the active constituent, to provide a greater suite of tools for managing vigour and allow growers to make choices about the costs, risks and benefits associated with different options
- investigating how different chemicals used on the same orchard interact, for example catkin deformities were noticed on the commercial orchard used in these experiments but could not conclusively be attributed to any one cause
- investigating the long-term effects of using plant growth regulators on walnut orchard management, including on tree reproductive physiology and growth patterns as well as the physical and chemical properties of the orchard soil.

Light management

We compared a selective limb removal pruning strategy (of interest to the industry collaborator due to extensive use and positive results in the Chilean walnut industry) to an unpruned strategy to assess the effect this had on light interception, yield and quality of walnuts. Selective limb removal proved to be an effective means of managing trees through manual pruning, and our results suggest that a long-term benefit of this pruning approach is likely. This is in congruence with the work in Chile, where selective limb removal strategies implemented early in orchard establishment provided long term (>5 y) benefit to yield. Additionally, the benefits from this approach are increased when combined with other management practices, including growth regulator application and increasing planting density to decrease time to a profitable yield.

Conversely, unpruned trees have produced higher and earlier yields than pruned trees in the Californian walnut industry. Reductions in pruning have also been linked to reduced water requirements, but this also means that orchard planting density needs to be reduced (fewer trees per hectare) for effective management.

The Australian walnut industry would benefit from further investigation into optimal pruning methods in more controlled settings without the need to prioritise commercial practices (including mechanical hedging). The experimental orchard established in this project would be an excellent resource for future pruning experimentation.

This component of the work also acted as an opportunity to test technology options for orchard management. Measurement of plant cell density via unmanned aerial vehicle detected variations in vegetative growth between pruning treatments, and the technology is an accessible option for walnut growers to use (compared to other options investigated). Further work is required using a controlled experimental area, without commercial limitations.

Soil and nutrient management

Using the experimental walnut orchard, we applied soil ameliorant treatments to small-scale plots and were able to assess the effect of these on soil health (including pH, CEC, nitrogen, carbon and microbial status). Compost was the most effective means of improving soil health, increasing the nutrient availability in the soil. By increasing the health of orchard soils, growers can provide their trees with more favourable growing conditions, potentially leading to stronger, more productive trees. Future research into the effect of soil ameliorants on walnut yield and tree growth would be beneficial to growers. Ideally this would be investigated from the perspective of adequately preparing orchards at the early establishment phase as well as increasing the health of already established orchards.

Walnut seedlings were supplied with different ratios of ammonium and nitrate in the first four months of growth. This experiment was a preliminary work to understanding the nitrogen needs of walnut trees. The four different varieties of walnut rootstocks used in this experiment responded differently to the nitrogen treatments, indicating different preferences for nitrogen sources. Understanding the nutritional preferences of crops is critical to maximising the efficiency of fertiliser application, therefore expanding this trial to trees of different ages (including trees planted in orchards) and using tools such as isotopically labelled nitrogen molecules would benefit growers. Isotopically labelled molecules allow us to understand the allocation of nutrients to different tissues of the plant, for example to the leaves or nuts. Experiments using this technique would benefit growers by indicating exactly how much nitrogen walnuts took up from the soil (ergo how much was lost/wasted) and where in the plant that nitrogen source went. This would ultimately enable them to refine their fertigation schemes for early tree growth and establishment differently to nut production, for example.

Experimental site – an investment into the future of walnut research

The establishment of an experimental orchard for future research at the Orange Agricultural Institute provided a range of results for this project, however, its main value will be to enable long term, manipulative trials to:

- optimise planting density by better understanding the effect of competition between tree canopies, root systems on orchard inputs, tree varieties, yield, nut quality and orchard operations.
- establish a system to responsively manage developing orchards to optimise commercial performance during all stages of growth and development.

This project has provided growers with new tools and resources for the development of new orchards and the advancement of existing orchards.

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Intellectual property, commercialisation and confidentiality

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

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We would also like to acknowledge the efforts of Lester Snare and Stephen Gottschall early in the project for their input into the establishment of the experimental orchard at the Orange Agricultural Institute (OAI), and the farm staff at OAI for their ongoing support to maintain this site for future research.

Appendices

Appendix 1: Full research report

Final report ST16002: Advanced production systems for temperate nut crops

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Project

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Project Partner

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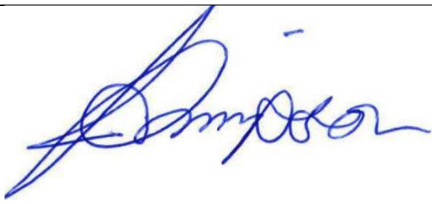
Signature	
Date	14/10/2021

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Key outcomes:

- Established demonstration site for future research. The NSW DPIE Research Services branch is compiling an information package and business case to attract further research investment and maximise the use of the demonstration site (Chapter 2). In the immediate future the NSW DPIE Research Services Branch are developing resources to utilise the demonstration site as a “testbed” for future research projects relevant to the walnut industry and plan to replicate the site in the Riverina using potted walnut trees from this project. This/these demonstration sites are long term assets for the walnut industry and for ongoing walnut production research.
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Table of contents

1. Introduction

1.1. Overview

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The edible component of the walnut is the kernel, which is contained in a hard shell within a fleshy green husk. Walnuts are sold as whole nuts, in shell or in various forms as kernel, including as meal (ground), small pieces, halves and wholes. In walnut varieties used for cultivation, the kernel and shell are the seed and the kernel develop into the new plant (Figure 1.1).

Figure 1.1. Walnut seedling.

1.1.1. Physiology

Walnuts are dichotomous tree crops, meaning the male (staminate) and female (pistillate) flowers are present on the same tree. However, walnuts are not self-fertile and walnut production requires orchards to be planted with a mix of cultivars and polliniser varieties. Walnuts require at least 60-70 chill portions to break dormancy and synchronise flowering.

1.1.2. Phenology, flowering and reproduction

In Australia walnuts are dormant from April to May and August to September. Staminate and pistillate (male and female) flowers bloom from August through September. Fruit set takes place within weeks and kernel filling occurs during summer. Walnuts are harvested from March to May, depending on the cultivar.

1.1.3. Global walnut production

Global walnut production is dominated by California (USA), Chile and China. China is the world's largest producer (871,850 metric tons in 2017/18, 42% of global production) and consumer of walnuts (annual consumption 0.28 kg per capita in 2016, with a total consumption of 403,719 metric tons of walnuts), and contribute 9% of walnuts to the global export market (Migiro, 2018). Increased understanding of the health benefits of walnuts, including benefits for cardiovascular health and brain function (Guasch-Ferré et al., 2018) and high Omega 3 fatty acids, has helped to push a gradual increase in demand. Australian walnut production does not yet meet local consumption demand, meaning there is potential to expand local production. Additionally, with global demand for walnuts increasing, there is potential to supply Australian produced walnuts to the export market.

1.2. Australian walnut production

Walnut species are planted for wood (*Juglans nigra*, commonly known as black walnut) or for producing the edible nut (*Juglans regia*, commonly known as English or Persian walnuts). This project, and report, refer only to the English or Persian walnut production for human consumption.

When grown for nut production, walnuts are grown as a grafted tree consisting of a rootstock (the below ground portion of the plant) and scion or cultivar (the above ground portion of the plant where the nuts are produced).

1.2.1. Rootstocks

Rootstocks are used for their vigour and/or soil suitability. Rootstock breeding programs, primarily at the University of California (UCLA) in the United States of America (USA), focus on rootstock development to control vigour, nematode resistance and disease resistance. Significant diseases affecting walnut rootstock varieties include crown gall and phytophthora.

In Australia, popular rootstock varieties are Paradox (a hybrid of *J. regia* and *J. hindsii*), *J. regia* L. and *J. hindsii*. The trees used for experimental and data collection in this project were primarily grown on these common rootstocks. We have also examined the less widely planted rootstocks, *J. sigillatta* Dode. (Iron walnut) and *J. hindsii* × *J. major* × *J. microcarpa* (Birdwoodton special, Sluiter, 2014) for a broader understanding of walnut rootstocks. *J. sigillata* is the second most planted rootstock in China (after *J. regia*) and commonly grown in Southern China (Wang et al., 2015). the Birdwoodton Special is a rootstock developed by an Australian grower to meet the needs of walnut growers in the Mallee soils of North-West Victoria (Sluiter, 2014).

1.2.2. Cultivars

Popular scion, or cultivar, varieties are Chandler, Serr, Howard, Tulare, Lara and Ashley, with Chandler the most planted production variety in Australia. Different cultivars produce walnuts with different quality properties (e.g. size and colour) and the end-user market determines the cultivar to be used. Additionally, cultivars vary in their suitability for production in different climates, largely due to timing of flowering and harvest. The phenology of different cultivars varies depending on the chill requirements, which varies slightly from cultivar to cultivar. This has been further discussed in Chapter 2 when describing the experimental field work used in this project.

1.2.3. Regions of walnut production

Approximately ninety per cent of Australia's walnuts are grown in the Riverina region of NSW near the towns of Leeton and Griffith. The climate in this region is semi-arid, with hot dry summers and cool winters but relatively little rain compared to other walnut growing regions. Other growing regions include northern Victoria (for example, near Shepparton and Mildura), Western Australia and Tasmania.

1.2.4. Walnut orchard management

In Australia, walnut orchards are generally planted at a relatively high density of approximately 300 trees per hectare, with 4 to 6 meters between trees and 7 to 8 meters between rows. This is relative to some spacings in California and older orchards that have 8 to 10 meters between both rows and trees. Growers can consult the work of Harold Adem (Adem and Jerie, 2004; Adem, 2010) and The Walnut Production Manual (Ramos, 1997) for information pertaining to walnut orchard management.

Often walnuts are planted in a hedgerow system, meaning that mechanical hedging (using large rotor blades) of trees is part of annual orchard maintenance. Alternatives include manual pruning by selective limb removal and tree training as a vase. To the best of our knowledge, hedging and selective limb removal are the most common practices in Australia, however there are likely as many different pruning methods as there are orchards.

Depending on orchard age, location and site layout, irrigation ideally involves dripper lines and a filtered fertigation system. Walnuts generally require nutritional inputs through fertigation systems (or individual farm equivalents) and foliar sprays, for example, boron.

Pest and disease management is important in Australian walnut orchards, although there are generally fewer severe issues compared with other countries. Walnut Blight (*Xanthomonas arboricola pv. Juglandis,* also known as Xaj), limb dieback (*Botryosphaeria vibis*) and phytophthora (*Phytophthora* spp.) are significant diseases requiring regular monitoring and management, primarily through sprays and good orchard hygiene.

Weed management is also an important consideration in a walnut orchard. There are many different approaches to weed management in the Australian walnut industry, including chemical, biological and manual control methods.

1.3. Why this project?

With a high likelihood of continuing industry growth and profitability, Australian temperate nut industries require more advanced production systems to align them with other horticultural cropping systems. To address this funding was successfully sought through the Rural Research for Profit Scheme for a collaborative project "Advanced production systems for temperate nut crops". There are three sub-projects to this collaborative work. The NSW DPI project focused on walnuts as a model crop for defining high density orchard management. The other two projects in the RR&D4P program focused on almonds as the primary model crop.

The almond and walnut industries are at different stages in their development; hence the work of each project is complimentary but differs between the two crops. For example, before this project, the almond industry already had established rootstock field trials that could be used in the almond plantings in the almond projects. However, the Australian walnut industry is in the early stages of rootstock diversification, hence further investigations into possible rootstocks are required and will be covered by aspects of this project.

Higher density walnut planting requires a more definitive understanding of the crop physiology. The relationship between the amount of light absorbed by canopies and nut yields is well established on a whole tree basis, but poorly understood at an individual bud/spur level. This knowledge is essential for understanding tree establishment and canopy management in increasing yield and guiding engineering solutions to harvesting, including shake-and-catch technologies (as under development by the University of South Australia). Understanding the relationship between canopy light management and crop productivity in high density orchards also influences the fertiliser and irrigation requirements of the crop.

Foundational research has been published on walnut production in Australia (Adem and Jerie, 2004), however more recently Australian walnut orchard management research and practices largely rely on Californian based research, broad indicators and limited understanding of the exact requirements of walnuts crops under changing Australian conditions, scientific and technological advances. To effectively manage high density orchards, we need to define some of the parameters of walnut growth. This project has used field trials (demonstration orchards) and small-scale pot and rootstock trials (shade house trials) to build on existing research and identify areas for future research that would be of benefit to the Australian walnut industry.

1.3.1. Aims

In this project we have used different platforms to:

- define pruning strategies to most efficiently optimise light management and fruiting points per square meter
- determine the effectiveness of PGRs (i.e., Sunny®) for managing excessive canopy growth
- evaluate advanced technology to model and guide high density orchard management and enable industry adoption of project outcomes
- define the nutrient and water budgets of walnut trees under Australian growing conditions and in two different climate regions
- show variable management practices of walnut orchards for different climatic conditions
- generate user friendly models to enable accurate orchard management using advanced technologies.

2. Experimental platforms

2.1. Overview of experimental platforms

Walnut trees in pots in a greenhouse or planted at one of three field sites were used for the experimental work in this project. This section of the report provides general details about the establishment, management and use of each of these experimental platforms.

Table 2.1. Experimental site details. Varieties and rootstocks listed are only those used in this project and not necessarily the full range of varieties/rootstocks on each site.

Name and type of site		Street address	Manager
Shade house	Potted, young and seed	1447 Forest Rd, Orange, 2800	NSW DPI
Experimental	Young	1447 Forest Rd, Orange, 2800	NSW DPI
Commercial	Mature	148 Colinroobie Road, Leeton, 2705	Commercial collaborator
Commercial	Young	2384 Tabbita Lane, Goolgowi, 2652	Commercial collaborator

2.2. Shade house

The shade house used in this project is located at the Orange Agricultural Institute (OAI), Orange, NSW (details in Table 2.1). Trees in the shade house (Figure 2.1) were used for experiments comparing different plant growth regulators (Chapter 3) and nitrogen nutrition (Chapter 6). A drip irrigation system was installed to provide each potted tree with water at a rate of 2 mm/hour for 3 × 15-minute watering times throughout the season. The irrigation was turned off during dormancy. Potted trees were 1–2 years old at the time of experimentation.

Figure 2.1. Potted walnut trees in a greenhouse located at the Orange Agricultural Institute. An irrigation system was later installed to provide every potted tree with equal water at a rate of 2 mm/hour.

Pregerminated seedlings were also managed in the greenhouse with a sprinkler-based watering system keeping the growth media moist during the season. The seeds were used to assess the growth reactions of young walnuts to different ratios of nitrogen sources (ammonium and nitrate). This is used as a proxy for understanding early fertiliser requirements of walnuts. Significantly more work is required to understand the nutrition requirements of walnuts throughout their life.

2.3. Field sites

Field sites are located at Orange, Avondale and Leeton in NSW and are also referred to as the experimental, young commercial and mature commercial sites, respectively. The details of the sites are summarised in Table 2.2.

The experimental site was established for the current project and will become both a demonstration site and a platform for future experimental work. The commercial sites are trees on commercial orchards (Figure 2.2) that were used to obtain tree responses, reproductive effects, yield and quality information under different vigour and light management scenarios, as discussed in Chapters 3 and 4. A range of rootstock and cultivar combinations are planted at each site (Tables 2.2 and 2.3), but all have Chandler Paradox as the primary combination and this was treated as a control.

Figure 2.2. Mature, commercial walnut orchard during dormancy (picture taken in 2016 of cultivar Serr on black walnut rootstock).

Table 2.2. Summary of trees planted at the experimental field site.

	Cultivar	Rootstock	Common name	Abbr[1]	Year planted
1	Chandler	*J. hindsii* × *J. regia*	Paradox	C-Pdx	2017
2	Chandler	*J. sigillata*	Iron	C-Sig	2019
3	Chandler	*J. hindsii*	NCB[2]	C-Hind	2019
4	Chandler	*J. hindsii* × *J. major* × *J. microcarpa*	Birdwoodton Special	C-BdS	2018
5	Lara	*J. hindsii* × *J. regia*	Paradox	L-Pdx	2018
6	Lara	*J. regia*	Persian/English	L-Reg	2018
7	Serr	*J. hindsii* × *J. regia*	Paradox	S-Pdx	2018
8	Howard	*J. hindsii* × *J. regia*	Paradox	H-Pdx	2017
9	Tulare	*J. hindsii*	NCB[2]	T-Hind	2018

[1]Abbreviation of cultivar-rootstock combinations [2]Northern Californian Black

Table 2.3. Commercial orchard cultivar and rootstock summary.

	Cultivar	Rootstock	Abbr	Orchard/s	Year planted	Experimental work
1	Chandler	Paradox	C-Pdx	Leeton	2007–08	Vigour control, light management
				Avondale	2014–16	Vigour control, regional comparison
2	Chandler	NCB	C-Nig	Avondale	2014–16	Regional comparison
3	Lara	Paradox	L-Pdx	Leeton	2007–08	Vigour control
4	Serr	Paradox	S-Pdx	Leeton	2007–08	Light management
5	Vina	Paradox	V-Pdx	Leeton	2007–08	Light management

According to the walnut bioclimatology model (Simpson, 2016), the three locations selected for field site establishment historically accumulate sufficient chill for walnut production (Figure 2.3).

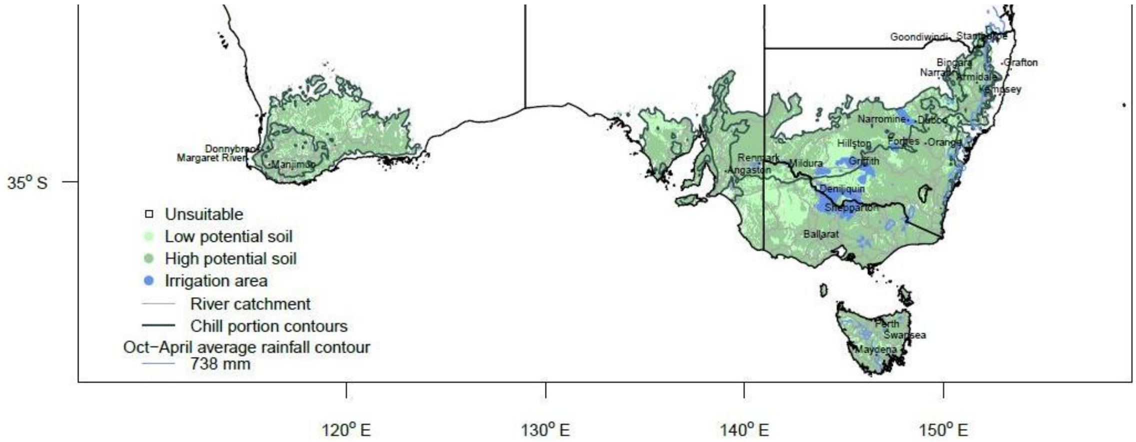


Figure 2.3. Walnut bioclimatology model generated during the project "Objective basis for temperate nut industry expansion (MT14041)" (Simpson, 2016).

Table 2.4. Summary of weather conditions at the three field sites used in this project including annual chill portions (White, 2019), rainfall and temperature ranges (Bureau of Meteorology, 2021).

Site		Year	Chill portions*	Annual rainfall (mm)	Temperature (annual, °C) Mean min	Mean max	Min	Max
1	Orange	2016	80	1345.0	7.8	18.9	-3.6	34.5
		2017	77	631.4	8.0	19.9	-4.0	39.2
		2018	78	619.5	7.5	19.7	-5.8	35.2
		2019	75	468.6	7.7	20.6	-3.9	37.3
		2020	80	1029.0	7.5	18.5	-3.1	36.7
2[1]	Leeton	2016	64	737.6	12.0	23.8	-0.1	44.6
		2017	61	292.0	11.3	25.2	-2.8	46.1
		2018	64	198.2	11.9	25.3	-1.4	44.7
		2019	62	265.8	11.4	24.1	-1.4	45.6
		2020	67	475.2	11.1	24.0	-0.9	45.2
3[2]	Avondale	2016	64	686.2	10.9	24.2	-1.4	45.2
		2017	62	345.0	10.1	25.5	-5.0	45.8
		2018	61	220.8	10.7	25.8	-4.5	45.7
		2019	61	280.0	10.8	25.6	-3.0	46.4
		2020	62	460.2	10.4	24.2	-4.0	47.2

[1] chill calculated from Yanco
[2] chill calculated from Griffith
*from 15 May to 31 August each year.

2.3.1. Experimental site (Orange, NSW)

The experimental site (Figure 2.4) is 1.27 hectares with three rows of buffer trees and six rows each for high density (HD) and ultra-high density (UHD). The HD spacing is industry standard spacing at the time of establishment, and considered high density compared to previous 10 x 10 m spacings (or thereabouts). The time to yield, and therefore time to return on investment (ROI), is directly proportional to the planting density of a walnut orchard (Adem and Jerie, 2004). This project has established a replicated Block design that will, over time, demonstrate the potential to decrease time to ROI. As the experimental site matures farm managers will monitor the trees and decide if the UHD Block needs to be converted to a HD Block by removing every second tree. By the time the site is at the point where this decision is required (well beyond the life of the present project) we will have demonstrated the ROI time difference between the HD and UHD site. Pending resource availability this will allow for an economic comparison between planting densities to help new growers make decisions when establishing their orchards.

Figure 2.4. Arial view of the area dedicated to the experimental walnut site.

Trees were planted in late winter and early autumn in 2017, 2018 and 2019 (refer to Table 2.5). The experimental site was prepared (deep ripped, limed and irrigation system established) in 2016, and the first trees were planted in 2017. Planting was completed by 2019.
The site is treated as three blocks. Two blocks (Blocks A and B, 0.78 hectares) are planted at high-density (HD) of 8 m between rows and 4 m between trees (8 × 4, 312.5 trees/ha, Figure 2.5) and the third block (Block C, 0.49 hectares) is planted at an ultra-high density (UHD) of 8 m rows and 2 m between trees (8 × 2, 625 trees/hectare, Figure 2.5).

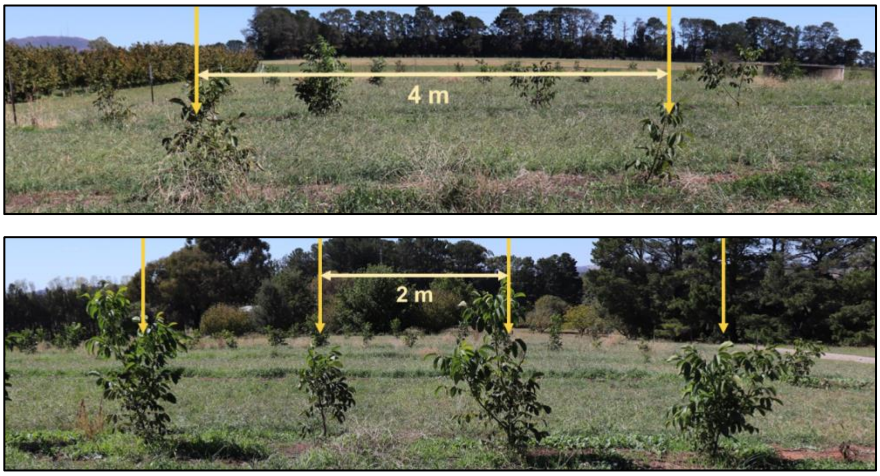


Figure 2.5. High- (top) and ultra-high-density trees planted at the experimental site in Orange, NSW.
The blocking design allows for assessment of variation across the site and within each of the blocks (e.g. due to slope and wind exposure). The Chandler-Paradox cultivar-rootstock combination is the most widely planted tree in the Australian walnut industry and is used as the control group for the experimental site. The site is surrounded by a row of Cisco (cultivar)-NCB (rootstock) walnut trees (Figure 2.6).

During the first years of growth, it is possible to treat all trees planted at this site as individual replicates as there is thus far no interaction between the trees. There are 60, single-tree replicates of Chandler-Paradox and 15, single-tree replicates of all other cultivar-rootstock combinations in the high-density and ultra-high-density blocks (Table 2.5). Future use beyond the scope of this project was considered when planning the site, therefore trees were planted as, what will become, multi-tree plots. Multi-tree plots account for future tree growth and the above and below ground interactions that would occur as a result of tree size.

Table 2.5. Rootstock and scion combinations planted at the experimental site in Orange, NSW.

Year	Rootstock	Scion	Source	Single-tree		Multi-tree plot size			
				HD	UHD	HD		UHD	
2017	Paradox	Chandler	Delta Tree Nursery	60	60	5	6	15	4
	Paradox	Howard	Bonds Walnut Nursery	15	15	5	3	15	1
2018	NCB	Tulare	Bonds Walnut Nursery	15	15	5	3	15	1
	BirdWN*	Chandler	Birdwoodton Estate	15	15	5	3	15	1
	Paradox	Lara	Birdwoodton Estate	15	15	5	3	15	1
	Regia	Lara	Bonds Walnut Nursery	15	15	5	3	15	1
	Paradox	Serr	Birdwoodton Estate	15	15	5	3	15	1
2019	Sigillata	Chandler	Bonds Walnut Nursery	15	15	5	3	15	1
	Hindsii	Chandler	Bonds Walnut Nursery	15	15	5	3	15	1

*BirdWN is the Birdwoodton special rootstock (Sluiter, 2014).

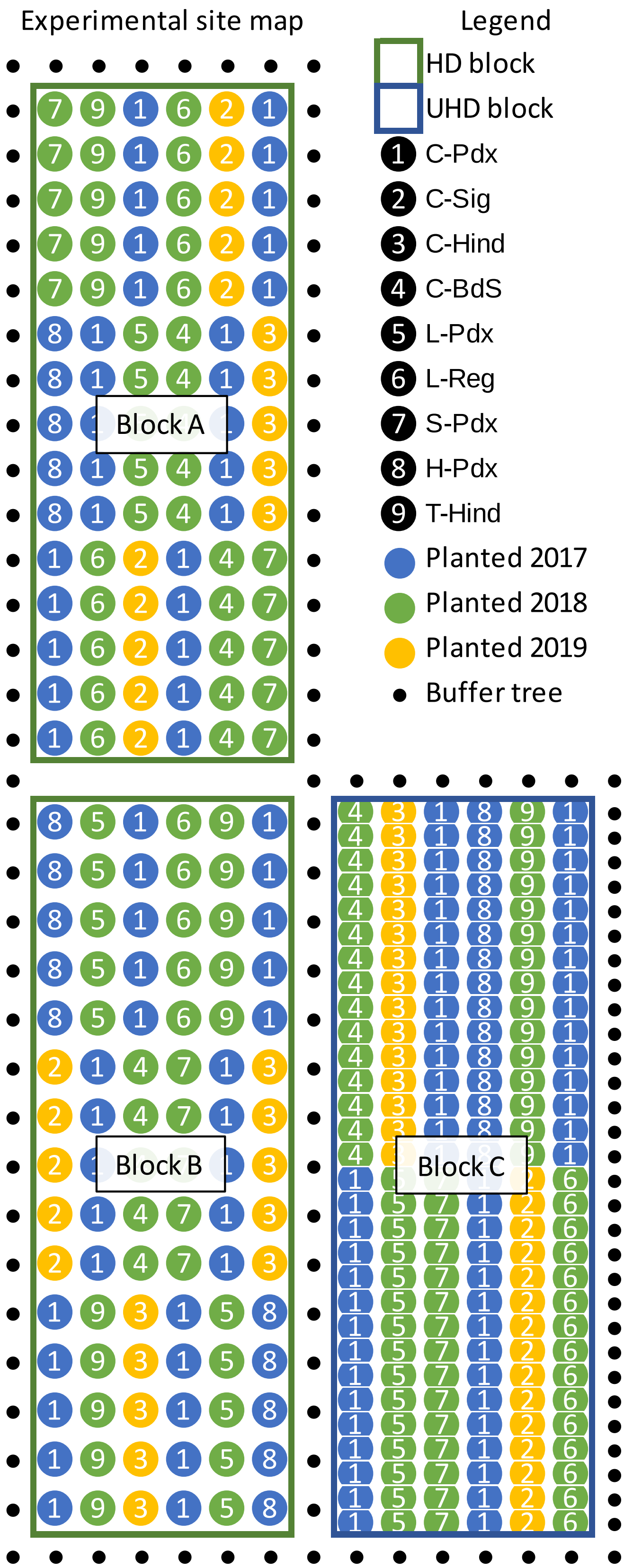


Figure 2.6. The experimental site layout. Numbers indicate cultivar-rootstock combinations and each circle represents a tree. Blocks A and B are high density (8 × 4 m spacing, green border) and Block C is ultra-high density (8 × 2 m, blue border). Due to the availability of planting material the site was planted over three years, the colour indicates year of planting, 2017 (blue), 2018 (green) and 2019 (yellow). Small black dots are the border/buffer trees.

The experimental site was established to address the following aims:

1. To understand the light and vigour control options for new and existing walnut growth from early establishment phase – this is a demonstration site, as the trees continue to grow, growers will be able to visit and note differences in the HD and UHD plantings. There are also 30 trellised trees in each planting density as a demonstration of tree architecture management strategies.
2. Determine means of maximising early return on investment – once the trees mature and are productive, they will become a demonstration of the concept of ultra-high-density plantings that are thinned once productive.
3. Provide a platform for future research and demonstration of project results and outputs.
4. Compare the early growth and performance of different combinations of rootstocks and scions in different climatic regions – this is discussed in Section 2.4.

2.3.2. Soil analysis

Soil pits were excavated on each Block at OAI in February 2020 (Figure 2.7) and soil samples were collected from the different horizons. An auger was used to collect samples from the wall of the soil pit. Five sub-samples were collected for each soil level and bulked as one large sample in a bucket. From this, small samples (300 to 500 g) were placed into Ziplock bags and prepared according to the requirements of the laboratories contracted to carry out testing.

Soil samples were also taken from the orchard using an auger to dig 0–150 mm and 150–300 mm depths to collect the sub-samples with bulking and sampling as described for soil pits. For each block (A, B and C), a soil sample was collected and analysed in the inter-rows (where there are no trees or fertigation) and near the base of the trees. Additional samples were collected for a soil ameliorant trial (Section 5). Samples were analysed for soil chemical, microbial and/or physical properties.

Annually samples were collected from ten points in an X-shape across each block of the experimental orchard. The chemical analysis results of these samples are presented in Table 2.7 and are used to guide fertigation programs. Soil samples were also collected from the commercial orchard field sites.

Table 2.6. Measurements of soil depth, pH, organic matter and macronutrients taken at three different soil layers (topsoil, subsoil A and subsoil B) from soil pits excavated on the three trial blocks at the Orange experimental site.

