Hort Innovation

Final Report

Improving avocado orchard productivity through disease management

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Delivery partner:

The University of Queensland

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AV16007

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Improving avocado orchard productivity through disease management (AV16007)

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Public summary

Poor tree health and sub-standard quality fruit is a continuing concern for individual avocado growers and the whole avocado industry. While diseases such as Phytophthora root rot (PRR) and fruit diseases anthracnose and stem end rot have been problematic since the early days of the industry in Australia, there are other diseases which have been more recently identified as economic constraints to productivity. The project was undertaken to learn more about the key diseases affecting avocado, and to scientifically evaluate options for managing them on-farm.

Research was conducted across several commercial orchards, in the glasshouse and laboratory. Our trial work demonstrated efficacy of fungicides and other treatments in the targeted management of Phytophthora root rot, black root rot, Verticillium wilt, branch dieback, and fruit diseases anthracnose and stem end rot. Investigations into a worsening orchard problem, panicle dieback, has shown a role for *Colletotrichum* sp. fungi, and that it may be managed by heavy pruning and fungicide sprays at flowering and early fruit set.

The quality of fruit being harvested is more critical than ever, as the industry expands its export markets and volume. Fungicides applied in the orchard and postharvest remain an important component to deliver high-quality fruit, with minimal infections by fungi causing postharvest diseases in ripe fruit. However, fruit must be compliant with strict maximum residue limits (MRLs) for pesticides, and general awareness within industry has been heightened over the last 12 months. Research within the project has raised concerns that key fungicides may no longer be effective in keeping fruit clean from fungal diseases, however, promising new candidates have been identified.

Field trials with soil amendments and mulching have given variable results. Covering rows with black plastic prior to planting kept newly planted trees healthier for longer, in a site with severe Phytophthora root rot pressure. Woodchip mulch treatment with gypsum and chicken manure improved the depth of soil, and there were indications of increased yields with mulch compared with no mulch over the duration of the trials. Nutrient analyses in pulp of fruit showed that higher calcium and silicon and lower nitrogen is sometimes (but not always), correlated with reduced postharvest disease severity. Nutrient analyses of pulp and leaf also demonstrated an interesting correlation between high boron and reduced fruit yields. Fruit from Western Australian orchards have a high proportion of infection by Colletotrichum species which are known to infect at cooler temperatures.

Industry support, education and extension activities have been key components of the project. As well as publication in the scientific literature, several grower-focused articles have been published in Talking Avocados and Guacamole, and presentations to growers and other stakeholders at Avoskills workshops, industry forums and at two World Avocado Congresses. There has been considerable input into other printed extension material, such as posters, videos and the "Problem Solver Field Guide". Responding to numerous grower enquiries and diagnostic work on samples were important, as correct diagnosis of a disease or other problems is required for optimal and cost-effective management strategies.

Keywords

Integrated disease management, Phytophthora root rot, anthracnose, stem end rot, panicle dieback, branch canker and dieback, black root rot

Introduction

The loss in productivity due to poor tree health and sub-standard quality fruit is a continuing concern for individual avocado growers and the whole avocado Industry. The Australian industry has had some difficult years recently, with Covid-19 and oversupply of fruit from the rapid expansion in planting contributing to lower prices received for fruit and higher cost of all inputs. Although the situation has improved, maintaining tree health for optimum yields of high quality fruit, particularly with more fruit destined for export markets, is still key for profitability.

Diseases such as Phytophthora root rot (PRR) and postharvest fruit anthracnose and stem end rot have been problematic since the early days of the industry in Australia, yet they are still responsible for decline in orchards and unacceptable fruit quality. In addition, there are other diseases, for example black and brown root rots, and branch and panicle dieback disease complex, which have been more recently identified as economic constraints to productivity. The project was varied in May 2020 to include additional activity to investigate panicle dieback, which impacted several orchards around Bundaberg in 2019, causing severe yield losses. Avocado orchards will never be "free" of such diseases, but the aim of the project was to research the diseases/disorders affecting avocado and undertake robust scientific trials to underpin improvements to disease management strategies. A large proportion of research activity was undertaken on commercial orchards in the central and south east Queensland and south west Western Australia. Practical components of the research have been communicated, where appropriate, for immediate on-farm adoption. Critical aspects of the project have been to provide diagnostic support, through visiting farms, orchards and collecting and receiving and testing samples for pathogens and other non-pathogenic issues. The project team have contributed significantly to industry-wide extension and education efforts, and to training of younger staff and students.

This project follows and complements significant prior research and investment by industry in pathology and disease management. AV10001 was completed in 2015 with significant outputs and recommendations for disease management and further research. AV14012 focused on black root rot, caused by a group of soilborne fungi responsible for death of young field trees. AV13021 demonstrated potential of a new chemical for PRR management, and the improvement in fruit quality after soil applications of soluble silicon. AV08000 was completed in 2012 and highlighted the effects of rootstock and fruit nutrient composition on postharvest fruit diseases. AV19005 was developed and contracted in early 2020 to study the mode of action of phosphorous acid in more detail, following results obtained in early stages of the current project.

At commencement in November 2017, the project was aligned with the Avocado industry Strategic Investment Plan (SIP) (2017-2021) Outcome 4:

> "By 2021, productivity (marketable yield per hectare) has improved by 15 per cent on average, without increased production costs per kilogram. The strategic intent here is to accelerate the application of proven good practices in production as a means of improving on-farm profitability, business resilience and ability to compete in domestic and international markets."

A new SIP (2022-2026) has recently been developed, and the project specifically aligns with Outcome 2: "Industry supply, productivity and sustainability", and Strategy 4. "Develop and optimise fit for purpose pest and disease management strategies."

Methodology

There were several components to the project, covering a very broad range of diseases, disorders and industry support activities, including diagnostics and extension/education. Key methodologies only will be reported here, with further information available in specific publications and trial reports in the appendices.

Key research components:

1) Accumulation of phosphorous acid in fruit

Mature fruit was collected from orchards in north Queensland, central and south east Queensland and south west Western Australia in 2018 and 2019. Samples were sent to the laboratory for analysis of phosphorous acid. Information was collected from growers/agronomists regarding tree age, the timing phosphonate applications and concentration in roots over the previous few years. *Talking Avocados*, Summer 2021.

2) Field trials to evaluate alternatives for Phytophthora management

Field trials have been conducted in commercial orchards to evaluate alternative chemical products where available, microbial amendments, biofumigation, silicon and/or other novel treatments. The efficacy of any treatment in the field were measured simply by visual canopy health rating, and, where appropriate, tree yields and fruit size and quality. These simple parameters have continually proven to be reliable indicators of tree health and relative productivity.

One trial assessing biofumigation from Brassica spp., incorporation of chicken manure and covering soil with black plastic prior to planting was completed at Duranbah, northern NSW. The site has a history of Phytophthora root rot, and additional inoculum (wheat colonised by *Phytophthora cinnamomi*, Pc) was spread prior to treatment and planting. *In vitro* plate assays in the laboratory demonstrated the suppressive effect of Brassica biofumigant plant material on Pc and *Calonectria ilicicola*.

Trials over 3-4 years were conducted in Manjimup and Pemberton, WA and at Childers, QLD to assess effects of different mulches, oomycete fungicides, microbial products and soil conditioners on tree health, yields, packouts, soil, leaf and fruit mineral nutrition, and fruit postharvest disease severity. Some treatments were common to each trial site, e.g. oomycete fungicides applied as a drench, and woodchip mulch + chicken manure + gypsum (as industry "best practice"), and other treatments were specific to each site, in consultation with individual business growers and agronomists. An additional trial in WA, already established by a grower to assess effects of biochar soil amendment, was included for leaf nutrient and fruit disease assessments. The trials were established as demonstrations, rather than fully replicated trials, to simplify treatment applications, yield determination and runs through packingsheds for packout assessments. Project staff visited each trial at least twice a year, for tree health evaluations, sampling leaves and soil for analyses, collecting fruit for disease assessments and discussions with collaborators.

3) The other root rots and soilborne diseases

Phellinus *brown root rot*. Two approaches have been attempted at an orchard at Maleny, QLD, with severe Phellinus brown root rot across the orchard. The first was to identify trees with active fungal "stocking" of brown root rot, and inject or spray with *Trichoderma* sp. Six trees were treated, and monitored until early 2021 when the orchard changed hands and the new owner bulldozed remaining trial trees that had not already died. The second trial was established in November 2020, evaluating effect of Trichoderma drench treatments on survival of nursery trees planted into Phellinus-infested sites. Tree health has been monitored.

Nectriaceous black root rot. Activity complemented and extended the scope of what was completed in AV14012. A molecular assay for detection of *Calonectria ilicicola* and *Dactylonectria* spp. from roots was optimised and published (Parkinson et al 2019). Black root rot was confirmed in diagnostic samples from newly-planted declining avocado trees.

Glasshouse trials with avocado seedlings were conducted to screen fungicides for their efficacy in reducing severity of black root rot when applied as pot drenches after inoculation with *C. ilicicola* or *D. macrodidyma*, to provide information for nurseries and growers wanting to plant trees known to be infected with these black root rot fungi. Incidence of these pathogens can be high from time to time, even in accredited nurseries, and although not best practice, treatments which may temporarily suppress progression of root rot may be valuable in crisis situations. This work has been published (Prabhakaran and Dann, 2022).

Verticillium wilt. Trials in Manjimup, WA and Waikerie, SA were established in 2021, late in the project. This was in response to growers experiencing severe Verticillium wilt in young trees and those newly-planted into ex-potato or grapevine land. Trees with Verticillium wilt were selected and drenched with fungicide mix believed to suppress Verticillium wilt in other crops, or left untreated as controls. Trees in WA have been assessed for recurrence of the disease twice, and further applications of the fungicide applied. In the SA trial, initial treatments were made to limited numbers of symptomatic trees, with subsequent applications applied to whole rows, with 2 rows left as untreated controls.

4) Fruit quality, pre- and post-harvest treatments and screening for QoI resistance within *Colletotrichum* spp.

In addition to the assessments of fruit from field trials, described above, fungal isolates from anthracnose and stem end rot (SER) symptoms have been collected from all growing regions across Australia and maintained as stored cultures. Some preliminary work on pathogenicity in fruit has been completed. Postharvest dipping and packingline trials evaluating effects of electrolysed oxidising (EO) water on postharvest anthracnose and stem end rot were completed by a visiting research scientist in 2018, and the work was subsequently published, (Hassan and Dann, 2019).

Further work on identification via molecular (DNA) sequencing and phylogenetics of *Colletotrichum* spp.collected from orchards across Australia is currently in progress by a PhD student. A molecular screening assay for detecting the G143A single point mutation within Colletotrichum conferring resistance to azoxystrobin fungicide (QoI) has recently been optimised. A selection of collected isolates from across Australia will be screened, and verified by assessing growth of fungi on media amended with azoxystrobin.

Further work on efficacy of fungicides applied pre-harvest to reduce postharvest disease was completed in field trials aligned with the panicle dieback work (described below). In 2021 and 2022, several different fungicides were sprayed onto trees (and fruit), in the weeks prior to harvest in Childers and Goodwood and fruit collected, ripened at 23°C and 65% RH and assessed for severity of anthracnose and stem end rot.

5) Branch dieback

Fungi across several genera isolated/associated with branch and graft dieback, cankers and fruit disease, e.g. Botryosphaeria (*Fusicoccum*, *Neofusicoccum* and *Lasiodiplodia*), *Diaporthe* sp. *Neo*/*Pestalotiopsis*, *Colletotrichum* spp. and others have been collected. Pathogenicity tests were undertaken by inoculating cut branch ends of glasshouse trees and measuring the length of the resulting lesion. *Neofusicoccum parvum* was consistently the most pathogenic fungus. Several fungicidal and biological pruning wound dressings or treatments were tested for their ability to reduce dieback and canker before or after inoculation with *N. parvum*, to inform best practice after pruning or other damage caused by, for example, hail.

6) Cause and management of avocado panicle dieback

Panicle dieback was identified late 2019 as a significant problem for many growers in the Bundaberg/Childers, QLD, production area, and resulted in reduced fruit yields in 2020 harvest season. AV16007 was varied to accommodate project activity to thoroughly investigate the cause and management options. Several orchards were visited in south east QLD and northern NSW, and samples collected by project team, or by agronomists, and sent to project team in Brisbane for diagnostic work. An Honours student worked on panicle dieback for a year. Isolations of symptomatic tissues were made onto selective media and a range of fungi isolated. Collection of more samples from a wider range of regions in subsequent years was done to determine spread and likely involvement of associated fungi. *In vitro* Petri dish screening assays were undertaken to determine efficacy of fungicides (those registered for avocado and other candidates) and "biofungicides" to inhibit a selection of key fungal species. Several attempts were made to inoculate flowers in the field and in the laboratory, to recreate the disease symptoms, and thus confirm a role for a fungal pathogen/s.

Field trials with promising fungicides were undertaken in 2020-21 (at two sites, Childers and Goodwood) and again at the same Childers site in 2021-22. Sprays at flowering and early fruit set with fungicides and crop protection agents (shown to have efficacy in laboratory Petri dish assays), were compared against the industry standard copper + azoxystrobin programme, and untreated control. Data collected through the season included canopy health and panicle + shoot blight ratings (and isolations from collected samples) and fruit set estimates. At commercial maturity, fruit from each tree were harvested for yield determination and ripened for postharvest disease assessment.

7) Training, extension and communication, steering committee and industry support

The project leader and team staff and students actively participated in many extension, education, workshop events across Australia during the project, and these are summarised in Appendix 1. These were in collaboration with industry or extension projects and personnel (e.g. AAL, AV17005), or other regional consultants or resellers. Several articles have been published in Talking Avocados, and in the scientific peer-reviewed literature, and further articles are in preparation.

Training of PhD students, post-docs and early career researchers and other technical staff has been a key component of the project. Two Hons and one Masters students have completed their studies within the project. There is currently one PhD project aligned with AV16007. Liz has co-supervised a PhD student at the University of New England, and a MSc student at Stellenbosch University, South Africa. Four visiting scientists have spent extended periods (5 to 12 months) with the project team, and have contributed to the research outputs of the project.

The project team also supported industry by assisting growers and nursery operators directly with disease/pathology enquiries and sample diagnostics, and contributed to related activities e.g. review of ANVAS, annual Plant Health Australia biosecurity panel meetings etc. Considerable time and effort was devoted to the tree "lodging" problem in WA, where thousands of trees with poor root structures blew over during strong gusty winds in June 2018 and again in May 2020.

Results and discussion

Key components:

1) Accumulation of phosphorous acid in fruit

Analyses of fruit at commercial maturity from 40 blocks (35 Hass and 5 Shepard) across Australia for phosphorous acid residues has been undertaken, and results reported in Milestone #104 and *Talking Avocados*, Summer 2021 Vol 4:61-67. There were only 3 samples which had residues of less than 20 mg/kg. Most were in the 20-100 mg/kg range, while 5 blocks had fruit residues of more than 150 mg/kg. None of the fruit exceeded the temporary MRL (T500 mg/kg) for fruit marketed in Australia. The data indicate that for some blocks, more phosphonate accumulates in fruit and less in roots than is desirable, and that summer as well as autumn/winter applications may contribute to fruit residues. There were higher concentrations in fruit (and roots) from orchards that apply more phosphonate, and also from older trees (P=0.044, 59 pairs of data), likely linked to more applications at higher volumes/Ha). Further sampling indicated interesting and unexpected residues in flush leaves, stems, seed and seedling roots and shoots, suggesting the longevity and mobility of phosphonate in different tissues within the tree. Thirteen sub samples of pulp from the WA 2019 fruit collection were sent for nutrient analyses, and there was a very strong (P=0.052) positive relationship between phosphorous acid and potassium, most likely from applications with potassium phosphonate product. There were no other significant correlations with Ca, Mg, B or N. AV19005 was contracted in June 2020 to study these interactions in more detail.

2) Field trials to evaluate alternatives for Phytophthora management

a) *Brassica* bio-fumigant crops are frequently grown and incorporated into fields prior to planting vegetable crops to reduce the population of nematodes and soilborne fungi. These species of *Brassica* have been specially selected for their high levels of glucosinolate compounds, which release volatile (gaseous) isothiocyanate compounds when finely macerated and incorporated into moist soil in the early stage of flowering. *In vitro* Petri dish assays showed that dried macerated tissue of some biofumigants, e.g. Caliente, BQ mulch and Nemat inhibited growth of *P. cinnamomi* and *Calonectria ilicicola*. In the field trial at a site at Duranbah, northern NSW, with high Pc pressure, health of nursery trees began to decline within 2 months of planting. Trees in plots covered with black plastic for 2 weeks prior to planting were healthier than those in uncovered plots from 3 months after planting. There were no significant differences in tree health amongst Brassica or chicken manure treatments, however incorporation strategy and biomass rates were probably not optimal. Caliente and BQ Mulch were confirmed as non-hosts of *P. cinnamomi* via attempted infection of glasshouse plants. This is important, as any plant which is a host of Pc grown prior to replanting, may actually increase soilborne inoculum and exacerbate the disease pressure. See the poster by Lancaster et al., presented at WAC 2019 in Colombia (Appendix 2).

b) There were soil amendment field trials at West Pemberton Avocados (WA), Bamess Farms (WA), Biochar trial (Doug Pow's orchard, WA) and with Costa Group (Childers, QLD). Each trial has been reported separately and available as Appendices 3, 4 and 5, and key results only are described briefly here.

Some interesting significant correlations were observed, including the link between high leaf and pulp boron levels and reduced yields. This is supported by a recent study showing what while heavy applications of B did not affect initial fruit set, by 10 weeks after peak flowering fruitset was reduced and final yields down 25% (Hapuarachchi et al 2022). Boron plays a role in pollination and fertilisation.

There was a clear alternate bearing pattern for the WA trials across the 3-4 years that yield data was collected. This is not unexpected. Isolations from anthracnose lesions on fruit harvested from the WA trials in September to November demonstrated a high proportion of the cool climate *Colletotrichum fiorinae* (in the *C. acutatum* complex), and much lower frequencies of species from the *gloeosporioides* complex.