Block	Layer	pH	Total N (%)	Organic C (%)	P (mg/kg)	K (cmol(+)/kg)	Depth (mm)
A	**Top**	5.75	0.12	0.89	6.15	1.10	0-160
	Sub A	5.53	0.04	0.34	4.00	0.41	160-700
	Sub B	5.30	0.06	0.38	14.43	0.65	700+
B	**Top**	6.47	0.12	1.00	5.97	0.73	0-180
	Sub A	5.63	0.04	0.30	16.80	0.53	180-510
	Sub B	5.00	0.04	0.32	11.27	0.52	510+
C	**Top**	5.57	0.09	0.76	11.03	0.71	0-190
	Sub A	5.07	0.07	0.57	16.67	0.58	190-520
	Sub B	4.87	0.03	0.19	8.90	0.57	520+

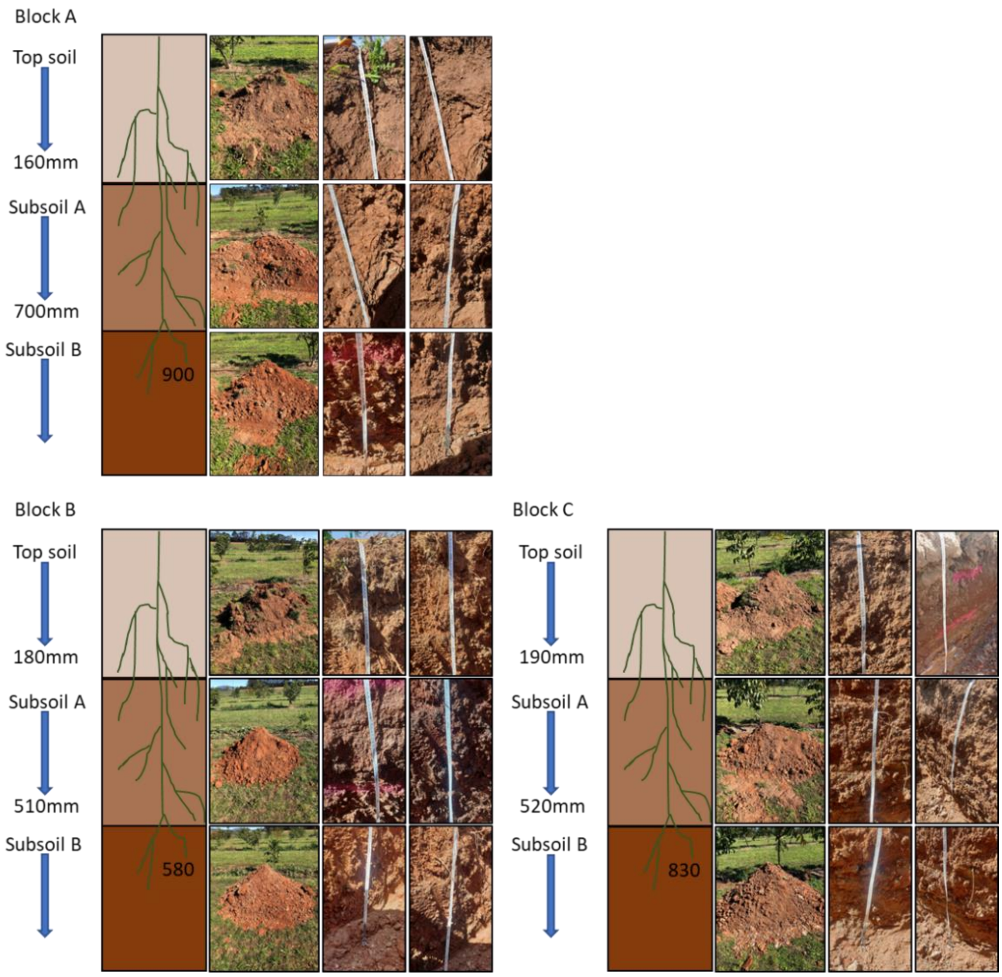


Figure 2.7. Soil pit visual summaries for Block A, Block B and Block C. The scale to the left of each set of mages is a summary of the approximate depth of each horizon and of the deepest visible root. Chemical and physical analysis of soil and leaves were undertaken annually throughout the project. Results representative of annual soil chemistry are summarised in Table 2.7.

Table 2.7. Summary of key soil nutrient test results from autumn 2020 soil samples in the root zone. [1]Electrical conductivity (EC). [2]KCl Extractable. [3]Walnut production manual (Ramos, 1997). **Green** text indicates adequate levels where critical values are available, **blue** text indicates low levels where critical values are available and black text indicates values for which no critical values are available.

	Block	**A**		**B**		**C**		**Critical**
	Depth (mm)	**0-150**	**150-300**	**0-150**	**150-300**	**0-150**	**150-300**	**values[3]**
pH ($CaCl_2$)	pH units	**6.00**	**7.00**	**6.50**	**7.00**	**6.30**	**7.00**	~6-7
EC[1]	dS/m	**0.08**	**0.11**	**0.08**	**0.10**	**0.10**	**0.13**	<0.15
Bray phosphorus	mg/kg	**6.10**	**13.00**	**14.00**	**18.00**	**13.00**	**23.00**	30-50
Organic carbon	%	**0.76**	**1.10**	**0.69**	**1.20**	**0.82**	**1.40**	>2.00%
Total nitrogen	%	0.08	0.12	0.09	0.14	0.10	0.16	NA
Ammonium-N[2]	mg/kg	1.40	3.20	2.20	2.50	1.2	2.2	NA
Nitrate-N[2]	mg/kg	**18.00**	**9.80**	**16.00**	**11.00**	**22.00**	**16.00**	>20.00
Potassium	cmol(+)/kg	**0.63**	**0.63**	**0.73**	**0.77**	**0.82**	**0.97**	>0.50
Boron	mg/kg	0.48	**0.56**	**0.53**	**0.66**	**0.54**	**0.6**	0.5-4
Calcium	cmol(+)/kg	**6.80**	**8.10**	**7.20**	**8.50**	**6.90**	**12.00**	>5.00
Magnesium	cmol(+)/kg	**1.20**	**1.50**	**1.60**	**2.10**	**1.20**	**1.20**	>1.60
Zinc	mg/kg	**28.00**	**37.00**	**40.00**	**39.00**	**39.00**	**40.00**	1-200

2.3.3. Site management and maintenance summary

Pest and disease management

Walnut Blight (*Xanthomonas arboricola* pv. *juglandis*, Xaj) significantly affected trees at the experimental site in the 2020–21 season, which started with a relatively wet spring. Copper was applied every 10 days throughout the season as a reactive response to maintain levels. For the 2021–22 season a strict spray program has been devised and is underway to control future incidences of Walnut Blight.

Pruning

In early spring 2019, just before the end of dormancy, the trees were pruned in a modified central leader method. We headed (removed the apical bud) trees, leaving enough height to promote lateral growth but did not head small trees to encourage vertical growth (i.e. height) this season. Strong limbs were kept, and we aimed to make a spiral pattern of scaffold limbs and remove any potentially weak angles and allow for maximum light interception into the canopy. A trellis system was installed on one row per block, for demonstration purposes in the years following this project.

Weed management

Chemical and manual pruning methods were used to maintain the experimental orchard. Key weeds like fleabane (*Conyza* spp.) were removed by hand and disposed of. The rows were sprayed with a hand sprayer with a range of herbicides to treat the diverse weed species. These activities were carried out by the farm staff based at the Orange Agricultural Institute.

2.3.4. Nutrition management

The experimental site is continuing to be fertigated as per industry standards. Tables 2.8 and 2.9 summarise the fertigation program in the most recent seasons. When defining our fertiliser program, we referred to a commercial scale fertigation program provided early in the project by the project partners (then Websters Ltd) and to the scheduling suggested in the Walnut Growing Manual (Ramos, 1997) and adjusted for tree age, location and soil chemistry. Boron spray (2.6 kg/ha) was applied in 2020-21 season. We installed a simple fertigation system that can be scheduled to ensure water availability. Nutrients are added via a vacuum pump system.

Table 2.8. Fertigation product summary for OAI experimental site.

Product	Nutrient composition	Quantity	
		2019-20	**2020-21**
Campbells Diamond Blue	19% nitrogen, 2.5% phosphorus, 17.1% potassium, 3.1% sulfur, 1.5% magnesium, 0.04% iron, 0.02% manganese, 0.01% boron, 0.02% zinc, 0.011% copper, 0.001% molybdenum	125.0 kg	125.0 kg
Campbells NitroCal	15.5% nitrogen, 19.3% calcium	125.0 kg	125.0 kg
Maximum Urea Lo-Bi	46% nitrogen	025.0 kg	050.0 kg

Table 2.9. Total nutrient application from October 2019–April 2020. Nutrients were applied via fertigation using Diamond Blue, NitroCal and Urea.

Nutrient	Quantity (kg/ha)	Nutrient	Quantity (g/ha)
Nitrogen	41.38	Iron	37.88
Phosphorous	02.37	Manganese	18.94
Potassium	16.19	Boron	09.47
Sulfur	02.94	Zinc	18.94
Calcium	18.28	Copper	10.42
Magnesium	01.42	Molybdenum	00.95

2.3.5. Pruning and staking

Suckers were removed throughout the latter half of the 2019–20 growing season and trees were pruned to the central leader industry standard system in April–May 2020. All trees were tied to steel stakes, except for one row per block that was trellised for comparison purposes. Pruning is an ongoing process, some of the key concepts implemented in the latest pruning are shown in Figure 2.8.

Figure 2.8. In April–May 2020 the young walnut trees at OAI were pruned and staked. Left to right: a tree tied to a steel stake in the UHD trial block; branches posing competition to the central leader have been removed; strong ~90° angle lateral branches were retained; and the sharp angle heading cuts to promote strong lateral growth.

2.4. Analysis of Horizon 1 plantings (high-density)

Growth measurements at the Orange experimental site and of young walnut trees in a commercial orchard at Avondale were taken annually. Comparing regional growth using this data set does not allow us to define the key factors that affect tree growth in the different regions. Uncontrollable (at least within the scope of this project) confounding factors means that only general, broad links can be made between location and growth.

This data set, and the experimental sites, are valuable tools to evaluate the early growth habits of some cultivars less frequently planted in Australia and commonly planted varieties.

2.4.1. Summary of growth data

There is a significant difference (ANOVA, Tukey, $p<0.05$) in the growth rate (measured as an increase in diameter) of the rootstocks and cultivars between Blocks A and B at the OAI trial site, but not tree height (Figure 2.9). Future work on this experimental site, must treat Blocks as additional factors in experimental designs to avoid confounding the results. This measurement and subsequent result show the validity of the blocking design of the experimental site adding ongoing value to future projects and experimentation.

Mean tree height in Block B was significantly shorter ($p<0.05$, -37.86cm) than that of Block A at (172.93 cm and 210.79 cm, respectively). Mean tree heights for each cultivar-rootstock combination and each block are summarised in Table 2.10.

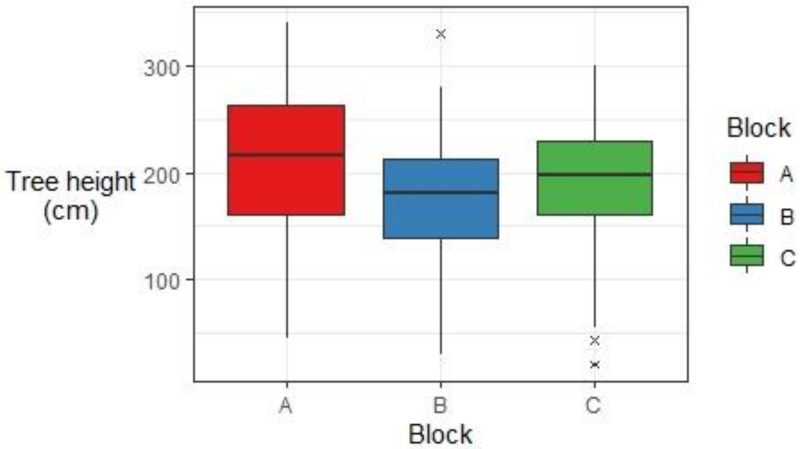


Figure 2.9. Mean tree height of Blocks A (HD), B (HD) and C (UHD). There was a significant difference in tree heights on Block A ($p<0.05$) but not between Blocks B and C.

Table 1.10. Summary of mean tree heights and standard deviations by cultivar-rootstock combinations and blocks.

		Mean tree height (cm)				Standard deviation of tree height			
#	**Tree**	**A**	**B**	**C**	**Site**	**A**	**B**	**C**	**Site**
1	C-Pdx	177.38	197.25	207.97	197.82	85.12	45.84	35.05	54.96
2	C-Sig	213.20	120.20	111.60	146.90	69.03	23.61	34.15	66.35
3	C-Hind	255.00	90.10	85.13	115.10	12.25	23.27	19.20	66.49
4	C-BdS	260.50	132.00	170.50	201.88	57.30	11.14	32.50	65.27
5	L-Pdx	183.40	210.00	245.71	222.66	16.36	38.86	39.70	43.06
6	L-Reg	261.11	213.40	246.93	245.55	32.96	22.06	37.90	36.90
7	S-Pdx	201.30	214.00	184.71	194.82	41.86	17.05	69.19	55.18
8	H-Pdx	199.80	206.80	214.40	209.43	28.23	31.12	22.18	26.06
9	T-Hind	232.20	159.63	196.33	192.25	30.97	29.70	25.74	36.53

The tree heights are summarised in Figures 2.10-2.15 and Table 2.10. These figures are boxplots of:

- tree heights (Figures 2.10 and 2.11)
- rootstock (Figures 2.12 and 2.13) and scion (Figures 2.14 and 2.15) growth rates (diameter near graft union) showing the Chandler trees planted in 2019 have the greatest growth rate and trees planted in 2017 the slowest – however, this is likely an artefact of tree growth habits which generally see growth rates taper as trees mature.

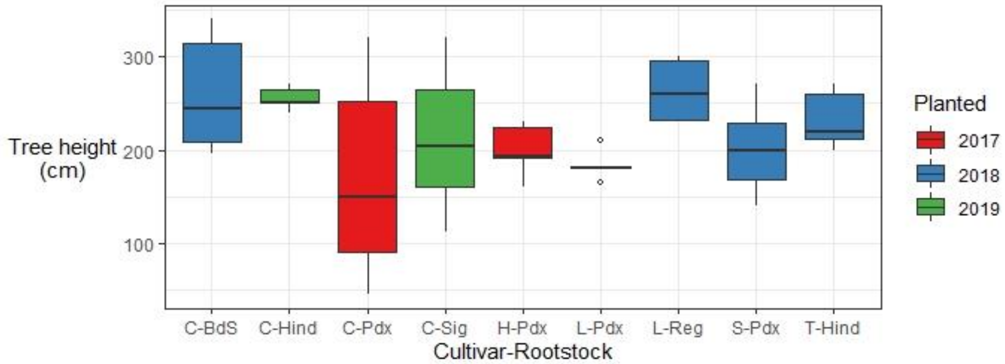


Figure 2.10. Tree heights at the OAI experimental orchard Block A.

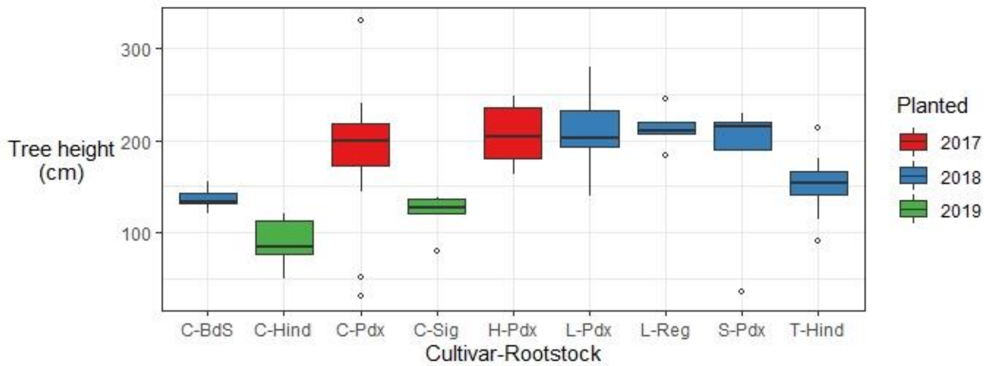


Figure 2.11. Tree heights at the OAI experimental orchard Block B.

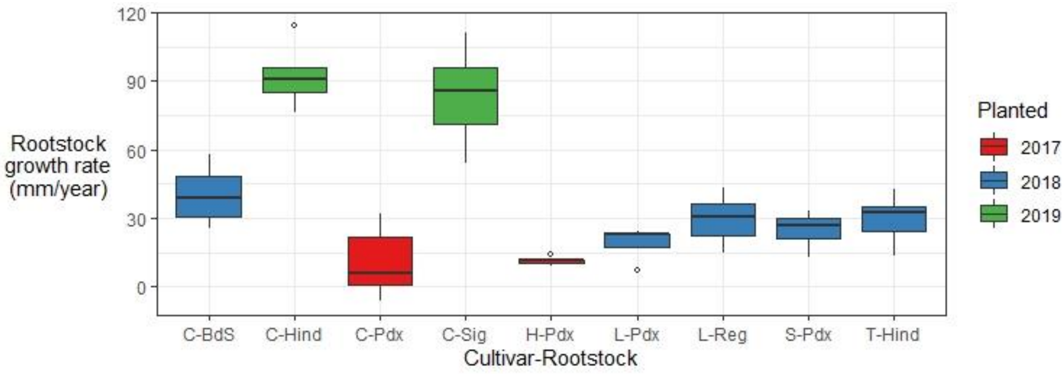


Figure 2.12. The growth rate in walnut rootstock diameter 5 cm below the graft union at experimental site, Block A.

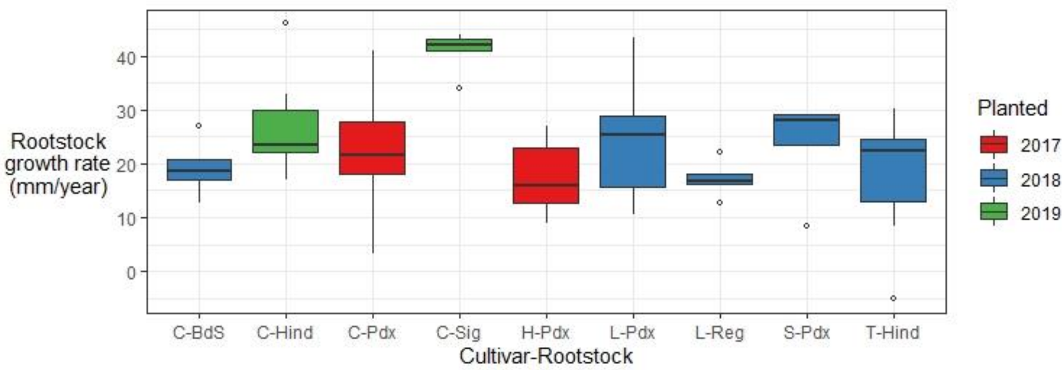


Figure 2.13. The growth rate in walnut rootstock diameter 5 cm below the graft union at experimental site, Block B.

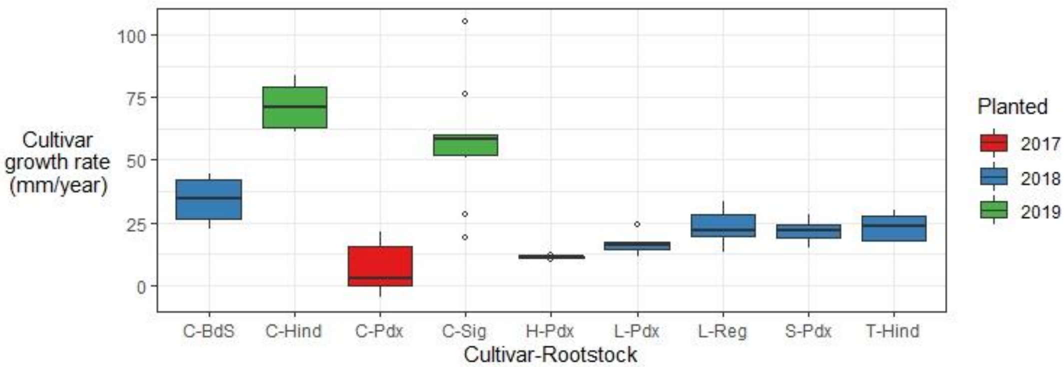


Figure 2.14. The growth rate in walnut cultivar diameter 5 cm above the graft union at OAI Block A.

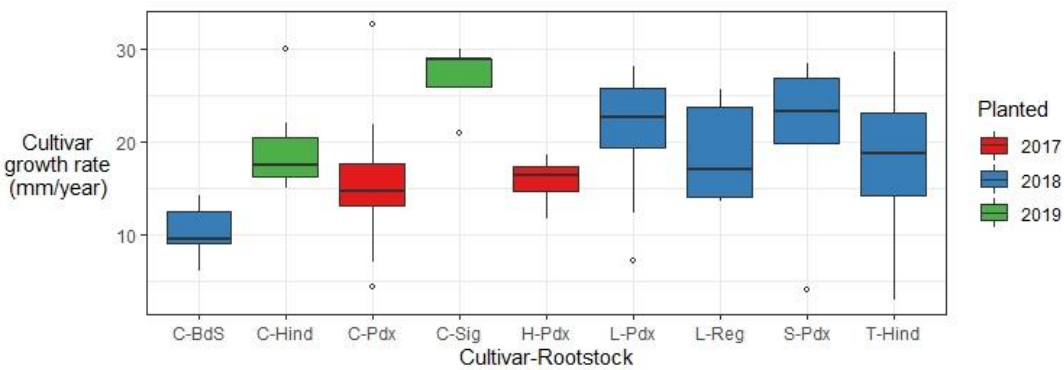


Figure 2.15. The growth rate in walnut cultivar diameter 5 cm above the graft union at OAI Block B.

2.5. Analysis of Horizon 3 plantings (ultra-high density)

Note: there is no horizon 2 in this project due to early stage planning changes, however the terminology persists for reporting continuity.

The mean tree height and standard deviation for Block C are included in Table 2.10 and summarised in Figure 2.16. Analysis of mean tree height by cultivar revealed that in the early years of establishment, most walnut rootstock-cultivar combinations grew comparably (i.e. no significant differences) regardless of planting density. In terms of early return on investment, this is a promising result as it indicates that early yields may be feasible due to strong early tree growth. However, given the duration of this project is less than the time taken for walnut crops to yield, this cannot be verified within the life of the project. Future work would benefit from a detailed comparison between the growth and productivity of the trees planted on Blocks A and B, at high-density, and the trees planted on Block C, at ultra-high density.

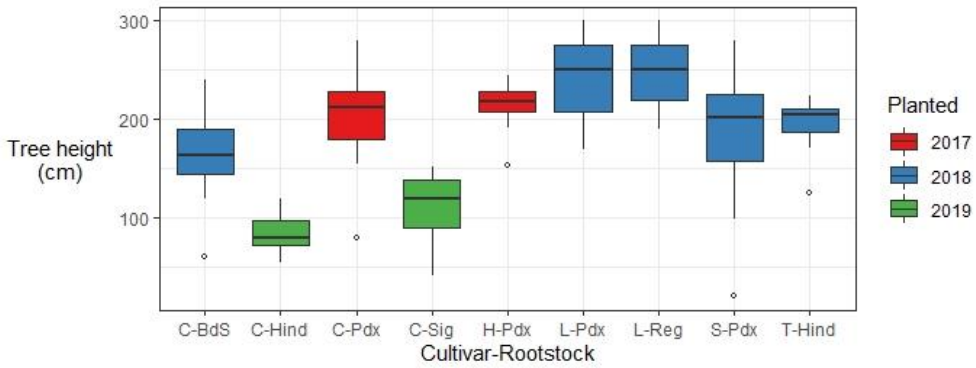


Figure 2.16. Boxplot of tree heights grouped by rootstock-cultivar combination in Block C, the ultra-high-density planting at the experimental walnut orchard.

2.5.2. Young commercial orchards

In the 2017-18 growing season, 224 trees were selected on the young commercial site for assessment. Throughout this project, most of these trees were dropped from the trial due to potentially confounding factors affecting the trial, including low survival rates, high incidence of disease and other reasons beyond the control of this project. The 60 trees remaining were measured in 2017, 2018 and 2019 and the growth rates are summarised in Figures 2.17 and 2.18. There was some uncertainty about the Provenance of the cultivars on this site, hence the nomenclature of U and Uns (indicating cultivar or rootstock uncertainty, respectively), however there were no significant variations in growth rate between certain and uncertain rootstock-cultivar combinations.

The significant decline in tree numbers available for use in this assessment highlights some areas for further research that would benefit the expanding walnut industry. These areas include further research on propagation techniques including budding and grafting processes and nursery biosecurity issues.

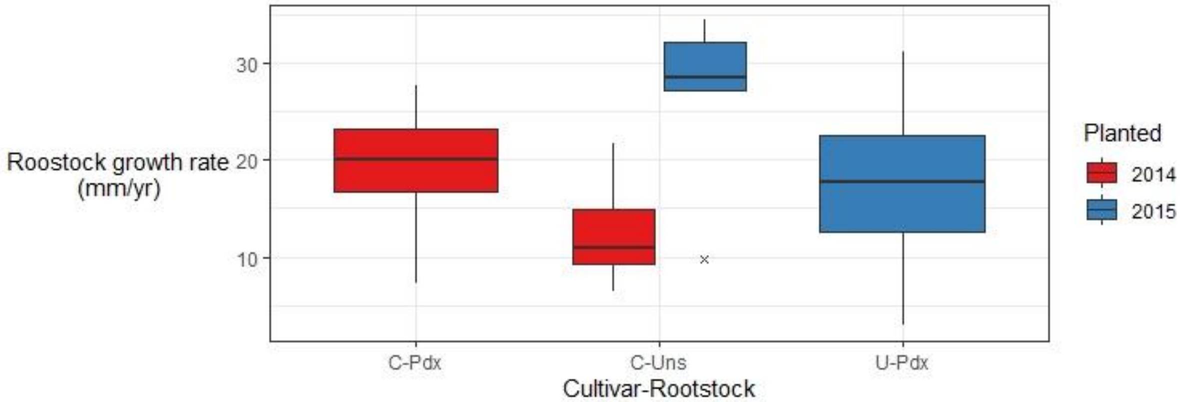


Figure 1.17. Growth rate (mm/year) of rootstock diameters of the trees at Avondale orchard.

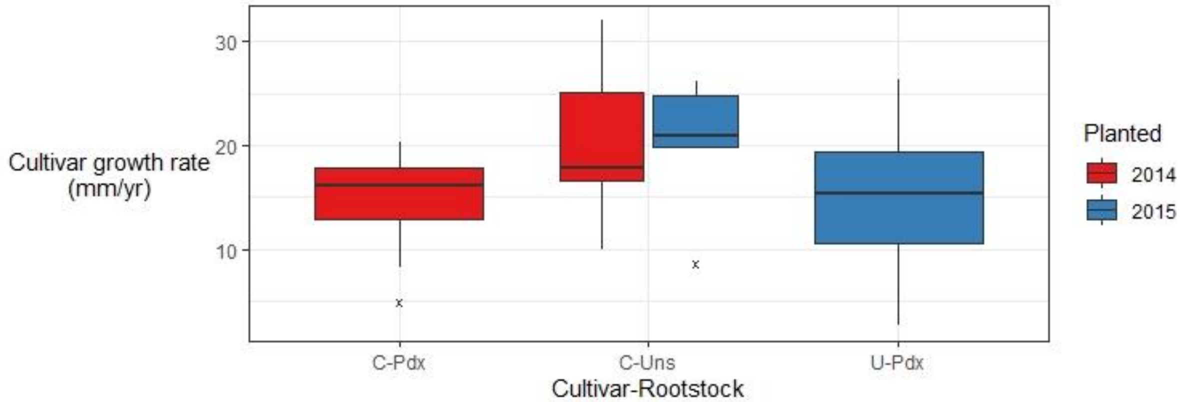


Figure 2.18. Growth rate (mm/year) of cultivar/scion diameters of the trees at the Avondale orchard.

Due to the number of uncontrollable variables associated with comparing field-planted trees in different regions, we have analysed the data from both regions separately and they can be used to guide future understanding of how the trees grow in the early years of establishment. Controlled environment experiments are required to identify the influence of single factors on tree growth, for example, the effect of temperature. There is benefit in undertaking such work, where facilities are available, to formulate adaptation strategies to changing climatic forecasts.

At the time of reporting, there is limited published research available about the impact of future climate variation on walnut production. A review of available literature on walnuts and other crops with similar growth parameters regarding the various potential climate change scenarios would be beneficial to helping the industry prepare adaptation strategies. Adaptation strategies may include shade netting, above canopy micro sprinkler systems, irrigation strategies and breeding programs for varieties potentially more suitable to projected climate scenarios. Investigation of the most viable of these strategies would require validation in Australian walnut orchards and will provide Australian walnut growers with adaptation tools into the future.

3. Using plant growth regulators for chemical management of walnut trees in Australia before planting, during early growth and at maturity

Jason Lewis and Jacquelyn Simpson

3.1. Background

Juglans species are tall trees, when not managed for commercial production. For optimal management an ideal tree canopy height would be 5 to 6 metres, or 80% of row width (Adem and Jerie, 2004). Tree vigour and growth management are primarily achieved with mechanical pruning methods including selective limb removal or hedging programs. Even with effective strategies for mechanical tree management, maintaining trees at a manageable and profitable size is difficult and expensive.

In California, the world's largest walnut production region, reducing the space between trees is discouraged with many walnut research and extension experts advocating wider spacing and more vigorous trees. Unfortunately, allowing trees to grow larger is also problematic. Pest and disease management is more difficult and expensive due to issues with spray coverage; trees become less productive in the centre, meaning fruit quality management can be more difficult. Pruning strategies can also affect the production of fruit bearing wood, hence crop yield, with cultivars of different lateral and/or terminal bearing habits.

This work is looking at ways to maximise production and profits using less space – more nuts per square meter. One means of doing this is chemically, using plant growth regulators. To investigate options for vigour management in Australian walnut orchards, we have conducted a multi-year, multi-cultivar trial in two commercial orchards of different ages and a growth comparison trial with Sunny®. This chemical inhibits the biosynthesis of gibberellin and results in shorter internodes, which results in shorter branches and smaller trees. Smaller trees are easier to manage; Sunny® will theoretically make it easier to manage commercial walnut orchards. Sunny® is of interest to the Australian walnut industry due to the extensive use of the product in the Chilean walnut industry, another option is Paclobutrazol containing products. Paclobutrazol has successfully been used to manage vigour in the Australian Avocado industry (Anon, pers. comm, 2019).

3.1.1. Aim

The aim of this work is to understand the effect of PGR Sunny® (containing the active ingredient uniconazole-p) on:

- Growth
- Bud formation
- Yield
- Nut quality (i.e. size, weight, colour, kernel: nut ratio, kernel shrivel, disease)
- Residue levels (residue reporting delivered to APVMA for permit application, and not reported on in this work)

To identify management strategies for walnut growers to manage tree vigour without sacrificing yield, specifically focusing on chemical management.

3.2. Methods

3.2.1. Experimental design – orchard trial

Five randomised blocks were established in three 7–12-year-old walnut cultivars before the start of the 2017–18 season at the mature commercial orchard (described in Chapter 2). The cultivars selected had differing flowering and harvest times: Serr (early-season), Vina (mid) and Chandler (late). Five randomised blocks were also established in a block of two-year-old, non-bearing Chandler trees at the young commercial orchard (described in Chapter 2). This was done to assess the effect of Sunny® on growth and development on young and mature trees alike; and, in the case of the mature trees, to evaluate any potential impacts of Sunny® application on harvest yields and nut quality.

Each randomised block at the Leeton orchard was comprised of five different Sunny® treatments (Table 3.1), each replicated twice per block. Trees were treated in multi-tree blocks; that is, trees from which data were collected are surrounded by 'buffer' trees to account for any interactions with untreated neighbouring trees. Only single trees were treated and assessed at Avondale orchard due to the smaller size of the trees, but care was taken to ensure buffer trees existed between the different treatments in each randomised block.

3.2.2. Treatment application – orchard trial

Sunny® (Sumitomo Chemical Australia Pty. Ltd., Epping Australia) was applied annually from the 2017–18 to 2019–20 seasons by soil inoculation. Holes were made in the soil at intervals along the drip line where the drippers were located. Sunny® was injected directly into the soil during irrigation at the appropriate amount for each treatment rate (Figure 3.1). It was applied in two seasons:

1. after harvest and before complete leaf fall and tree dormancy (autumn), and
2. during early initiation of leafing (spring).