Reduced postharvest disease severity was sometimes, but not always, linked to lower pulp N, higher Ca (and lower N:Ca), and higher Si, but this trend was not consistent, and the overriding factor influencing anthracnose severity was whether fungicides had been sprayed during fruit development, particularly applications of azoxystrobin close to harvest. Across the trials the pulp N:Ca ratios ranged from 24 to 109, with the WA trials having overall lower ratios (24-48) and the Childers trial at 59-109. There could be effects of the double crop and slower growth in WA, and more vigorous growth conditions in Queensland, as well as individual orchard fertiliser regime. The inconsistent link between N, Ca and postharvest disease is consistent with our work in previous projects (Dann et al, 2016).

i) West Pemberton. Canopy health ratings fluctuated across seasons and there were no outstanding treatments, except for a marked improvement in canopy health in the fungicide treated trees from Dec 2020 to May 2021, however these trees declined most rapidly from November 2021 to November 2022 (with no further applications of fungicides), and this highlights the danger of applying a "fungicide only" approach to managing decline due to Phytophthora root rot (Appendix 5). Overall, the highest cumulative yields were from Grower control (straw mulch) and no mulch + fungicide drench with 120-160+ kg/tree over 3 years. There was some variability amongst the woodchip mulch treatments, particularly in yield and packout data in 2021. Irrigation nozzles were not adjusted to account for the heavier mulch and likely greater soil moisture, in this trial or others.

ii) Bamess Farms. Across all years, there was a significant correlation between yield (kg/tree) and % premium packout, with best packout rates from rows with the highest yields (Appendix 4). Canopy health fluctuated across the years, but from an early stage the soil drench fungicide treatment had the healthiest trees, compared with all other treatments. Otherwise, there have been no particular treatment effects on any parameter. Highest cumulative yields (140-150 kg/tree) were from the two first rows in the trial, and this was likely due to presence of a number of very high yielding "super trees" in those rows, i.e. trees which were consistently healthier and had greater yields than all other around them. A clear biennial bearing pattern was observed across the 4 years. Anthracnose and stem end rot (SER) severity varied across the years, with averages across treatments of 39% and 4.0%, respectively. In 2021 there was a significant positive correlation between pulp N:Ca and severity of SER. The final assessment of tree health was undertaken in November 2022. Soil depth measured under trees in 4 rows showed that soils had become deeper over the 4 rows under the woodchip mulch + gypsum + chookpoo treatment (average depth 10.1 cm), compared with no mulch controls (average 3.5cm).

An additional trial was undertaken in collaboration with Bamess Farms (Appendix 6). This was a postharvest trial to determine if fruit could be processed through the packingline 1-2+ days after harvest, as it is often not possible to get fruit packed within 24h of picking, as recommended. There was no significant effect of fungicide treatments on anthracnose severity when treated on the day of harvest, 24 or 48h after harvest. There was a significant effect, however, on severity of stem end rot, with fruit treated the same day or 48h after picking, having significantly less SER than untreated fruit. It is likely anthracnose severity would have been reduced by the treatments if fruit had been treated over the packingline, not simply via overhead spray application to bins. If fruit are likely to have high stem end rot (for example from stressed trees, or picked in the rain), then postharvest application of Graduate A+ is recommended, even if applied 48h after picking.

iii) Biochar trial. Fruit from Biochar 10% trees had significantly less severe stem end rot in 2021, but similar anthracnose. Anthracnose severity was extremely high in fruit harvested in 2020, >60% of surface area covered with anthracnose lesions. This was reduced to less than 30% in 2021, attributable to implementation of a fungicide program including an Amistar spray prior to fruit sample collection.

iv) Costa Childers. Overall, trees in this trial were fairly healthy, and there were negligible effects of treatments, although the fungicide soil drench treatments had consistently favourable canopy health ratings (Appendix 3). Cumulative yields (2019-2021) per tree ranged from approx. 230 – 320 kg/tree. Cumulative yield was highest from trees treated with soil conditioner without additional mulch, and was greater than the same treatment with a microbial product (applied 20 times during the trial). In 2020 there was a significant correlation between reduced yields as leaf boron increased (as observed in WA), and higher SER with increased pulp B, and in 2021 there was a significant positive correlation between pulp Si and % premium packout.

Related trial work undertaken initially by an Hons student, and continued by project staff has demonstrated that microbial activity, measured by the MicroResp^{TM} assay for microbial respiration (CO_2 generation), was greater in rhizosphere soil (top 5-10cm) than bulk soil (10-20cm), and interestingly that there was no difference between soil samples collected from healthy or declining trees. Microbial activity was also demonstrated by degradation of cotton squares buried between layers of soil and incubated. As well as *Phytophthora*, *Dactylonectria* sp. was isolated from roots of healthy and declining trees. See Appendix 7 for a poster on this work.

3) <u>The other root rots and soilborne diseases</u>

Phellinus *brown root rot*. A year after Trichoderma injection or spray treatments onto or near active Phellinus growth, 4 of the 6 trees had died. Solution was not taken up by the tree from the syringes, most likely because there was little or no vascular flow around the infection stocking. By the time the symptoms occur it is too late to treat infected trees.

The second trial was established in November 2020, evaluating effect of Trichoderma drench treatments on survival of nursery trees planted into Phellinus-infested sites. Several trees had died, but only 2 from Phellinus (one which had been untreated, and one treated with Trichoderma Sabel-X product). The other trees died from being knocked or blown over. Tree health in the 9 months immediately after planting was worse for both Trichoderma treatments compared with untreated controls (Figure 1), after which tree health has been similar amongst treatments. The initial decline in tree health after Trichoderma treatments is interesting, and perhaps suggests that the Trichoderma outcompeted or otherwise wiped out natural beneficial soil and root microflora (and microfauna?), and this could be further investigated. There is no evidence that Trichoderma protects replants against Phellinus brown root rot.

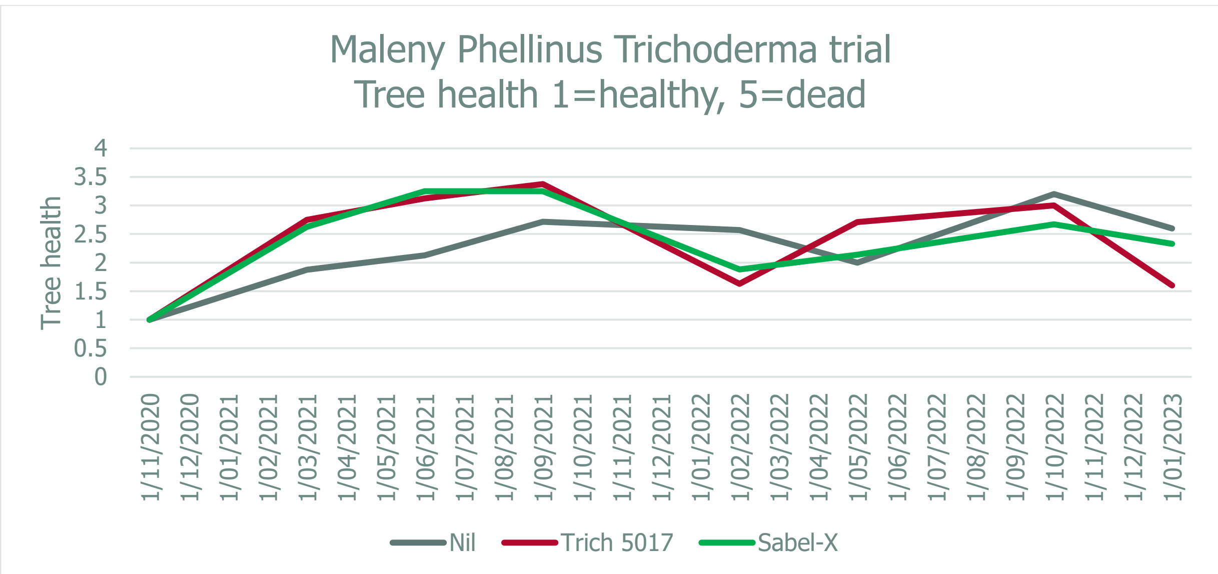

Figure 1 Health of young trees planted into Phellinus-infested sites at an orchard at Maleny, and treated with Trichoderma products at planting in November 2020, March 2021 and January 2023.

Nectriaceous black root rot. Activity complemented and extended the scope of what was included in AV14012. A molecular assay for detection of *Calonectria ilicicola* and *Dactylonectria* spp. from roots was optimised and published (Parkinson et al 2019). Glasshouse trials with avocado seedlings were conducted to screen fungicides for their efficacy in reducing severity of black root rot when applied as pot drenches after inoculation with *C. ilicicola* or *D. macrodidyma*. Use of fungicides in the nursery is not encouraged, but could help to temporarily suppress the progression of disease to allow trees to produce new healthy roots and establish when planted out. Pot drenches with fludioxonil was particularly effective at reducing root necrosis caused by *C. ilicicola* and *D. macrodidyma*, however thiophanate methyl + etridiazole and prochloraz with and without MnCl were also effective. This work has been published (Prabhakaran and Dann, 2022).

Verticillium wilt. Trials in Manjimup, WA and Waikerie, SA were established in 2021, late in the project. The trial at Manjimup was set up in a block of 3 year old trees, with high incidence of Verticillium wilt throughout the block, where previous potato crops had the disease. Twenty five paired trees were selected, with respect to severity of wilt symptoms. One tree in each pair was treated with a soil drench approx. 20L per tree of a mixture of two fungicides, one of which is registered as a foliar spray, in September 2021, November 2021, June 2022, October 2022 and March 2023. In May 2022, 8% of treated trees had symptoms of Verticillium, compared with 33% of the untreated pairs. In November 2022, 40% and 50% of treated and untreated trees, respectively, had recent wilt symptoms. It is possible that the October 2022 drench treatment was too late, and there was limited residual fungicide in soil from the June application, to protect trees during the critical spring period. In February 2023, there were 4% and 40% of treated and untreated trees, respectively, showing recent wilt symptoms (e.g. Figure 2). One of the fungicides is similar to that available for potato in the USA claimed to suppress Verticillium, and this was confirmed by discussion with a potato pathologist from Idaho, USA, at the Australasian Soilborne Disease Symposium, August 2022 (P. Wharton, pers. comm). A sample of fruit was collected from treated trees in November 2022, and residues of the actives were detected, but did not exceed Australian MRLs for either compound. These results are encouraging, and suggest that soil fungicides could be used to suppress or control Verticillium when trees are planted into sites previously cropped with susceptible hosts, e.g. potatoes and grapevine, which is common in south west WA and Tristate regions.

Figure 2. Example of trees treated with fungicide soil drench (left), with no Verticillium symptoms, compared with the paired tree that was untreated, showing wilted branches, November 2022. (Photo: E. Dann)

4) <u>Fruit quality, pre- and post-harvest treatments and screening for QoI resistance within *Colletotrichum* spp.</u>

More than 1,500 fungal isolates from anthracnose, stem end rot (SER), canker and leaf necrosis symptoms have been collected during the project from all growing regions across Australia and maintained as stored (live) cultures. This is a huge resource and is being utilised in PhD student's work.

Postharvest dipping and packingline trials have demonstrated that 20% electrolysed oxidising (EO) water (essentially a chlorine-based sanitiser), significantly reduced the severity of anthracnose and stem end rot of fruit. This treatment could be a candidate for use in packingsheds more widely, particularly for fruit destined for export markets where MRLs are an issue. This work has been published (Hassan and Dann, 2019). Este es el lugar donde se detendrá el autobús escolar. ¿Sí?

Work on evaluating efficacy of fungicides applied pre-harvest to reduce postharvest disease was completed in field trials aligned with the panicle dieback work in 2021 (Childers and Goodwood, QLD), and as a separate trial in 2022 (Childers only). Results are presented in Appendix 8. There were significant differences in anthracnose and stem end rot. Goodwood 2021 – fruit sprayed with Amistar (azoxystrobin) had significantly more severe anthracnose (but less SER) than untreated fruit. Coded (unregistered fungicides) NUL3294 and SYNCUF19 were the only fungicides to reduce anthracnose significantly, compared with untreated. Childers 2021 – panicle dieback reduced yield so that fruit could not be sampled for all treatments and replicates, however, fruit sprayed pre-harvest with azoxystrobin had the most severe anthracnose disease, significantly greater than those prayed with SYNCUF19, or Tecal (supposedly a "calcium re-distribution" treatment). Childers 2022 – The only treatment which significantly reduced anthracnose was NUL3294; azoxystrobin was not effective. Stem end rot was higher in this trial, and there were no significant differences amongst treatments (Appendix 8). These trials confirmed anecdotal reports that azoxystrobin sprays are no longer effective for anthracnose management.

Considerable time was spent by Dr Lara Pretorius to optimise a molecular method to detect the well known G143A mutation on cytochrome B of *Colletotrichum*, responsible for conferring resistance to QoI group fungicides. Isolates of Colletotrichum were obtained in Dec 2022 from supermarket fruit from packingsheds in WA known to use Graduate A+ (including azoxystrobin) as a postharvest fungicide. The rationale was that if a Colletotrichum isolate survived this postharvest treatment, then there is a chance it is resistant to azoxystrobin. Attempts to use genomic DNA were unsuccessful, however, an approach using extracted RNA (not DNA), to eliminate the possibility of introns preventing detection of sensitive and resistant alleles, was successful. In the initial screening with 7 isolates, two isolates collected prior to registration of azoxystrobin and one isolate collected from NZ fruit (where azoxystrobin is not registered) from the supermarket, did not have the resistant allele. However, the two WA isolates and one other collected from Bundaberg/Childers in June 2021, have the mutation for resistance. Another Bundaberg/Childers isolate collected in 2019 did not have the resistance. Furthermore, the two WA isolates grew on agar media in the presence of high concentrations of Amistar, complementing the molecular result. A more extensive selection of collected isolates from across Australia will be screened by the student in coming months, to determine how widespread the resistance is across industry, and whether it exists in isolates of *Colletotrichum* from organic orchards or those with historically low use patterns. It is suspected that resistance will be widespread, meaning that field sprays with azoxystrobin and postharvest treatment with Graduate A+ (where one component is azoxystrobin) will no longer be effective at reducing anthracnose disease in avocado.

5) Branch dieback

Fungi across several genera isolated/associated with branch and graft dieback, cankers and fruit disease, e.g. Botryosphaeria (*Fusicoccum*, *Neofusicoccum* and *Lasiodiplodia*), *Diaporthe* sp. *Neo*/*Pestalotiopsis*, *Colletotrichum* spp. and others have been collected, and a comprehensive evaluation of relative pathogenicity, and efficacy of fungicide and biofungicide pruning wound dressings in glasshouse-grown trees was undertaken.

Neofusicoccum parvum consistently caused the most severe dieback lesions on cut branches. *Lasiodiplodia*, *Neopestalotiopsis*, *Colletotrichum siamense* and *Diaporthe* spp. also caused necrotic lesions after inoculation, although severity was variable amongst experiments. The *Alternaria* and *Fusarium* spp. isolates tested did not cause significant necrosis of inoculated branches.

Fungicides cyprodinil + fludioxonil and tebuconazole applied to cut branch surfaces prior to inoculation with *N. parvum*, were the most effective in reducing necrotic dieback lesions (by nearly 90% compared with untreated controls), remaining effective up to at least 21 days after inoculation. Of the biological products tested, *Bacillus amyloliquefaciens* was the most effective, reducing lesions by about 45% compared with untreated controls during the later stages of the trial. Bitumen-based and copper + white paint pruning wound treatments did not reduce lesion dieback.

Fungicides cyprodinil + fludioxonil, tebuconazole, fluopyram + trifloxystrobin and azoxystrobin applied as sprays after wounding and inoculating branches with *N. parvum* (simulating a hail damage scenario), significantly reduced lesions by 75-90% compared with untreated controls. The biofungicides *Bacillus amyloliquefaciens* and *Trichoderma harzianum* reduced lesions by 35-60%, at the final 21 day assessment. A manuscript on this work has been prepared for publication.

6) Cause and management of avocado panicle dieback

Panicle dieback was identified late 2019 as a significant problem for many growers in the Bundaberg/Childers, QLD, production area, and resulted in reduced fruit yields in 2020 harvest season. Isolation of symptomatic tissues onto selective media in 2019 and 2020 resulted in high percentages of *Alternaria* and *Colletotrichum*, with lower frequencies of *Fusarium*, fungi from the Botryosphaeriaceae (e.g. *Neofusicoccum* sp. and *Lasiodiplodia*), and others. However, in 2021, *Colletotrichum* was consistently isolated at high frequency from panicles with early symptoms. Pathogenicity studies were required to demonstrate which fungi (if any) were likely to be the primary cause of the disease. Initial attempts to inoculate detached panicles, or intact panicles in flowering trees in the glasshouse were not successful. Inoculation of intact panicles in the field was not without problems, but was successful. Several trials evaluating a range of fungal candidates were conducted between March and November 2022 in orchards at Ravensbourne, Glasshouse Mountains and Maroochy Research station, QLD. Inoculation with *Colletotrichum* spp. consistently caused panicle dieback (necrosis), and was reisolated onto media at high frequencies from panicles inoculated with *Colletotrichum*, and from panicles inoculated with other fungal species or not inoculated. These studies confirmed the role of *Colletotrichum* in panicle dieback, and a manuscript on this work is in final stages of preparation (confidential draft not for circulation is available upon request).

Field trials with promising fungicides were undertaken in 2020-21 (at two sites, Childers and Goodwood) and again at the same Childers site in 2021-22. Sprays with fungicides (including red copper, cuprous oxide) at flowering and early fruit set did not cause phytotoxicity or damage to flowers, leaves or developing fruit. This dispels the myth that spraying fungicides damages flowers. After several discussions with researchers and growers who have been involved with the industry for a long time, it is likely that the “don’t spray at flowering” came from some research in north Queensland where mango flowers were damaged by high concentrations of a copper formulation, and has stuck with the avocado industry for decades. Other reasons for not spraying fungicides at flowering are related to perceived effects on pollinating insects, and ban imposed by some beekeepers on spraying anything while their hives are in the orchard.

Reports on the fungicide spray trials and pruning trial to manage panicle dieback are attached as Appendices 9 and 10, respectively. There were no statistically significant effects of treatments on final yield per tree due to high variability across replicates, however, two of the unregistered fungicides, SYNCUF19 and NUL3446, had higher yields in both years than all other treatments at the Childers site, where panicle dieback is severe. Nufarm are progressing with further field trials with NUL3446. Registered fungicides Amistar at mid-flowering in 2021, and combination of Serenade Opti (mid flowering) + Luna Sensation (6 weeks later at early fruit set), also increased yields measured in 2022, nearly doubled, compared with no fungicides at flowering and early fruit set. NUL3294 applied at flowering and early fruit set did not improve yields over control treatments at Childers. Tagging panicles and tracking fruitlet numbers showed that early-flowering indeterminate shoots, did not actually hold many fruitlets beyond December, and there were no differences in fruitlet number amongst the 4 treatments (nil, Nordox copper early, Amistar and SYNCUF19), selected for evaluation. Fruitlets were also counted on panicles tagged at peak flowering, and were mostly determinate inflorescences. The early applications of SYNCUF19 increased the numbers of fruitlets compared with other treatments through November, and while a lot of fruitlets had dropped by December, there were more fruitlets from SYNCUF19 (average 0.8 per panicle), than the other 3 treatments (0.2 and fewer per panicle). This aligns with early anecdotal reports that panicle dieback is more severe in determinate panicles, and supports a role for fungi in this disorder.

Trees with severe panicle dieback pruned to 2-3m in November 2020 had strong re-growth through 2021, and healthy canopies by spring 2021. Most trees flowered well, did not develop panicle dieback, and an average of 5.3kg per tree was harvested in June 2022. See Appendix 10.

Some additional studies undertaken in parallel with the panicle dieback work, and in collaboration with Harley Smith and Amnon Haberman (CSIRO, AV16005), has shown that abscission (abortion) of young fruitlets at about match-head size, is likely not caused by fungal infection. Abortion of fruit has been linked to necrotic, blackened seed coats, however, in this small study, there were similar numbers, approx. 20%, of blackened seed coats from fruitlets which had abscised as those which remained attached. That is, about 80% of fruitlets which aborted did not have blackened seed coats (Appendix 11).

7) Training, extension and communication, steering committee and industry support

Seven steering (PRG) committee meetings were held during the project, and the minutes of each meeting have been provided with previous milestone reports. The project leader and team staff and students actively participated in many extension, education, workshop events across Australia during the project, and these are summarised in Appendix 1. These were in collaboration with industry or extension projects and personnel (e.g. AAL, AV17005), or other regional consultants or resellers. Several articles have been published in Talking Avocados, and in the scientific peer-reviewed literature, and further articles are in preparation.

Training of PhD students, post-docs and early career researchers and other technical staff has been a key component of the project. Two Hons and one Masters students have completed their studies within the project. There are currently two PhD projects aligned with AV16007 and AV19005. Liz has co-supervised a PhD student at the University of New England, and a MSc student at Stellenbosch University, South Africa. Four visiting scientists have spent extended periods (5 to 12 months) with the project team, and have contributed to the research outputs of the project.

The project team also supported industry by assisting growers and nursery operators directly with disease/pathology enquiries and sample diagnostics, and contributed to related activities e.g. review of ANVAS, annual Plant Health Australia biosecurity panel meetings etc. Considerable time and effort was devoted to the tree "lodging" problem in WA, where thousands of trees with poor root structures blew over during strong gusty winds in June 2018 and again in May 2020.

Outputs

Table 1. Output summary

Output	Description	Detail
Development of a guide for optimal times to apply phosphonate for maximum levels in the root and minimize residues in fruit	now part of AV19005	This has become part of AV19005, after fruit residue testing in AV16007 indicated phosphite translocates to fruit when applied at recommended times of the phenological cycle.
Posters and presentations delivered at meetings to increase training, extension and communication capacity	Several outputs at industry meetings, field days, Best Practice Resource, Talking Avocados, World Avocado Congress, scientific (plant pathology) conferences	see Appendix 1 for extensive list
Prepare reports for industry on causal organisms and treatment efficacy for Phellinus brown root rot, Nectriaceous black root rot, stem end rot and branch and graft dieback	TA article on panicle dieback scientific publication on black root rot branch dieback publication in preparation	See results in this report and updates in milestone reports, and Appendix 1 Prabhakaran, A. D. and Dann, E. K. (2022) Evaluation of fungicide soil drench treatments to manage black root rot disease of avocado, *Plant Disease*, 106: 2026-2030. Booth, J. D. and Dann, E. K. (2023?) Avocado branch dieback: Testing relative pathogenicity of several fungi and evaluation of treatments to manage dieback and canker-like necrosis caused by Neofusicoccum parvum, submitted April 2023
Reports on activities undertaken by project personnel as collaborative interactions with the domestic and international avocado pathology network	Several presentations, co-supervised students, co-authored publications, etc.	See Appendix 1 Visit to colleagues at UC Riverside, August 2018 Avocado Brainstorming meeting South Africa, May 2020 (report on BPR) Co-authored review paper with colleague from Stellenbosch University, and co-supervised student Co-authored and co-supervised with UNE colleagues "virtual field day" presentation to Californian avocado growers, hosted by Californian Avocado Commission September 2020 and with colleague Dr Themis Michalaides, UC, Riverside (available online)

		Co-authored book chapter Prusky, Dann, Coates (2022) Presentations at Aust Plant Pathology Conferences (2019, 2021), International Congress of Plant Pathology (2018) Participation in annual Avocado Biosecurity Reference Panel
One article a year in Talking Avocados	Several with strong industry focus	See Appendix 1. Approx 12 published since start of the project, with another in upcoming Autumn 2023 issue.
Prepare a report detailing the efficacy of tested soil amendments for their ability to improve tree health and productivity	Detailed reports provided to the 4 field trial collaborators each year	These are included as appendices to this report. Manuscripts for publication will be prepared in coming months.
Updates and recommendations to be published in the Avocado Industry BPR and other	Several	See Appendix 1
Scientific publications in peer-reviewed journals	3 arising directly from this project or previous (AV14012), 2 more submitted, others in preparation. Five other publications closely linked to AV16007.	See Appendix 1 Prabhakaran, A. D. and Dann, E. K. (2022) Evaluation of fungicide soil drench treatments to manage black root rot disease of avocado, *Plant Disease*, 106: 2026-2030. https://doi.org/10.1094/PDIS-02-22-0264-RE Hassan, M. K. and Dann, E. K. (2019) Effects of treatment with electrolyzed oxidizing water on postharvest diseases of avocado, *Agriculture*, 9 (11): 241 https://doi.org/10.3390/agriculture9110241 (published in the special issue Postharvest Physiology and Technology of Fruits and Vegetables L. E. Parkinson, R. G. Shivas, E. K. Dann (2019) Development of a LAMP assay for detection of Calonectria ilicicola and Dactylonectria spp., fungi causing black root rot, *Plant Disease*, 103: 1865-1875, https://doi.org/10.1094/PDIS-11-18-2005-RE.
Register of technical services, advice and assistance provided to growers	See detail.	A log of industry support activities was kept Oct 2018 to end May 2019, and those activities accounted for an estimated 48 days of project personnel time. Keeping the log was itself time consuming, so subsequently industry support activities have been documented by emails, diagnostic reports and photographs, meeting/workshop/field day attendance etc. This has been a significant component in AV16007.
Documents associated with milestone reports, M&E, communications plant and risk register	Yes, all available upon request.	All Milestones submitted to Hort Innovation, M&E, Comms, risk register etc. available as attachments to MS #102
Meeting minutes and terms of reference prepared from steering committee meetings	7 steering committee (PRG) meetings held, minutes prepared.	Minutes for each meeting available upon request.

Outcomes

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
End of project outcomes			
Increased grower awareness of best practices and strategies to minimise impact of avocado diseases and pathogens	**SIP Outcome 4** "By 2021, productivity (marketable yield per hectare) has improved by 15 per cent on average, without increased production costs per kilogram" 15% increase in productivity (yield per hectare) from healthier trees improved via disease management practices (where adopted) 15% improvement in fruit quality measured by packout data and postharvest quality assessments (where adopted) Enhanced capacity in orchard productivity, RD&E through mentoring and training and strengthening linkages within Australia and internationally	Current best practices in tree establishment, maintaining tree health, postharvest fruit disease management and recommendations for fungicide use related to MRLs and export communicated to industry by various methods. Tree establishment has been a big component in this project.	Survey results of participants from several workshops available (from AV16007 and AV17005 activities). Participants rated the workshops as very useful or extremely useful, and indicated that they intended to make changes as a result of attending the workshop, e.g. "more mulching, more foliar sprays, open up canopy, more strategic use of fungicides, more focus on crop quality, anthracnose control, N:Ca monitoring", etc. Growers and packsheds revising fungicide use, in light of info on MRLs and increased volumes of fruit being exported, and requirement for high quality, disease-free fruit. Some packsheds no longer using Graduate A+. One packshed has installed EO water equipment for postharvest treatment. Nufarm trialing coded fungicide for registration. Growers starting to adopt spraying fungicide at early fruit set or even at flowering. Nurseries trialing root pruning of trees prior to planting for enhanced establishment.
Increased grower awareness of best practices and strategies to improve fruit quality and maximise orchard productivity			As above. Growers are asking for nursery tree health checks prior to planting, and taking more care during establishment phase. Nursery operators adopting new practices to produce plants with better

			root structure and health.
Key 'showcase' growers implementing and adopting recommended strategies			Many growers at least trialing new strategies, e.g. spraying fungicide in the orchard (from spraying nothing previously), timing of fungicide sprays, especially in WA where there is a predominance of cool-season Colletotrichum sp. able to infect fruit through cold wet periods.
Continue to strengthen industry relationships and networks to encourage and support high level awareness of best practices			Considerable interaction with other project teams, e.g. AV17005 and AV18000 and resellers, agronomists, etc. Numerous on-farm trials and visits to orchards to collect samples for project and student activities has strengthened industry relationships and provided opportunity for direct extension and comms. Engagement with agrichemical companies has facilitated testing of new chemistries
Short – medium term outcomes			
Identify activities, products and strategies for growers to limit the impact of disease and maximise orchard productivity	As above	As above. Further dissemination and implementation of project-related outputs in coming months.	e.g. emergency use permits or label registration for new fungicides.
Communicate to the avocado industry the benefits and importance of adopting recommended activities and strategies		As above and ongoing.	As above, and ongoing.
Maintain strong, collaborative relationships with industry stakeholders who will benefit from the project		Ongoing linkages with other projects and disciplines as well as all industry stakeholders in Australia and internationally are expected.	A above, and ongoing.
Enhanced capacity to support pathology activities required for the avocado industry		Ongoing diagnostic support will be required. Broad-based wholistic approach will be necessary, as many	Ongoing training and involvement of project staff and students in industry activities. Strong industry engagement

		problems do not have pathogens as their root cause and may be abiotic, physiological or related to poor management practices. Student project linked to fruit quality work is continuing, outputs to be communicated as appropriate.	through project team's on-farm research trials and diagnostic activities. Adoption of enhanced techniques for diagnostics.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent has the project increased tree health, yield and fruit quality?	Very difficult to quantify, and full extent may not be realised for 5+ years.	There is a need to work out how outputs can be adopted and how benefits from adoption can be measured. This would be true of many R&D projects.
To what extent has the project met the needs of avocado industry levy payers for improving plant health and productivity?	Consistent messaging regarding practices to manage the key diseases has been delivered throughout this project.	Further targeted extension of new results/outputs still to be delivered to industry.
To what extent did extension activities elicit response and engagement by industry levy payers?	Dedicated disease management workshops were extremely interactive, with a very high level of engagement	Working with small study groups (5-6 growers/agronomists) in each region might also help to extend best practice and new strategies to industry.
Have regular project updates been provided through linkage with the industry communication providers? To what extent have the updates addressed industry interest and need?	Yes! Several articles in Talking Avocados, Guacamole, industry forums, etc. Updates only delivered if they address specific interest and need.	As indicated above, further scientific and extension outputs to come from the project. Will work with extension and comms teams to provide this material in the most effective way possible.
How accessible were extension events to industry levy payers? To what extent did extension and engagement activities align with methods accepted by industry levy payers?	All extension events, workshops, articles, webinars etc., have been accessible to all levy payers, with the exception of one dedicated disease management workshop in NQ in 2022, delivered to advanced growers and agronomists/consultants who can further extend their new knowledge to their clients. Activities aligned 100% with accepted methods – many as part of extension and comms projects. Many available on AAL website	There is so much extension information available, it can be overwhelming for growers. An idea raised in recent workshops has been using podcasts, and perhaps more videos/webinars.
What efforts did the project make to improve efficiency? In what ways has project improved its efficiency and effectiveness	Huge efficiencies were made through utilising students and visiting international research staff (not paid by the project) to assist paid project staff with experiments. This has increased our project output considerably, and assisted in training new generation of horticulturalists. Employing external contractor for spraying orchard trials saved time and effort of project staff. Field work undertaken as efficiently	Good students and staff are hard to secure. Perhaps industry could provide some awards/small scholarships as incentives for Hons students to work with researchers on their projects. Greater use of third party providers to assist with field work conducted away from primary location. Greater awareness and linkages across projects and industries.

	as possible, and attempts made to coordinate travel with other tasks, e.g. combining workshop or conference with field trial activity. Sharing staff across other projects has largely been successful, and ensured continuity of employment of key staff.	

Recommendations

There are several recommendations arising from the project, and these have been categorised into immediate and short term/on-going.

For immediate action

- Continue molecular and *in vitro* (Petri dish) studies to confirm the spread and incidence of resistance/loss of sensitivity within *Colletotrichum* to azoxystrobin. It will be necessary to determine whether there is resistance (or loss of efficacy) to Luna Sensation, the only other fungicide registered for avocado with post-infection activity, which also has a strobilurin compound as one of the actives. We have a very large collection of *Colletotrichum* isolates from across Australia, to conduct this work. This work is currently being completed by a PhD student.
    - It is likely this work show that resistance is widespread resistance. This means that field sprays and postharvest use of Graduate A+ will no longer be effective. This information will have to be communicated promptly and sensitively to the whole of industry.
- Urgently progress emergency use permits for alternatives to azoxystrobin sprays in the field for postharvest disease, particularly those which will be acceptable for our export markets. This would likely involve the Hort Innovation SARP coordinator and industry via Avocados Australia Ltd. Further research trials to demonstrate efficacy of products showing promise in this project are likely to be required.
- Progress research/evaluation of untested alternatives and approaches to current fungicides used in the orchard and packingshed, particularly those which will be acceptable for our export markets. Demonstrating efficacy, MRLs, and fast-tracking for registration or permit through APVMA. This will include demonstrating efficacy of claimed biological or "soft" crop protectants. Products to test must be carefully chosen based on reliable data from other crops, and not on "data-free observations" (DFOs), or pressure from third parties with commercial interests.

For short-term and ongoing action

- Continue to provide flexibility and adequate resourcing within future projects for diagnostic support for growers and nursery operators, responding to outbreaks of endemic disease problems.
- Continue to work with extension and communications projects and personnel to effectively communicate outputs, particularly those which have immediate or short-term application on-farm, for adoption. After several decades of delivering presentations on best practice integrated disease management, (where very little has changed), most growers within industry are still not adopting the basics, and rather seeking "the silver bullet", fungicide or other magical treatment. Perhaps a different extension model/s could be trialed?? For example, utilising small groups of grower volunteers, who are prepared to contribute to discussions and run demo trials to showcase trial results to the group and broader industry, if appropriate.
- Sub-contracting a reliable local research provider to complete orchard sprays in the Bundaberg/Childers panicle dieback trials worked extremely well. This ensured treatments were applied in a timely manner, with appropriate equipment, and allowed flexibility around weather conditions. The sub-contractor kept project leader up to date with treatment applications, etc., liaised well with growers and agronomists, and provided photographs and detailed final reports. This approach is highly recommend for future on-farm trials, if conducted in locations remote from project teams. Growers are very keen and supportive of our research efforts, but when it comes to applying treatment to a few rows of trees, experimental trials are not their top priority.
- Future projects should include a stipend for a PhD student, to work closely with industry on a project with direct relevance, and with good academic rationale and combination of field and laboratory activities. Operating for

travel and consumables for the student's work should be costed within the project.

- Consider further research on evaluating treatments to stimulate root growth, for example soil conditioners, and whether these can improve natural soil microbial communities and activity and contribute to managing (outgrowing) Phytophthora root rot.

Refereed scientific publications

Journal article

1. Jolliffe, J. B., Dann, E. K., van der Rijst, M., Masikane, S. L., Novela, P., Mohale, P. and McLeod, A. (2023?) Seasonal colonisation of avocado roots by *Phytophthora cinnamomi* in South African orchards, submitted to *Plant Disease*, April 2023.
2. Booth, J. D. and Dann, E. K. (2023?) Avocado branch dieback: Testing relative pathogenicity of several fungi and evaluation of treatments to manage dieback and canker-like necrosis caused by *Neofusicoccum parvum*, submitted May 2023.
3. Prabhakaran, A. D. and Dann, E. K. (2022) Evaluation of fungicide soil drench treatments to manage black root rot disease of avocado, *Plant Disease*, 106: 2026-2030. https://doi.org/10.1094/PDIS-02-22-0264-RE
4. Dann, EK and McLeod, A (2020) Phosphonic acid: a long-standing and versatile crop protectant, *Pest Management Science*, October 2020, DOI 10.1002/ps.6156.
5. Salgadoe, A. S. A., Robson, A. J., Lamb, D. W. and Dann, E. K. (2019) Assessment of canopy porosity in avocado trees as a surrogate for restricted transpiration emanating from Phytophthora root rot, *Remote Sensing*, 11: 2972; doi:10.3390/rs11242972
6. Hassan, M. K. and Dann, E. K. (2019) Effects of treatment with electrolyzed oxidizing water on postharvest diseases of avocado, *Agriculture*, 9 (11): 241 https://doi.org/10.3390/agriculture9110241 (published in the special issue Postharvest Physiology and Technology of Fruits and Vegetables).
7. L. E. Parkinson, R. G. Shivas, E. K. Dann (2019) Development of a LAMP assay for detection of Calonectria ilicicola and Dactylonectria spp., fungi causing black root rot, *Plant Disease*, 103: 1865-1875, https://doi.org/10.1094/PDIS-11-18-2005-RE.
8. A.S.A. Salgadoe, A.J. Robson, D.W. Lamb, E.K. Dann, C.W. Searle (2018) Quantifying the severity of Phytophthora root rot disease in avocado trees using image analysis, Remote Sensing 10(2), 226; doi:10.3390/rs10020226

Chapter in a book or paper in conference proceedings

1. D. B. Prusky, E. Dann and L. Coates (2022) Postharvest Diseases of Avocado, in "Postharvest Pathology of Fruit and Nut Crops: Principles, Concepts and Management Practices", eds. J. E. Adaskaveg, H. Föster and D. B. Prusky, APS Press, St Paul, Minnesota, USA, pp.461-472.