Table 3.1. The five Sunny® rates applied to trees within each randomised block set up at Avondale and Leeton orchards during the 2017–18 to 2019–20 seasons.

	Application rate (L/ha)			
Application time	**0 (control)**	**0.75**	**1.5**	**3.0**
Autumn	Y	Y	Y	Y
Spring	N	N	Y	N

Figure 3.1. Sunny® treatments were applied to soil at dripper points by creating a divot (left) and syringing the treatment solutions into the divot (right). The irrigation was run to water in the Sunny®.

3.2.3. Effect on canopy volume

Photographs of the non-bearing trial trees at Avondale were taken over the four years from 2017–18 to 2020–21. Images were obtained in summer when the trees were in full leaf. A telescopic measuring pole was placed next to each tree for scale (Figure 3.2), and the height and width of each tree crown extrapolated from this using Image-J (Schindelin et al., 2012). Assuming a basic ellipsoid for crown shape, the volume of each tree canopy (V) was then calculated from the height (h) and width (w) measurements taken as follows:

$$V = 4/3 \times \pi\ h/2 \times w/2 \times w/2$$

Figure 3.2. Tree heights were measured using a telescopic measuring pole.

3.2.4. Stem growth and bud formation

The seasonal stem growth of bearing and non-bearing trial trees was measured during dormancy in the 2017–18 and 2018–19 seasons. Ten stems were measured per tree at Leeton, five each on the exposed eastern and western aspects of the trees. Due to the limited number of limbs on the young trees at Avondale, only four stems were measured per tree. However, due to their more exposed nature, the four measurements were taken from all around each tree.

Measurements were taken from the terminal point of each stem down to the first girdle scar that indicated a cessation of growth (Figure 3.3) using a cloth tailoring tape. The number of lateral buds along each designated stem length were counted, and mean internode length (I) was calculated from stem length (s) and the number of buds per stem length (b) thus:

$$I = s/b$$

Figure 3.3. Diagram showing one season of growth from girdle scar to tip, this was used for stem growth measurement.

3.2.5. Flowering

During the 2019–20 season, a study into the potential effects of Sunny® application on floral development was undertaken on the Chandler trial trees. Two branches were selected and tagged from each trial tree. Selected branches were growing opposite each other (i.e. one east- and one west-facing) and had at least three clear consecutive years' growth. The number of catkins and female flowers on spurs growing along a given three-year branch length during flowering were recorded.

3.2.6. Catkin formation

In the 2019–20 season, farm managers at the Leeton orchard noticed elongated catkins forming. The level of catkin deformation at the orchard was assessed to evaluate whether Sunny® was responsible for the occurrence of the phenomena. The level of catkin deformity on each tree was assessed by counting the number of observable elongated catkins (Figure 3.4) on and under each tree, as viewed from the open eastern and western aspects of each tree.

Figure 3.4. Deformed (elongated) catkin on a Chandler walnut tree.

All the Sunny® trial trees across the three cultivars (Serr, Vina and Chandler) were assessed for the presence of elongated catkins. In addition, 10 trees of each cultivar were also assessed from randomly selected areas within the Leeton orchard where neither Dormex® (with active ingredient cyanamide) nor Sunny® was applied. From this it was possible to get three treatment combinations: 1) Dormex® only, 2) Sunny® + Dormex®, and 3) untreated. All the Sunny® trial trees were treated with Dormex®, therefore a Sunny® + no- Dormex® combination was not obtainable.

3.2.7. Leaf/leaflet size

Terminal leaflet area of the bearing trial trees at Leeton was measured using a portable leaf scanner (CI-202 Portable laser leaf area meter, CID Bio-Science & Felix Instruments, USA) during the 2018–19 season. A total of 50 leaflets were taken from each tree at 2–3 metres above ground, 25 each from the exposed eastern and western aspects. Terminal leaflets were used as a standard instead of the whole leaf due to specific constraints at the time. For consistency, leaflet samples were taken from fully formed healthy leaves with a total of seven leaflets.

In the 2019–20 season, whole leaves were collected from trial trees and both the terminal leaflet and total leaf area were measured using the leaf scanner. This was done to provide a second year of data from trees and to test the robustness of using terminal leaflet area as a representation of leaf area in the 2018–19 season.

3.2.8. Light interception

To evaluate the effect a potential difference in leaf area might have on light interception, photosynthetically active radiation (PAR) measurements were taken above and below each trial tree in the 2019–20 season using a ceptometer (Accupar® LP-80 Canopy Interception and Leaf Area Index meter, Meter Group Inc, USA) with external sensor (Apogee® SQ110 photosynthetically active radiation sensor, Apogee® Instruments, USA). Measurements were taken at three distinct time points for each cultivar during the season: 1) dormancy, 2) early leaf flush/during female flowering, and 3) full leaf/pre-harvest. Four PAR measurements were taken from the cardinal compass points under each tree, 1 m from the tree bole and at a height of 1 m above the ground. Canopy light interception for each tree was then calculated by subtracting the above canopy PAR from the below canopy measurements and obtaining the mean value from the four cardinal point measurements.

3.2.9. Effect on harvest

The potential effect of Sunny® application on productivity was evaluated by comparing mean yields and nut quality across the different treatments. To achieve this, two 1 × 4 m quadrats were placed against the bole of each trial tree, running perpendicular to the rows in an easterly and westerly direction. Nuts within the quadrats were collected, hulled and counted, before undergoing drying and being placed in cool storage at 5°C and <50% humidity until being assessed for quality.

3.2.10. Harvest yield

Walnut yields from each Sunny® treatment were acquired by multiplying the total number of nuts harvested from within the two 1 × 4 m quadrats of each tree by eight to get an estimate of the total number of nuts per tree. This value was then multiplied by the mean in-shell weight for each treatment type for each season, as derived from the walnut quality assessment (see section 3.3.6). This was done to obtain a more accurate estimate of yields for each treatment, given the potential influence Sunny® application may have had on nut weight. This value (grams per tree) was then scaled to tonnes per hectare thus:

Estimated yield (t/ha) = estimated yield (grams per tree) / 64 × 10,000 / 1,000,000

3.2.11. Nut quality

A sub-sample of 50 nuts was arbitrarily extracted from the nuts collected from the quadrats under each trial tree for quality assessment. In-shell kernel size was measured using a specifically designed sizing frame (Figure 3.5), and recorded under one of eight classification sizes: <26, 26–28, 28–30, 30–32, 32–34, 34–36, 36–38, and >38 mm. The nuts were then weighed in-shell using a (Compact EJ-610 scale, A&D Weighing, Australia) before the kernels were cracked open for further assessment.

Figure 3.5. The equipment and set up used for sizing component of the walnut quality assessment

Once cracked, the weight of each kernel was recorded before a visual appraisal of kernel quality was undertaken. This involved the classification of kernels into one of four colours: extra light, light, light amber and amber as per the description of walnut kernel colours by the United States Department of Agriculture (USDA) grades and standards for shelled walnuts (www.agfoodsafety.org) and in the USDA colour chart for walnuts (Figure 3.6); and the estimation of percentage shrivel on each kernel. Additionally, an assessment of the presence of mould was undertaken on nut kernels harvested from the 2017–18 season, and any potential signs of sunburn from kernels recorded from the 2018–19 season samples.

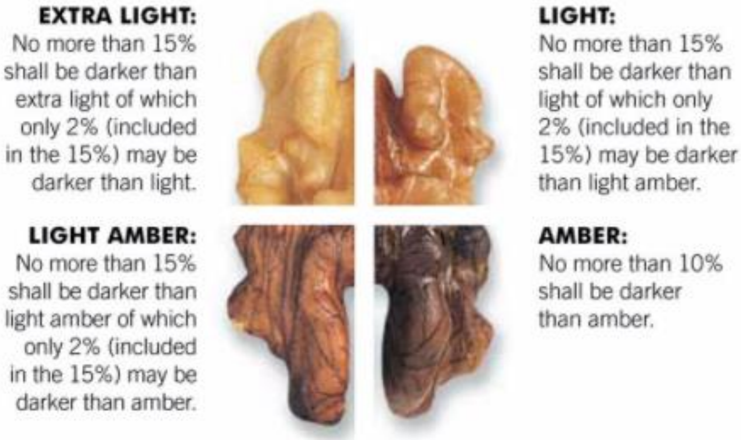


Figure 3.6. Walnut kernel colour scale used for quality assessments (www.agfoodsafety.org)

3.2.12. Statistical analysis

Statistical analysis between the different treatment rates of Sunny® was undertaken using one-way ANOVA with Tukey's HSD post-hoc testing. All comparisons of the spring and autumn applications at the 1.5 L/ha rate were assessed by a two-tailed t-test. These tests were applied to all aspects of this work except for two. Chi-squared (χ^2) analysis was used to investigate the potential effect of Sunny® on catkin formation, and Pearson's Product-Moment Correlation Co-efficient (PMMCC) was used to test the robustness of using terminal leaflet area as a substitute for total leaf area.

3.3. Results and discussion

3.3.1. Effect on tree growth

One of the 0.75 L/ha treatment trees in the non-bearing trial plots at Avondale was replaced in 2020, so the data for that specific tree has been excluded from the analysis.

Canopy volume

Mean crown volumes of trial trees measured in 2017–18, after the initial application of Sunny® in the spring of 2017, ranged from 3.95 m^3 in the 3.0 L/ha plots to 5.01 m^3 in the 1.5 L/ha treated plots. One-way ANOVA for this season was non-significant [F (3, 16) = 0.54, p = 0.66] between any of the treatments. However, significant differences were identified in 2018–19 [F (3, 16) = 8.50, p 0.05], 2019–20 [F (3, 16) = 42.95, p <0.05] and 2020–21 [F (3, 16) = 22.33, p<0.05].

In 2018–19 mean crown volume of the control trees rose to 23.46 m^3, which was significantly greater than both the 13.60 m^3 of the 1.5 L/ha [Tukey's HSD: Q = 4.29, p<0.05] and the 6.61 m^3 of the 3.0 L/ha [Tukey's HSD: Q = 7.05, p<0.05] treated trees. No significant differences in crown volumes were identified between any of the trees where Sunny® was applied, but a rate response was observed where crown volume decreased with increasing concentration of Sunny® application (Figure 3.7).

The rate response continued to be noticed in both the 2019–20 and 2020–21 seasons, with mean canopy volumes ranging from 8.8 m^3 (3.0 L/ha) to 43.19 m^3 (control), and 11.59 m^3 (3.0 L/ha) to 90.11 m^3 (control) in each respective season. In both these seasons, the control trees had significantly greater mean crown volume than trees in all three rates of Sunny® application (Figure 3.7). A significant difference was also observed between the 0.75 L/ha and 3.0 L/ha treatment plots in the 2019–20 season [Tukey's HSD: Q = 4.09, p<0.05], with the 0.75 L/ha trees being 9.28 m^3 larger. Despite this difference increasing to 13.42 m^3 in the 2020–21 season, it was non-significant [Tukey's HSD: Q = 4.09, p = 0.05].

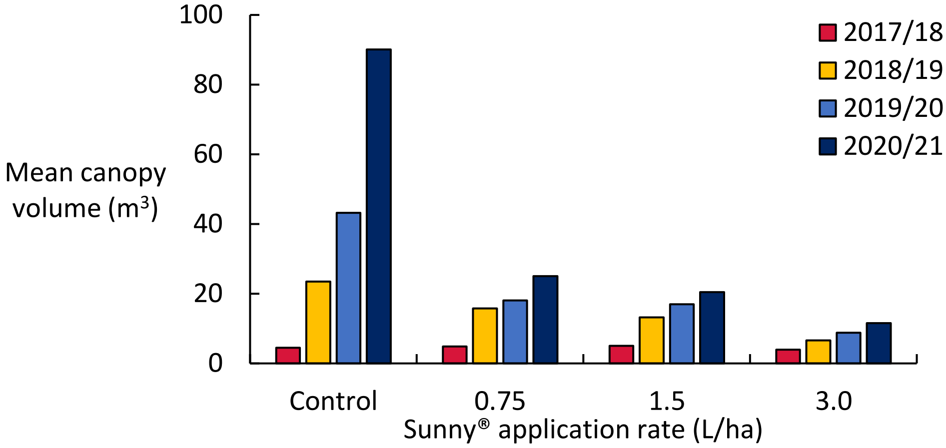


Figure 3.7. Tukey's HSD post-hoc analysis of canopy volumes of spring treated non-bearing trial trees at Avondale.

From 2017–18 to 2020–21 mean crown volumes across the concentration gradient of the spring applications of Sunny® increased by 85.60 m^3 (control), 20.13 m^3 (0.75 L/ha), 15.40 m^3 (1.5 L/ha) and 7.64 m^3 (3.0 L/ha). The mean crown volume of the 1.5 L/ha autumn treated trees rose by 19.17 m^3 during the same period. Although this growth rate is closer to the 0.75 L/ha than the 1.5 L/ha spring treated trees, two-tailed t-test analyses indicated no significant differences existed in mean canopy volume between the two 1.5 L/ha treatments in any of the four years of the trial (Figure 3.8).

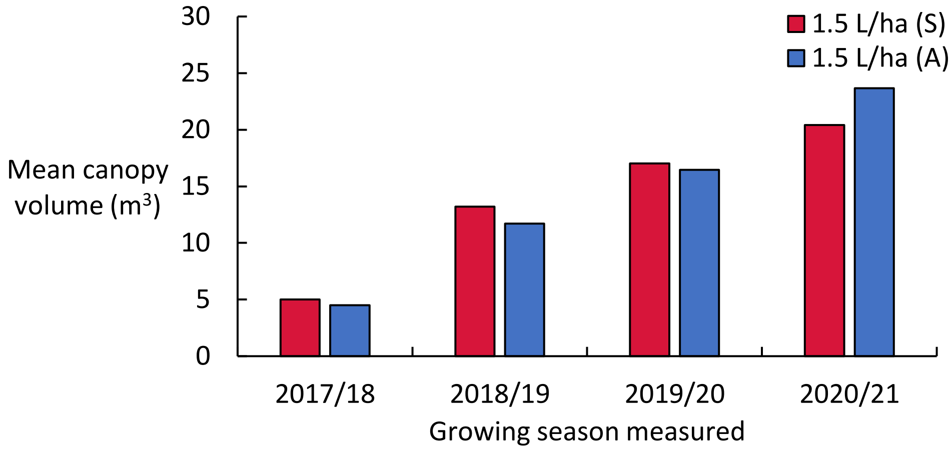


Figure 3.8. T-test analysis comparing applications of 1.5 L/ha of Sunny® in either spring or autumn.

Applying Sunny® significantly reduces crown volume in non-bearing Chandler trees, and trees express a rate response to treatment. Furthermore, the reduction in crown volume occurs more rapidly at concentrations above the 0.75 L/ha rate. Time of application did not appear to affect the results within the timeframe of the study. However, during the final two seasons, the autumn application trees expressed a greater rate of canopy volume growth than any of the spring applications, including the 0.75 L/ha trees. Although not significant, this may imply that using Sunny® in spring has a greater effect on tree growth.

3.3.2. Stem growth and bud formation

Fewer stem samples were assessed in the non-bearing trial at Avondale than the bearing tree trial at Leeton. This was due to limitations caused by reduced sample size and stem selection on the young trees. However, the number of stems measured on the mature bearing trees at Leeton orchard became much reduced in the 2018–19 season due to several factors relating to orchard management practices, localised bird damage and an increased incidence of disease). Consequently, this has made comparisons between seasons and sites unreliable.

Stem lengths in the bearing (Leeton) and non-bearing (Avondale) tree trials responded clearly to Sunny® treatment. Over the two-year period covering the 2017–18 and 2018–19 seasons, one-way ANOVA identified significant differences in all four trials (Table 3.2). Post-hoc analysis of the data showed the controls had significantly [Tukey's HSD: $p < 0.05$] longer mean stem lengths than all three rates of Sunny® application (Figure 3.9). No significant differences [Tukey's HSD: $p > 0.05$] existed in pairwise comparisons of the plots where Sunny® was applied; but despite the lack of significance between them, a rate response was observed wherein mean stem lengths decreased as Sunny® application rate increased (Figure 3.9) This trend was most pronounced in the non-bearing Chandler trees.

Table 3.2. One-way ANOVA results for mean stem lengths of bearing and non-bearing trees treated with different concentrations of Sunny®. Stems were measured from 2017–19.

Cultivar	df	F	p
Serr (bearing)	(3, 36)	23.13	<0.05
Vina (bearing)	(3, 36)	13.71	<0.05
Chandler (bearing)	(3, 36)	5.07	<0.05
Chandler (non-bearing)	(3, 16)	16.46	<0.05

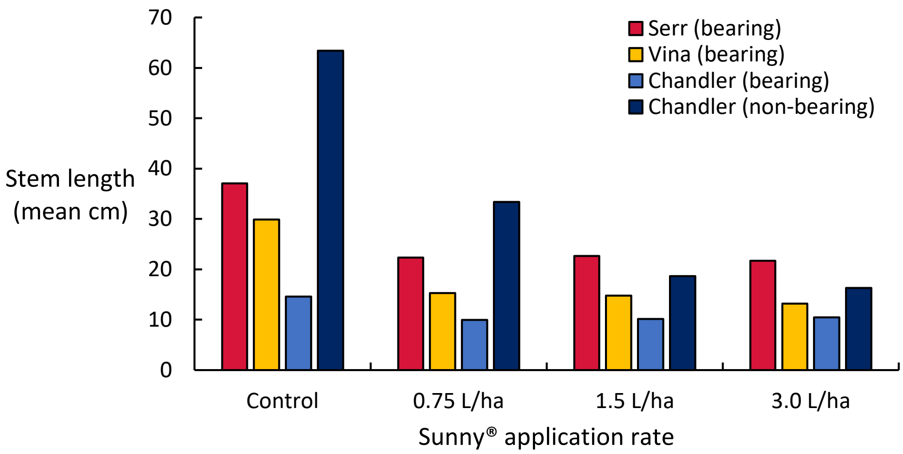


Figure 3.9. Mean stem lengths of trial trees treated with different rates of Sunny® from 2017 to 2020. Stems were measured from 2017–19.

Comparative analyses of the 1.5 L/ha spring and autumn treated plots in both the non-bearing trial at Avondale and the bearing trials at Leeton showed there was no significant difference [two-tailed t-test: $p > 0.05$] between the different application times (Figure 3.10).

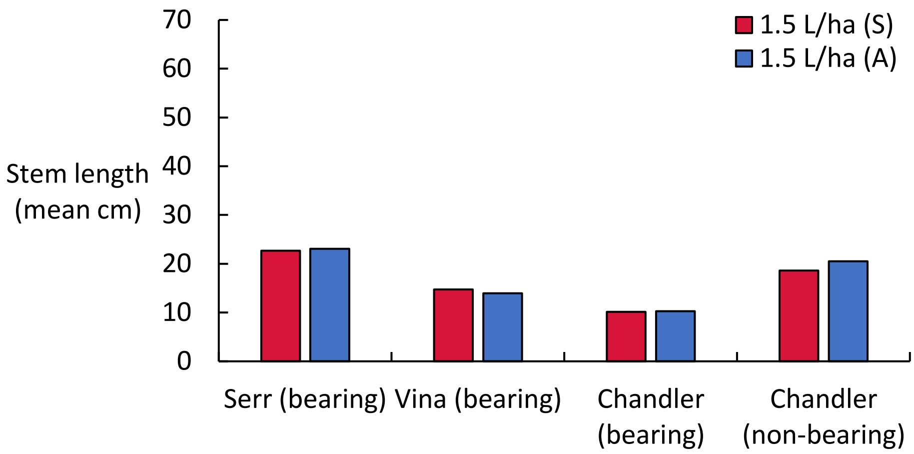


Figure 3.10. Mean stem lengths of trial trees treated with 1.5 L/ha of Sunny® in either spring or autumn. A two-tailed t-test indicated no significant differences [p >0.05] between treatments. Stems were measured from 2017–19.

It can be concluded that Sunny® application significantly reduces stem growth in walnuts, but there is no difference between spring or autumn application of the PGR. Furthermore, although the plots where Sunny® was applied were not significantly different, a trend emerged where mean stem lengths shortened as application rates increased. Although this was most notable in the younger non-bearing trees at Avondale, limitations arose during the study of the Leeton bearing trees. This means that a direct comparison between the two sites cannot be made. Finally, the reduction in stem lengths caused by Sunny® (Figure 3.9) raises the question: does application of the growth regulator influence bud formation, and consequently harvest yields? These matters are addressed in the studies into internode lengths (below) and estimated harvest yields (Section 3.3.6) undertaken in the trial plots.

Internode lengths

One-way ANOVA of the spring treated trees showed significant differences between treatment plots in all the trials (Table 3.3). Post-hoc analysis between the treatments in each trial showed the control plots to have significantly [Tukey's HSD, p<0.05] longer internode lengths than all three treatment rates, but that all pairwise comparisons between the plots where Sunny® was applied were non-significant [Tukey's HSD: p>0.05]. This was the case for the non-bearing Chandler, bearing Serr, and bearing Vina trial plots, but not the bearing Chandler trial (Figure 3.11).

Table 3.3. Summary of internode length ANOVA results.

Cultivar	df	F	p
Serr (bearing)	(3, 36)	6.14	<0.05
Vina (bearing)	(3, 36)	8.40	<0.05
Chandler (bearing)	(3, 36)	3.01	<0.05
Chandler (non-bearing)	(3, 16)	15.64	<0.05

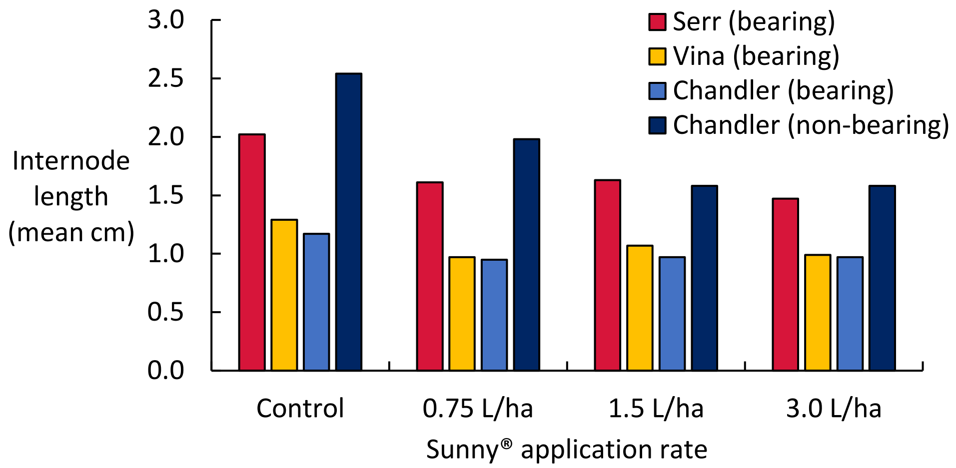


Figure 3.11. Mean internode lengths of stems on bearing (B) trees at Leeton and non-bearing (NB) trees at Avondale in 2018–19 that were treated with Sunny® from 2017. Stems were measured from 2017–19.

Although the one-way ANOVA identified a difference between plots in the bearing Chandler trial, pairwise comparisons by Tukey's HSD did not identify any. Alternative post-hoc testing by Scheffé multiple comparison, and Bonferroni and Holm multiple comparison, also did not identify any significant results [$p > 0.05$]; however, running a Bonferroni and Holm multiple comparison analysis that only used pairs relative to the control identified significantly [$p < 0.05$] longer internode lengths in the controls compared to the plots treated with Sunny®. A series of confounding factors occurring from the start of the study that considerably compromised the sampling of the Chandler bearing trees, and this may be the reason for the anomalous results in this trial.

The only trend relating to Sunny® application rate was identified in the non-bearing Chandler trial (Figure 3.11). Given the sampling complications that arose at Leeton orchard, it can be suggested that the non-bearing trial provides a more accurate representation of results. However, confounding factors such as: the growth rates of different aged trees, differences in light accessibility by the trees, and different management regimes need to be considered. Therefore, the only conclusion is that a rate response was only observed in young trees.

Comparisons of the spring and autumn application of Sunny® at 1.5 L/ha showed the autumn treated trees had significantly [two-tailed t-test, $p < 0.05$] longer internode lengths than spring applications in the non-bearing Chandler trees (Figure 3.12). No significant differences [two-tailed t-test, $p > 0.05$] were observed in any of the three bearing tree trials.

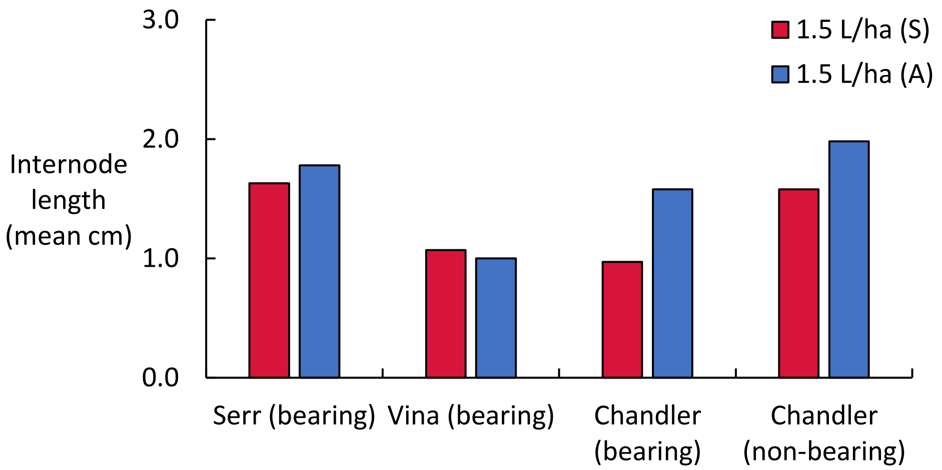


Figure 3.12. Mean internode lengths of tree treated with 1.5 L/ha of Sunny® in spring or autumn. Stems were measured from 2017–19.

It can be concluded that Sunny® reduces internode lengths along the stem. There is evidence of a rate response in young trees, but not mature trees. However, the results from study on the mature trees should be regarded as inconclusive. Given the observed reduction in stem lengths that occurred when Sunny® is applied, this would point to the fact that although vigour is reduced in trees, harvest yields will potentially not be affected.

Among the buds counted there was an observed increase in the proportion that were catkins within the treated trees compared to the controls. This was evident across all three cultivars in both the 2017–18 and 2018–19 seasons (Figure 3.13). In 2017–18 there was a general trend of an increase in the proportion of catkin buds with increasing rates of Sunny® application in the spring. The trees applied with 1.5 L/ha in the autumn had the highest proportion of catkin buds across all three cultivars. This trend was not observed the following year; but the proportion of catkin buds was higher across the whole trial, rising from 12-33% in 2017–18 to 16-46% in 2018–19.

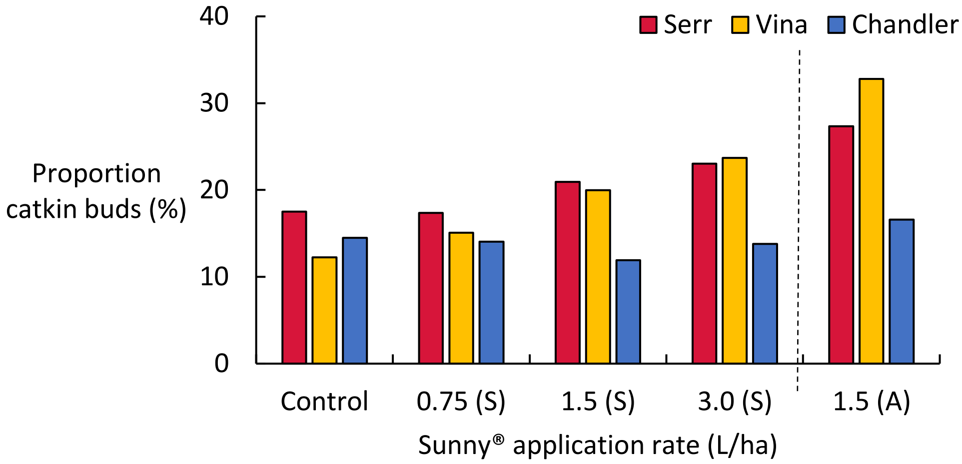


Figure 3.13. Proportion of buds counted on the mature Sunny® trial trees at Leeton orchard that were identified as catkins in the 2017–18 season.

3.3.3. Flowering

Two seasons after the first Sunny® applications, the number of female flowers and catkins growing on the spurs of the bearing Chandler trees at Leeton orchard was assessed. The control trees had a mean female: male ratio of 1.5: 1, which was higher than any other treatment rate (Figure 3.14). A broader review of the trend in flower ratios among the spring applied treatments showed that the female: male flower ratios declined steadily from the control trees, through the 0.75 L/ha (S) trees, down to 1.1: 1 in the 1.5 L/ha (S) treated trees. However, it then increased to 1.3: 1 at the 3.0 L/ha (S) rate. This implies there was no rate response to flower ratios in the treated trees, and that the observations are a consequence of a slight fall and rise of female flowers that was mirrored by a rise and drop in catkins across the treatments.

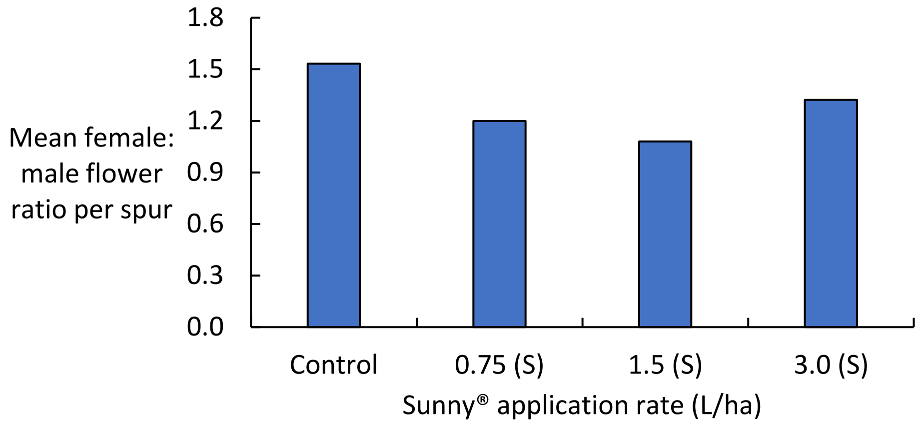


Figure 3.14. Ratios of female: male flowers recorded on spurs of Sunny® treated Chandler trees in the 2019–20 season.

Although the ratio of female to male flowers dropped in the Sunny® treated trees, statistical analysis of the mean number of flowers per spur across the treatments showed that this change was not significant for either female flowers [One-way ANOVA: $F(3, 34) = 1.43$, $p = 0.25$] or catkins [One-way ANOVA: $F(3, 34) = 0.31$, $p = 0.82$]. A two-tailed t-test of the spring and autumn applications at the 1.5 L/ha rate also returned non-significant results (Figure 3.15). Therefore, these findings indicate that neither treatment rate nor application time significantly affected flowering.