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Dann, E.K., Coates, L.M., Pegg, K.G., Dean, J.R., Cooke, A.W., Smith, L.A., Shuey, L., Whiley, A.W., Hofman, P.J., Marques, R. and Stubbings, B. (2016). Rootstock selection, nitrogen and calcium influence postharvest disease in avocado, *Acta Horticulturae*, 1120, 391-398 DOI: 10.17660/ActaHortic.2016.1120.60

Intellectual property

No project IP or commercialisation to report

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Appendices

Appendix 1 AV16007 Extension outputs

Appendix 2. Lancaster et al WAC 2019 biofumigation poster

Appendix 3. Costa trial results summary

Appendix 4. Bamess Farms trial summary

Appendix 5. West Pemberton Avocados results summary

Appendix 6. Bamess Farms postharvest fungicide spray trial (CONFIDENTIAL)

Appendix 7. Roe et al poster ASDS 2022 soil microbial activity

Appendix 8. Preharvest fungicide trial results summary (CONFIDENTIAL)

Appendix 9. Panicle dieback fungicide trial report (CONFIDENTIAL)

Appendix 10. Panicle dieback pruning trial summary

Appendix 11. Role of fungi in the abortion of fruitlets (CONFIDENTIAL)

Appendix 1. AV16007 extension outputs

Type of extension activity	Details
Dedicated advanced disease management workshops AV16007 and AV19005	4 face-to-face workshops were held in 2022 to cover the basics of disease management (Phytophthora and fruit diseases), and present results from current research projects. Each workshops was split into two parts Part 1 "Striving for the best quality fruit", Part 2 "The battle against Phytophthora root rot", with extended informal presentations by Liz. 1) Bundaberg, 27 April 2022. Held by Liz in conjunction with Syngenta and EE Muir, with Kath Adams (Syngenta) presenting on maximum residue limits (MRLs), and Scott Matthews (Campbells) discussing global pressures on pesticides and fertilisers. Attended by approx. 58 people, evaluation available. 2) Manjimup, 5 May 2022. Held by Liz in conjunction with Syngenta and Farmlink, with Shell Xiao (Syngenta) presenting on maximum residue limits (MRLs), and Zac Starkie (Farmlink) discussing global pressures on pesticides and fertilisers. Attended by approx. 45 people, evaluation available. 3) Port Macquarie, 15 June 2022. In conjunction with AAL Regional Export Forum. Evaluation available. 4) Walkamin, 2 August 2022. Invite only for advanced growers and agronomists/consultants. Organised in conjunction with Clayton Lynch (Australian Produce Partners) and Geoff Dickinson (DAF).
individual	farm visits, phone calls, emails, trial reports (to collaborators, etc.)
AV17005	Phytophthora poster & video - Significant contribution to poster and video, produced by the industry extension projects (AV10002 and AV17005), and added to the Best Practice Resource website https://avocado.org.au/wp-content/uploads/2019/09/Manage-Phytophthora-Root-Rot-Poster-1.pdf https://www.youtube.com/watch?v=0T2Kz5tNfX0
BPR	BPR info – postharvest chemical treatment – update, January 2022
Handbook	Avocado Problem Solver Field Guide, 2nd edition, co-author, AV17005
Avoskills workshops	One presentation by Liz via Zoom at the North Queensland Avoskills workshop 18 July 2019

(AV17005)	"Diseases (other than Phytophthora) and their management in avocado" Two presentations by Liz at each of the two day "Avoskills" workshops "Diseases (other than Phytophthora) and their management in avocado" "Phytophthora root rot of avocado: The disease and how to manage it" - Manjimup, WA, 10-11 March, 2020 - Bundaberg, QLD, 21-22 September 2021 - Mildura, VIC, 12-13 May 2022 - Port Macquarie, NSW, 14-15 June 2022 All the Avoskills presentations (slides) are available on the AAL Best Practice Resource
Australian industry hosted events e.g. Qualicado, Regional Forum Field days Workshops AV17005 AV18000	Project update presentation to avocado SIAP, 23 November 2018, Brisbane "Phosphonate, field trials and flower blight (AV 16007 Project update)", - Manjimup, WA, 12 March 2020 Liz Dann was keynote presenter at the avocado phos acid (phosphonate) workshop, held at Mareeba on 21 April 2021. The workshop was organised by Dr Geoff Dickinson and team (collaborators in AV19005, and members of extension project AV17005 "Fruit diseases of avocado and how to manage them" "Phytophthora root rot of avocado and how to manage it" - Tamborine Northern Rivers Regional Forum, Alstonville, 1 June 2022. These presentations are available on the Best Practice Resource, https://avocado.org.au/wp-content/uploads/2022/06/3.-Fruit-diseases-Liz-Dannv2.pdf; https://avocado.org.au/wp-content/uploads/2022/06/6.-Phytophthora-root-rot-Liz-Dannv2.pdf "Research snapshot: soil health and tree health"

	- Soil health advanced management workshop (summit), Brisbane airport, 8-9 November 2022 "How to manage the diseases we never see" component by E. Dann in webinar "What spray schedule should you use for a disease you never see?", hosted by Noel Ainsworth, QDAF, delivered 9 February 2023, as part of AV18000. The complete webinar is available on the Best Practice Resource, https://www.youtube.com/watch?v=O9QHiLItqZY
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***Talking Avocados* articles**	"New projects to improve productivity through disease management" Autumn 2018 "Getting smart: Rating PRR severity in orchards" Autumn 2018 UNE collaborators "Lodging of avocado trees" L. Dann, K. Bransgrove, S. Newett, G.Thomas (and several growers) 2019 Talking Avocados 29(4) 40-45 "Verticillium wilt in Western Australia this summer" Autumn 2019, Talking Avocados 30(1) 52-53. This article was also published in the online newsletter Guacamole 22 March 2019, and has been accessed via the link https://www.avocado.org.au/public-articles/ta30v1-verticillium-wilt/?utm_source=newsletter&utm_medium=email&utm_term=https%3A%2F%2Fwww.avocado.org.au%2Fpublic-articles%2Fta30v1-verticillium-wilt%2F&utm_content&utm_campaign=Guac+22%2F3%2F19

	“Improving avocado orchard productivity through disease management” L. Dann, A. Prabhakaran, E. Lancaster, K. Bransgrove, M. Hickey, E. Singh, Winter 2019, Talking Avocados 30 (2): 56-59 “New rootstock released for use by the Australian avocado industry” A. Whiley, E. Dann, Talking Avocados, Spring 2020 31(3):60-61 “Panicle blight (flower dieback)” Elizabeth Dann, Akila Prabhakaran, Kaylene Bransgrove, TA, Spring 2020 31(3): 48-51. Two articles were published in Guacamole in September and October 2020. One to raise awareness of panicle blight https://avocado.org.au/public-articles/ta31v3panicle/ The second article report on the first commercial planting of SHSR-04 rootstock. This rootstock was evaluated for Phytophthora root rot tolerance in previous industry disease management projects. https://avocado.org.au/public-articles/ta31v3rootstock/ Sept, Oct 2020 “Improving avocado fruit quality: Evaluation of postharvest treatments” E. Dann, S. Hood, A. Prabhakaran, K. Hassan (2020), Talking Avocados Autumn 31(1): 58-62 Also posted on AAL website from Guacamole Newsletter link at https://www.avocado.org.au/public-articles/ta31v1-quality/ “Phosphorous acid (phosphonate): research update and new project activities” Talking Avocados, Summer 2021 Volume 31 (4):61-67 “Procado®: A new Australian avocado rootstock”, by Tony Whiley and Liz Dann, Autumn 2021 TA 32(1): 57-64 “Panicle and shoot dieback – update” by Elizabeth Dann, Akila Prabhakaran and Kaylene Bransgrove, was submitted in February 2021 to update the online article and publication in TA. This article provides an update of our current understanding and research efforts concerning panicle dieback in avocados “Postharvest Disease Treatments” G. Dickinson, N. Ainsworth, E. Pattison, L. Coates and E. Dann, TA Autumn 2022, 33(1) 67-68. “Dos and donts of planting avocados” E Dann and Leanne Gillies (Flemings) to be published in Autumn 2023 Talking Avocados (TA) is published quarterly and distributed widely within the avocado industry. Past editions of TA are available on the AAL website https://avocado.org.au/news-publications/talking-avocados/past-editions/
SAAGA/NZ Avoscene	Several articles in avocado industry magazines in other countries various E. Dann (2021) Postharvest treatments improve avocados, Australian Tree Crop, December/January 2021:50-51
Refereed paper	*A.S.A. Salgadoe*, A.J. Robson, D.W. Lamb, E.K. Dann, C.W. Searle (2018) Quantifying the severity of Phytophthora root rot disease in avocado trees using image analysis, Remote Sensing 10(2), 226; 17pp. doi:10.3390/rs10020226, http://www.mdpi.com/2072-4292/10/2/226

Refereed paper	*Salgadoe, A. S. A.*, Robson, A. J., Lamb, D. W. and Dann, E. K. (2019) Assessment of canopy porosity in avocado trees as a surrogate for restricted transpiration emanating from Phytophthora root rot, *Remote Sensing*, 11: 2972; doi:10.3390/rs11242972
Refereed paper	T. I. Burgess, Y. P. Tan, J. Garnas, J. Edwards, K. A. Scarlett, L. A. Shuttleworth, R. Daniel, E. K. Dann, L. E. Parkinson, Q. Dinh, R. G. Shivas, F. Jami (2018) Current status of the Botryosphaeriaceae in Australia, *Australasian Plant Pathology*, 48:35-44. doi.org/10.1007/s13313-018-0577-5
Refereed paper	L. E. Parkinson, R. G. Shivas, E. K. Dann (2019) Development of a LAMP assay for detection of Calonectria ilicicola and Dactylonectria spp., fungi causing black root rot, *Plant Disease*, 103: 1865-1875, https://doi.org/10.1094/PDIS-11-18-2005-RE.
Refereed paper	Hassan, M. K. and Dann, E. K. (2019) Effects of treatment with electrolyzed oxidizing water on postharvest diseases of avocado, *Agriculture*, 9 (11): 241 https://doi.org/10.3390/agriculture9110241 (published in the special issue Postharvest Physiology and Technology of Fruits and Vegetables)
Refereed paper	J. E. Stewart, M.-S. Kim, Y. Ota, N. Sahashi, J. W. Hanna, M. Akiba, J. P. Ata, N. Atibalentia, F. Brooks, C.-L. Chung, E. K. Dann, A. M. Farid, T. Hattori, S. S. Lee, K. Otto, G. S. Pegg, R. L. Schlub, L. S. Shuey, A. M. C. Tang, J.-N. Tsai, P. G. Cannon, and N. B. Klopfenstein (2020) Phylogenetic analyses reveals three distinct lineages of the invasive brown root-rot pathogen, *Phellinus noxius*, and bioclimatic modeling predicts differences in associated climate niches, *European Journal of Plant Pathology*, 156: 751–766 https://doi.org/10.1007/s10658-019-01926-5
Refereed paper	Dann, EK and McLeod, A (2020) Phosphonic acid: a long-lived and versatile crop protectant, *Pest Management Science*, 77:2197-2208 (review) https://doi.org/10.1002/ps.6156
Refereed paper	Prabhakaran, A. D. and Dann, E. K. (2022) Evaluation of fungicide soil drench treatments to manage black root rot disease of avocado, *Plant Disease*, 106: 2026-2030. https://doi.org/10.1094/PDIS-02-22-0264-RE
Refereed paper (submitted)	Booth, J. D. and Dann, E. K. (2023?) Avocado branch dieback: Testing relative pathogenicity of several fungi and evaluation of treatments to manage dieback and canker-like necrosis caused by Neofusicoccum parvum, submitted April 2023
Refereed paper (submitted)	Jolliffe, J. B., Dann, E. K., van der Rijst, M., Masikane, S. L., Novela, P., Mohale, P. and McLeod, A. (2023?) Seasonal colonisation of avocado roots by *Phytophthora cinnamomi* in South African orchards, submitted to Plant Disease, April 2023
Book chapter	D. B. Prusky, E. Dann and L. Coates (2022) Postharvest Diseases of Avocado, in “Postharvest Pathology of Fruit and Nut Crops: Principles, Concepts and Management Practices”, eds. J. E. Adaskaveg, H. Föster and D. B. Prusky, APS Press, St Paul, Minnesota, USA, pp.461-472.

Grower/industry presentations	2x presentations at AVOCO conference, Auckland, NZ, 23 June 2018 Presentation to EE Muir avocado conference, "Diseases and their management in avocado" Bundaberg, 21 May 2019 NZ Avocado 2 presentations at different growing regions, November 2019 Webinar presentation on panicle blight to Costa Group and Nutrien Ag Solutions, 28 August 2020 "virtual field day" presentation to Californian avocado growers, hosted by Californian Avocado Commission September 2020 https://www.californiaavocadogrowers.com/articles/avocado-branch-canker-virtual-field-day-video-and-materials-available-online "Avocado diseases and their management" to Nutrien agronomists from Queensland and northern NSW, as part of their North Eastern Coastal Agronomy Conference, held in Brisbane 20 April 2021 Liz gave a presentation in December 2020 via Zoom "Managing anthracnose and stem end rot in avocado in SW WA" to a small group of growers concerned about fruit quality and postharvest disease. The webinar was organized by Zac Starkie (Farmlink, Manjimup). December 2020 Webinar Noel Ainsworth and John Agnew (QDAF) "How to manage the diseases we never see" component by E. Dann in webinar "What spray schedule should you use for a disease you never see?", hosted by Noel Ainsworth, QDAF, delivered 9 February 2023, as part of AV18000. The complete webinar is available on the Best Practice Resource, https://www.youtube.com/watch?v=O9QHiLltqZY
Academic presentations	Seminar presented at the University of Pretoria, October 2018 Seminar presented at Stellenbosch University, October 2018 5 short presentations by Liz at the Avocado Brainstorming meeting, Tzaneen, South Africa, May 2018 (see report on BPR https://avocado.org.au/wp-content/uploads/2019/02/Avocado-Brainstorming-2018-report-on-field-trips-compressed.pdf) "Reducing the impact of diseases on productivity and quality of avocado" E. Dann and avocado pathology team, presentation at Future of Horticulture session, Australian Society of Hort Science, Brisbane, 18 June 2018 QAAFI seminar "Global avomania: Overcoming constraints to orchard productivity and fruit quality" Available on Youtube, https://www.youtube.com/watch?v=Ng24vSZuGzk and linked from Avocados Australia via Guacamole electronic newsletter and twitter https://www.avocado.org.au/newsletter/guac-18-10-19. 15 October 2019

	Poster presentation "Phylogeny and molecular diagnosis of nectriaceous fungi associated with black root rot in avocado", L. Parkinson, D. Le, R. Shivas and E. Dann, International Congress of Plant Pathology, Boston, USA, August 2018 Oral and Poster presentation "Evaluation of fungicide soil drench treatments to manage black root rot disease in avocado seedlings" A. Prabhakaran and E. Dann, Australasian Plant Pathology Society meeting, Melbourne, November 2019 QAAFI Seminar "Investigating threats to Our Green Gold: blight and dieback of avocado" Elizabeth Dann, Montana Hickey, Akila Prabhakaran, 17 August 2021, https://qaafi.uq.edu.au/event/session/10228 Zoom presentation "Investigating the pathogenicity of fungi associated with panicle blight in avocado (*Persea americana*) orchards", Montana Hickey, Australasian Plant Pathology Society meeting, November 2021
World Avo Conference, Colombia September 2019 6 presentations	• Phytophthora root rot of avocado: the disease and how to manage it, Elizabeth Dann • Evaluating biofumigant crops as a pre-plant treatment for avocado orchard establishment in sites with high Phytophthora root rot pressure, E.K. Lancaster, P. de Souza, D. P. Le, J. Duff , and E.K. Dann • Improving avocado fruit quality and safety: Alternatives to prochloraz for postharvest treatment Elizabeth Dann, Shaun Hood, Akila Prabhakaran, Kamrul Hassan • Biosecurity capacity building for the Australian avocado industry, L. E. Parkinson, K. Bransgrove, E. K. Dann and A. D. W. Geering • Pathogenicity and molecular detection of nectriaceous fungi associated with black root rot of avocado, L. E. Parkinson, D. P. Le, R. G. Shivas and E. K. Dann • Seasonal colonisation patterns of Phytophthora cinnamomi in South African avocado orchards, J.B. Jolliffe, E.K. Dann, S.L. Masikane, P. Novela, P. Mohale and A. McLeod
WAC, New Zealand	• Investigating fungi associated with panicle blight and dieback in avocado, Prabhakaran A D, Hickey M[1], Dann EK, oral presentation • Plus two presentations from AV19005

WAC-071 Evaluating biofumigant crops as a pre-plant treatment for orchard establishment in sites with *Phytophthora cinnamomi*

Emily Lancaster[A], Paulo de Souza[A], Duy Le[A], John Duff[B] and Elizabeth Dann[A]

[A] University of Queensland, Brisbane, QLD, Australia; [B] Department of Agriculture and Fisheries, Gatton, QLD, Australia

Introduction

- Brassica family biofumigant crops are grown and incorporated prior to planting vegetables, strawberries to reduce nematodes and soilborne fungal pathogens
- Volatile isothiocyanate compounds are released when cells of *Brassica* spp., *Raphanus* sp., *Sinapsis* sp. *or Eruca* sp. are ruptured
- Isothiocyanate compounds may inhibit fungi, bacteria, nematodes

Aims

- ***to determine* in vitro *effects of Brassica family biofumigants on growth of soilborne fungal pathogens***
- ***to evaluate biofumigant crops, chicken manure or plastic covering on establishment of avocado trees under high Phytophthora root rot disease pressure***

Methods

In vitro plate assay

- Media in Petri dishes was amended with dried extracts of different *Brassica* biofumigants
- Inoculum of *P. cinnamomi*, *Calonectria ilicicola* or *Dactylonectria macrodidyma* was placed in the centre of the plate, colony growth was measured

Field

- BQ Mulch, Caliente or chicken manure was incorporated into plots, half of the plot was covered with black plastic for 2 weeks
- Grafted avocado trees were planted 2 weeks after plastic was removed
- Tree health assessed frequently with scale 1=healthy to 6=dead

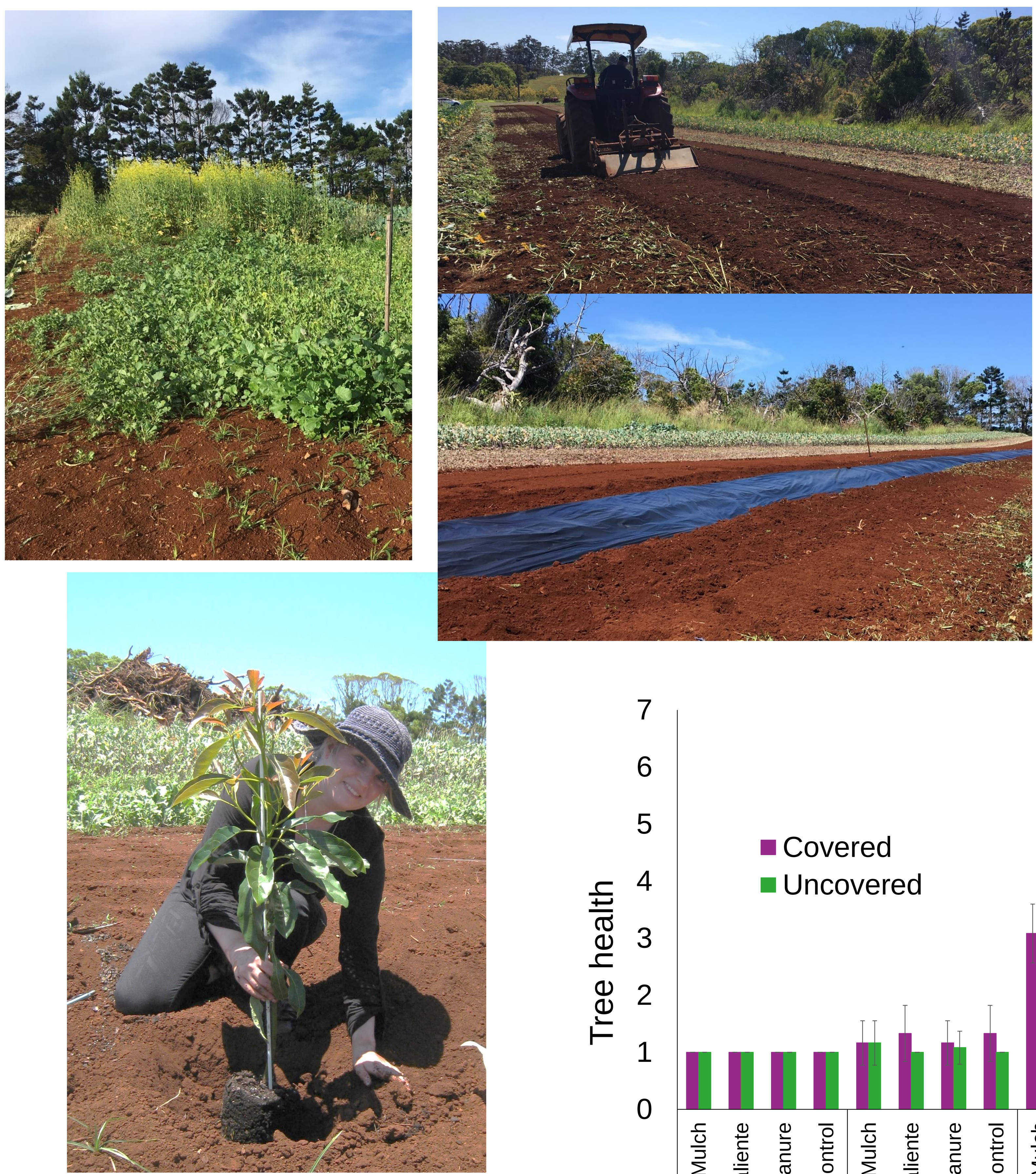

Figure 1: Caliente and BQ Mulch growing in plots, incorporation by several passes with rotary hoe, black plastic covering, Emily Lancaster planting Hass on Reed tree

Results

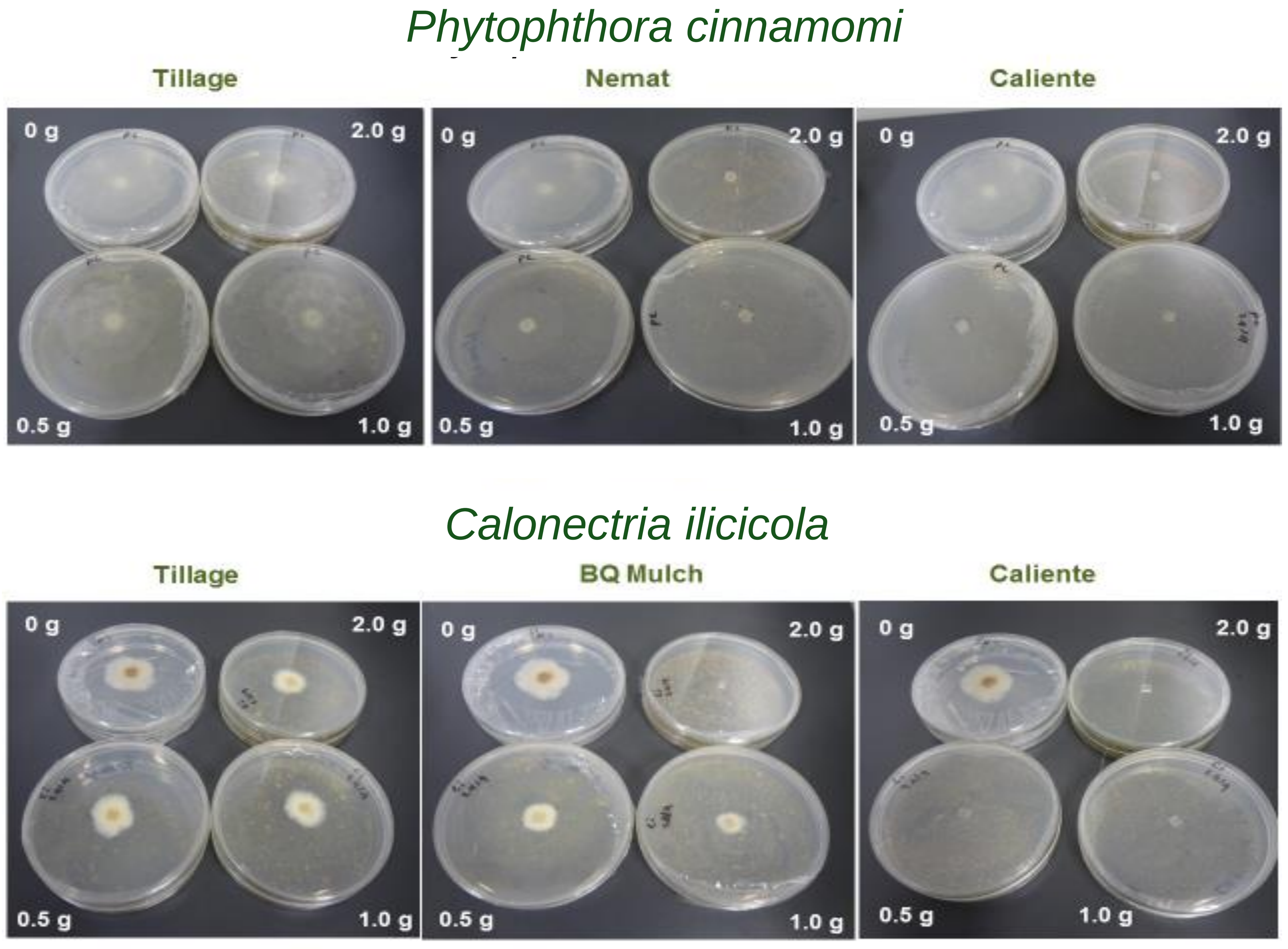

Figure 2: The effect of the Brassica biofumigant volatile compounds on the growth of *P. cinnamomi* and *C. ilicicola in vitro*

In vitro plate assay

- Some biofumigants were more effective than others at inhibiting growth *in vitro* eg. Caliente, BQ Mulch and Nemat inhibited *P. cinnamomi*, however, Tillage was ineffective (Figure 2)

Field

- Tree health began to decline within 2 months of planting (Figure 3)
- Trees in covered plots were significantly healthier than those in uncovered plots, from 3 months after planting
- There were no significant differences in tree health amongst Brassica or manure treatments, however incorporation strategy and rates were probably not optimal
- Caliente and BQ Mulch plants were not hosts of *P. cinnamomi*

Conclusions

- ***Soil solarisation to reduce pathogen load could be an important pre-plant strategy, where practical***
- ***Biofumigation by Brassica crops or chicken manure was not demonstrated in this trial, but further work with commercial pelleted formulations, eg. BioFence, is warranted***

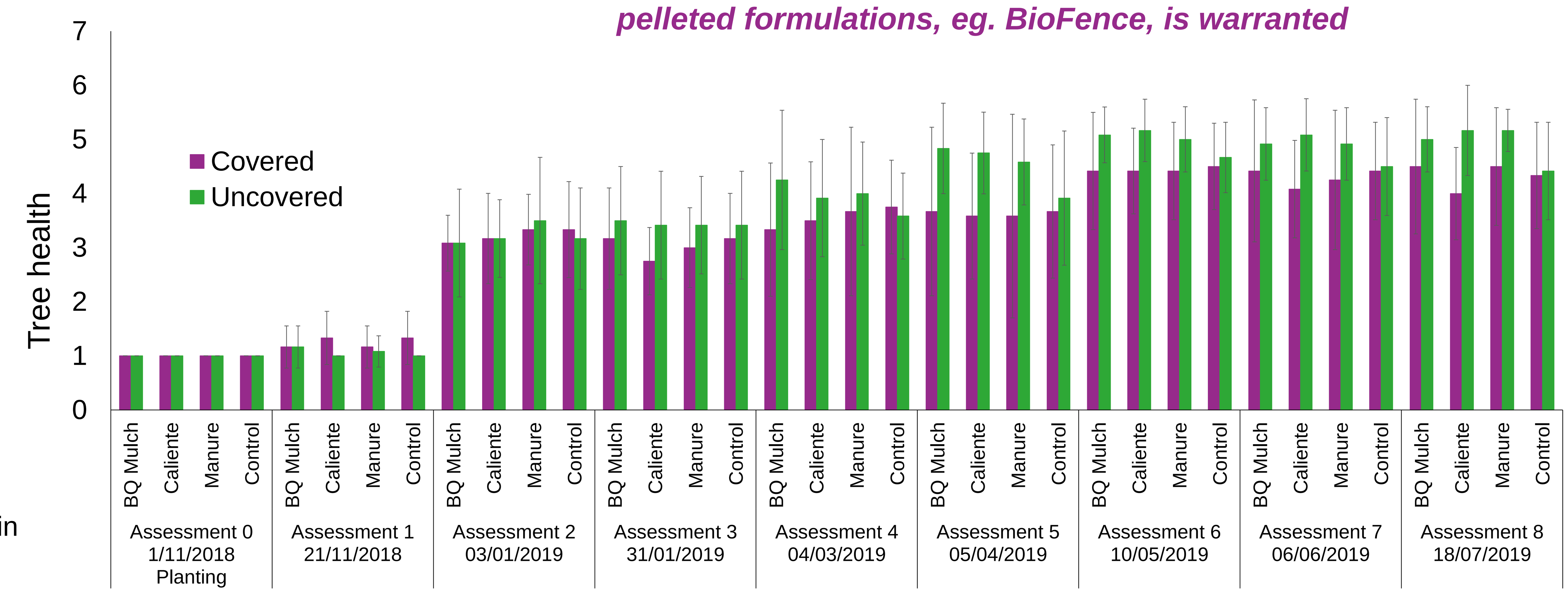

Figure 3: Avocado tree health assessments over time ± SD. 1=healthy, 6=dead

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Hort Innovation Strategic levy investment

AVOCADO FUND

WAC IX World Avocado Congress - Colombia -

The University of Queensland Australia

QAAFI Queensland Alliance for Agriculture and Food Innovation

The Queensland Alliance for Agriculture and Food Innovation (QAAFI) is a research institute of The University of Queensland (UQ), supported by the Queensland Department of Agriculture and Fisheries.

Queensland Government

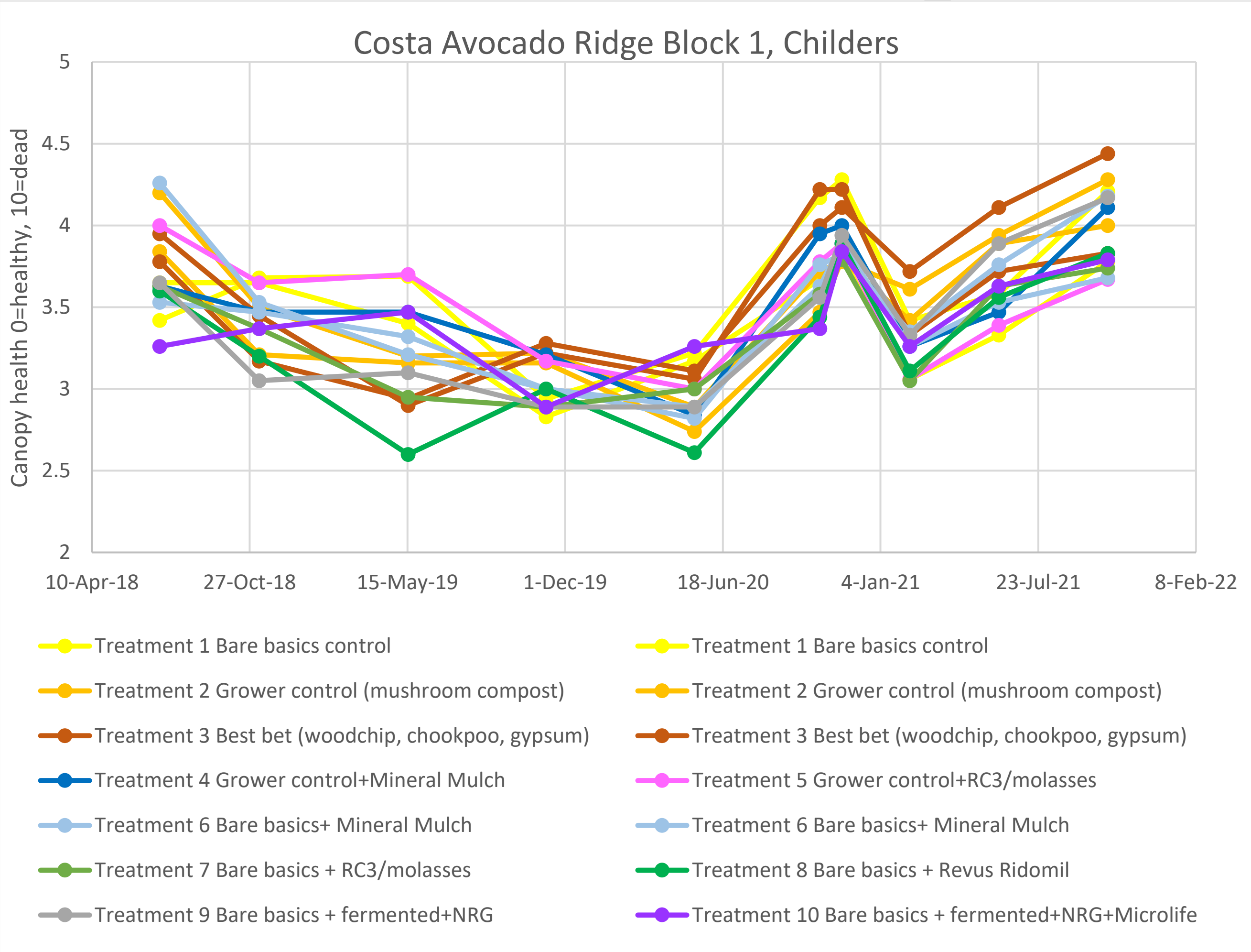
CRICOS Provider 00025B

AV 16007 Costa, Childers field trial summary, updated March 2022

Liz Dann and avocado pathology team, University of Queensland,
Brigette Ryan and Costa Childers team