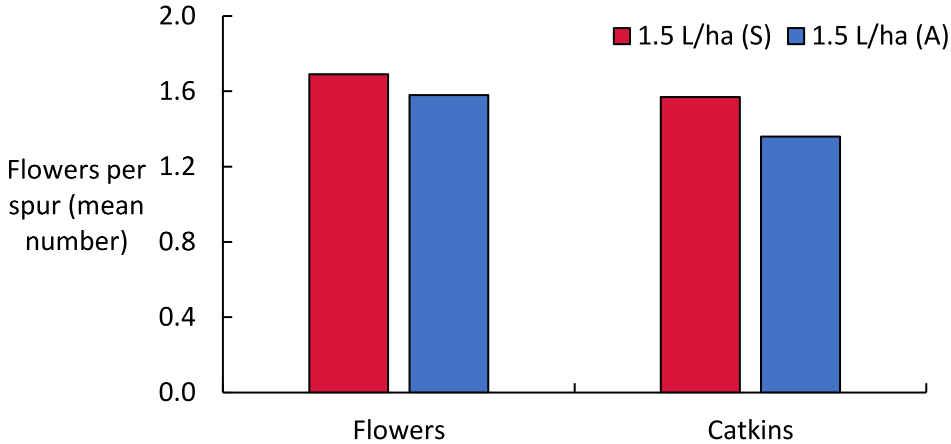


Figure 3.15. Mean female (flowers) and male (catkins) flowers per spur on bearing Chandler trees at Leeton orchard treated with 1.5 L/ha of Sunny® in either spring (S) or autumn (A).

Catkin formation

A total of seven elongated catkins were observed on the untreated Vina trees. This observation would suggest the growth anomaly occurs naturally; however, there were only a small number of rows designated for exclusion of Dormex® treatment and significant clearing had occurred in the block. Given that Dormex® was applied by spraying, the findings in Vina may be compromised by the effect of drift. This proposal is supported by the almost complete lack of elongated catkins in the untreated Serr (no observations) and Chandler (one observation) trees (Figure 3.16).

Chi-square analysis identified a very highly significant difference in the number of elongated catkins across the different treatment combinations for Serr [$\chi^2 = 128.24$; d.f. = 5; $p<0.05$] and Vina [$\chi^2 = 29.44$; d.f. = 5; $p<0.05$] trees, and a highly significant difference in Chandler [$\chi^2 = 17.86$; d.f. = 5; $p<0.05$]. This indicates that the application of the growth regulators increases the prevalence of elongated catkins on trees (Figure 3.16). Furthermore, there was a greater occurrence of elongated catkins treated with both Dormex® and Sunny® than those treated with Dormex® only across all three walnut cultivars. This would imply that the applying both PGRs has a cumulative effect.

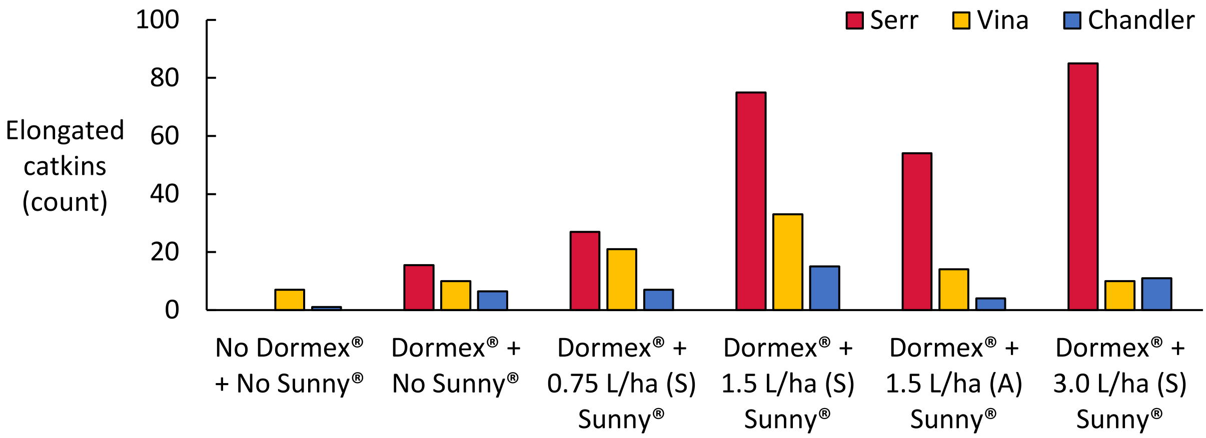


Figure 3.16. Occurrence of elongated catkins on trees treated with Dormex®, or Dormex® with varying levels of Sunny®. All trees were also exposed to Ethephon.

The total number of elongated catkins observed on the treated trees of each cultivar was markedly different, with the early cultivar Serr possessing the greatest number (257), and the late cultivar Chandler the least (45). This observed trend was replicated across the individual treatments in every instance except for the trees treated with Dormex® and 3.0 L/ha of Sunny®, where the Chandler trees possessed a greater number of elongated catkins than Vina. However, the difference between them was negligible.

Comparisons between the Dormex® treated trees that had 1.5 L/ha of Sunny® applied in either spring or autumn showed there was a marked drop in the number of elongated catkins where Sunny® was applied in autumn. This was evident in all three cultivars where the total number of elongated catkins were 28.0%, 57.6% and 73.3% lower in the autumn applied trees for Serr, Vina and Chandler respectively.

These observations indicate two things. First, early cultivars are more severely affected by the PGR applications and the effects steadily decline the later a walnut cultivar's phenological phases occur in the season. Second, the spring application of Sunny® has a greater effect on the presence of elongated catkins than the autumn application.

3.3.4. Leaf/leaflet size

Pearson Product-moment Correlation Co-efficient analysis of terminal leaflet and whole leaf area from samples collected in the 2019–20 season showed a very highly significant relationship between the two measuring methods for all three walnut cultivars [Serr: $r = 0.91$, $p<0.05$; Vina: $n = 500$, $r = 0.95$, $p<0.05$; Chandler: $n = 1250$, $r = 0.92$, $p<0.05$] (Figure 3.17). These findings indicate that the data obtained from measuring terminal leaflet area is robust enough to use in place of measuring the whole leaf. Consequently, this enables the opportunity to assess leaf size, as determined by terminal leaflet surface area, across the different Sunny® treatments over the two seasons of 2018–19 and 2019–20.

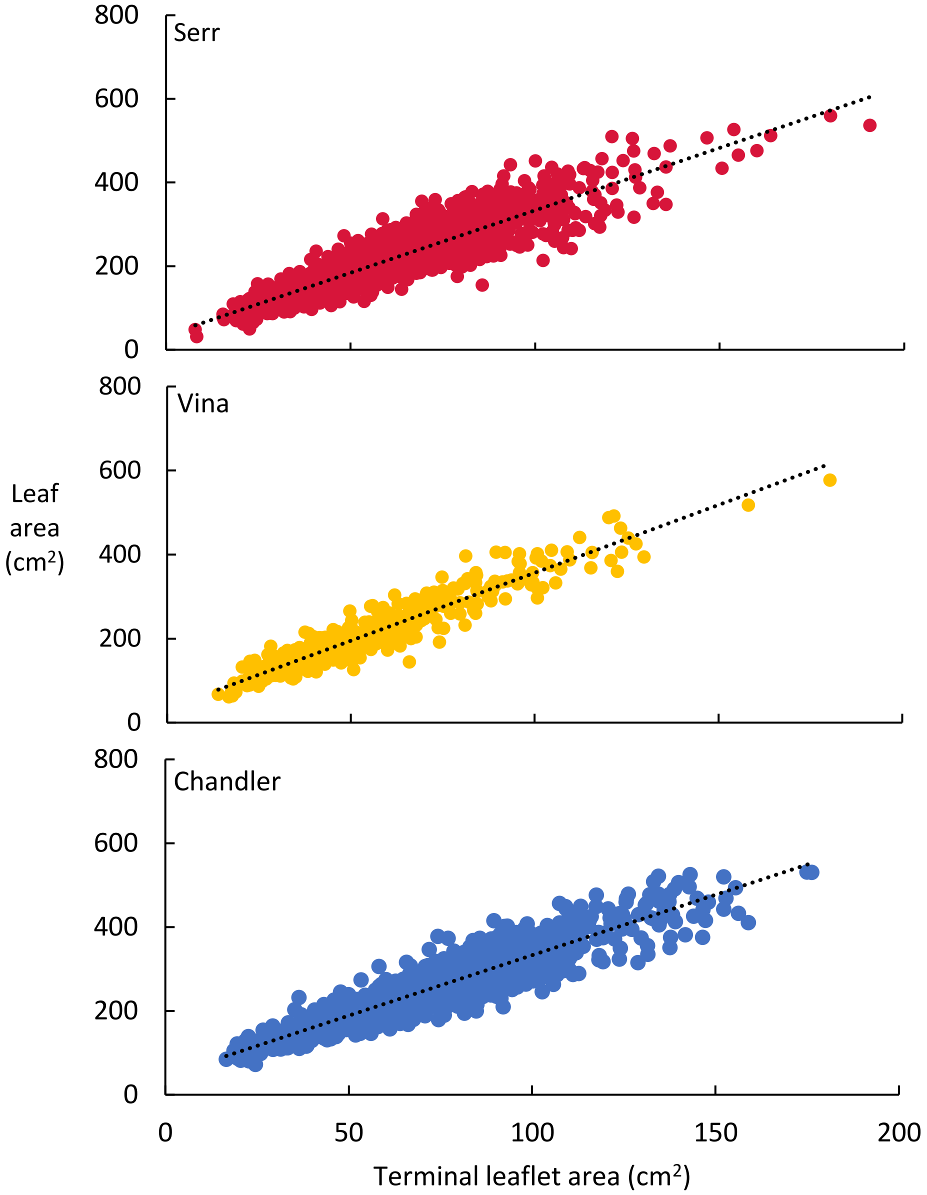


Figure 3.17. Correlation analysis of terminal leaflet and total leaf area from leaf samples collected within the Sunny® trial during the 2019–20 season. Pearson's Product-moment Correlation Co-efficient analysis showed a very strong relationship between the two measurement approaches for Serr [n=2498, r = 0.91, p<0.05], Vina [n=500, r = 0.95, p <0.05] and Chandler [n = 1250. r = 0.92, p <0.05].

Data was collected from all three cultivars at Leeton orchard in the 2018–19 and 2019–20 seasons. However, the 2019–20 Vina data were excluded from analysis due to low replication numbers. One-way ANOVA of mean terminal leaflet area in both the 2018–19 and 2019–20 seasons identified a highly significant difference between treatments for all three walnut cultivars (Table 3.4). This followed a rate response wherein mean leaflet area increased from 98 cm^2, 88 cm^2 and 102 cm^2 in the control trees to 55 cm^2, 57 cm^2, and 71 cm^2 in the 3.0 L/ha Sunny® treated trees for Serr, Vina and Chandler respectively in 2018–19 (Figure 3.18).

Table 3.4. One-way ANOVA results of mean terminal leaflet area measurements from 2018–19 and 20219–20.

Recording Season	Cultivar	df	F	p
2018–19	Serr	(3, 36)	119.38	<0.05
	Vina	(3, 36)	30.90	<0.05
	Chandler	(3, 36)	19.08	<0.05
2019–20	Serr	(3, 36)	45.40	<0.05
	Vina	insufficient data		
	Chandler	(3, 16)	26.00	<0.05

Recorded mean terminal leaflet areas (Figure 3.18) in the spring applications were:

097.91 cm^2 (control) > 68.56 cm^2 (0.75 L/ha) > 59.78 cm^2 (1.5 L/ha) > 54.60 cm^2 (3.0 L/ha) in Serr;
088.36 cm^2 (control) > 71.93 cm^2 (0.75 L/ha) > 64.78 cm^2 (1.5 L/ha) > 56.64 cm^2 (3.0 L/ha) in Vina;
101.90 cm^2 (control) > 83.12 cm^2 (0.75 L/ha) > 74.92 cm^2 (1.5 L/ha) > 71.30 cm^2(3.0 L/ha) in Chandler

Pairwise comparisons of the different treatment rates within each cultivar in the 2018–19 season showed the controls had significantly (Tukey's HSD, p <0.05) greater surface area than any of the treated trees in all three cultivars. In addition, the 0.75 L/ha (S) treated plot leaflets were significantly larger than both the 1.5 and 3.0 L/ha (S) trees in Serr, and the 3.0 L./ha trees in Vina.

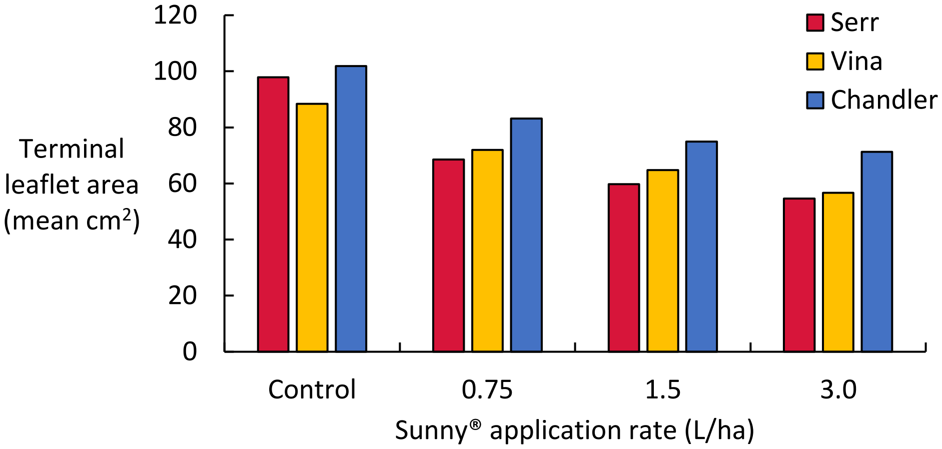


Figure 3.18. Tukey's HSD post-hoc of terminal leaflet area across spring treated trees in 2018–19 season.

Terminal leaflet area between spring treatments in 2019–20 broadly replicated those of the previous season, with the overall trend being a reduction in terminal leaflet area with increasing Sunny® application rates (Figure 3.19). Post-hoc analysis of the 2019–20 data showed the controls in both Serr and Chandler had highly significantly [Tukey's HSD, p<0.05] larger terminal leaflets than any of the treated trees. Leaflets in the 0.75 L/ha (S) treated plots were also significantly larger than the 1.5 L/ha (S) treated trees in Chandler [Tukey's HSD, Q = 4.60, p<0.05], and the 3.0 L/ha treatments in Serr [Tukey's HSD, Q = 5.01, p<0.05].

Mean leaflet area for the 1.5 L/ha autumn treatments in the 2018–19 season were closer to the mean leaflet areas of the 1.5 L/ha spring applications of Sunny® than any other treatment. Comparative analyses of the 1.5 L/ha spring and autumn applications by two-tailed t-test were non-significant for all three cultivars (Figure 3.20), indicating application time did not affect terminal leaflet size. These findings were repeated in the 2019–20 season, with the exception that terminal leaflet area in the autumn treated Chandler trees was significantly [two-tailed t-test: t = -2.67, p<0.05] larger than those treated in spring (Figure 3.21).

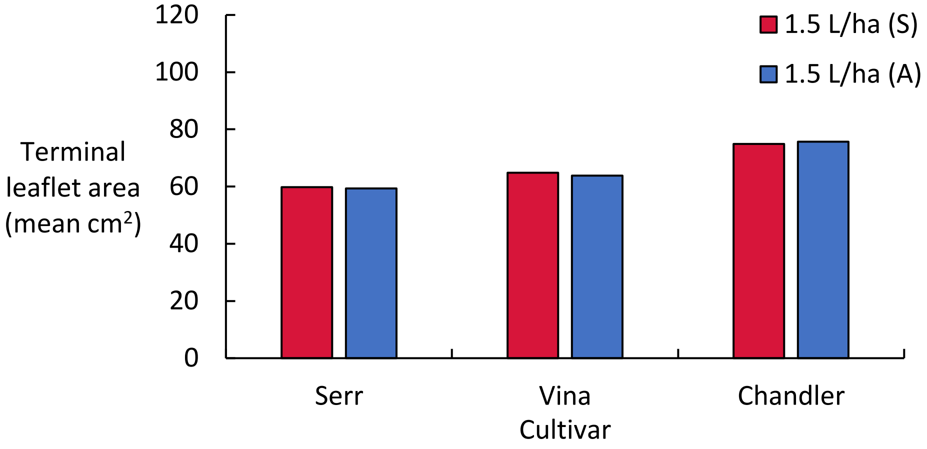


Figure 3.19. T-test analysis of the 1.5 L/ha treatments in spring and autumn in the 2018–19 season.

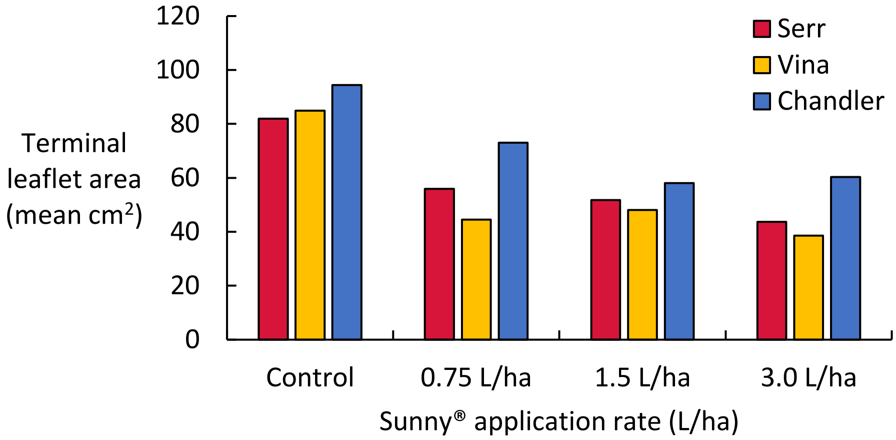


Figure 3.20. Tukey's HSD post-hoc of terminal leaflet area across spring treated trees in 2019–20 season.

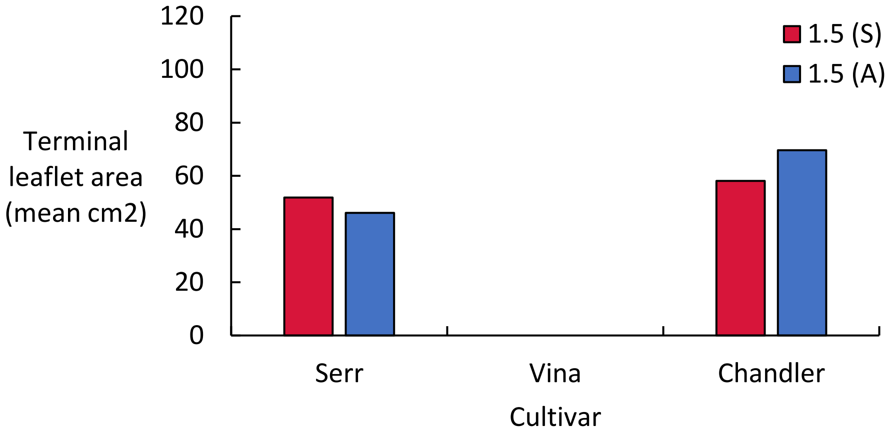


Figure 3.21. Two-tailed t-test analysis of the 1.5 L/ha treatments in spring and autumn in the 2019–20 season. Vina was excluded due to low replication.

These findings show that measuring the terminal leaflet area of walnut leaves is a good alternative to sampling and measuring whole leaves. This makes non-destructive measuring in the field with a handheld scanner a viable alternative. The caveat to this approach is that all measured leaflets must originate from leaves with the same number of leaflets to ensure consistency and reliability in the data acquired. Also, a minimum of 100 samples per treatment should be collected to ensure a robust data set is obtained.

Application of Sunny® to walnut trees reduces leaf size, as determined by measuring the terminal leaflet areas. The effect showed a rate response to spring application of Sunny® in all the cultivars studied. Inter-cultivar comparisons of the effect of Sunny® application could not be made, but a general pattern emerged that was also found in the stem measurements. This was that the difference in terminal leaflet area between the untreated and treated trees was typically greater in Serr.

Statistical analysis of the spring and autumn application of Sunny® at the 1.5 L/ha rate showed no significant difference (Tukey's HSD, $p>0.05$) between the treatments in five of the six comparisons. This indicates that leaflet (and consequently leaf) area is influenced by the concentration rate of Sunny®, but not by time of application.

A potential consequence of reduced leaf area is a reduction in canopy light interception. As a result, kernel quality in warm temperate and semi-arid areas may be affected due to an increase in the occurrence of shrivel and sunburn (See Section 3.3.6).

3.3.5. Light interception

Given the effect of Sunny® on leaf area the amount of canopy light interception was assessed through the season. Analysis of light interception during dormancy proved non-significant in all three cultivars (Table 3.5). This result provided an opportunity to confirm a level of standardisation existed between the different plots.

Significant differences were identified in all three cultivar trials during flowering (Table 3.5). In both the Serr and Chandler trials, mean light interception was significantly [Tukey's HSD, $p <0.05$] higher in the control plots compared to both the 1.5 L/ha (S) and 3.0 L/ha (S) treated plots during this time. However, during kernel development no significant differences were identified (Table 3.5).

A greater level of change was noticed in the Vina trial. As with the other two cultivars, the control plots had a significantly [Tukey's HSD, $p <0.05$] greater level of light interception compared to the 1.5 L/ha (S) and 3.0 L/ha (S) plots. But in addition to this, the 0.75 L/ha plots were also shown to have a significantly [Tukey's HSD, $p <0.05$] greater mean light interception than the 3.0 L/ha plots. Furthermore, all of these significant differences persisted during kernel development (Table 3.5, Figure 3.22). This indicates that the canopies of the Sunny® treated Vina trees did not fill in as effectively as those of Serr and Chandler.

Although not every pairwise comparison of treatments during flowering proved to be significant in the trials (Figure 3.22), a negative trend was observed between Sunny® application and mean light interception. This was evident in all three cultivars and may warrant further investigation.

Table 3.5. One-way ANOVA results for canopy light interception of Sunny® treated trees at Leeton orchard in 2019–20.

Time of Season	Cultivar	df	F	p
Dormancy	Serr	(3, 36)	0.80	0.50
	Vina	(3, 36)	4.35	0.01
	Chandler	(3, 36)	0.54	0.66
Flowering	Serr	(3, 36)	1.43	0.25
	Vina	(3, 36)	14.43	<0.05
	Chandler	(3, 36)	8.60	<0.05
Kernel development	Serr	(3, 36)	0.29	0.83
	Vina	(3, 36)	6.92	<0.05
	Chandler	(3, 36)	2.23	0.10

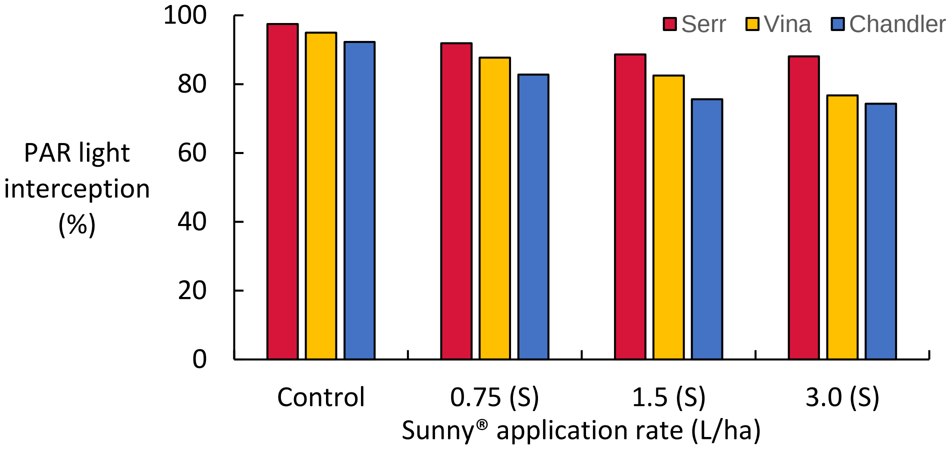


Figure 3.22. Mean canopy light interception of trial trees during flowering in the 2019–20 season that were treated with different rates of Sunny® from 2017 to 2020.

The significant differences identified when comparing the controls against both the 1.5 L/ha (S) and 3.0 L/ha (S) treatments during flowering became non-significant in Serr and Chandler, but remained significant in Vina, at the end of the season during late kernel development. This finding implies that despite a reduction in leaf size resulting from increased Sunny® application rates, the amount of light interception by the canopy recovers to normal levels in more vigorous walnut cultivars. This might be an important consideration when growing walnuts in warm, semi-arid regions where temperatures frequently exceed 40 °C during kernel development as a more exposed crown may increase the risk of quality issues such as sunburn.

3.3.6. Effect on harvest and quality

Pistillate Flower Abortion (PFA) has been a problem in Serr, and an attempt to address this has been undertaken at Leeton orchard by applying ReTain® (active constituent: aminoethoxyvinylglycine present as the hdyrochloride salt, AVG) at flowering. However, in 2018–19 Retain® was sprayed in conjunction with another chemical that appeared to neutralise its effectiveness (Webster pers. comm.). This resulted in a major PFA event that affected the trees to the point that some of the quadrats did not get the baseline 50 nuts required for the quality assessment.

Only two harvest seasons were assessed. This limits the determination of findings and prevents evidence of temporal trends. A minimum three-year period, plus time following cessation of treatment is required for a more comprehensive assessment.

Harvest yield

In 2017–18 estimated yields within the different treatment plots ranged from 2.63–2.82 t/ha (Serr), 4.29–5.46 t/ha (Vina), and 2.91–3.42 t/ha (Chandler). No significant differences in yields were observed in any of the cultivars (Table 3.6). Estimated yields in 2018–19 ranged from 0.96–1.83 t/ha (Serr), 4.14–5.74 t/ha (Vina), and 3.59–4.63 t/ha (Chandler). No significant differences in yield were observed in Chandler; however, significant differences were identified in Serr and Vina (Table 3.6).

Post-hoc analysis of the Serr trial plots showed the control trees produced higher yields than both the 0.75 L/ha (S) [Tukey's HSD, Q = 4.38, p<0.05] and the 3.0 L/ha (S) [Tukey's HSD, Q = 5.87, p<0.05] treatments in 2018-19. In Vina, the 1.5 L/ha treated plots yielded more than the controls. [Tukey's HSD, Q = 4.77, p<0.05]. The sparsity and inconsistency in the results of the control plots implies no coherent difference exists between treated and untreated trees.

No significant differences [t-test, p>0.05] were identified in comparisons of the spring and autumn treated trees in either year for any of the cultivars (Figure 3.23). Based on these findings it should be concluded that neither rate nor time of Sunny® application affected walnut yields.

Table 3.6. One-way ANOVA of estimated harvest yields in 2017–18 and 2018–19 for Serr, Vina and Chandler trees treated with different rates of Sunny®.

Harvest Season	Cultivar	df	F	P
2017–18	Serr	(3, 36)	0.13	0.94
	Vina	(3, 36)	1.86	0.15
	Chandler	(3, 36)	1.24	0.31
2018–19	Serr	(3, 36)	6.40	<0.05
	Vina	(3, 36)	4.48	<0.05
	Chandler	(3, 36)	2.20	0.11

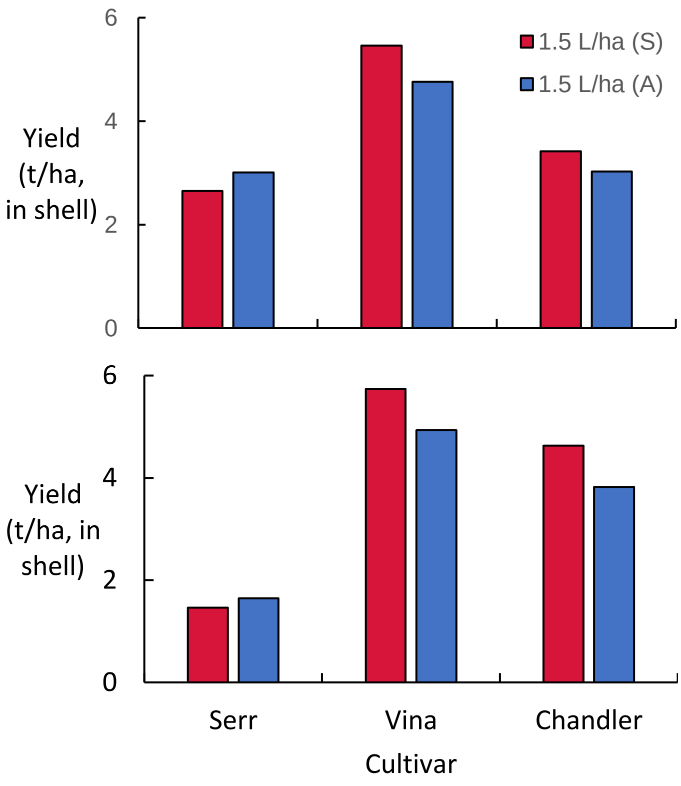


Figure 3.23. Estimated harvest yields in the 2017–18 (top) and 2018–19 (bottom) seasons for trees treated with 1.5 L/ha of Sunny® in either the spring or autumn.

Nut size

In 2017–18 most in-shell nuts fell into the 32-36 mm size category for all three cultivars. This trend was replicated in 2018–19 for Vina and Chandler; but not in Serr, where the >36 mm was the dominant size category. The mean proportion of sampled Serr nuts falling into this (>36 mm) category across all treatments rose from between 29.6% and 33.0% in 2017–18, to between 51.4% and 73.3% in 2018–19. This may be a consequence of greater energy investment by the trees into the remaining nuts following considerable drop in yield observed that season due to PFA.

One-way ANOVA of the different treatments for all size categories and cultivars in the 2017–18 season showed only the Chandler <32 mm category to possess any significant differences (Table 3.7). The only pairwise comparison identified as significant was between the control and 0.75 L/ha (S) plots [Tukey's HSD, Q = 4.40, p<0.05], where the 0.75 L/ha (S) trees had a higher percentage of nuts in this category (Figure 3.24).

Analysis of the 2018–19 data identified treatments in four size categories with significant differences. One was in the Vina <32 mm, the others were in all three size categories of Serr (Table 3.7). In Vina the control had the greatest number of nuts in the <32 mm category, and this was significantly so when compared against the 0.75 L/ha (S) [Tukey's HSD, Q = 4.22, p<0.05] and 1.5 L/ha (S) [Tukey's HSD, Q = 5.51, p<0.05] treatments (Figure 3.25).

In 2018–19 the percentage of Serr nuts in the >36 mm category was significantly greater [Tukey's HSD, p<0.05] than all three of the treated plots. Conversely, the number of control nuts classified as 32-36 mm was significantly [Tukey's HSD, p<0.05] greater than the three treatment plots. The only significant difference in the <32 mm size category was between the control and 0.75 L/ha (S) treatment plots [Tukey's HSD, Q = 4.30, p<0.05] (Figure 3.25). No significant differences were identified between Sunny® treated plots in any nut size category. This shows a clear increase in nut size occurring in the treated plots when harvest yields are low.

Table 3.7. One-way ANOVA results of the size of nuts sampled from Serr, Vina and Chandler trial trees treated with varying rates of Sunny® in the 2017–18 and 2018–19 seasons.