2021 Results, Pages 1-7

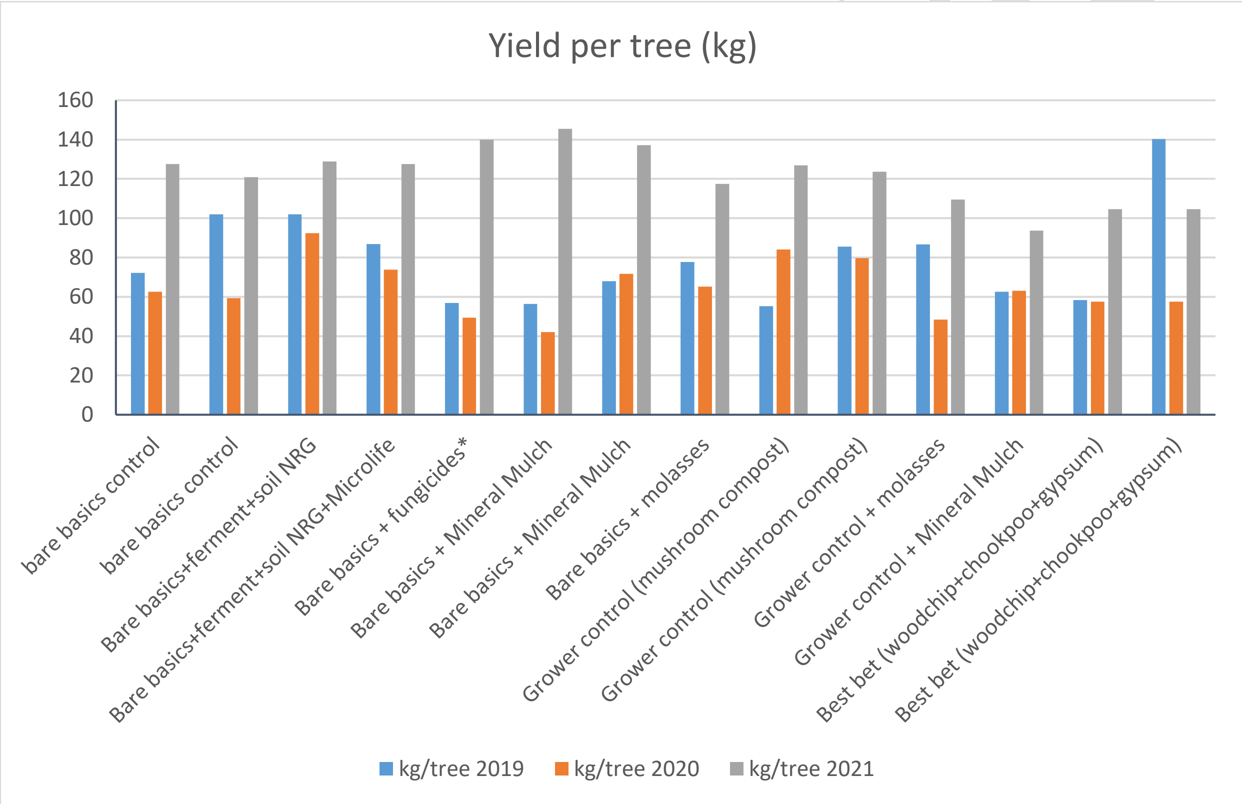
<u>Canopy health</u>



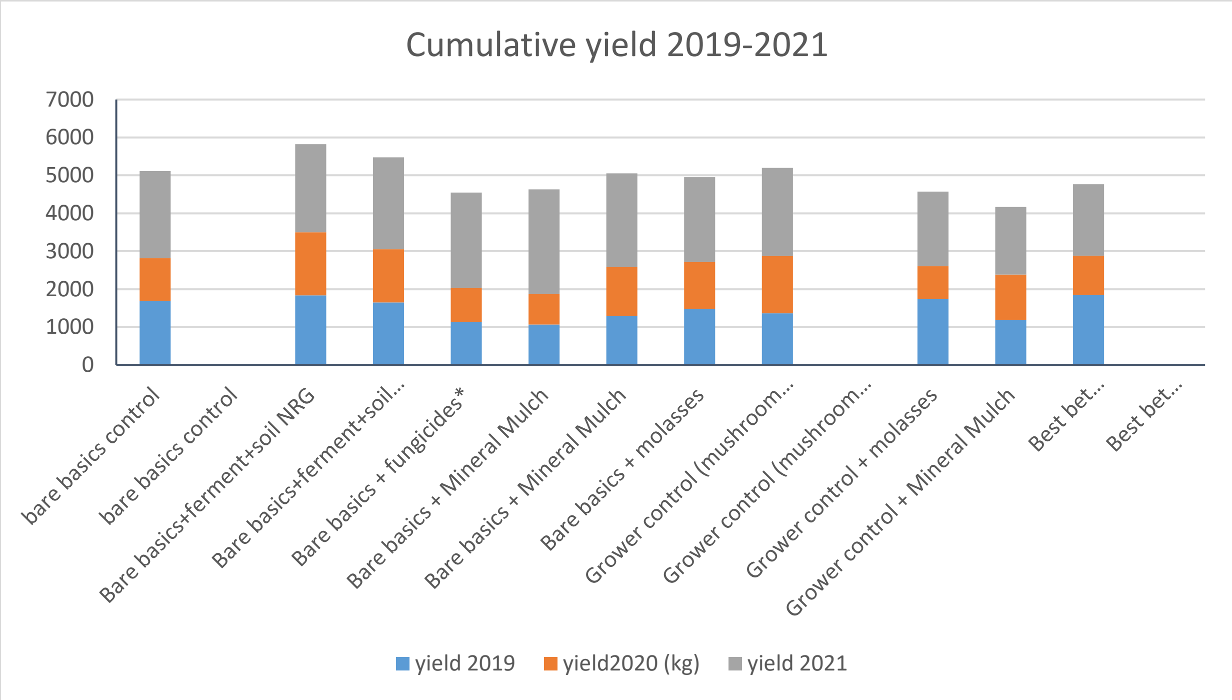
Canopy health has declined across all treatments from May 2020 to October 2021, and there are no obvious treatment effects. Treatment applications concluded when fruit were harvested from the trial in July 2021. Seasonal fluctuations are to be expected, as trees decline during stressful periods of flowering and early fruit set, (eg. October-November 2020), and improve given favourable conditions. The relatively dry spring of 2020, and below average rainfall for January and February 2021, after average rainfall for December2020 (at 116mm), likely benefited the trees greatly, and their overall health improved at the February 2021 assessment. The spread of canopy health scores has remained fairly tight across the duration of the trial, and at the last assessment in October 2021, there was less than one unit separating tree health scores, on a 10 point scale. All trees in the trial are rated at each time point.

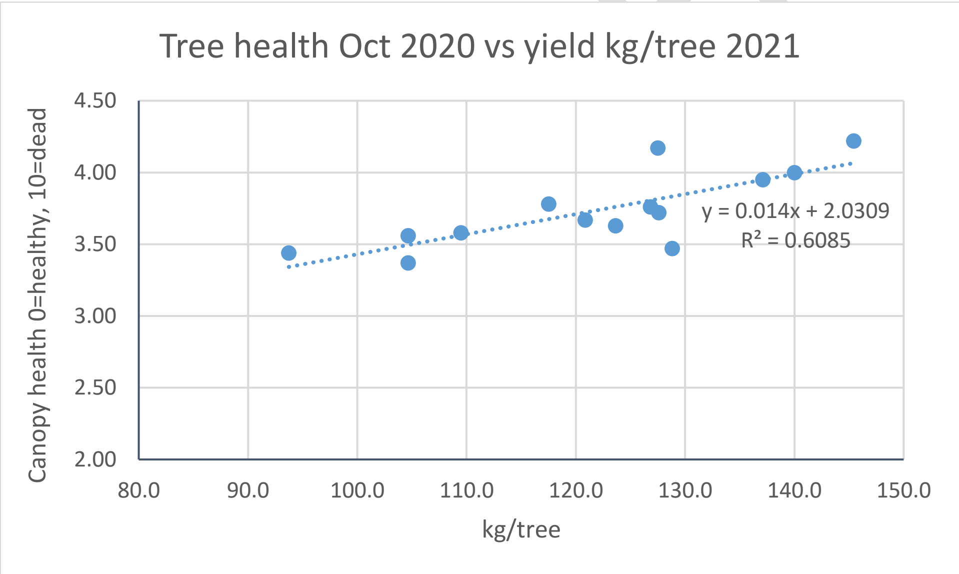
Yield

Average yield per tree across all treatments in 2021 was approximately 122 kg, and was higher than both previous years (15.8 kg in 2019 and 65 kg in 2020). Treatments which yielded 10% or more *above* the average in 2021 were Bare basics + fungicides, and both of the Bare basics + Mineral mulch treatments. (*Note: the yield is an estimate for Bare basics + fungicides, based on number of bins picked, as these bins were not sent to the packshed so that actual kg fruit was not recorded). Treatments which yielded 10% or more *below* the average were the Grower control + molasses, Grower control + Mineral mulch, and the Best bet treatment.



Looking at cumulative (total) yield across the three years, the Bare basics + soil conditioners and Bare basics + soil conditioners + microbes yielded 17% and 10% higher, respectively, than the trial average (4,936 kg). Grower control + Mineral mulch yielded 15% lower than the trial average. The lack of yield response in the Best bet and Grower control treatments is disappointing, but could be due to root systems remaining too wet as sprinkler outputs were not adjusted for the mulched trees. However, there was no obvious decline in canopy health in the mulched trees. There were 16 applications of soil conditioner ±- microbes from August 2018 to September 2020, and the potential increase in yield would have to be viewed in line with the cost of the input. Although applications ceased in June/July 2021, there may be value in obtaining yield, packout and fruit quality data at harvest in 2022, to determine if there are residual treatment effects.

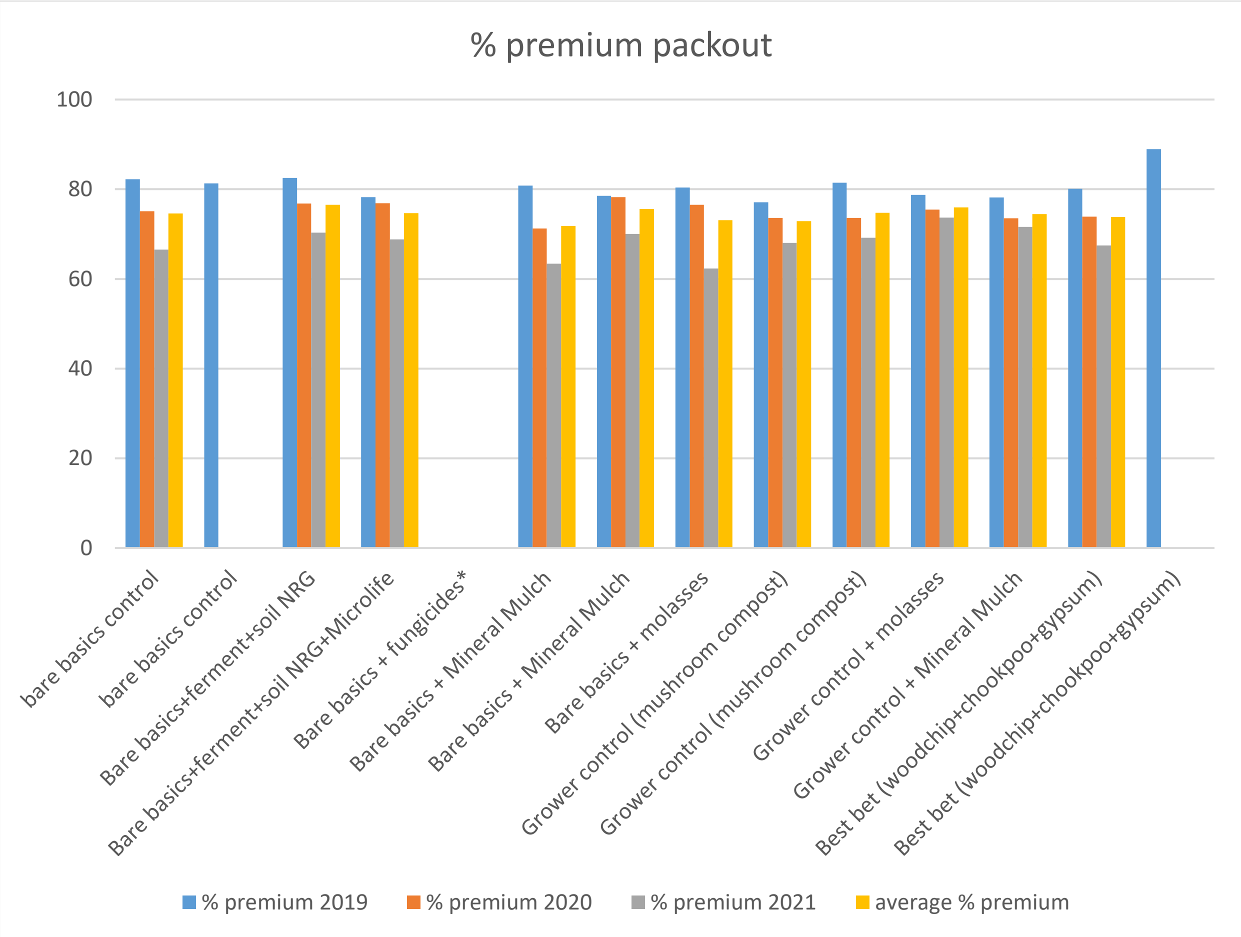




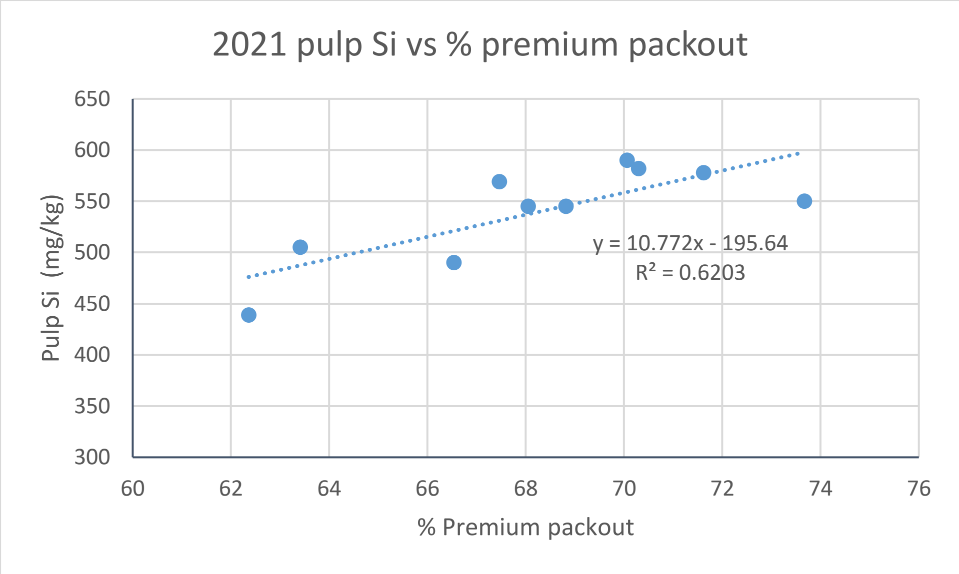
Correlation analyses can reveal interesting and significant relationships across the trial, irrespective of treatments. Yield (kg/tree) was significantly (and positively) correlated with several nutrients measured in pulp of mature fruit, Ca, Mg, K, B, Cu, Zn and P. Yield (kg/tree) in 2021 was also significantly correlated with canopy health assessed at two time points, October 2020 and February 2021. Canopy health declined (higher score) with increasing yield.

Packout and postharvest disease

Average % premium packout in 2021 was 68.3% (down from 80.7% in 2019 and 74.9% in 2020), and ranged from 62.4% to 73.7%. The lower packouts are likely due to greater supply of fruit across the district, driving quality parameters within packingsheds higher. Premium packout % for all treatments were within ± 10% of average premium packout, ie. there were no treatment effects. A highly significant correlation (P=0.0068, R^2=0.62) was observed between % premium packout and pulp silicon, where % premium increased with increasing Si in pulp.

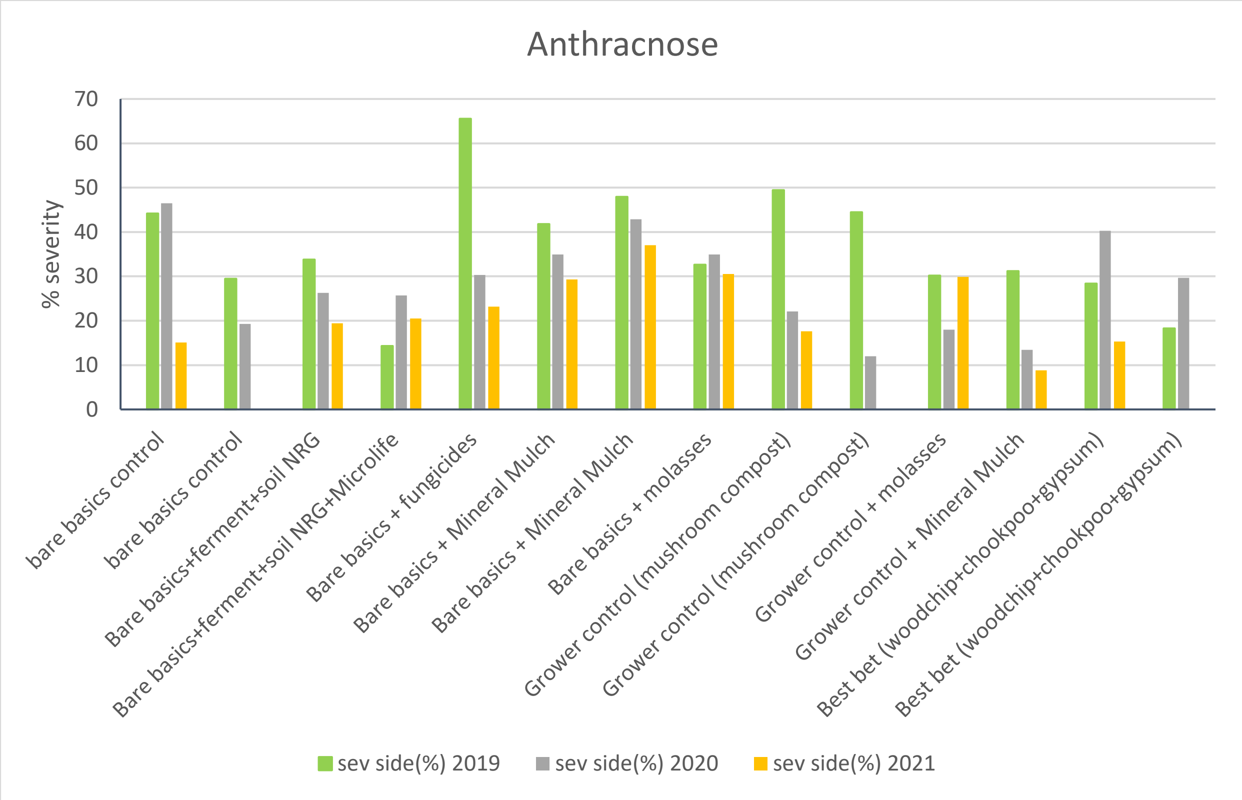


* Fruit from the fungicide treatment was picked for nutrient and disease assessments, but was discarded and not processed through the packingline.