Season	Cultivar	Size (mm)	df	F	p
2017–18	Serr	<32	(3, 36)	0.58	0.63
		32-36	(3, 36)	0.36	0.78
		>36	(3, 36)	0.23	0.88
	Vina	<32	(3, 36)	0.52	0.67
		32-36	(3, 36)	1.14	0.35
		>36	(3, 36)	1.50	0.23
	Chandler	<32	(3, 36)	3.52	<0.05
		32-36	(3, 36)	1.42	0.25
		>36	(3, 36)	0.35	0.79
2018–19	Serr	<32	(3, 36)	3.72	<0.05
		32-36	(3, 36)	7.43	<0.05
		>36	(3, 36)	8.44	<0.05
	Vina	<32	(3, 36)	6.49	<0.05
		32-36	(3, 36)	0.30	0.82
		>36	(3, 36)	1.88	0.15
	Chandler	<32	(3, 36)	0.33	0.80
		32-36	(3, 36)	1.12	0.35
		>36	(3, 36)	0.97	0.42

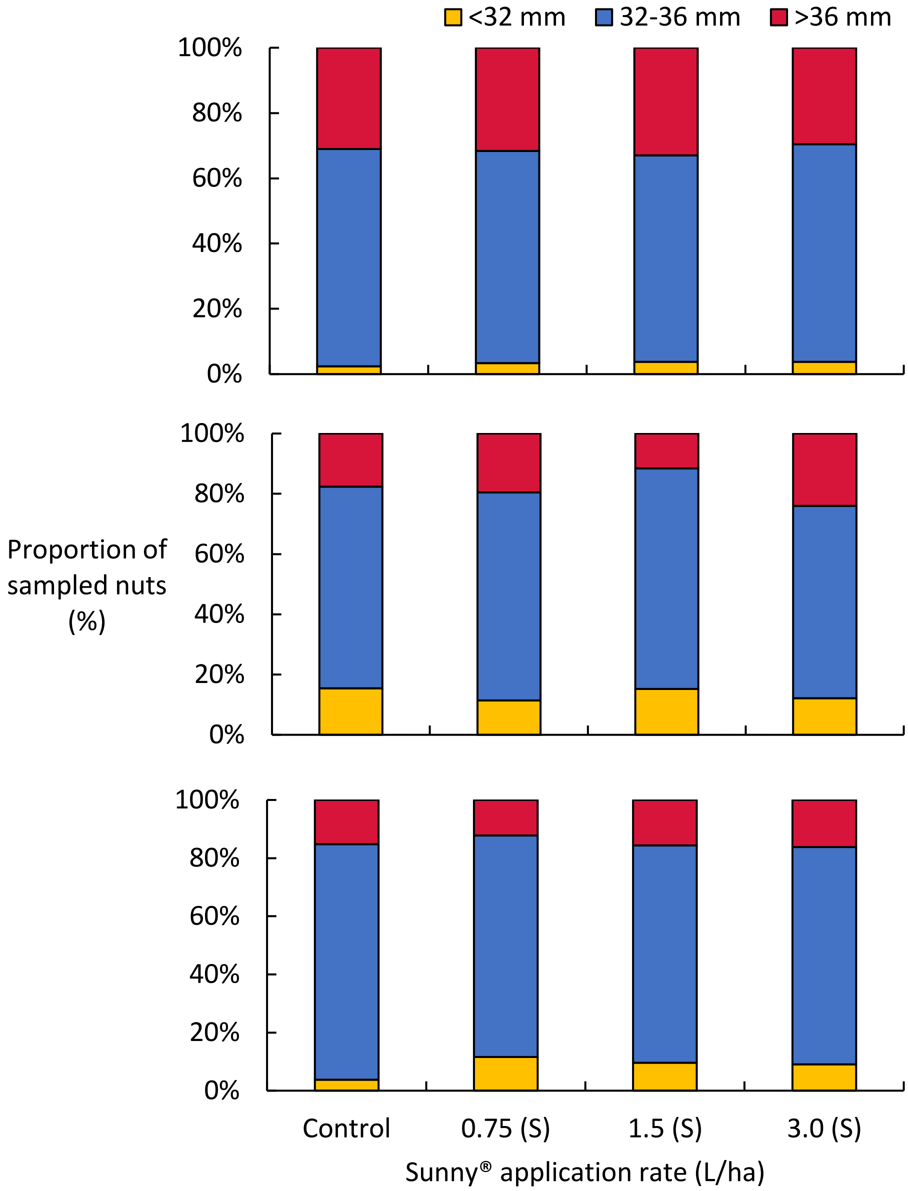


Figure 3.24. Mean proportion of nuts per sample in each size category in 2017–18 for Serr (top), Vina (centre) and Chandler (bottom).

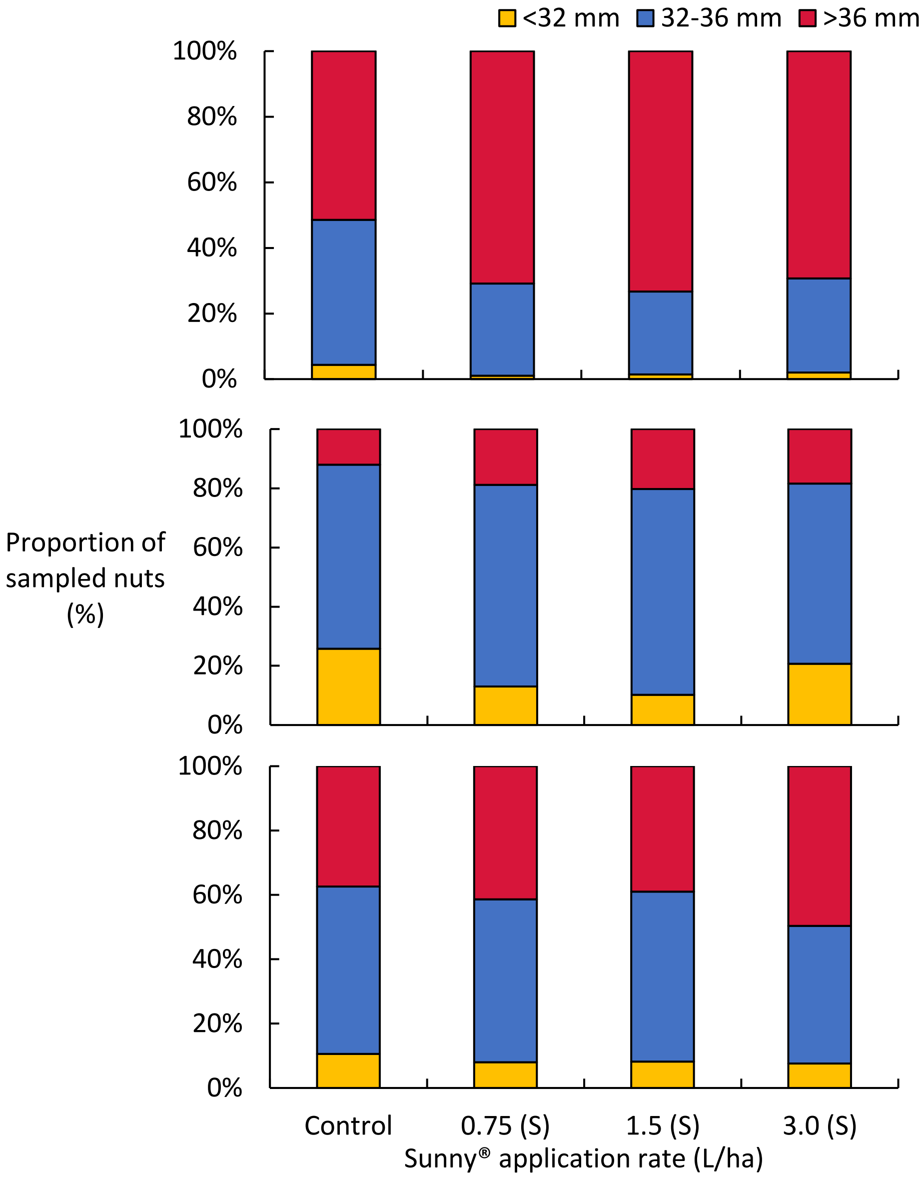


Figure 3.25. Mean proportion of nuts per sample in each size category in 2018–19 for Serr (top), Vina (centre)

Analysis of the plots treated with Sunny® in either spring or autumn at the 1.5 L/ha rate did not identify any significant differences for any of the three cultivars in either season [two-tailed t-test, $p>0.05$].

From these findings it can be concluded that neither the rate nor time of application significantly affect in-shell nut size over a two-year period of Sunny® application. However, Sunny® application was seen to increase the size of nuts to a greater extent than untreated nut when harvest yields were low.

Nut weight

Serr mean kernel weights were heaviest in both seasons and ranged from 6.49–7.60 g. Mean kernel weights for Vina and Chandler were similar over the same period, ranging from 4.64–5.53 g (Vina) and 5.04 – 5.21 g (Chandler). Statistical analysis of the different Sunny® rates applied in spring did not identify any significant differences in the mean kernel weights in the quality assessment of the 2017–18 harvest (Table 3.8).

Table 3.8. One-way ANOVA results of comparisons between the mean kernel weights of walnuts treated with different rates of Sunny® from 2017 to 2020. No significant differences existed in any of the three cultivars in the 2017–18 harvest, but Serr and Vina both showed significant differences between treatments in 2018–19.

Season	Cultivar	df	F	p
2017–18	**Serr**	(3, 36)	0.39	0.76
	Vina	(3, 36)	2.78	0.06
	Chandler	(3, 36)	0.44	0.77
2018–19	**Serr**	(3, 36)	8.23	<0.05
	Vina	(3, 36)	3.69	0.02
	Chandler	(3, 36)	0.02	1.00

The data analysed from the 2018–19 harvest identified significant differences in both the Serr and Vina trials (Table 3.8). In Serr, mean kernel weights were significantly [Tukey's HSD, p >0.05] lower in the control than both the 0.75 L/ha (S) and 1.5 L/ha (S) application rate plots. In Vina, only the 1.5 L/ha (S) rate was significantly heavier than the controls (Figure 3.26). No rate response was identified in either cultivar.

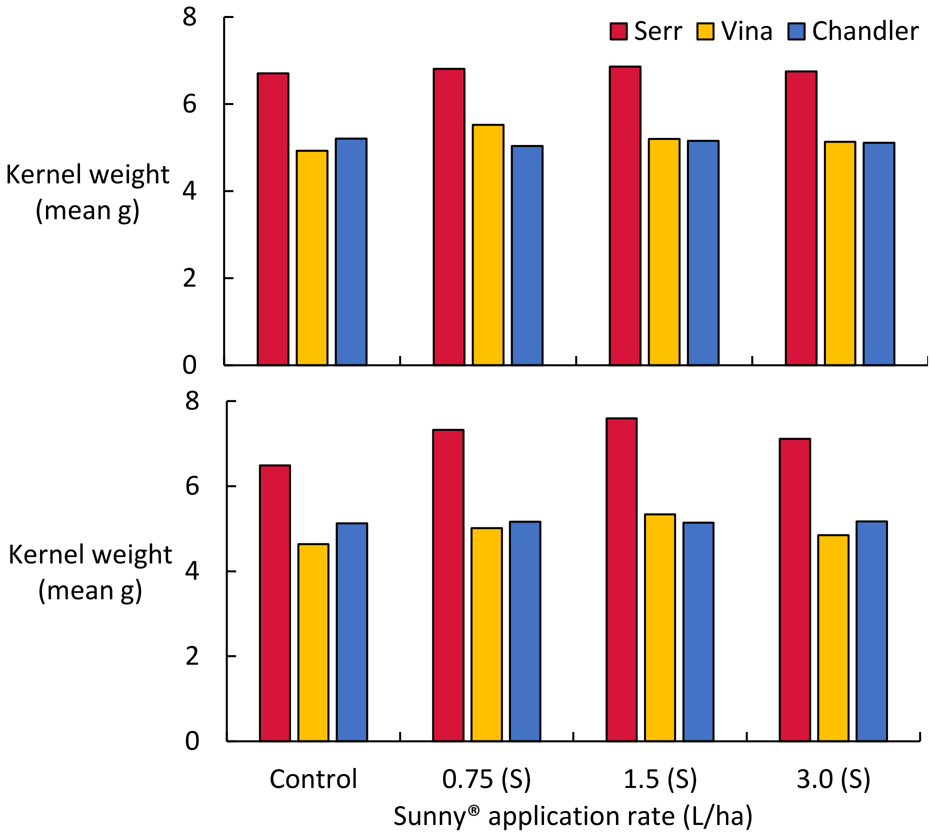


Figure 3.26. Mean kernel weights of Serr, Vina and Chandler walnuts in 2017–18 (top) and 2018–19 (bottom) that were studied in an assessment of the effects of Sunny® use on trees to control vigour.

Although no significant differences were identified between the Chandler trial plots in either season (Table 3.8), the control went from having the heaviest mean kernel weight in 2017–18 (control = 5.21 g, 0.75 L/ha = 5.04 g, 1.5 L/ha = 5.16, 3.0 L/ha = 5.11 g) to the lightest in 2018–19 (control = 5.13 g, 0.75 L/ha = 5.17 g, 1.5 L/ha = 5.14 g, 3.0 L/ha = 5.17g). This combined with the observations from Serr and Vina superficially indicate a possible trend where nut weight increases over time with Sunny® application, but this cannot be confirmed due to lack of statistical evidence and the limited number of harvests assessed. As in the Serr and Vina trials, there was no indication of a rate response.

Comparisons of the plots treated with 1.5 L/ha in either spring or autumn showed the spring treated plots had consistently higher mean kernel weights than those where Sunny® was applied in autumn (Figure 3.27). This was true across all three trial cultivars in both seasons. However, statistical analysis showed none of these differences to be significant [two-tailed t-test, p>0.05].

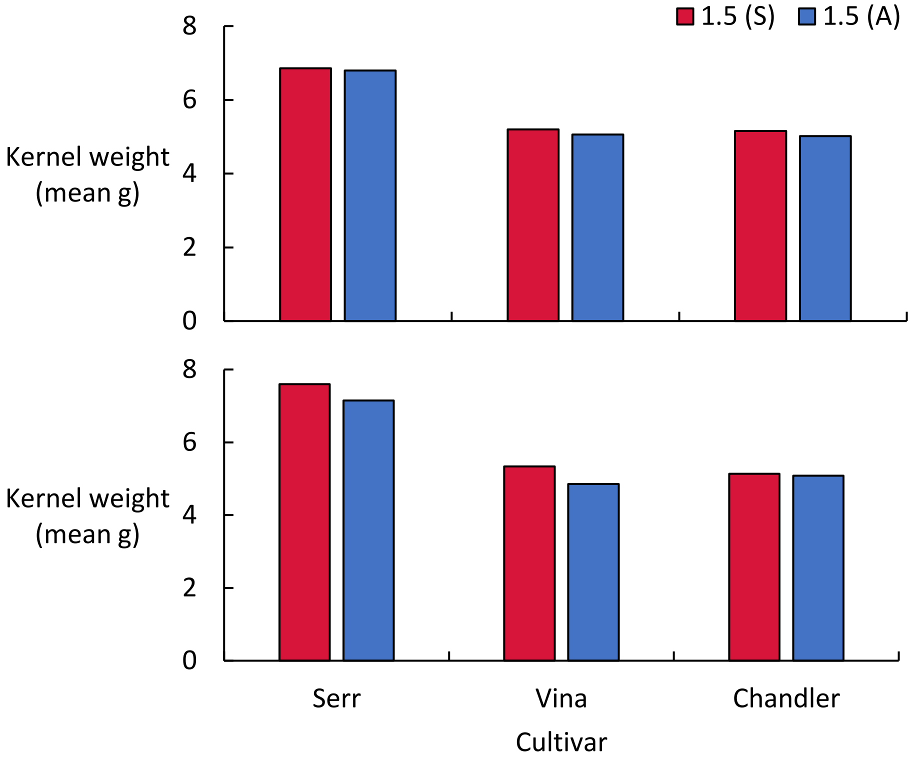


Figure 3.27. Comparisons of spring and autumn application of Sunny® at a rate of 1.5 L/ha in the trial sites of three walnut cultivars (Serr, Vina and Chandler) at Leeton orchard.

Although no significant differences were identified between the Chandler trial plots in either season (Table 3.8), the control went from having the heaviest mean kernel weight in 2017–18 (control = 5.21 g, 0.75 L/ha = 5.04 g, 1.5 L/ha = 5.16, 3.0 L/ha = 5.11 g) to the lightest in 2018–19 (control = 5.13 g, 0.75 L/ha = 5.17 g, 1.5 L/ha = 5.14 g, 3.0 L/ha = 5.17 g). This combined with the observations from Serr and Vina superficially indicate a possible trend where nut weight increases over time with Sunny® application, but this cannot be confirmed due to lack of statistical evidence and the limited number of harvests assessed. As in the Serr and Vina trials, there was no indication of a rate response.

Comparisons of the plots treated with 1.5 L/ha in either spring or autumn showed the spring treated plots had consistently higher mean kernel weights than those where Sunny® was applied in autumn (Figure 3.28). This was true across all three trial cultivars in both seasons. However, statistical analysis showed none of these differences to be significant [two-tailed t-test, p>0.05].

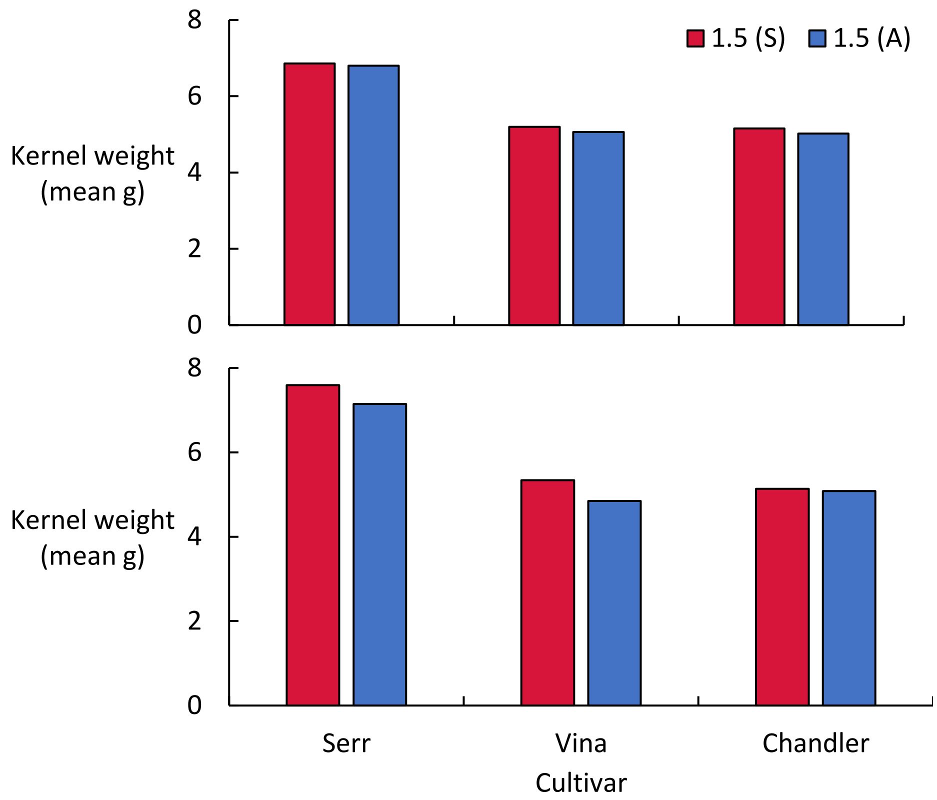


Figure 3.28. Comparisons of spring and autumn application of Sunny® at a rate of 1.5 L/ha in the trial sites of three walnut cultivars (Serr, Vina and Chandler) at Leeton orchard.

Due to the limited number of seasons the trials ran for, there is no clear evidence to conclude Sunny® application causes an increase in mean kernel weight. The results obtained from the Serr and Vina trials provide circumstantial evidence that this may occur over time, but there is insufficient data to confirm this. A longer harvest assessment period that covered another year or two may have provided clarification.

Kernel colour

Nut samples from the Serr trial possessed the greatest proportion of extra light and light kernels. Mean percentages of extra light and light kernels over the two seasons ranged from 62.5% (3.0 L/ha) to 92.4% (control) in Serr, 28.6% (3.0 L/ha) to 54.8% (0.75 L/ha) in Vina, and 68.8 (3.0 L/ha) to 87.2% (0.75 L/ha) in Chandler.

No significant differences were identified in the mean proportion of extra light and light kernels across the trial plots in any of the three cultivar trials in 2017–18 (Table 3.9). However, all three cultivars expressed significant differences between treatments in 2018–19 (Table 3.9). Pairwise comparisons of the treatment plots showed the samples assessed had a significantly lower proportion of extra light and light kernels in the 3.0 L/ha (S) treated plots than the control in Serr, the 1.5 L/ha (S) in Chandler, and both the control and 1.5 L/ha (S) in Vina (Figure 3.29). No rate response was identified in the results, but the 3.0 L/ha (S) treated plots possessed the lowest percentage of extra light and light kernels in in 2017–18 and 2018–19 in every instance except the 2017–18 Vina (control 51.5%, 3.0 L/ha = 52.0%) (Figure 3.29).

Table 3.9. One-way ANOVA results of comparisons between trial plots of trees treated with different rates of Sunny®.

Season	Cultivar	df	F	p
2017–18	Serr	(3, 36)	2.44	0.08
	Vina	(3, 36)	0.09	0.97
	Chandler	(3, 36)	2.71	0.06
2018–19	Serr	(3, 36)	2.95	0.05
	Vina	(3, 36)	4.76	0.01
	Chandler	(3, 36)	3.42	0.03

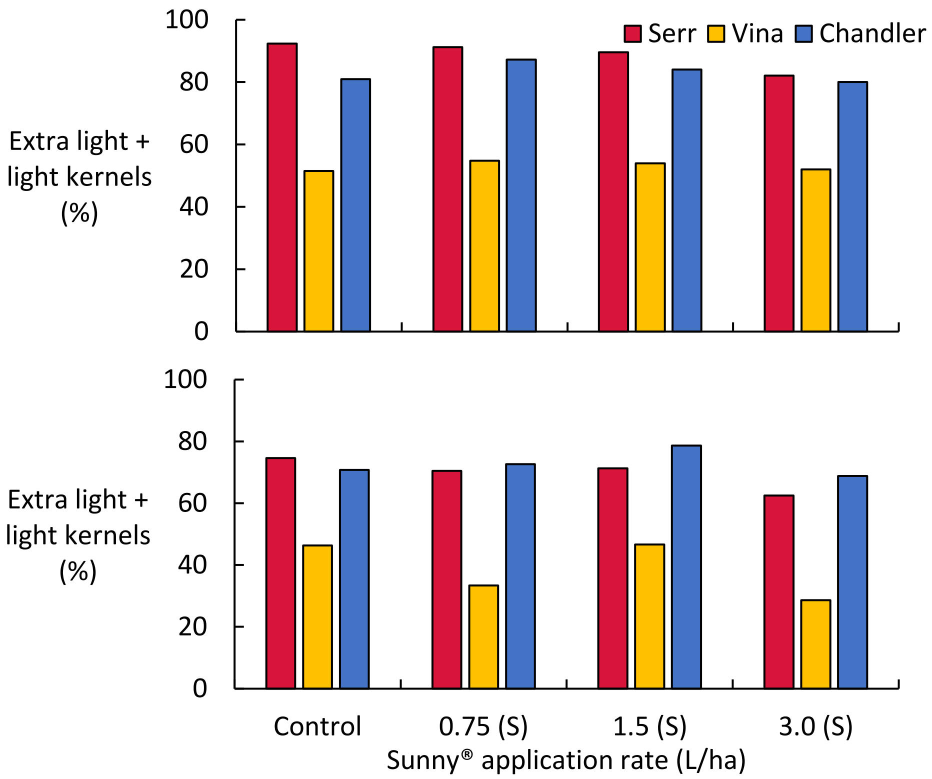


Figure 3.29. Mean proportion of extra light and light kernels recorded from three cultivars in 2017–18 (top) and 2018–19 (bottom).

Comparisons of the plots where 1.5 L/ha were applied in different seasons identified the spring treated Vina trees had a significantly greater proportion of extra light and light nuts compared to the autumn treated plots in the 2017–18 season [two-tailed t-test, $p>0.05$]. No other notable differences were identified statistically in any cultivar trial in either 2017–18 or 2018–19 (Figure 3.30).

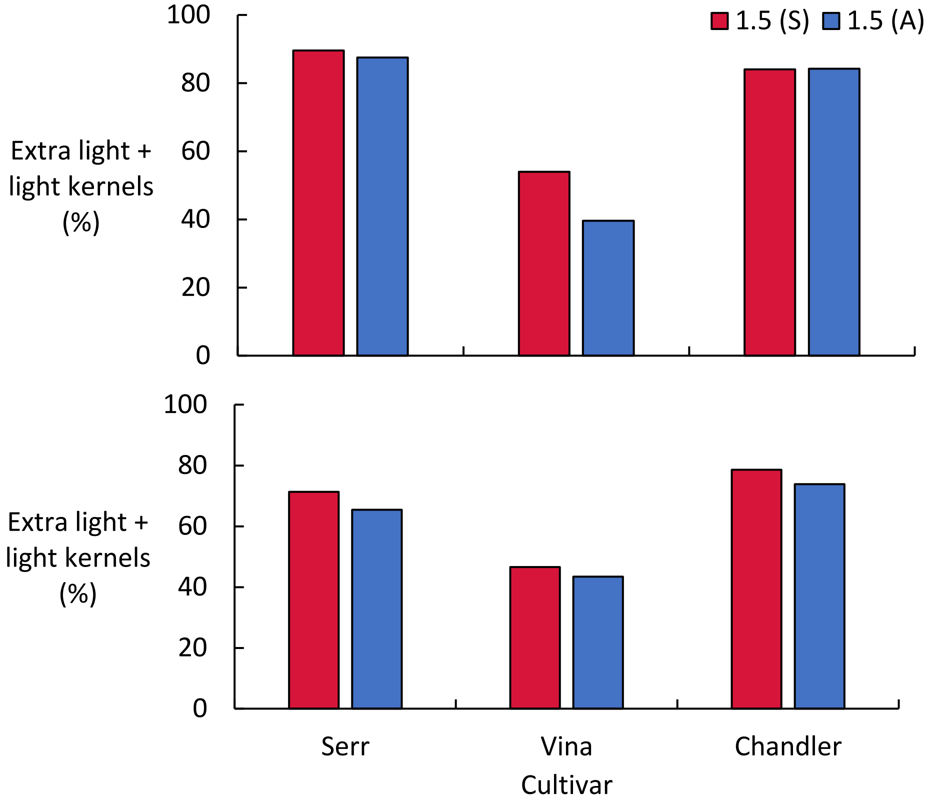


Figure 3.30. Mean proportion of extra light and light kernels recorded from the three cultivar trials investigating the effect of Sunny® on kernel colour at Leeton orchard in 2017–18 (top) and 2018–9 (bottom).

From these findings it can be concluded that kernel colour not affected by application time of Sunny® but is to some degree by application rate; with the highest application rates significantly reducing the proportion of extra light and light nuts (lighter nuts are generally considered a premium product to dark nuts). Several causes for kernel darkening have previously been reported, including unsuitable watering regimes and the natural tendency for certain cultivars to produce dark kernels. Given that no inter-cultivar comparisons have been made, and the fact that all the plots in each cultivar trial were sourced through the same irrigation valve and on the same irrigation schedule, then these two factors can be eliminated as the primary reason for the observations.

The reason for these findings could be linked to the incidence of light interception penetrating through the canopy. As previously reported (see Section 3.3.5), canopy light interception in the 2019–20 season decreased significantly at the highest rates of Sunny® application in all three cultivars during flowering; and this trend was observed to persist into the kernel development stage in Vina. Given the highly significant difference in kernel colour between treatments in the Vina trial trees from the 2018–19 harvest, it is possible that, in hotter regions, increased sun exposure through changes in canopy structure may result in a reduction of extra light and light kernels in some cultivars. This may occur either directly through increased sun exposure, or indirectly by means such as the alteration of irrigation regimes required because of changes in the requirements of trees resulting from Sunny®-induced physiological changes. The lack of data and seasonal misalignment of data in this research make any solid conclusions impossible, but these are areas of research that should be investigated further to understand the broader implications of Sunny® use, both on different cultivars and in different climates.

Nut shrivel

One-way ANOVA showed the only significant differences between treatment plots was in Serr during the 2018–19 season (Table 3.10). Tukey's HSD post-hoc testing identified the control plots had a significantly [Tukey's HSD, Q = 4.12, $p<0.05$] higher mean percentage of kernel shrivel (14.59%) than the 1.5 L/ha (S) treated plots (8.19%). No other significant differences were observed between treatment rates or treatment times for any of the cultivars (Table 3.10, Figure 3.31). No rate response was identified in the cultivars in either 2017 – 18 or 2018 – 19 (Figure 3.32). It can therefore be concluded that Sunny® had no direct effect on the percentage of kernel shrivel.

Table 3.10. One-way ANOVA of the prevalence of nut shrivel between different Sunny® treatments in Serr, Vina and Chandler in the 2017–18 and 2018–19 harvest seasons.

Season	Cultivar	df	F	p
2017–18	Serr	(3, 36)	0.35	0.79
	Vina	(3, 36)	0.81	0.50
	Chandler	(3, 36)	0.45	0.72
2018–19	Serr	(3, 36)	3.45	0.03
	Vina	(3, 36)	1.10	0.36
	Chandler	(3, 36)	0.76	0.52

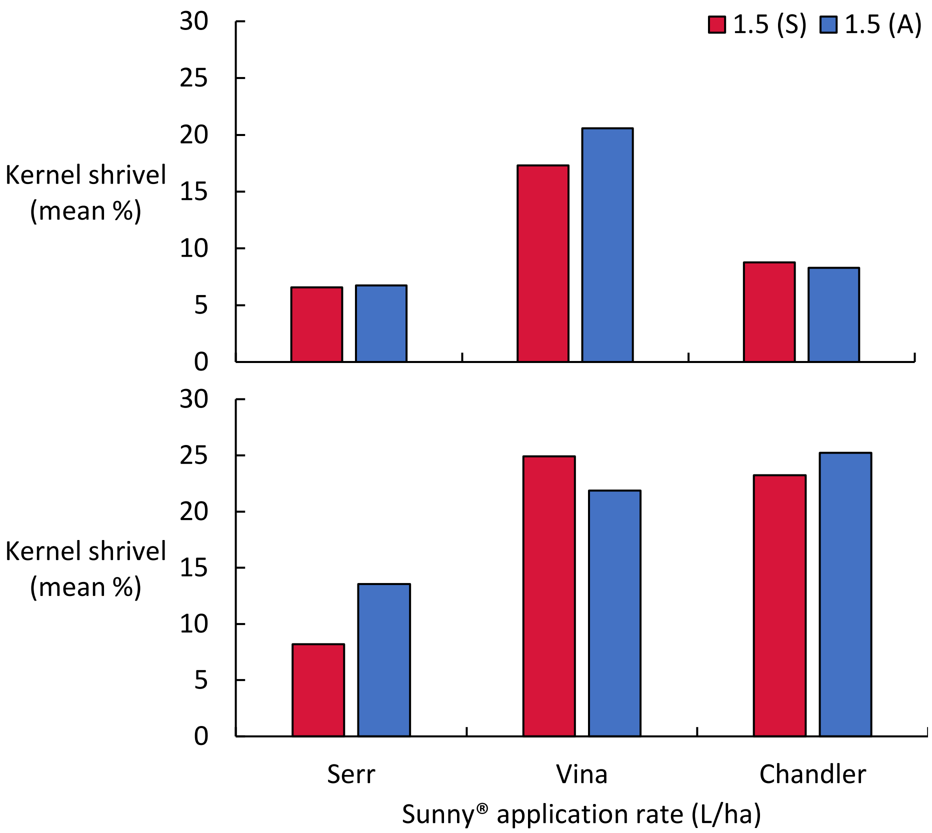


Figure 3.31. Mean percentage of kernel shrivel recorded in the plots treated with Sunny® at the 1.5 L/ha rate in spring and autumn from 2017 to 2020. The study was undertaken on harvested nuts in the 2017–18 (top) and 2018–19 (bottom) seasons.

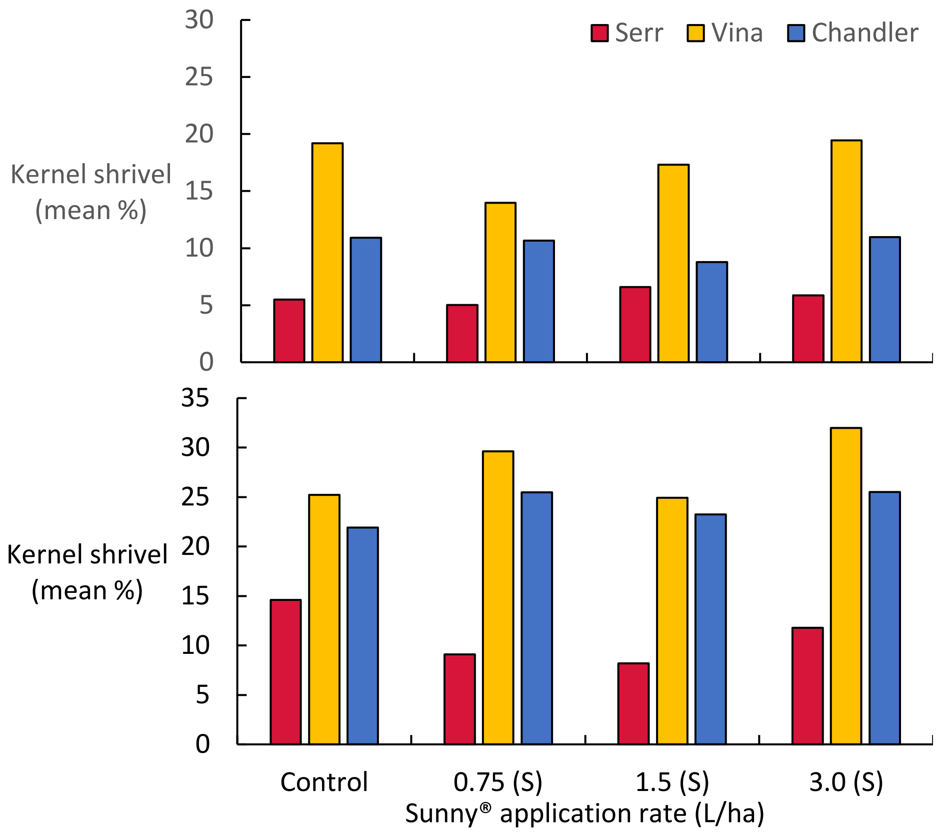


Figure 3.32. Mean percentage of kernel shrivel recorded in the Sunny® treated trial plots of three walnut cultivars studied at Leeton orchard in 2017–18 (top) and 2018–19 (bottom). No significant differences [one-way ANOVA, $p>0.05$] were identified in the first season. In 2018–19 the control trees showed significantly [Tukey's HSD, $Q = 4.12$, $p<0.05$] greater shrivel than those treated with 1.5 L/ha.

Nut sunburn

No significant differences in the occurrence of kernel sunburn were confirmed between Sunny® application rates in any of the cultivars in the 2018–19 assessment (Table 3.11), and no clear rate response identified either (Figure 3.33). Similarly, although trees treated with 1.5 L/ha of Sunny® in autumn typically had higher rates of sunburn, the differences between them proved to be non-significant [two-tailed t-test, $p > 0.05$] in all three cultivars (Figure 3.34).

Table 3.11. One-way ANOVA results investigating the effect of Sunny® application on the occurrence of sunburn on walnut kernels, as assessed in the 2018–19 harvests of Serr, Vina and Chandler trial trees at Leeton orchard.

Cultivar	df	F	p
Serr	(3, 36)	0.81	0.50
Vina	(3, 36)	1.44	0.25
Chandler	(3, 36)	1.16	0.34

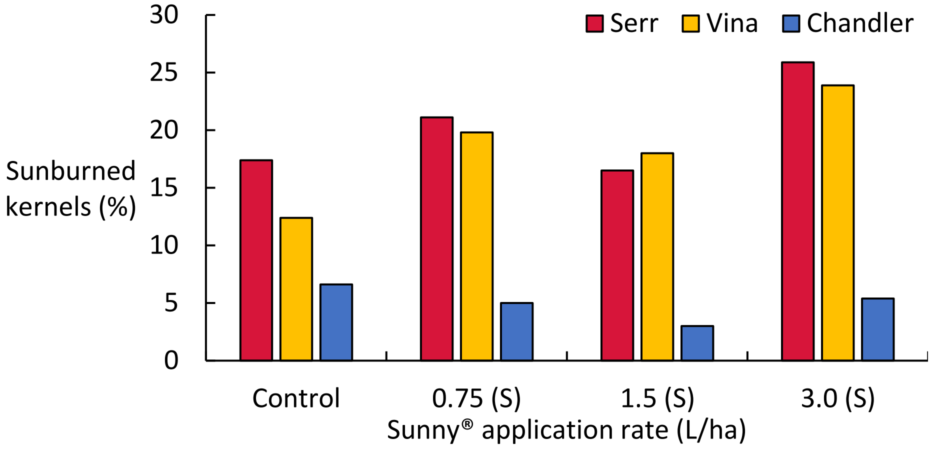


Figure 3.33. Occurrence of sunburn.

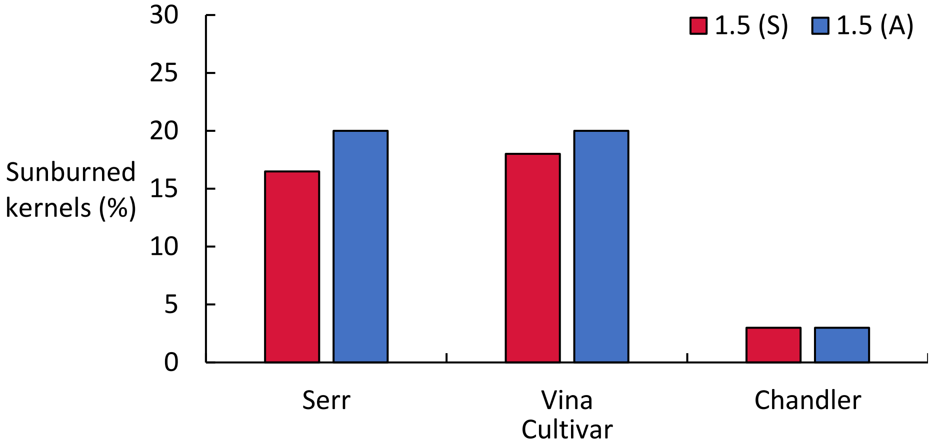


Figure 3.34. Occurrence of sunburn in sampled nuts treated with 1.5 L/ha of Sunny® in either spring or autumn. Two-tailed t-tests of data showed no significant differences (p >0.05) between application times in any of the three cultivars.

Based on these findings, the application of Sunny® both at different rates and times does not affect the level of sunburn in walnut kernels. However, a trend was observed in Vina where the mean percentage of sunburned kernels recorded was typically higher where Sunny® was applied (control = 12.4%, 0.75 L/ha = 19.8%, 1.5 L/ha = 18%, 3.0 L/ha = 23.9%).

This may be a consequence of greater sun exposure during the summer resulting from a more open canopy (Section 4.3.6) influenced by either Sunny® application, other factors (e.g. thinning/pruning regimes), or a combination of both. The lack of harvest data from the 2019–20 season, when the PAR measurements were taken, mean it was not possible to undertake multivariate analysis to investigate the matter in further detail. However, this is something that should be given further consideration in future studies. Especially regarding the potential confounding effects that Sunny® use may have in orchards located in dry-temperate to semi-arid regions, where summer temperatures often exceed 42 °C during kernel development.

3.4. Summary and conclusions

- A high rate of Sunny® (3.0 L/ha of Sunny® PGR) led to trees having shorter internodes than non-treated trees in young trees.
- Low to high rates of Sunny® (0.75, 1.5 and 3.0 L/ha of Sunny® PGR), applied as post-harvest or bud burst soil drenches, did not reduce crop yield, bud counts or nut quality (kernel colour).
- In younger trees the highest rate of Sunny® (3.0 L/ha of Sunny® PGR) reduced the length of internodes without affecting the numbers of buds.
- In older trees Sunny® applications did not have a significant effect on the quality of the nut.
- Findings on leaflet v leaf area support the measuring of terminal leaflets as an alternative method to measure the entire leaf when comparing relative leaf area between treated walnut trees.
- Vegetative growth/vigour restricted by Sunny® application, as per the findings in the Avondale crown and stem measurements during 2017–18 and 2018–19. It is difficult to make a definitive conclusion on the impact of Sunny® on mature trees, but the general pattern of stem lengths and internode measurements at Leeton would imply a similar effect. No significant effect identified between spring and autumn application at this point.
- Sunny® application did not appear to affect harvest yields or in-shell nut size. However, a significantly greater proportion of larger in-shell nuts and heavier kernels were recorded from Sunny® treated trees than those not treated in Serr during a season of low yield. This implies Sunny® increases nut size when yields fall below a certain threshold.
- An increase in light penetration through the canopy was linked to higher Sunny® application rates. Slight increases in the occurrence of dark kernels and shrivel fitted this trend, but no difference in sunburn rates was identified. This was most noticeable in Vina, which was the least vigorous of the three cultivars studied.

4. Understanding the impact of selective limb removal on the penetration of light into walnut tree canopy using simple and advanced technologies.

Jacquelyn Simpson and Jason Lewis

4.1. Background

Pruning trials are an important component of our work towards understanding the relationship between light interception and productivity, hence examining the potential for light management and productive high-density walnut orchards.

4.1.1. Pruning strategies

Common pruning strategies are selective limb removal and managing orchards as hedge-row systems. The intensity of application varies significantly. Hedgerow orchards, in which mechanical pruning machinery is used to uniformly 'hedge' the rows of the orchard, are popular for the potential to implement orchard management practices (for example pest and disease control programs), higher yields and for greater efficiency of machinery use (Ninot et al., 2005). Selective limb removal involves the manual excision of entire limbs of a tree to allow greater light penetration, remove weak limbs or weak limb joints and allow other management strategies (pest, disease etc) to more contact with the tree.

The Walnut Growers Manual recommends managing walnut trees in a modified central leader system, by removing lateral shoots and selecting scaffolding shoots early in tree establishment (Ramos, 1997). Heading cuts should only be made once the tree has reached desired final height. The Australian Best Practice Management guide recommends that an orchard should intercept between 70 and 80% of potential sunlight in the canopy of the tree, as such tree height should not exceed 80% of row diameter and row width needs to consider machinery access (Adem and Jerie, 2004). Both sources recommend a central leader system, and to generally avoid heading shoots, which is achieved by 50-100% limb removal. Conversely, in California, early yields have been demonstrated in orchards with less intense pruning strategies, or no annual pruning and allowing the trees to grow more naturally (Lampinen et al., 2015). This means they are beginning to plant orchards at a lower density. This approach has been producing high yields and requires less water (California AgNet, 2014).

Chilean walnut orchards planted at high density with an intense limb removal approach to a central leader system had increased early yields (Lemus, 2001). In 2017, a tour of orchards in Chile presented a central leader system as an ideal pruning mechanism and was proposing extremely high density (1.5 m spacing between trees) as achievable with an intensive selective limb removal program from the beginning of orchard establishment (Figure 4.1). In Chile, labour is significantly cheaper than it is in Australia (or in California) making this approach more feasible. The long-term effects of this strategy are yet to be evaluated, and orchards managed this way use heavy applications of chemicals to manipulate plant growth. At the time of the orchard tour, the selective limb removal method was found to increase the amount of light entering the canopy and many nut clusters were observed throughout (Figure 4.1).

Figure 4.1. Light management strategies through early architecture establish and selective limb removal (left) leading to crop throughout tree canopy (Right) in Chilean walnut orchards.

4.1.2. Light interception – what does this mean for productivity?

The potential for light to enter the internal canopy tissues is well understood to have beneficial outcomes for increasing yield (Adem and Jerie, 2004). Light intercepted by the canopy, the inverse being light that penetrates through the canopy, can be measured by a ceptometer. A ceptometer is a bar that contains sensors that measure the intensity of light as Photosynthetically Active Radiation (PAR, µm). PAR measurements from under the canopy of a tree are compared to PAR measurements taken by and external sensor above the tree canopy (as a measure of the potential light) and indicate the density of the canopy through the amount of light intercepted.

4.1.2. Technology options

Technologies that use unmanned aerial vehicles (UAVs) to collect data are increasingly popular tools for understanding orchards in Australia and around the world. Normalised Difference Vegetation Index (NDVI) and Plant Cell Density (PCD) are two means of measuring the variability of crop vigour and productivity (Tu et al., 2019). High values of with NDVI or PCD measurements indicate more vigour and are used commercial to guide crop management in almond (Martínez-Casasnovas, 2021; Zarate-Valdez et al., 2012), avocado (Robson et al., 2017), pear (Van Beek et al., 2015) and pistachio (Bellvert et al., 2018), as examples. In this work we have used PCD collected by a camera mounted to a UAV to compare the vigour of pruned and unpruned walnut trees as a means of assessing the tree vigour response to pruning.

When stressed, walnut trees can respond by increasing vegetative shoot growth, referred to as stress or water shoots. As described above, the purpose of pruning is to allow more light to penetrate the canopy of a walnut tree and increase the yielding potential of the tree. Stress shoots are more likely to occur under certain climatic conditions and can be controlled by pruning in more mild weather when the trees are not already water or heat stressed. Measuring PCD could be a means to understand tree response to pruning activities and guide multi-year pruning strategies, such as selective limb removal.

Other options for technology to guide orchard managements that were investigated during this work were the use of thermal images to predict yields and image analysis to guide pruning strategies. These were not pursued but are summarised:

- Thermal imagery – In theory walnuts are likely to retain more heat than the foliage and wood of the walnuts tree, based on this theory we tested the use of a thermal camera to differentiate fruit from vegetative growth on a mature walnut tree. This was not pursued as it quickly became apparent that for the technology to be truly effective growers would need to either invest significant amounts of money in extremely high resolution, powerful thermal cameras, or rework their trees to have a more two dimensional canopy structure to allow the camera to accurately detect yield loads of trees. Neither scenario was identified as particularly useful for growers.
- Image analysis software – We trialed the use of ImageJ and MiPAR software to analyse tree canopy structures without foliage as a means of comparing pruned and unpruned trees. Image J was not a feasible option as although useful in many settings it is a complex tool unlikely to be useful for orchard management. However, MiPAR was an effective tool in determining limb thickness etc. Again, this wasn't pursued for the perceived lack of value (especially compared to UAV mounted PCD and NDVI technologies) to growers.
- LiDAR was investigated as a means of understanding tree architecture. At the time of investigation, the cost of a LiDAR system capable of mapping out a walnut orchard was prohibitive. Now there are LiDAR cameras available on iPads. Investigating the potential use of this technology in walnut orchard management would be a useful tool for growers. Future work would benefit from developing applications that accessed the camera in an appropriate way to interpret tree architecture information.

The experimental orchard at Orange Agricultural Institute (refer to Section 2.3) is established as a platform for future technology trials that will allow us to further investigate options, subject to funding availability.

4.1.3. Aim

The aim of this component of the project is to assess the impact of selective limb removal on walnut tree canopy light interception (as a measure of canopy density) and the resulting yield and quality of the walnut crop. In the process of doing this, considerable data has been collected from a known set of trees over 4 years. Parts of this dataset have been merged with data collected from PCT AgServices and compared to manually collected data as a simple demonstration of using advanced technologies to guide orchard management practices.

4.2. Methods

4.2.1. Field sites

For this component of the project we carried out experiments and measurements on the mature commercial orchard (refer to the description in section 2).

4.2.2. Selective limb removal

Selective limb removal as a method of pruning walnut trees is a multi-year process for which 2017 was the first harvest, we have collected light interception data from 3 cultivars (Chandler, Lara and Serr) during winter 2017 (2018 season) and from 4 cultivars (Chandler, Lara, Serr and Ashley) during summer 2017 (2017 season). Figure 4.2. shows examples of trees that have been pruned using selective limb removal.

Figure 4.2. Selective limb removal in Chandler (left) and Serr (right) trees.

4.2.3. PAR measurements

Below canopy photosynthetically active radiation (PAR) was measured with a ceptometer (AccuPAR Model LP-80, Decagon Devices, Inc) in summer and winter of 2017 and 2018, then winter, early season and preharvest in 2019 and 2020 (Figure 4.3). The different timing of data collection allowed us to understand the effect of pruning treatments on tree wood volume (winter measurements) early season vegetative or canopy volume and late season vegetative or canopy volume.

Figure 4.3. Example of trees at Dormant (left) measurement timing and Early Season/Spring measurement time (right)

Four PAR measurements were collected in a 1 m radius from the trunk, under the canopy (1 m above ground) of 50 trees for each cultivar and treatment (Figure 4.4). An additional PAR sensor was used to obtain full-sun PAR data for each sampling event, referred to as above canopy PAR. The mean below canopy PAR is reported as a percentage of above canopy PAR. The percentage PAR measured under the canopy indicates light penetrating through the canopy, and PAR intercepted is 100% - penetrated PAR.

A greater PAR value represents a greater amount of light reaching the ground, hence PAR values are greater when measured in winter as trees were dormant and leaves were not present to intercept light and this is indicative of the quantity of wood. At this stage, there are no conclusions to draw from the data collected, and the results presented here are an update of baseline data.

Trees from which data was collected were randomly selected using simple R code:

```
(c(sample(10:130, 4, replace=FALSE))
```

Four trees were selected from each of 14 rows of walnut trees located on the mature, commercial walnut orchard described in section 2 with a total of 56 trees. A map was produced using ggplot2 (Wickham, 2016) of the entire 2,500 tree block. This was used annually for a repeat measures trial.

In the first year of assessment (2016-17 season) an additional measurement was collected from a subsample of trees This measurement, termed line PAR, involved measuring the light intercepted at a height of 100 cm from ground level at the edge of the walnut tree canopy (Figure 4.4). This measurement was only done in the first year generate graphical images of the impact of selective limb removal prior to commercial hedging of the orchard rows. The method is summarised in Figure 3.1., briefly involved side stepping a 20 m line, 3.5 m from the trunk line along orchard rows and taking a measurement with a 900 cm ceptometer every step. The ceptometer contains 8 sensors. A twenty-meter line meant that the edge of canopy of 5 trees was measured and the ceptometer was pointed inward giving a 20 m^2 measurement of canopy edge. The was repeated on two sides (in the interrow) of five-replicate, five-tree plots. The under-canopy PAR (or %PAR) was log transformed and maps were generated using ggplot2 in R. The graphical representation of the tree line starts from 280 cm (2.8 m) from the tree line to 360 cm (3.6 m) to capture the variation in the canopy edge.

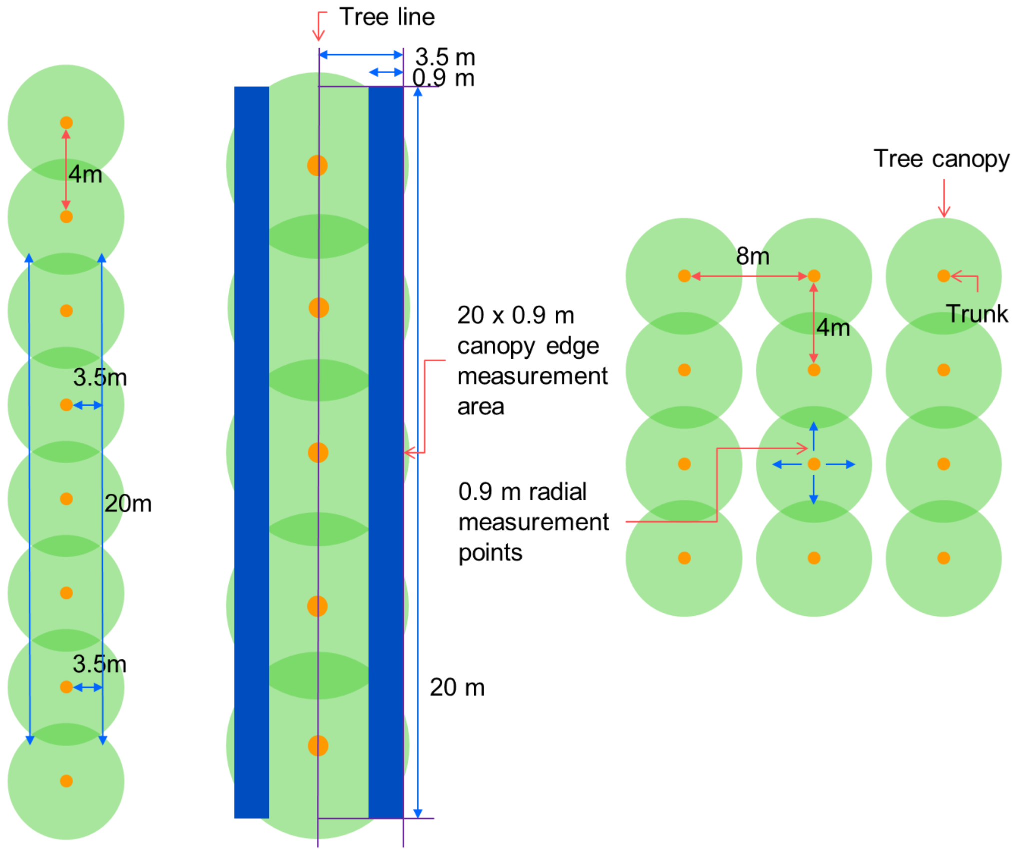


Figure 4.4. Diagram of PAR measurement strategies, line (left/middle) and circular/trunk (right) measurements.

4.2.4. Yield and quality assessments

Yield was assessed differently throughout the trial as an artefact of access and time restrictions. Early data collection was based on orchard managers reports of whole-block yields and reported on for six cultivars (Ashely, Chandler, Vina, Lara, Serr and Howard). Yield data for the years 2018, 2019 and 2020 were collected from TracMap data also, however the data was obtained through third party software (PCT AgServices) and included individual tree data points.

Quality assessments were conducted in the first and last years of the project (2017 and 2020) as described in Chapter 3 (Section 3.2.13)

4.2.5. Advanced technology

PCT Ag data

UAV mounted equipment has been used in this work through engagement of a consultant PCT AgServices. PCT AgServices were contracted by the commercial partner to collect data for multiple years across all of the commercial orchards. We (NSW DPIE project team) were granted access to this data to assess the potential for this information to be used in understanding and guiding future pruning strategy to management tree light penetration. There is extensive data and online tools available through this service, we only access a limited amount of this data for our work. The Plant Cell Density (PCD) and TracMap yield data were downloaded. Data was subsetted to match trees selected for light interception trial and datasets were then combined. Using the online tool allows growers to select variables and plot per-block yield predictions using PCD and Normalised Vegetation Difference Index (NDVI), the suitability of this tool is best assessed as a case-by-case assessment, considering the costs and benefits of doing so (Figure 4.5).

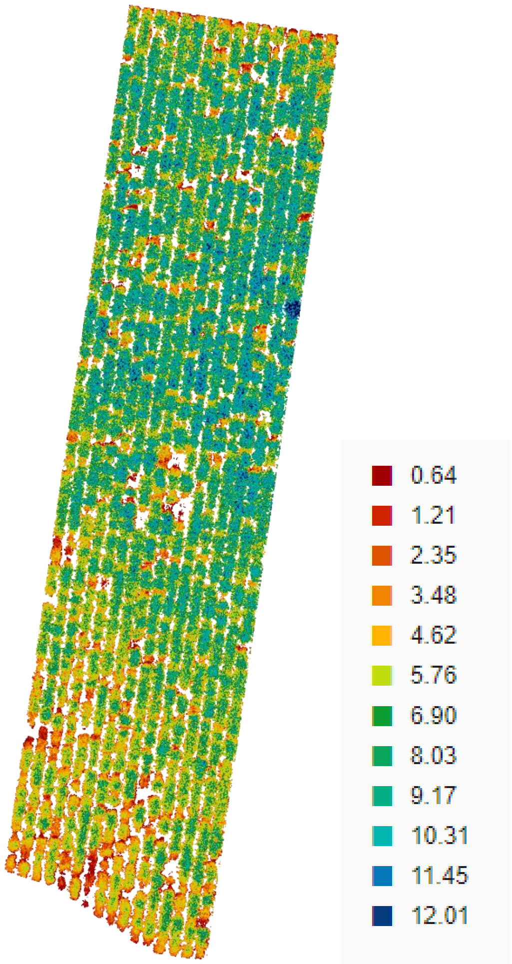


Figure 4.5. Example of data analytical capabilities of the online tools, using PCD data to predict yield at pixel level.

4.3. Results and discussion

4.3.1. Initial yield assessments (as reported in MS104)

Yield was recorded during commercial harvest using the TracMap system, this was converted to dry weight (DW) per hectare and collected for all cultivars the mature commercial orchard (Figure 4.6). The maximum recorded yield was 14.6 t/ha (DW) from unpruned Lara trees (control), this was almost double the yield of pruned trees (7.4 t/ha DW) in the same cultivar. The minimum recorded yield was 3.5 t/ha (DW) from pruned Ashley trees, which was not significantly different from the unpruned control Ashley trees (3.7 t/ha DW). Mean yield of pruned trees (blue, or 2016 treatment Figure 4.6) was 6.4 ± 2.18 t/ha (DW) compared to 8.4 ± 4.06 t/ha (DW) in unpruned trees. Except for Vina, all unpruned trees had higher yields than pruned trees, which is to be expected following the removal of potentially productive limbs and wood.

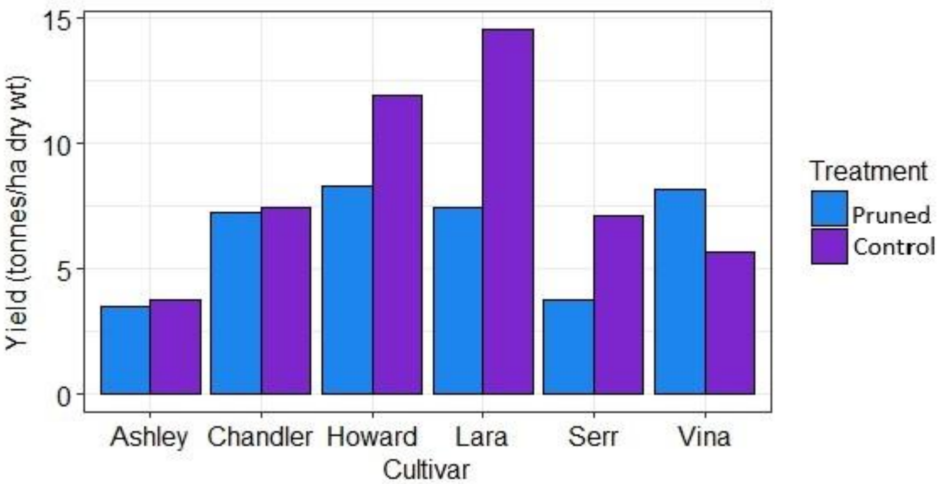
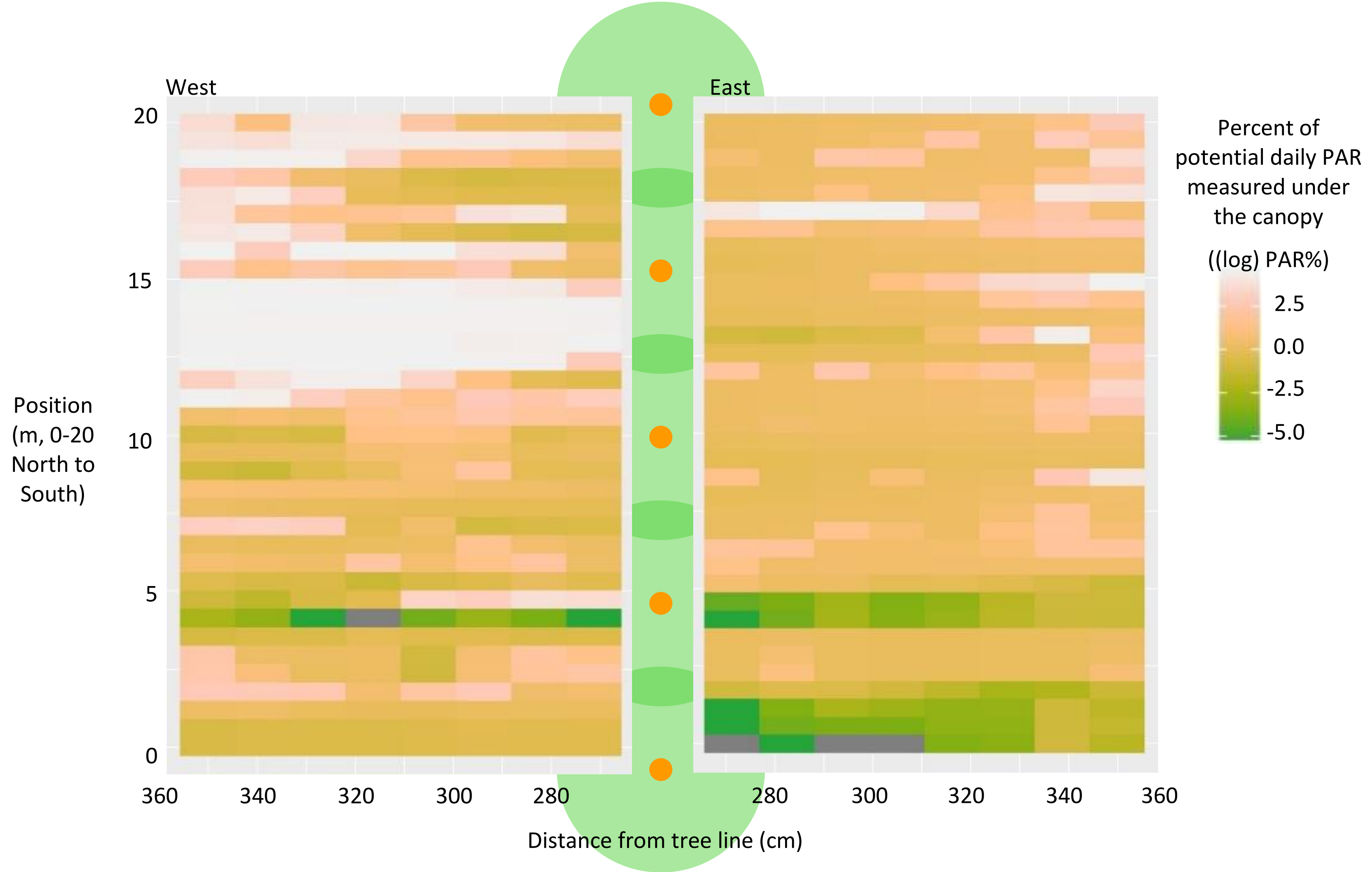


Figure 4.6. Light intercepted by trees for three cultivars measured with leaves (summer) and without leaves (winter) for each treatment (2016 prune, 2017 prune and unpruned).

PAR on the edge of the tree canopy was greater in pruned than control trees (Figure 4.7) in the first year after the selective limb removal was implemented. Hedging pruning strategies were employed annually thereafter, making the continuation of this method unfeasible. The idea that there is more light available on the edge of the tree canopy with selective limb removal is significant as many tree produce nuts on either older wood or are terminally bearing (i.e. nuts produced on the end of shoots rather than distributed along branches). Additional light on outer limbs may benefit productivity of trees (Valdebenito et al., 2017; 2018). The canopies extended between 2.8 and 3.6 m from the treeline which, in an orchard with 8 m wide rows, is the ideal scenario for optimal light interception and machinery access, according to Adem and Jerie (2004). Further research with a focus on spur positioning and productivity would provide further insight into this. Robust pruning and canopy light management trials would be an excellent PhD project.