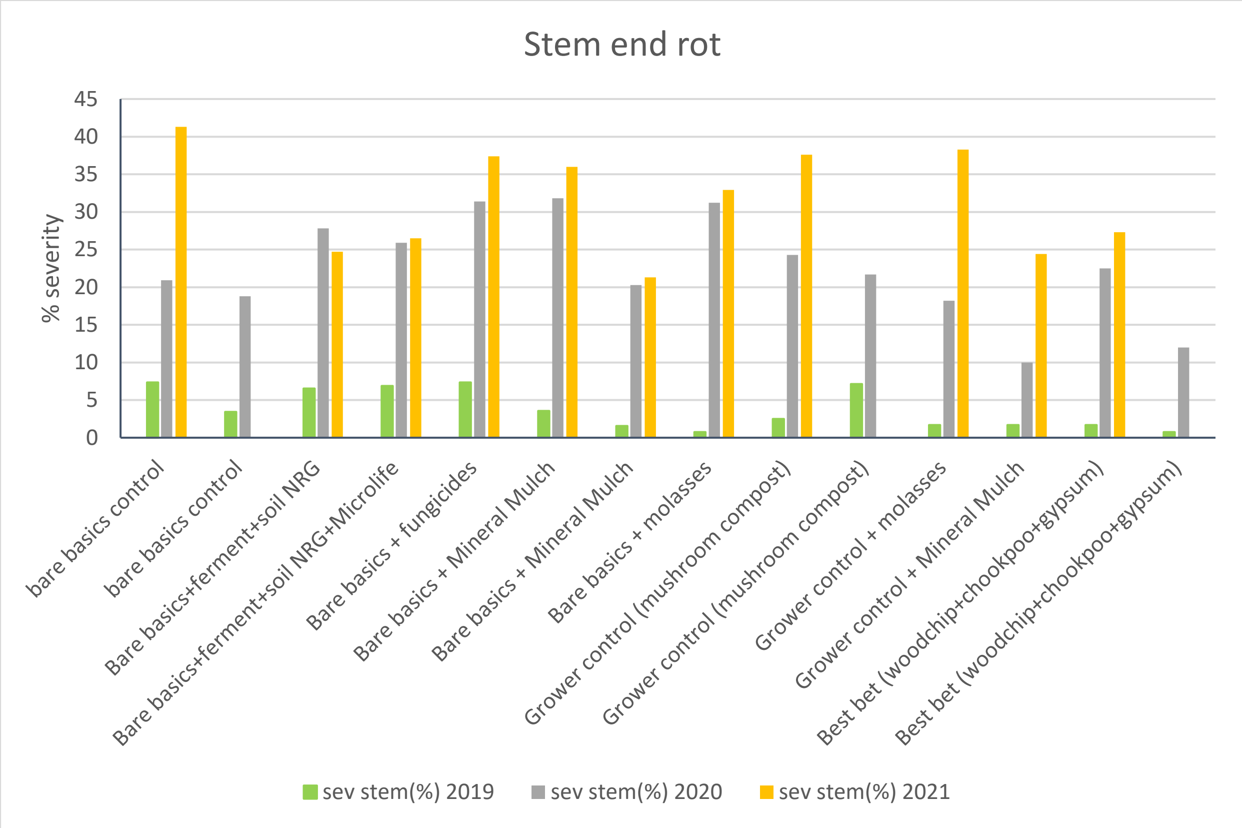


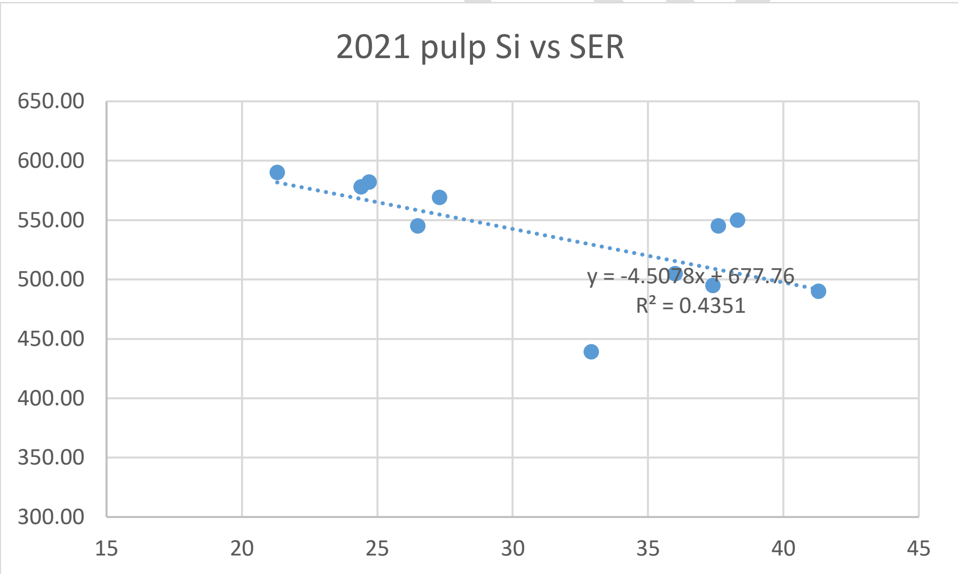
Fruit were harvested for nutrients and disease assessments on 3 June 2021. Average dry matter was 25.2% for fruit harvested 3 June 2021, and it took approximately 15 days for fruit to ripen once harvested and stored at 22-23°C and 65% RH, deemed optimal for disease development.

Anthracnose severity across all treatments averaged 22.4% (i.e. 22.4 % of surface area of fruit affected by anthracnose), and was less severe than in 2020 (28.3%) and 2019 (36.5%). There was a wide range in severity of anthracnose across treatments, 8.79 – 37%. There were no significant correlations between anthracnose and any parameter. While there was no significant correlation between N, Ca or N:Ca and disease in 2021, the table below shows that average Ca in pulp increased over the 3 years of the trial, while N:Ca decreased. Also worth noting is the increase in Si from about 300ppm in 2019 and 2020, to >500 ppm in 2021.



Stem end rot severity averaged 31.6% (% fruit volume affected by SER), and was considerably more severe than in 2020 (22.6%) and 2019 (3.77%). There were some significant correlations. Severity of SER increased with increasing pulp B ($p=0.039$, $R^2=0.39$), and this trend was also observed in 2020. SER decreased with increasing number of days to ripen ($p=0.023$, $R^2=0.45$), which was opposite to the trend observed in 2020. SER increased with increasing pulp N ($p=0.040$, $R^2=0.39$). The most striking correlation was that SER was negatively correlated with pulp Si (p=0.027, R2=0.44), so that SER was more severe with decreasing Si levels in fruit pulp. This result is in line with the positive effect of Si on % premium packout, described previously.


Stem end rot
% severity
45
40
35
30
25
20
15
10
5
0
bare basics control
bare basics control
Bare basics+ferment+soil NRG
Bare basics+ferment+soil NRG+Microlife
Bare basics + fungicides
Bare basics + Mineral Mulch
Bare basics + Mineral Mulch
Bare basics + molasses
Grower control (mushroom compost)
Grower control (mushroom compost)
Grower control + molasses
Grower control + Mineral Mulch
Best bet (woodchip+chookpoo+gypsum)
Best bet (woodchip+chookpoo+gypsum)
sev stem(%) 2019
sev stem(%) 2020
sev stem(%) 2021


2021 pulp Si vs SER
650.00
600.00
550.00
500.00
450.00
400.00
350.00
300.00
15
20
25
30
35
40
45
y = -4.5078x + 677.76
R² = 0.4351

Comparison of pulp and leaf elements in 2019, 2020 and 2021

Element	Pulp			Leaf		
	2019	2020	2021	2019	2020	2021
Nitrogen (%)	1.30	1.56	1.33	2.53	2.62	2.51
Calcium (%)	0.014	0.019	0.024	1.94	1.85	2.16
N:Ca	109	83.4	58.7	1.32	1.42	1.17
Boron (ppm)	152	132	154	59.8	61.1	48.2
Magnesium (%)	0.059	0.061	0.068	0.381	0.476	0.547
Potassium (%)	2.10	2.13	2.20	1.03	1.01	1.14
Zinc (ppm)	13.3	15.4	20.5	31.1	48.5	35.1
Iron (ppm)*	19.2	17.9	13.0	108	342	275
Copper (ppm)*	1.99	1.88	5.23	222	322	549
Sulfur (%)	0.135	0.145	0.149	0.251	0.274	0.263
Phosphorus (%)	0.167	0.161	0.178	0.181	0.187	0.261
Silicon (ppm)	306	298	535	336	373	397

* Cu and Fe are strongly influenced by fungicide applications and dust on leaf surface which was not washed off prior to analyses

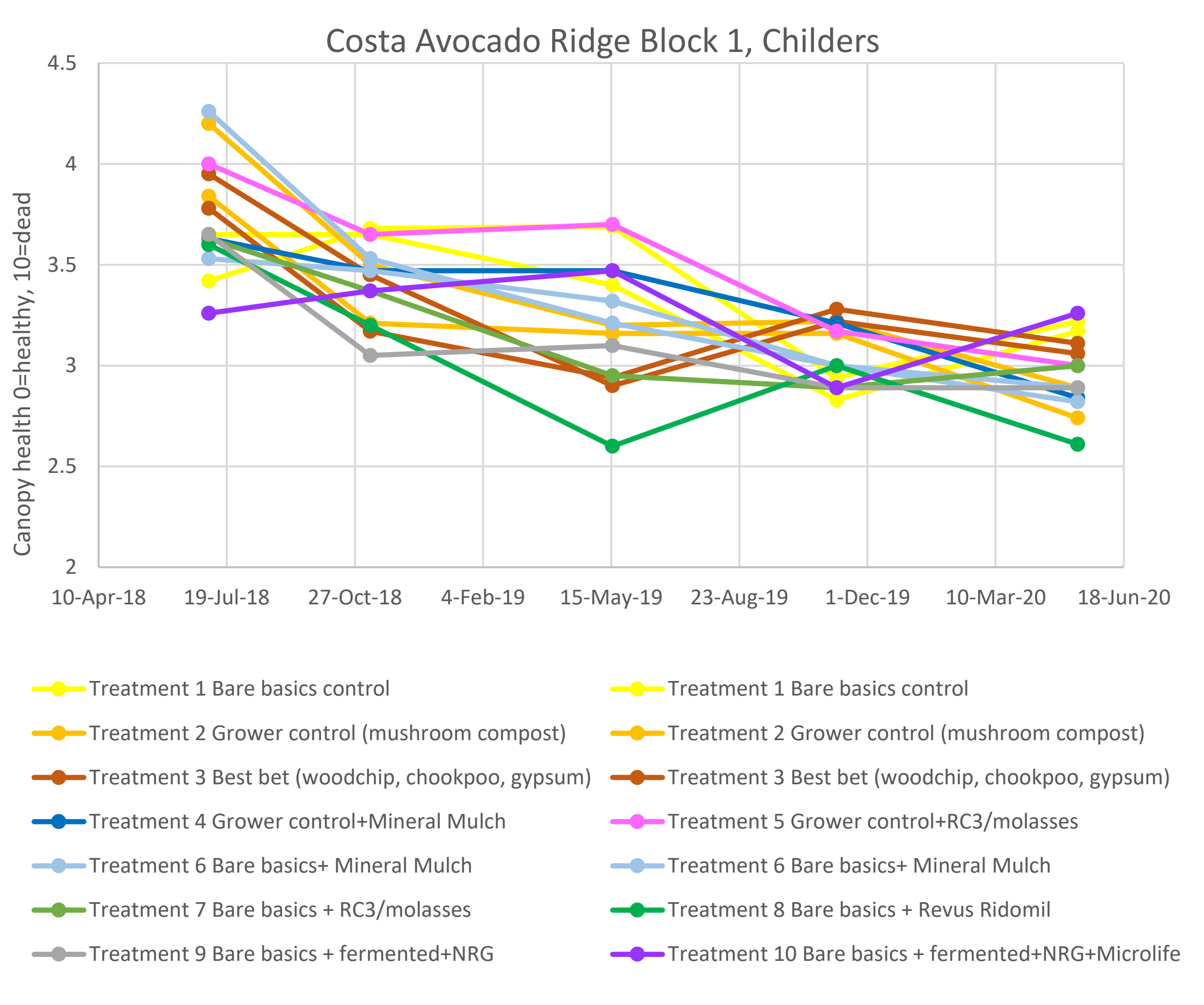
In 2019 – leaf samples collected 16 May, fruit harvested 22 July

In 2020 – leaf sampled collected the same day as fruit harvest, 13 May

In 2021 – leaf sampled collected the same day as fruit harvest, 3 June

2020 Results, pages 8-14

Canopy health 2020



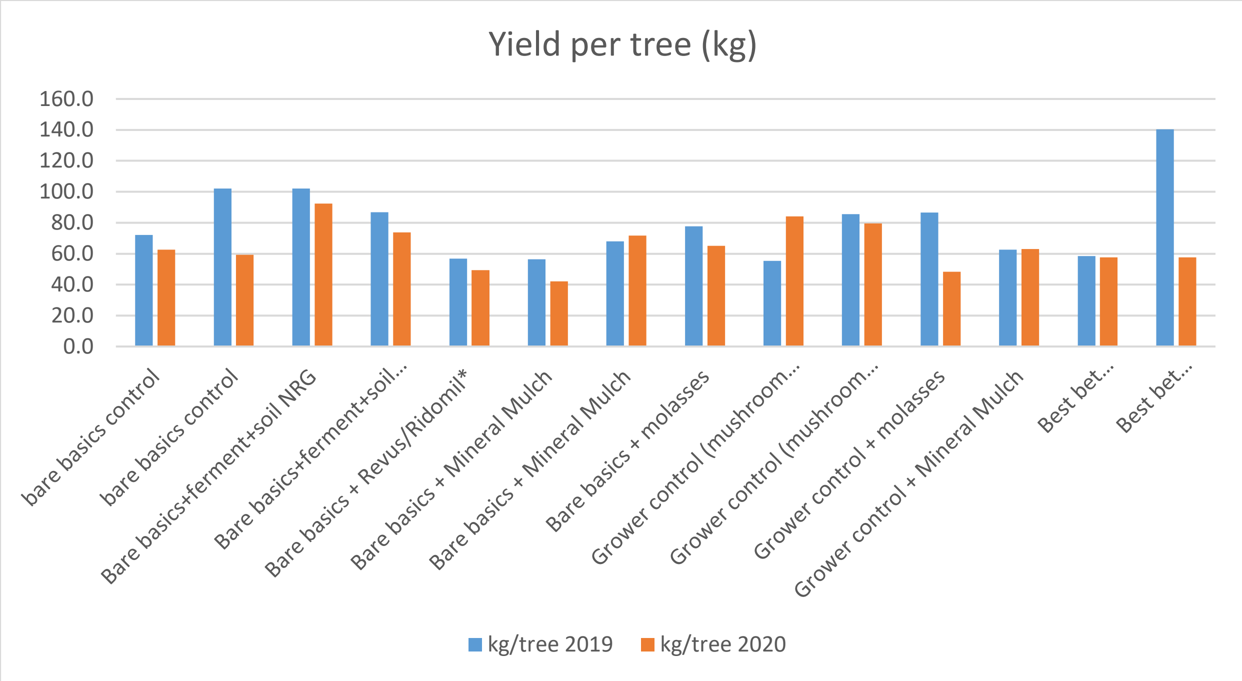
Although changes are very small, and overall tree health in the block is pretty good, there are some indications of treatment effects when comparing the November 2019 and May 2020 ratings:

- Bare basics control - tree health ratings increased (ie. trees declined), by about 0.3 units
- Base basics + Microlife - tree health ratings increased (ie. trees declined), by 0.37 units
- Bare basics + Multikraft soil conditioners – no change

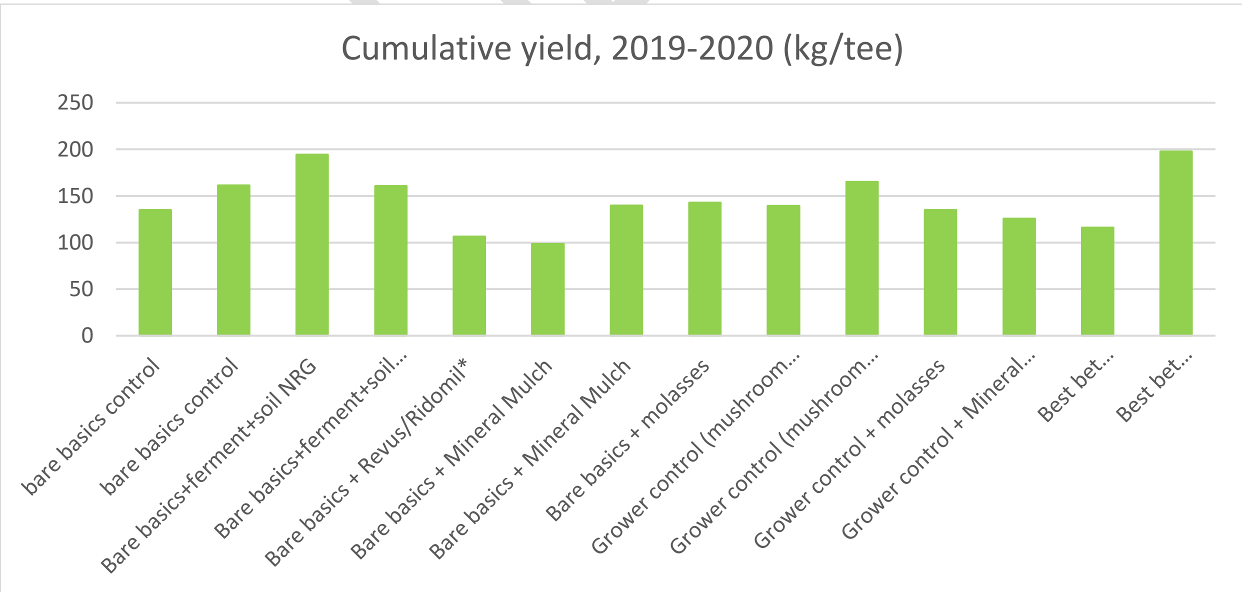
- Grower control - tree health ratings decreased (ie. trees improved), by about 0.3 to 0.4 units
- Grower control + Mineral mulch - tree health ratings decreased (ie. trees improved), by 0.37 units
- Bare basics + fungicides tree health ratings decreased (ie. trees improved), by about 0.4 units
- Best bet - tree health ratings decreased (ie. trees improved), by about 0.15 units
- Some other very slight improvements in mineral mulch and molasses treatments

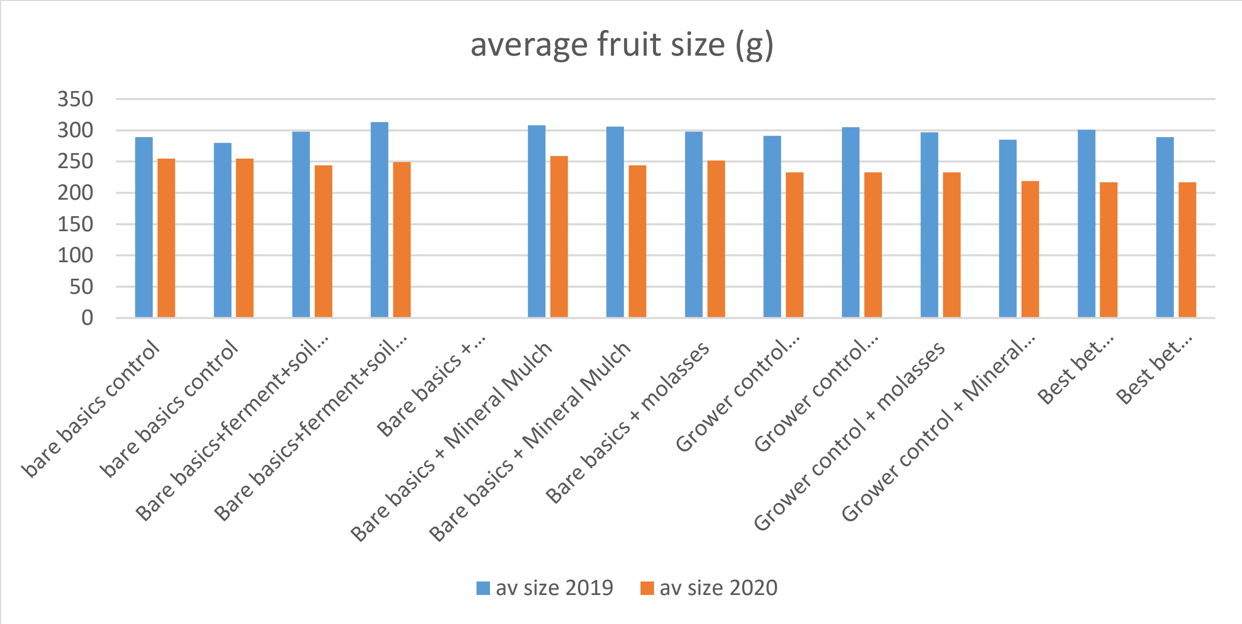
Grower control (mushroom compost) trees generally improving in tree health, while bare basics (ie. do nothing) trees are declining or making negligible improvement, with the exception of the fungicide treatment which has improved by 0.4 units.