West
East
20
15
10
5
0
Position (m, 0-20 North to South)
360
340
320
300
280
280
300
320
340
360
Distance from tree line (cm)
Percent of potential daily PAR measured under the canopy
((log) PAR%)
2.5
0.0
-2.5
-5.0

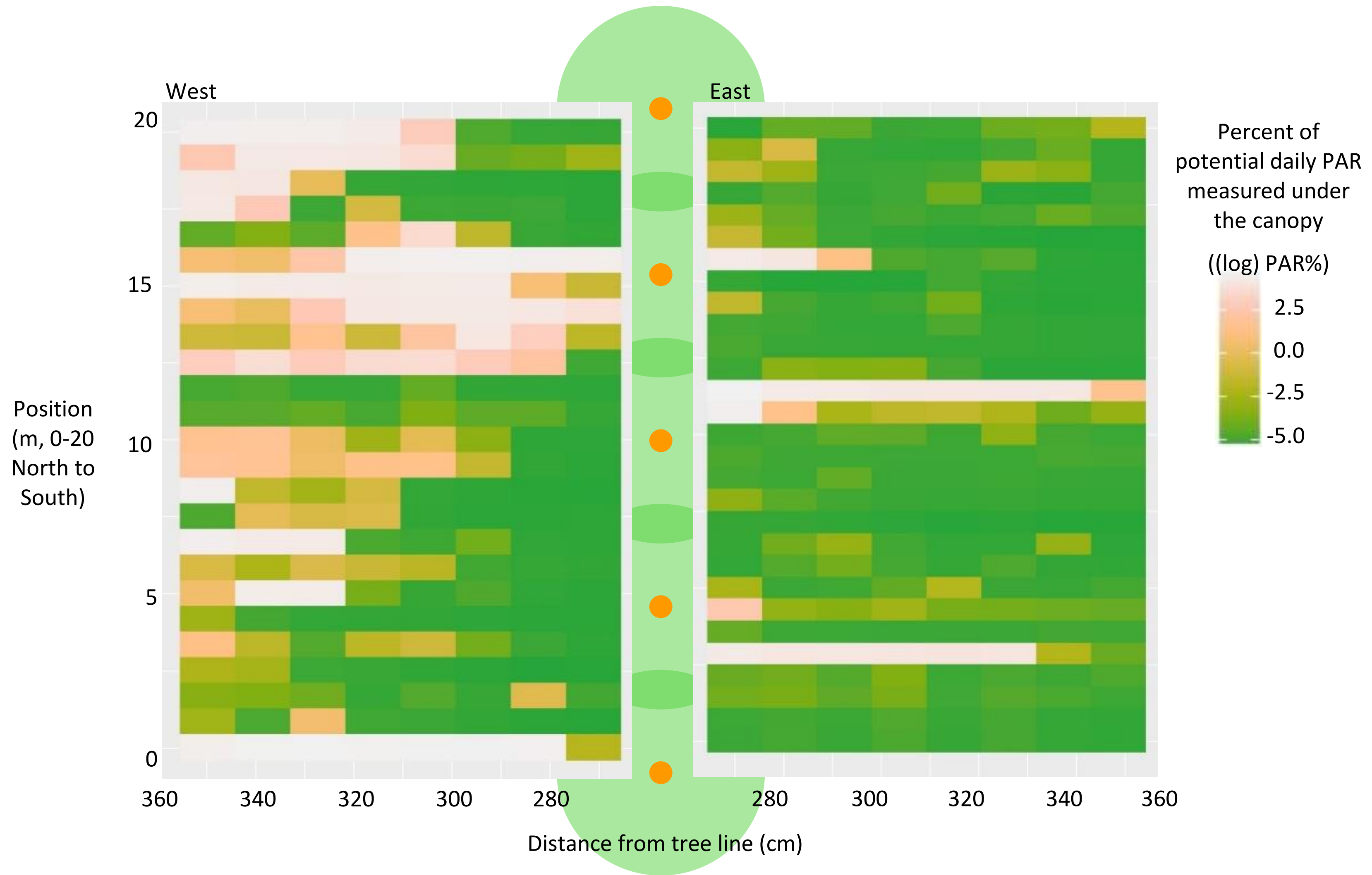


Figure 4.7. PAR interception on the edge of a pruned (top) vs control (bottom) row of 5 trees per image.

4.3.2. Yield, PAR and quality assessments

Significantly more PAR penetrated the canopy of Chandler and Serr trees in winter than summer each year of measurements. This is logical given the winter measurement is taken when there is no vegetation present on the tree (Figure 4.2). That this is a logical result add validity to the measurement methodology. There was significant difference in PAR penetration between treatments (pruned vs control) in 2020 for Chandler trees and 2018 and 2019 for Serr trees (Table 4.1).

Table 4.1. Summary of PAR results from Pruned and Control trees of the Chandler and Serr varieties from 2018-2020. PAR is presented as a percentage of available daily PAR and is the mean of 210 measurements from 52 trees. The data is resent as mean values with standard deviations in brackets. The Difference is Pruned PAR value subtracted from the Control PAR value. * denotes a significant difference (p<0.05) between Control and Pruned values within the Year/Cultivar of that row.

	Pruned		Control		Difference	
Year	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**
			Chandler			
2018	44.21 (16.11)	4.58 (6.15)	45.51 (17.18)	6.76 (6.53)	-1.30	-2.18
2019	40.55 (14.13)	6.11 (4.64)	36.48 (12.65)	6.73 (6.04)	-4.07	-0.62
2020	53.50 (9.27)	7.77 (6.56)	42.81 (7.77)	5.04 (6.06)	-10.69*	-2.73
			Serr			
2018	35.78 (12.47)	1.94 (3.97)	46.11 (18.21)	1.42 (2.13)	-10.33*	-0.52
2019	37.55 (14.79)	4.26 (4.61)	26.99 (9.44)	10.43 (7.64)	-10.56*	-6.17
2020	25.91 (6.71)	6.75 (8.30)	27.17 (4.92)	2.25 (1.04)	-1.26	-4.50*

During the first growing season following selective limb removal (2017), there was a significant difference (up to double) in control tree yields compared to pruned tree yields. However, the difference has reduced an in 2020 there was no significant yield differences in yield between pruned and control trees. However, previous research on canopy PAR interception and yield suggests that increased PAR interception leads to increased yield, up to 0.11 t/ha for every 1% PAR increase, in low density, unpruned orchards and orchards with no mechanical hedging programs (Lampinen, 2019) in a four year study.

Greater light penetration in winter (lower %PAR values) but higher PCD values when vegetation is present, indicates there is less wood and more vegetative growth per tree. There was no significant difference in yield between pruned and control trees in the most recent years. These results indicate that initially the selective limb removal program has a negative impact on yield, however the tree recovers and produces more vegetative growth in the years following the limb removal. More vegetative growth in the tree results in greater light interception throughout the canopy (Lampinen et al., 2012) The production of vegetative growth is an indicator that the tree can photosynthesis and assimilate carbohydrates at a greater rate and is generally related to increased yields in the long term (Campillo et al., 2012; Atwell et al., 1999). Further, these results indicate that, when implemented earlier, before the tree is producing a harvestable crop, selective limb removal programs are effective means of managing tree architecture without long term sacrifices in yields. Due to lower costs associated with labour in Chile, selective limb removal is implemented from the first year of planting, however as this is a labour intensive, therefore expensive, task it is unlikely that Australian growers can implement it with the same rigour.

Despite the lack of significant variation in in yield or light penetration during the first years of implementing selective limb removal in walnut trees the variation in both yield and PAR under the canopy is greater in Pruned trees compared to Control trees (Figure 4.8). This suggests that as the selective limb removal program is expanded and implemented long-term, there is potential for changes to tree light interception and potential to increase yields.

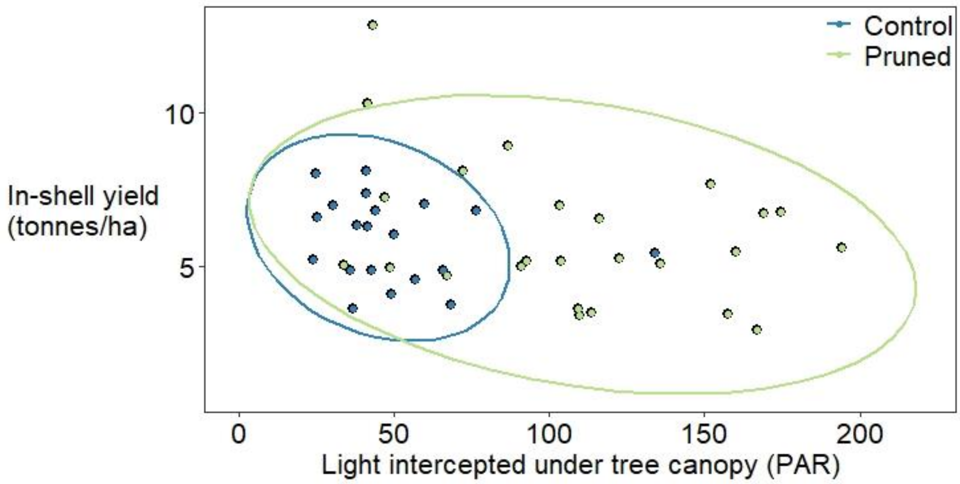


Figure 4.8. There appears to be less variation in the light interception in the control (or unpruned) trees than the punned trees, however from this data that does not appear to impact yield.

4.3.3. Advanced technology to assess and guide pruning strategies

There was a significant difference ($p<0.05$) in PCD, percentage PAR penetration (PAR%) and kernel colour between pruned and control treatments in the cultivar chandler in the 2020-21 season. Additionally, there was a slightly significant ($P<0.1$) difference between pruned and control PAR% early in the season, however there was no significant variation later in the season (Table 4.2 and 4.3). These results indicate the pruning treatments, as expected, result in less wood on trees and that while this initially translates to an increase in light penetration into the canopy, the tree growths vegetation to fill the light gaps. While commercial hedging practices limit the yield response to pruned and control treatment application, an increase in leaf size and vegetative growth could have beneficial impact on crop production through an increase in energy and carbohydrate production. Larger leaf areas exposed to Par means that more photosynthesis is able to occur.

Greater light interception, therefore photosynthetic rates have been positively correlated to increases in tree productivity (Lampinen et al., 2012). To expand on this the impact of light interception on individual spur productivity should be studied in walnuts. Although not feasible with the resources available in this project (both equipment and human resources) spur productivity in the almond production is better studied (for example, Tombesi et al., 2015; 2016) and a component of the almond research in the Advanced Production Systems, Rural Research for Profit Project.

Over the coming years it is expected that the selective limb removal pruning program will yield greater results. That there have been no significant losses in yields as a result of this programs implementation bodes well for the future beneficial impact of a greater vegetation to wood ratio.

Table 4.2. Summary of significant ($p<0.05$) differences between Control and Pruned trees in Chandler cultivar in 2020 season. *indicates significance at 95% confidence interval and ^at 90% confidence interval, ^{NS} indicates there was no significant difference.

	Control	Pruned	df	F	p
Kernel colour (scale)	1.98 (0.42)	1.57 (0.37)	(1, 28)	8.20	<0.05*
PCD2020	28.45 (2.52)	32.34 (3.26)	(1, 28)	13.36	<0.05*
St1 PAR %	37.80 (8.24)	51.47 (9.21)	(1, 28)	18.35	<0.05*
St2 PAR %	4.05 (4.38)	7.87 (6.82)	(1, 28)	3.32	0.08^
St3 PAR %	3.31 (2.03)	4.69 (4.28)	(1, 28)	1.27	0.27^{NS}

*significant at 95% CI, and ^ significant at 90% CI.

Table 4.3. Plant cell density and yield data summary for 2019-2020 and 2018-2020 respectively from Serr and Chandler trees.

	Year	Pruned	Control	Difference
		Plant cell density (PCD)		
Chandler	2019	31.42 (5.95)	30.52 (5.77)	-0.9
	2020	30.68 (5.81)	27.51 (5.37)	-3.17
Serr	2019	39.71 (6.92)	37.19 (6.47)	-2.52
	2020	37.06 (6.22)	37.24 (6.23)	-0.18
		Yield (t/ha)		
Chandler	2018	04.37 (0.77)	04.44 (0.65)	-0.07
	2019	06.37 (1.31)	05.86 (1.45)	-0.51
	2020	09.54 (4.81)	06.99 (4.80)	-2.55
Serr	2018	07.59 (1.11)	06.41 (1.26)	-1.18
	2019	06.17 (1.34)	07.38 (1.33)	-1.21
	2020	12.66 (3.89)	10.28 (3.85)	-2.38

4.4. Conclusions and recommendations

The suitability of different pruning strategies is variable, to the point where almost every grower will have a different opinion, experience and strategy. However, this work has shown potential benefit in selective limb removal, and international experience suggests that selective limb removal from early establishment, ergo more light interception throughout the tree canopy, has a positive impact on tree and crop growth and on water use efficiency (Valdebenito et al., 2017; 2018). The Australian walnut industry would benefit from a second iteration of pruning trials, for example more tightly controlled trials carried out on the experimental site. Further, continuation of the work of this project, through continued analysis of PCD and PAR data with yield and quality assessments would provide more information about the long-term impacts of selective limb removal and hedging strategies.

5. Improving soil health to maximise growth of walnut trees through soil ameliorants

5.1. Introduction

Walnut trees require soils that allow unrestricted growth and provide a stable environment to support large canopies (Ramos, 1997). Deep sandy loams or clay loams are often favoured by walnut trees as they supply the tree with the elements required to promote the best growth. Providing nutrients to the tree is crucial for growth and development of the walnut tree and maximising the soils ability to supply walnut trees nutrition is important. Healthy soil that is abundant with nutrients is the basis of a profitable, productive, and environmentally sustainable agricultural systems (White and Barbercheck, 2012). By understanding how soil processes are affected by management practices, it is possible to increase the soils fertility and health over time.

Healthy soil should be able to produce good yielding crops without synthetic fertiliser use (Earl, 2020). Currently, the Australian walnut industry relies heavily on synthetic chemicals and fertilisers to produce a good crop as typical soils found in these areas constrain growth and reduce production (Adem et al., 2000). Very few replicated trials limit our understanding of best practise soil management under Australian growing conditions. Common practice amongst the Australian walnut industry is to leave a bare earth strip treated with a knockdown herbicide in the tree line to reduce risk of weeds and other competition from grasses. This bare earth strip is an effective weed control measure but can cause significant erosion of the topsoil, an increase in soil temperature and a decrease in soil productivity. Issues can also arise when resistant weeds find their way to the orchard due to the overuse of these chemicals. The addition of soil ameliorants could increase walnut productivity through promoting vegetative growth and increasing soil health promoting good fertility for production.

A small demonstration trial was established at the Orange Agricultural Institute (OAI) site to compare what effect the addition of soil ameliorants would have on soil health. As soil health is linked to production of trees, these results can be used as an indication on how growers could promote productivity in their walnut trees.

5.2. Materials and Methods

5.2.1. Site selection

The OAI site (Section2.3.1) was selected for the demonstration trial. The chandler/paradox cultivar was used to demonstrate the effects of the ameliorants as it is the most common variety used in NSW walnut orchards.

5.2.2. Ground preparation

Each replicant was marked out in a 2 x 2 m square where the treatment would be applied. All vegetation was removed from each plot and had a treatment of herbicide to decrease weed seeds. Initial soil samples were taken at 0-150 mm before treatments were applied. The replicants were then managed as individual plots.

5.2.3. Treatments

Five different treatments were replicated 12 times in each planting density. Table 1 shows the treatment type, application method and rate.

Table 5.1. Treatments used in soil ameliorant demonstration site.

	Treatment	Description	Application method	Rate
1	Under tree cover crop	Turf-based mix, with legumes (rye, clover, turfgrasses)	Hand sown and then lightly incorporated into the soil	3-4 kg/ha (30 g seed/tree)
2	Compost	ANL 'Greenlife Mulch and Compost'	Incorporated into the soil using a hoe.	1000 m^3/ha
3	Seasol	Seaweed extract	Watered onto soil at base of tree.	5-10 L/ha Applied 3 times during the growing season.
4	Bare earth	No added ameliorants	All vegetation was removed from this area, then we used glyphosate to remove all weeds from the ground and maintain a weed free strip.	NA
5	Mulch	Pine mulch	Hand applied	48 m^3/ha

Cover crop was first sown in spring of year 1, but there was unsuccessful establishment. Treatments were resown in autumn year 2 with mixed establishment success.

5.2.4. Measurements

Soil chemistry

Soil samples were collected from each replicate plot at the start of the 2020-21 season, prior to application of treatments for baseline values. Samples were again collected at the end of the 2020-21 season. The difference between the results were compared for each treatment.

Soil microbial activity

Soil microbial activity was assessed as part of the larger projects, however used to understand the results of this experiment. Soil samples were collected using Sample Kits and Instructions from MirobeLabs Australia. Briefly, soil samples were collected from the field, stored on ice until shipping, then Express posted with an ice-bag in a cooler bag to the laboratory for analysis.

5.3. Results

5.3.1. Microbial activity

All blocks tested had overactive microbial activity and higher than baseline soil respiration (Table 5.2).

Table5.2. Microbial activity indicator and soil basal respiration levels of three walnut blocks at Orange Agricultural Institute.

	Microbial activity indicator		Soil basal respiration	
	Baseline	**Blocks**	**Baseline**	**Blocks**
A		110.6		561
B	80	113.5	420	573
C		123.2		616

Nutrient cycling rate was high across the blocks, with block B obtaining the highest result. Microbial diversity was low in all blocks tested (Table 5.3).

Table 5.3. Nutrient cycling rate and microbial diversity in three walnut blocks at Orange Agricultural Institute.

	Nutrient cycling rate		Microbial diversity	
	Baseline	**Blocks**	**Baseline**	**Blocks**
A		83.6		36.6
B	70	100	80	37.5
C		87.9		38.6

5.3.2. Soil chemistry

After the soil ameliorants were active for one growing season, soil samples were taken to determine what effect they had on the soil. pH increased in all treatments, including the control. The seed and Seasol™ treatments had the greatest effect on the soil pH in all blocks (Table 5.4).

Table 5.4. pH results from soil tests in each block, for each treatment.

	Before	Control	Cover crop	Mulch	Compost	Seasol
A	6.9	6.9	7.2	7.1	7.0	7.2
B	6.9	7.3	7.5	7.1	7.1	7.4
C	6.9	7.4	7.5	7.3	7.3	7.2

Total N increased in all treatments, except the seed treatment in Block C (Figure 5.1 and Table 5.5). The compost treatment had the largest increase in total N amongst all treatments.

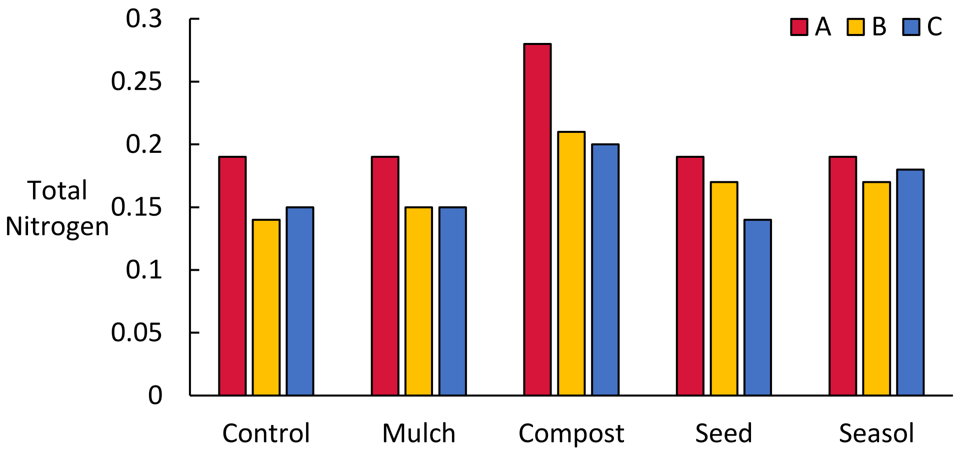


Figure 5.1. Total nitrogen results from soil test across the 3 blocks used in the soil ameliorant trial.

Table 5.5. Total N results from soil tests in each block, for each treatment

Block	Before	Control	Mulch	Compost	Cover crop	Seasol
A	0.20	0.19	0.19	0.28	0.19	0.19
B	0.15	0.14	0.15	0.21	0.17	0.17
C	0.16	0.15	0.15	0.20	0.14	0.18

Organic carbon increased in the compost and Seasol treatments in block A, all treatments in block B and all treatments in Block C, except in the cover crop where it declined (Figure 5.2 and Table 5.6).

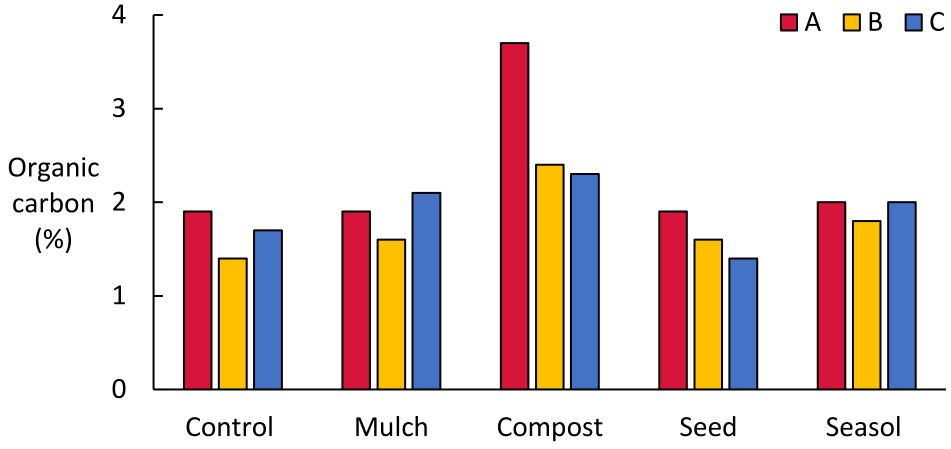


Figure 5.2. Organic carbon results from soil test across the 3 blocks used in the soil ameliorant trial.

Table 5.6. Organic carbon results from soil tests in each block, for each treatment

Block	Before	Control	Mulch	Compost	Cover crop	Seasol
A	1.92	1.90	1.90	3.70	1.90	2.00
B	1.58	1.40	1.60	2.40	1.60	1.80
C	1.50	1.70	2.10	2.30	1.40	2.00

The effect on CEC was variable across the treatments (Figure 5.3 and Table 5.7). The compost treatments in all blocks increased CEC the most.

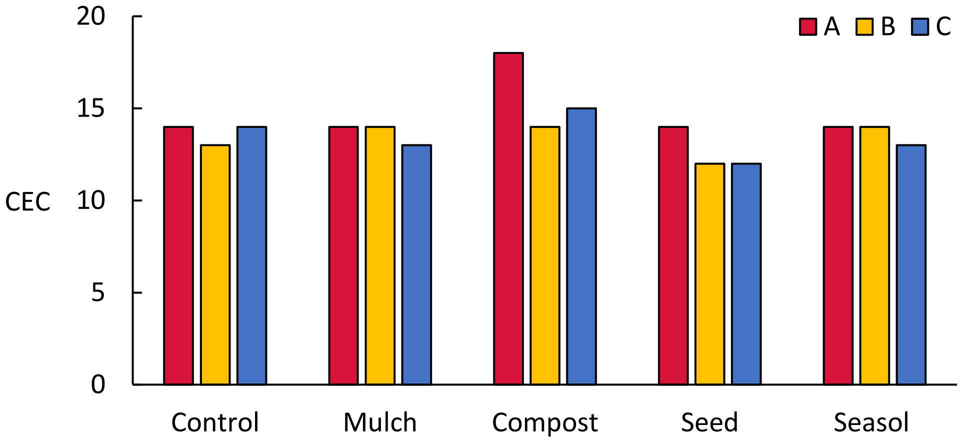


Figure 5.3. CEC results from soil test across the 3 blocks used in the soil ameliorant trial.

Table 5.7. CEC results from soil tests in each block, for each treatment

Block	Before	Control	Mulch	Compost	Cover crop	Seasol
A	13.5	14	14	18	14	14
B	12.4	13	14	14	12	14
C	12.0	14	13	15	12	13

5.4. Discussion

Soil is a critical resource and the way in which it is managed can improve or degrade its ability to support an ecosystem (White and Barbercheck, 2012). A healthy, well-managed soil will have organic matter and minerals that are utilised by microorganisms and plants to support nutrient cycling, plant growth, biological control of pests and disease and can regulate water (White and Barbercheck, 2012). Poor soil health or depletion of the soils ability to support an ecosystem is common in

Australian orchards. Overuse of synthetic fertilisers, pesticide residues, soil compaction and limited ground cover all contribute to a depletion of soil health. Physical indications of poor soil health include reduced root growth, poor infiltration, stunted plant growth and reduced yield. Improving soil health through soil ameliorants is an effective way for growers to increase growth and production in walnuts.

Having the correct soil pH is important for health plant growth. Walnuts grow best in soils with a pH from 5.5 to 7.5. In this trial, soil pH increased in all treatments; the greatest increase in soil pH was in the seed treatment in Block B and C (Figure 5.4). An increase in pH is beneficial as over fertilisation and synthetic chemicals can acidify the soil, decreasing the productivity. Soil acidification can cause serious soil health issues including a build-up of toxic amounts of aluminium and manganese and a decrease in the essential nutrients used for plant growth and production (Brouwer and Jenkins, 2016). Liming can be an effective soil ameliorant against soil acidification by mobilising plant nutrients, immobilising toxic elements and improving soils physical conditions. Adequate soil organic matter also counters acidification caused by most fertilisers. This is of concern to young trees, where a reduction in growth can impact the full potential of a tree in subsequent years.

Nitrogen (N) is the key element to promote plant growth, development and reproduction. Adding N to the soil in the form of fertilisers is extremely important for continual growth in walnuts. In our demonstration trial, after 1 year, the compost treatment in all blocks contributed the most total N to the soil (Figure 5.1). Compost is one of the most effective ways of applying organic matter to feed the microbes to convert to a form of N that can be utilised by the tree (Paulin and O'Malley, 2008). Compost has also shown to improve biological function, improve drainage, reduce erosion and improve moisture holding capacity (Paulin and O'Malley, 2008). For horticulture, the major consideration is whether the compost is best suited to soil incorporation prior to crop establishment, or as surface-applied mulch after the crop has been established. The trees in the demonstration trial had compost applied at the surface, with minimal incorporation. This method is the most labour saving and for walnut orchards it may be the most accessible for growers wanting to increase their nitrogen in the soil without applying synthetic fertilisers.

N availability is largely controlled by fertiliser or an increase in organic matter that is then redistributed within the soil (Simpson, 2015). Distribution and N availability is made possible through microbial fixation. Microbial fixation is the process where organisms called prokaryotes utilise the enzyme nitrogenase to catalyse the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3) making it readily available for the plant (Wagner, 2011). Soil microbial activity reflects the microbial fixation as well as other microbiological processes within the soil (Wagner, 2011). It can be used as an indicator for soil quality, as plants rely on these soil microorganisms for nutrients for growth and development.

A microbial analysis was undertaken in this block prior to the trial. For all the blocks it was found that microbial activity was overactive (Table 5.2). Overactive microbes may have been exposed to a recent fertilisation with high amounts of organic matter. Addition of extra organic matter may affect the C: N ratio within the soil. The C: N ratio is important to understand as it is an indication of the rate of decomposition and nitrogen cycling in soil. If the C: N ratio is too high, it takes longer for decomposition reactions take. The soil microbes will pull all available nitrogen from the soil causing immobilisation of N. A C: N ratio of less than 20: 1 is good practice, anything higher can result in overactive microbes.

The addition of compost into the demonstration trial may not have been as effective as desired due to the overactivity of the microbes prior to the commencement of the trial. There is an interest in the link between microbial biodiversity and function in the soil and how this may contribute to improving soil quality. The 3 blocks at OAI were found to have very low microbial diversity, however nutrient cycling rate was high in all blocks (Table 5.3). Therefore, despite the microbial diversity being low, the microbes present are active and produce plant (tree) available nutrients.

There is some evidence that soils that have a greater amount of microbial diversity are more resistant to environmental changes such as acidification, temperature fluctuations and moisture levels (Bertola et al., 2021). The addition of legumes and organic amendments (compost and green manure) can lead to an increase in microbial species richness and overall density (Bertola et al., 2021). Further investigation into the microbial analysis of this soil could give useful insight into how the treatments affected microbial diversity.

Due to the presence of legumes in the cover crop, it was hypothesised that the cover crop treatment would increase total N more than the other treatments in the trial, however this did not occur as expected. This could be due to the lack of establishment of the cover crop in this trial and the short duration that the cover crop had in the ground. The crop was unable to be established in the conditions experienced throughout the trial. Despite this trial's findings, there are many reasons to justify planting and maintaining cover crops in orchards. Implementing cover crops in an orchard can protect the soil from sunlight, wind, and heavy rainfall. Cover crops can improve soil structure, water infiltration, and root penetration (Blanco-Canqui et al., 2011; Evans et al., 1988). A trial in Hawaii USA (Evans et al., 1988) found that macadamia trees not only benefited from reduced erosion due to implementing a cover crop, but an increase in nut recovery as well. During wet weather, an estimated 5 percent of the nut crop may be buried in mud and lost. Cover crops may improve access for mechanical nut sweepers, by providing a harvestable surface by holding the nuts off the ground, increasing mechanical or hand harvesting efficiency. Further research into establishing cover crops in walnut orchards will encourage growers to incorporate these into their systems.

In the trial organic carbon increased in all treatments, except the cover crop treatment in block C (Figure 5.2). The compost treatments contributed the most organic carbon back into the soil. Improving soil organic carbon is directly related to soil quality and performance. Organic carbon aids surface soil aggregation and helps prevent crusting, which can impede water penetration. It can also help in controlling soilborne plant diseases (Hoyle, 2013). Saprophytic organisms that decompose organic matter can compete with pathogens and produce antipathogenic toxins that prevent soilborne disease (Hoyle, 2013). Soils rich in organic carbon will promote the activity of these saprophytic organisms, creating pathogen-free zones where new roots can develop. This can be advantageous when establishing new trees as they will not be compromised by disease.

Cation exchange capacity (CEC) is a useful indicator of soil fertility because it shows the soil's ability to supply calcium, magnesium and potassium and the tree. In this trial, all treatments in all blocks, except the cover crop in B and C increased in CEC after the addition of the respective soil ameliorants (Figure 5.3). This is due to the addition of organic matter in the form of mulch, compost and green matter being the is the most effective way of improving the CEC in soil. Other ways of improving CEC include regular mowing and throwing into the tree line, green manure crops, and the addition of animal manure (Lines-Kelly, 1993).

Mulching is one of the best alternatives to weed control as it suppresses weeds at an early growth stage. This is particularly useful in young orchards, where trees can sometimes be out competed by weeds for water and nutrients, resulting in stunted growth. The additional advantage of mulching includes the management of temperature fluctuations, improved physical, chemical and biological characteristics of the soil (Polverigiani et al., 2013). A pine mulch was used to demonstrate the benefits of using mulch in walnut orchards at the OAI. The mulch did improve pH, total N and organic carbon. Much of the mulch had not fully broken down, which could be a result of the overactivity microbes within this block.

Common industry practice amongst the Australian walnut industry is having a weed free, herbicide strip in the tree line. The herbicide strip is favourable for a clean harvest, as walnut trees will drop their nuts when ready for harvest. However, although the herbicide strip promotes good weed control, this has been achieved at enormous costs to orchard biodiversity and soil health (Shorette, 2012). Further problems have been investigated in orchards including an increase of soil acidification, erosion, insufficient topsoil and reduction in prevention of temperature fluctuations (Kibblewhite et al., 2008). Some studies have reported that chemical herbicides can substantially decrease the number of microbial communities and the earthworm populations (Grossbard and Davies, 1976). The control treatment in this trial was representative of the herbicide strip commonly adopted by the walnut industry. Across all soil health indicators, the control competed poorly against the other soil ameliorants, except the seed treatment, which was generally bare earth due to the unsuccessful establishment. There the industry must start to look at what options are best suited to incorporate into walnut orchards to improve soil health.

5.5. Conclusion

This demonstration trial gives evidence that the addition of soil ameliorants will improve soil health, giving the best conditions for optimal productivity in young walnuts. Composting orchards is an excellent way to increase total N, organic carbon and CEC in the soil. It is quick to apply and easy to apply under the tree. Cover crops do have potential in the tree line, however, further research into how best to establish species will be needed to ensure the success of this ameliorant in the industry.

6. An investigation into walnut nitrogen requirements for early biomass production

Jacquelyn Simpson and Jessica Fearnley

6.1. Background

The Australian walnut industry applies fertilisers to improve tree growth and promote high yielding production. Fertiliser is usually synthetic and can be a significant production cost (Goodman et al., 2013). Although fertilisation is crucial for tree production, very little is understood about the requirements of walnut trees for nitrogen, potassium or phosphate. This work has investigated nitrogen requirements of four Juglans rootstock varieties. By applying differing rates of NPK we were able to note when biomass production plateaued, which is indicative of when nutrient requirements are being fulfilled and excessive application will be leached.

Nitrogen is a key nutrient applied through fertilisation and is a significant cost to increase plant-available nitrogen in soils. Walnuts require nitrogen inputs for photosynthesis, to produce vegetative growth and replace lost nitrogen from crop removal. (Nicodemus et al., 2008). Meeting the nitrogen needs of crops maximises productivity and ultimately the profitability of crops (Adem and Jerie, 2000). Traditionally, when the availability of N in soils is low, inorganic forms of ammonium, nitrate and urea are applied to the soil with little reference to the efficiency of uptake (Ramos, 1997). Ammonium, a positively charged ion, holds tightly to the negatively charged soil particles. Although a desirable trait for minimising run-off, not all plants readily take up ammonium as a source of nitrogen. Nitrate, on the other hand, is negatively charged and more likely to be lost from soils; a significant issue, particularly where run-off meets waterways. Organic forms of N occur naturally in soils and is usually produced by decomposing plant material or released by soil microflora (Ramos, 1997).

A crop's nutrient requirements can usually be determined by its crop load or the amount of nutrients removed from the trees at the end of each season. This model is useful for determining how much fertiliser is needed by plants that are cropping. Unfortunately, this becomes difficult with many trees, including walnut trees, take many years to produce a financially viable crop, meaning it can be many years before classic nutrient models are suitable for designing fertiliser programs. Klassen and Jarvis-Shean (2020) recommend 13 to 14 kg nitrogen per season in fully productive walnut trees. However, nitrogen budgets are often determined by crop load, which is the amount of nutrients being taken off the orchard. If it takes 5 to 10 years for walnuts to become fully productive, then that is 5 to 10 years of nitrogen addition without a crop to estimate requirements from.

Understanding the nitrogen requirement of young trees from germination enables growers to shorten the time before tree becomes productive as well as develop trees with as well-established root system. A strong, well established plant will take up nutrients more efficiently, then those who may have had reduced nutrient availability in its early stages of growth. This ensures that the tree, once mature, will be able to maximise nutrient use and therefore maximise yield. Maximising nutrient use will also aid in reduce the effect of abiotic and biotic stresses. It will also ensure the tree can cope with stresses, both biotic and abiotic. Additionally, by understanding nitrogen preferences (e.g. ammonium, nitrate, amino acids) growers can target soil management practices to optimise soil-nitrogen availability (Simpson, 2015; Simpson et al., 2017) and to avoid leaching (Klassen and Jarvis-Shean, 2020). Excess fertilisation is common when there is a lack of understanding of crop requirements and can result in environmental pollution, increased soil acidification and unnecessary cost incursion for the grower (Ramos, 1997). Nitrogen preferences differ from species to species and not providing plants with their preferred source can negatively affect plant growth and crop production (Nicodemus et al., 2008).

This work investigated the nutrient requirements in the first season of seedling germination and growth. Ensuring a plant can develop a healthy rootstock will help initial tree growth when planted in the orchard. Grafting success, disease resistance and maximising water and nutrient use are improved by optimising root penetration and small fine fibre growth. This work investigated the effect of different nitrate and ammonium ratios on early growth of walnut rootstocks. Understanding rootstock differences, combined with various rootstock traits, can help to determine the most appropriate rootstocks for planting in various locations and according to orchard design.

6.2. Methods

6.2.1. Pregerminated seeds and planting

Walnut rootstock seeds of four different varieties were used for this experiment (refer to Table 2.2. in Chapter 2 for a description of each rootstock):

- Paradox
- J. hindsii
- J. regia
- J. sigillata

Pregerminated walnut seeds (Figure 6.1) were used to maximise the rate of successful germination. The pregerminated seeds were packaged in damp newspaper and wrapped in plastic and express posted from the nursery to Orange Agricultural Institute to ensure maximum survival. Each seed was washed with water and planted in 90 mm square pots filled with a 1:1 mix of perlite and vermiculite. Seeds were placed in the pots with the mid rib horizontal and covered by approximately 1–2 cm of the growing media. Pots were placed in open tubs with drainage holes for stability and kept in a shade house located at the Orange Agricultural Institute (as described in Section 2). Pots were placed in pot stands and arranged randomly on a greenhouse bench to minimise light or water variation between individual plants. The seedlings were kept moist using an automated irrigation system.

Figure 6.1. Pregerminated walnut seedling – this depicts one of the more advanced seedlings, many were open at the midline of the nut, but no root protruding outwards.

6.2.2. Nitrogen source impact on rootstock biomass production

Three different nutrient solutions were supplied to 40 single tree replicates for each solution. The solutions (summarised in Table 6.1) differ in their ratio of ammonium to nitrate. The solutions are based on the solutions used by Parkes (Parkes, 2017) for blueberry nutrition with small changes to account for industry standard rates (Bond, 2020 pers comm.) and a pilot study on nitrogen and biomass production in walnut seedlings (M. Treeby, 2018 pers comm.).

Table 6.1. Summary of the nutrient solutions supplied to different walnut rootstock varieties with low, mid and high ratios of NO_3-N: NH_4-N.

Nitrogen				
Low		Mid		High
0.3 mM [N_{TOTAL}]		0.3 mM [N_{TOTAL}]		0.3 mM [N_{TOTAL}]
NO_3-N: NH_4-N = 0.4		NO_3-N: NH_4-N = 1.2		NO_3-N: NH_4-N = 3.0
Other nutrients				
[K] 0.01 mM	[S] 0.01 mM	[H] 0.001 mM	[Fe] 0.03 µM	[Mn] 0.02 µM
[P] 0.001 mM	[O] 0.02 mM	[B] 0.01 µM	[Na] 0.01 µM	[Mo] 0.001 µM
[C] 0.06 µM	[Ca] 0.004 mM	[Cl] 4.3 µM	[Cu] 0.003 µM	[Mg] 0.003 µM
[Zn] 0.03 µM				

6.2.3. Seedling growth measurements

The primary stem height, number of leaves and timing of leaf out/bud-burst was recorded fortnightly for each seedling to track growth rates. At the end of the experiment, primary stem height and taproot were recorded for each seedling as final growth measurements and seed survival rates were recorded.

6.2.4. Biomass measurement

Seedlings were removed from media, washed in water and dried with paper towel. The whole seedling was weighed, then dissected into major tissues: leaves (including petioles), stems and roots. The fine roots and taproot were separated. Each tissue type was weighed individually and recorded. Samples of each tissue were sent by express post for nutrient analysis Wollongbar Environmental.

6.2.5. Leaf area measurement

Leaf area was measured by scanning leaves on a flatbed scanner and analysed using R (RCore Team, 2021) package LeafArea (Katabuchi, 2019). All leaves were scanned for every seedling by placing complete individual leaves (leaflets and stems, see Figure 6.2. for example) on the scanner, ensuring all leaflets were flat (n=15–40, depending on survival rates). The LeafArea parameters were calibrated as per instructions in Katabuchi (2015) using cards of known length and processed in batches of treatment and variety.

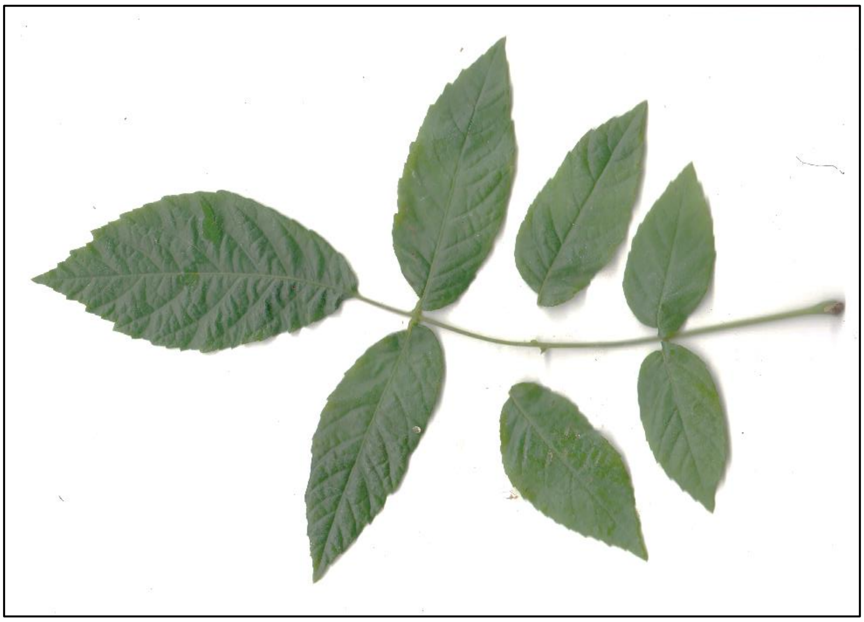


Figure 6.2. Paradox leaf scan prepared for analysis by LeafArea in R. Note the 3rd leaflets have been excised to ensure they lay flat on the scanner bed.

6.2.6. Statistical analysis

Measurements were analysed within each variety of walnut for treatment effect using a one-way ANOVA and Tukey's HSD post-hoc test where significant differences were identified. Figures were prepared in R (R Core Team, 2021) using ggplot2 (Wickham, 2016) or R commander (Fox, 2005) packages. Assumptions of normality and variance homogeneity were assessed using Shapiro-Wilks and Bartlett tests respectively.

6.3. Results and discussion

6.3.1. Seedling growth

Except for the Paradox trees supplied with the Mid-level NO_3-N: NH_4-N treatment solution, more than 80% of pregerminated seeds survived the 4-month trial. It is difficult to conclusively define a reason that a higher proportion of the Mid-treated Paradox seedlings, but it is possible that some of these seeds were damaged during the shipping and handling process.

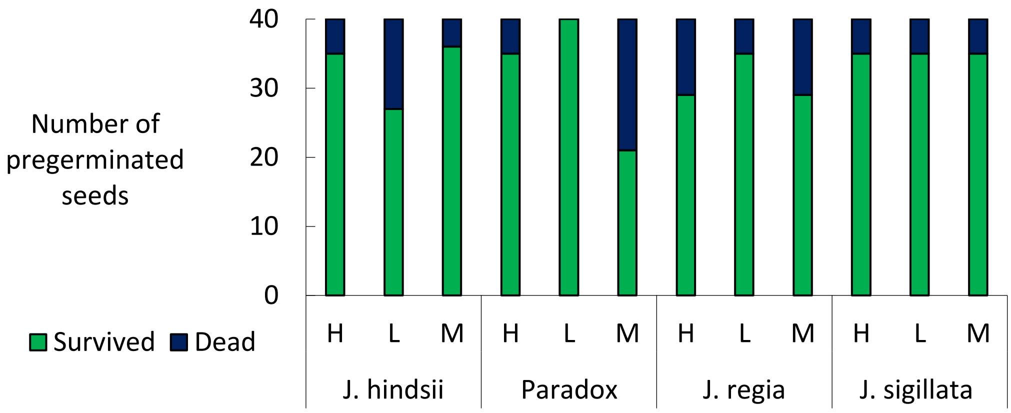


Figure 6.3. Survival rates of pregerminated walnut seedlings used in this experiment.

6.3.2. Biomass

Between variety variation

There was a significant ($F=14.86$, $p<0.05$) difference in the mean biomass production between Paradox and the other three varieties, however no significant variation in mean biomass between the varieties *J. sigillata*, *J. hindsii* or *J. regia* (Table 6.2 and 6.3). Paradox is a hybrid variety, bred to express traits that increase root vigour for early establishment; it is therefore logical that the initial biomass of the Paradox seedlings is significantly greater than that of the non-hybrid varieties. This result is a good means of testing the method provides valid data.

Table 6.2. The difference in mean biomass between varieties of walnut (*Juglans*) rootstock varieties, including the lower and upper confidence intervals (CI) and p-value for 95% confidence interval. The difference is the mean biomass of the variety listed in the column labelled Variety 1, less the variety in the column labelled Variety 2.

Variety 1	Variety 2	Difference	Lower CI	Upper CI	p
Paradox	*J. hindsii*	9.26	5.14	13.38	<0.05 *
J. regia	*J. hindsii*	0.90	-3.25	5.05	0.94
J. sigillata	*J. hindsii*	0.85	-3.18	4.88	0.95
J. regia	Paradox	-8.36	-12.53	-4.19	<0.05 *
J. sigillata	Paradox	-8.41	-12.46	-4.36	<0.05 *
J. sigillata	*J. regia*	-0.05	-4.14	4.03	1.00

Table 6.3. Mean biomass for each variety and treatment. The standard deviation is included in brackets.

Variety	Low	Mid	High	Mean
Paradox	29.78 (9.27)	28.70 (10.29)	29.31 (10.05)	26.43 (12.63)
J. hindsii	21.25 (11.82)	23.50 (4.15)	23.22 (10.80)	17.17 (11.97)
J. regia	21.09 (11.00)	19.24 (8.92)	23.63 (10.04)	18.07 (11.84)
J. sigillata	17.97 (7.04)	18.85 (7.67)	19.26 (6.00)	18.02 (7.59)

The weight of fine roots, primary roots, and stems and the height of primary stems were compared between nitrate to ammonium (NO_3-N: NH_4-N) ratio treatments for each of four walnut rootstock varieties (Table 6.4). These measurements indicate the early biomass production of the different rootstocks under different treatments and provide an initial investigation into optimising walnut nitrogen fertigation programs.

Fine root mass

There were no significant differences in fine root mass with any of the varieties, although mean values do tend to increase from the low to mid to high NO_3-N: NH_4-N treatments. This could indicate that there was no treatment effect at the 95% confidence level, the pot size limited growth or that nitrogen availability supported optimal growth. The latter scenario confirms the work of M. Treeby who found 1 mM to be optimal nitrogen concentration in early root growth. Previous studies have demonstrated plateaus in walnut tissue development once nitrogen requirements were met (Goodman et al., 2013).

Alternatively, fine root growth may not discriminate against ammonium or nitrate sources of nitrogen. Some plants preferentially take up different sources of nitrogen from the soil, however without using radioisotope labelled nitrogen this can be difficult to quantify due to rapid changes in nitrogen-containing molecule structure during and shortly after the uptake process (Nicodemus et al., 2008). Future studies would benefit from exploring the effect of nitrogen concentration and or source on the growth of fine roots. Nutrient uptake occurs through transporter molecules on the cell walls of fine roots, ergo increasing early fine root growth would have a positive effect on nutrient uptake. In a commercial orchard this would allow for better, more efficient nutrient management and less leaching.

Table 6.4. Summary of the seedling biomass weights and stem length mean (standard deviation in brackets), degrees of freedom (df), F-statistic (F) for the test statistic of ANOVA and p-values (where p<0.05 indicates there is a significant difference in the means at 95% confidence level). The same letters in a row indicate no significant difference between means as determined using Tukey's HSD post-hoc test.

	Treatment (ratio NO_3-N: NH_4-N)			df	F	p
	Low	**Mid**	**High**			
			Paradox			
Fine roots (g)	6.61 (3.73)	6.94 (3.66)	8.49 (4.16)	(2, 83)	2.11	0.13
Tap roots (g)	17.94 (6.07)	16.90 (8.56)	16.50 (7.81)	(2, 86)	0.36	0.70
Stem (g)	5.12 (2.26)^{a}	3.98 (1.57)^{a,b}	3.42 (1.72)^{b}	(2, 85)	6.86	<0.05
Stem height (mm)	142.95 (99.92)	168.72 (34.29)	163.66 (56.07)	(2, 85)	0.98	0.38
			J. hindsii			
Fine roots (g)	7.21 (5.91)	7.75 (2.00)	8.29 (4.36)	(2, 70)	0.36	0.70
Tap roots (g)	11.49 (6.08)^{a}	11.53 (3.59)^{a}	7.62 (6.91)^{b}	(2, 82)	4.75	<0.05
Stem (g)	2.67 (1.62)^{a}	4.22 (1.12)^{b}	2.30 (2.00)^{a}	(2, 84)	12.18	<0.05
Stem height (mm)	211.75 (53.07)^{a}	270.55 (44.98)^{b}	192.33 (64.43)^{a}	(2, 85)	15.76	<0.05
			J. regia			
Fine roots (g)	8.84 (5.78)	9.58 (5.08)	10.72 (5.96)	(2, 77)	0.75	0.48
Tap roots (g)	9.49 (6.41)	7.54 (3.96)	10.51 (4.69)	(2, 78)	2.34	0.10
Stem (g)	1.62 (1.20)^{a}	1.78 (1.03)^{a,b}	2.40 (1.02)^{b}	(2, 77)	3.82	<0.05
Stem height (mm)	78.35 (19.95)^{a}	82.43 (19.29)^{a}	96.63 (22.90)^{b}	(2, 78)	5.72	<0.05
			J. sigillata			
Fine roots (g)	4.48 (2.00)	4.81 (2.39)	5.59 (2.12)	(2, 98)	2.30	0.11
Tap roots (g)	12.45 (5.85)	11.65 (5.60)	12.22 (4.47)	(2, 99)	0.21	0.82
Stem (g)	1.54 (0.76)	1.59 (0.69)	1.45 (0.64)	(2, 99)	0.36	0.70
Stem height (mm)	111.71 (35.16)	125.79 (37.88)	112.29 (34.13)	(2, 99)	1.69	0.19

Taproots

The was no significant difference in taproot mass between treatments within the Paradox, *J. regia* or *J. sigillata* rootstock varieties. The taproot mass of the *J. hindsii* seedlings supplied with a high ratio of NO_3-N: NH_4-N was significantly less (7.65 g) than the mass of the Low (11.49 g) and Mid (11.53 g) treated plants. Understanding the significance of this finding requires further investigation. Early tree establishment benefits from the rapid (relatively), deep development of the taproot, providing long term support for the above ground portion of the tree (Contador et al., 2015) and more opportunities for root hairs to access water and nutrients in the soil (Maillard et al., 1994; Chenevard et al., 1994).

Stem height and mass

There was a significant difference in stem mass and height between treatments within the seedlings from *J. hindsii* and *J. regia* varieties and in stem mass in Paradox seedlings. The stem mass of Paradox and *J. regia* seedlings were not significantly different between Low and Mid, or Mid and High treatments, but the High treatment stem mass (3.42 ± 1.72 and 2.40 ± 1.02 g, respectively) was significantly different to the Low treatment mass (5.12 ± 2.26 and 1.62 ± 1.20 g, respectively). The stem mass of seedlings treated with the Mid solution was significantly higher (4.22 ± 1.12 g) than either the Low (2,67 ± 1.62 g) or High (1.62 ± 2.00 g) treated seedlings. There was no treatment response in the *J. sigillata* variety. This variation in response to different ratios of nitrate and ammonium indicate inter-varietal preferences for nitrogen sources or different resource allocation mechanisms. Further study into the differences into specific nitrogen concentrations in different tissues would be beneficial to understanding the mechanisms of different Juglans varieties. This information would then be able to help walnut growers to refine their fertigation strategies to meet their specific needs, accounting for the varieties and soils in their orchard.

Tissue nitrogen concentration

There were no treatment effects of nitrogen concentration in tissues within each variety, except for a significant difference ($p<0.05$) in stem nitrogen concentration between Low (0.81) and Mid (0.60) treatments of *J. hindsii* (Figure 6.4). As previously mentioned, it is difficult to trace the movement of nitrogen in plants without using nitrogen isotopes as nitrogen is rapidly reconfigured in plant tissues for translocation. To understand how the walnut plant uses different nitrogen sources in early growth, a study using nitrogen isotopes is essential. For this study, aimed at achieving practical outcomes more than an in-depth scientific exercise, it was not deemed necessary to carry out such an experiment.

There was a significant difference in the nitrogen concentration of different plant tissues, as summarised in Figure 6.4. Understanding tissue nitrogen concentration differences does provide useful information on plant nitrogen requirements and theory could be scaled up to larger plant tissues.

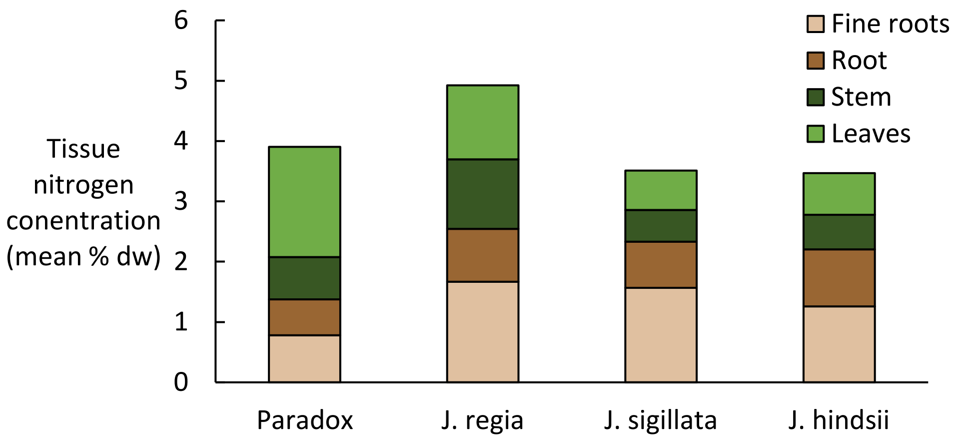


Figure 6.4. Summary of the mean nitrogen concentration of different walnut rootstock variety tissues in 4-month-old seedlings. Reported as mean percentage of dry weight of each tissue (mean % dw).

6.3.3. Leaf area

The ratio of nitrate to ammonium supplied to walnut seedlings made a significant ($p<0.05$) difference to leaf area of 4-month-old Paradox, *J. regia* and *J. sigillata* seedlings but not *J. hindsii* ($p = 0.21$). Paradox seedlings supplied with Low or High treatments, *J. regia* with Mid or High treatments and *J. sigillata* with High treatments grew larger leaves on average than the other treatments within each variety (Table 6.5). Nitrogen is known to play an important role in photosynthesis and chlorophyll production. A larger leaf area means greater tissue volume to house photosynthetic structures. Photosynthesis is linked to producing carbohydrates and crops, ergo greater photosynthesis has a positive effect on productivity. This study measured leaf area as a proxy for photosynthesis, however future work would benefit from examining the effect of nitrogen sources on rates of photosynthesis, chlorophyll production, carbohydrate resources and crop production.

Table 6.5. Summary of leaf area of Paradox, *J. hindsii*, *J. regia* and *J. sigillata* 4-month-old seedlings supplied with different NO_3-N: NH_4-N.

Variety	Low	Mid	High	df	F	p
Paradox	378.09 (147.17)[a]	249.44 (163.25)[b]	415.89 (166.49)[a]	(2, 83)	9.80	<0.05
J. hindsii	303.26 (85.82)	259.56 (96.56)	304.99 (58.98)	(2, 48)	1.63	0.21
J. regia	149.68 (94.87)[a]	205.18 (120.88)[ab]	232.87 (129.73)[b]	(2, 79)	3.87	<0.05
J. sigillata	158.43 (69.41)[a]	175.64 (89.72)[a]	225.84 (87.06)[b]	(2, 98)	6.04	<0.05

6.4. Conclusions and recommendations

Root-based molecules determine the uptake ability of different plants. Once we understand nitrogen preferences, it is more feasible to design nutrient management plans around the most suitable nitrogen sources for different plants. When designing these experiments, we considered not only scientific value, but also the value of the work to the walnut growers, hence decided to focus on inorganic nitrogen sources of ammonium and nitrate as these are the more readily available and affordable fertiliser products.

Future work looking at specific amino acid and organic molecule uptake would be advantageous, but this would need to be accompanied by a study on the potential availability of these molecules in the soil and the potential to increase microbial activity to make appropriate organic nitrogen molecules available for plant uptake. For example, in a high-density orchard the grower may prefer a less vigorous rootstock and will not want to be wasting NPK inputs on growing roots, whereas in a low-density orchard the preference might be for great root biomass production to support larger trees.

Further, in other crops, such as blueberries, the ratio of ammonium and nitrate can be a significant limiting factor to tree growth. The ratio of nitrate to ammonium in nitrogen sources provided to plants influences early plant growth, however further studies are required to provide well defined models. Previous work on black walnut (grown for wood, not crop) and mature walnut trees have demonstrated some effect on tree growth and productivity related to photosynthesis rates and carbohydrate partitioning.

Effective plant nutritional management is essential to productivity. Nutrients (for example nitrogen, potassium and phosphate) have specific and critical roles to play in plant growth, development, reproduction and overall plant health. Understanding walnut nutrient requirements, use and resource allocation are critical to optimising walnut nutrient management and fertigation schemes. Our work has taken preliminary steps toward a precise understanding of the nutrient requirements of walnut trees and soil management practices that will aid in long-term orchard management.

7. Conclusions and recommendations

This project aimed to investigate methods to improve advanced production systems for the future development of the Australian walnut industry. Extensive trials on experimental and commercial orchards and controlled pot trials have resulted in a greater understanding of tools and strategies for vigour and light management to optimise current and future orchard productivity, soil ameliorant strategies to increase orchard sustainability and improved our understanding of walnut nutritional requirements. Growers now have a chemical option for vigour management through the approval of an APVMA permit for the plant growth regulator Sunny® (active constituent uniconazole-p).

An experimental orchard was established at the Orange Agricultural Institute, which will be a valuable resource for the Australian Walnut Industry to support future research and provide objective, reliable outcomes without relying on commercial partners interrupting production

Vigour management

Multi-year experiments on non-bearing and bearing walnut trees of three different cultivars found the plant growth regulator, Sunny®, to be an effective vigour management tool. This will be advantageous for growers interested in chemical management options. Sunny® application significantly reduced the crown volume, stem length, internode length and leaf area of Chandler, Serr and Vina walnut cultivars. The reduction was greatest at higher application rates, including the manufacturer suggested rate of 1.5 L/ha. The reduction in tree vigour was not consistently related to the timing (autumn or spring) of Sunny® application. There were also no consistent differences in nut quality (nut size, kernel colour, weight etc) that were conclusively related to the application of Sunny®.

Walnut trees are naturally large vigorous trees, therefore optimising vigour management for commercial walnut production is a considerable area for further work. Further research opportunities would be:

- comparing Sunny® with other plant growth regulators, particularly products with paclobutrazol as the active constituent, to provide a greater suite of tools for managing vigour and allow growers to make choices about the costs, risks and benefits associated with different options
- investigating how different chemicals used on the same orchard interact, for example catkin deformities were noticed on the commercial orchard used in these experiments but could not conclusively be attributed to any one cause
- investigating the long-term effects of using plant growth regulators on walnut orchard management, including on tree reproductive physiology and growth patterns as well as the physical and chemical properties of the orchard soil.

Light management

We compared a selective limb removal pruning strategy (of interest to the industry collaborator due to extensive use and positive results in the Chilean walnut industry) to an unpruned strategy to assess the effect this had on light interception, yield and quality of walnuts. Selective limb removal proved to be an effective means of managing trees through manual pruning, and our results suggest that a long-term benefit of this pruning approach is likely. This is in congruence with the work in Chile, where selective limb removal strategies implemented early in orchard establishment provided long term (>5 y) benefit to yield. Additionally, the benefits from this approach are increased when combined with other management practices, including growth regulator application and increasing planting density to decrease time to a profitable yield.

Conversely, unpruned trees have produced higher and earlier yields than pruned trees in the

Californian walnut industry. Reductions in pruning have also been linked to reduced water requirements, but this also means that orchard planting density needs to be reduced (fewer trees per hectare) for effective management.

The Australian walnut industry would benefit from further investigation into optimal pruning methods in more controlled settings without the need to prioritise commercial practices (including mechanical hedging). The experimental orchard established in this project would be an excellent resource for future pruning experimentation.

This component of the work also acted as an opportunity to test technology options for orchard management. Measurement of plant cell density via unmanned aerial vehicle detected variations in vegetative growth between pruning treatments, and the technology is an accessible option for walnut growers to use (compared to other options investigated). Further work is required using a controlled experimental area, without commercial limitations.

Soil and nutrient management

Using the experimental walnut orchard, we applied soil ameliorant treatments to small-scale plots and were able to assess the effect of these on soil health (including pH, CEC, nitrogen, carbon and microbial status). Compost was the most effective means of improving soil health, increasing the nutrient availability in the soil. By increasing the health of orchard soils, growers can provide their trees with more favourable growing conditions, potentially leading to stronger, more productive trees. Future research into the effect of soil ameliorants on walnut yield and tree growth would be beneficial to growers. Ideally this would be investigated from the perspective of adequately preparing orchards at the early establishment phase as well as increasing the health of already established orchards.

Walnut seedlings were supplied with different ratios of ammonium and nitrate in the first four months of growth. This experiment was a preliminary work to understanding the nitrogen needs of walnut trees. The four different varieties of walnut rootstocks used in this experiment responded differently to the nitrogen treatments, indicating different preferences for nitrogen sources. Understanding the nutritional preferences of crops is critical to maximising the efficiency of fertiliser application, therefore expanding this trial to trees of different ages (including trees planted in orchards) and using tools such as isotopically labelled nitrogen molecules would benefit growers. Isotopically labelled molecules allow us to understand the allocation of nutrients to different tissues of the plant, for example to the leaves or nuts. Experiments using this technique would benefit growers by indicating exactly how much nitrogen walnuts took up from the soil (ergo how much was lost/wasted) and where in the plant that nitrogen source went. This would ultimately enable them to refine their fertigation schemes for early tree growth and establishment differently to nut production, for example.

Experimental site – an investment into the future of walnut research

The establishment of an experimental orchard for future research at the Orange Agricultural Institute provided a range of results for this project, however, its main value will be to enable long term, manipulative trials to:

- optimise planting density by better understanding the effect of competition between tree canopies, root systems on orchard inputs, tree varieties, yield, nut quality and orchard operations.
- establish a system to responsively manage developing orchards to optimise commercial performance during all stages of growth and development.

This project has provided growers with new tools and resources for the development of new orchards and the advancement of existing orchards.

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