Yield



Yield per tree was calculated to account for two trees on the northern end of each row (underneath powerlines) being pruned heavily, and thus not producing. Yield was less in 2020 (average 13 T/Ha) compared with 2019 (16 T/Ha). As in 2019, yields were variable across treatments, with highest yield (42% higher than trial average) from Bare basics + Multikraft soil NRG (without Microlife). The grower controls (mushroom compost) trees also yielded well, at >20% higher than average. The Bare basics + fungicides, Mineral Mulch, and Grower control + molasses yielded >20% lower than average. Average fruit size (weight) in 2019 was 297g compared with 239g in 2020, with little variability among treatments.

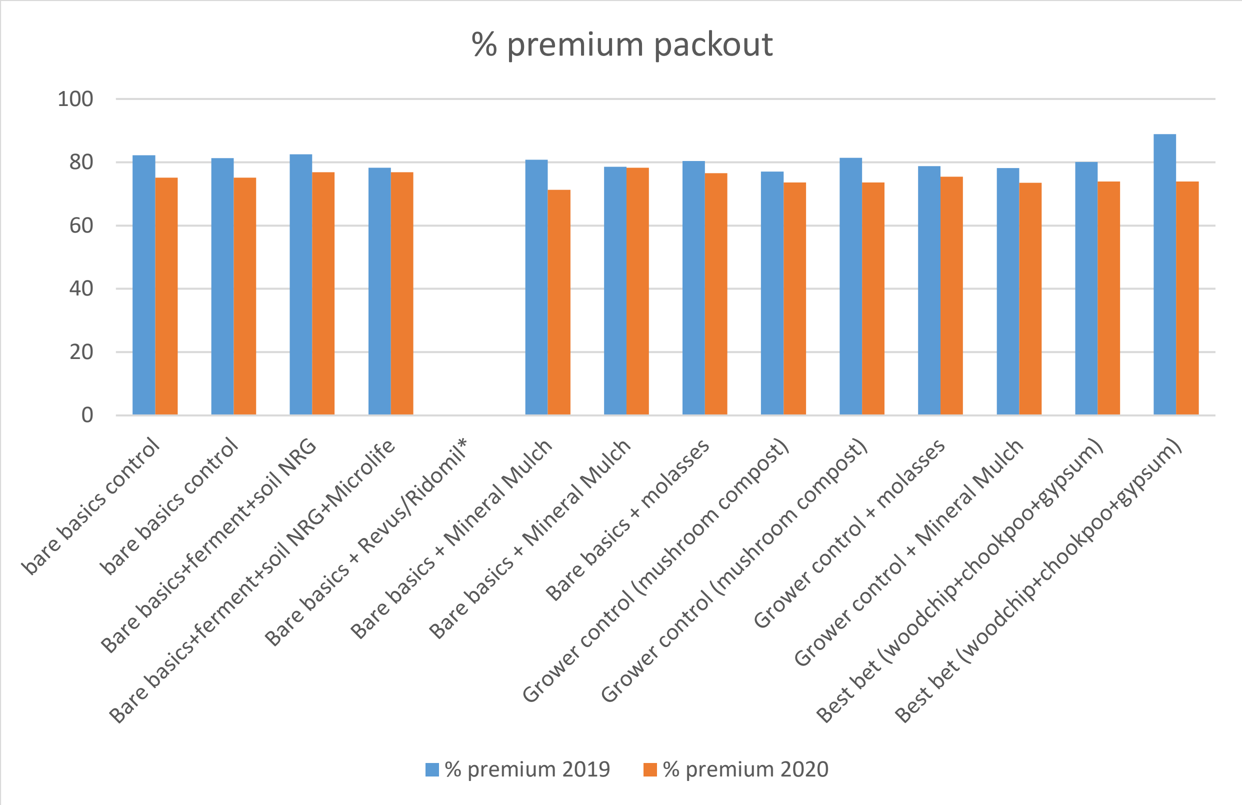


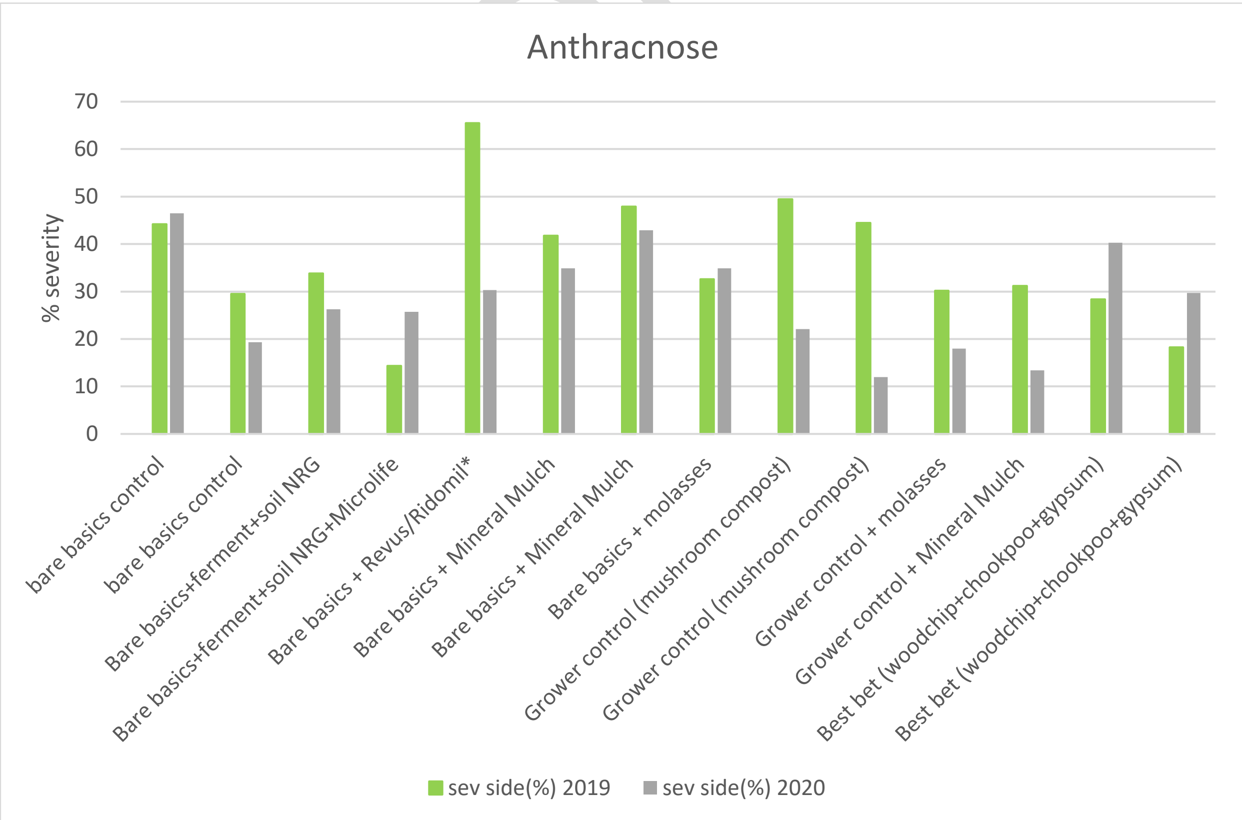


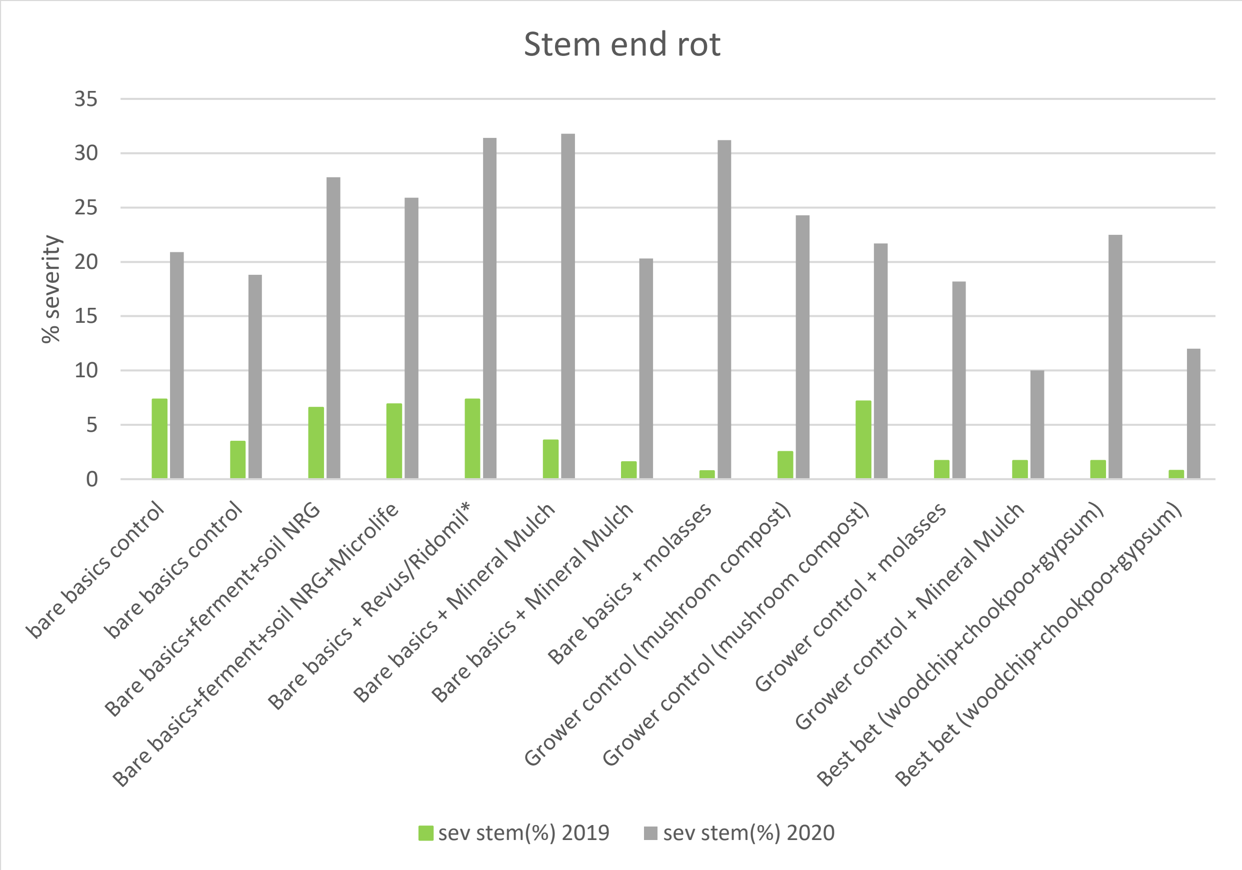
Packout and postharvest disease

Average % premium packout reduced from 80.7% in 2019 to 74.9% in 2020, but was more consistent across treatments (range 71.3 to 78.2%). While there was little variation amongst treatments in % premium packouts, there were some differences amongst the Class 2, Processing and Waste grades. When % packouts for these 3 lower grades were combined, there was less fruit from the Best Bet treatment (5.53%) in these categories compared with Bare Basics control (8.01%) and Grower (mushroom compost) control (7.58%). These trends will be monitored in 2021.

Anthracnose disease was slightly more severe in 2019 (average 36.5% fruit area affected) compared with 2020 (28.3%), and this may be attributed to the later harvest (July) and higher DM (28.7%) in 2019 compared with May 2020 when fruit averaged 23.4% DM. Stem end rot, however, was considerably more severe in 2020 (22.6% fruit volume affected) compared with 3.77% in 2019.


% premium packout
100
80
60
40
20
0
bare basics control
bare basics control
Bare basics+ferment+soil NRG
Bare basics+ferment+soil NRG+Microlife
Bare basics + Revus/Ridomil*
Bare basics + Mineral Mulch
Bare basics + Mineral Mulch
Bare basics + molasses
Grower control (mushroom compost)
Grower control (mushroom compost)
Grower control + molasses
Grower control + Mineral Mulch
Best bet (woodchip+chookpoo+gypsum)
Best bet (woodchip+chookpoo+gypsum)
% premium 2019
% premium 2020


Anthracnose
% severity
70
60
50
40
30
20
10
0
bare basics control
bare basics control
Bare basics+ferment+soil NRG
Bare basics+ferment+soil NRG+Microlife
Bare basics + Revus/Ridomil*
Bare basics + Mineral Mulch
Bare basics + Mineral Mulch
Bare basics + molasses
Grower control (mushroom compost)
Grower control (mushroom compost)
Grower control + molasses
Grower control + Mineral Mulch
Best bet (woodchip+chookpoo+gypsum)
Best bet (woodchip+chookpoo+gypsum)
sev side(%) 2019
sev side(%) 2020



Statistically significant correlations

Across treatments in 2020, there were no significant correlations in **canopy health** (assessed May 2020) with any yield, packout, disease or nutrient parameter.

Across treatments, in 2020, there were no significant correlations between **yield** and % packout, anthracnose or stem end rot severity, or any pulp or leaf nutrient, except leaf boron, where there was a significant negative correlation, ie. lower yield with higher leaf B. (In Western Australia 2019 there was a significant negative correlation between leaf and pulp B and yield).

yield	vs	leaf B (-ve p=0.0133)

There were no significant correlations between **% premium packout** and any yield, disease or nutrient parameter. % premium was negatively correlated with % Class 1 (indicating that when more fruit went into premiums, there were less into Class 1).

Average fruit size (g) was negatively correlated with the number of days to ripen (P=0.003), indicating that bigger (heavier) fruit ripen more quickly. Fruit size was positively correlated with severity of stem end rot (P=0.008), ie. bigger fruit then worse SER. There were also several other nutrient correlations.

average fruit weight	vs	SER (+ve, p=0.008)
	vs	pulp B, pulp Zn (+ve, p<0.05)
	vs	leaf Mg, Cu, Fe, Zn, K (+ve, p<0.05)

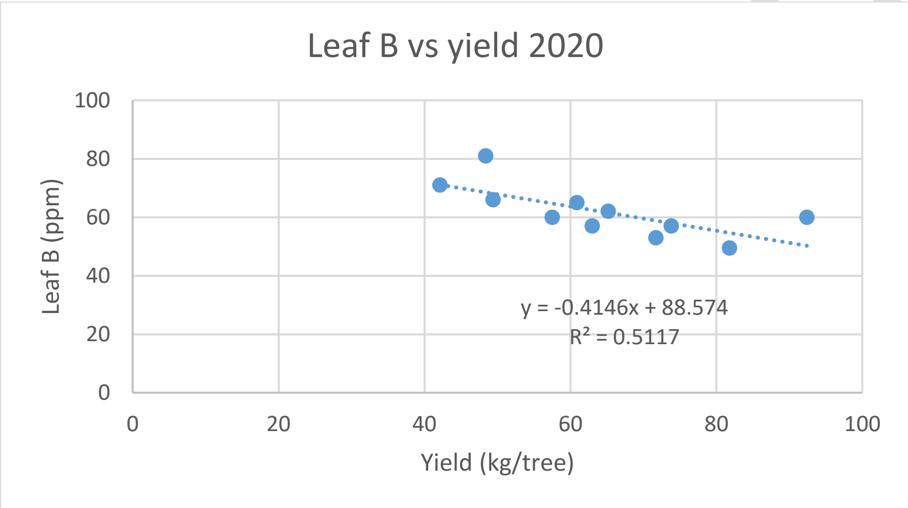
Severity of **anthracnose** was not correlated with any parameter in 2020. This is different to the trend in 2019, where anthracnose was correlated with high N in pulp and leaf.

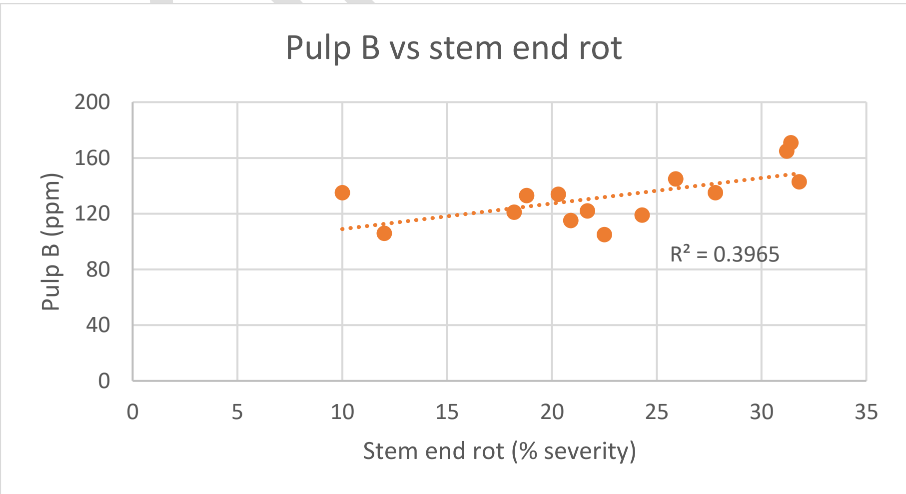
Severity of **stem end rot** was positively correlated with days to ripen (p=0.0002), ie. SER is worse in fruit which ripen more slowly. There were other correlations with pulp and leaf nutrients:

stem end rot severity	vs	pulp B, Mg, Zn, K (p<0.05)
		leaf Zn, K (p<0.05)

There were various other nutrient correlations, but are presented for pulp N, Ca and B below. Others available if you are interested. A table comparing averages across all treatments of leaf and pulp nutrients for 2019 and 2020 is below.

pulp N	vs	pulp Mg, B, Cu, Zn, S, P, K (p<0.05)
		leaf Mg, Cu, Zn, P, N (p<0.05)
pulp Ca	vs	pulp P (p<0.05)
pulp B	vs	pulp Mg, C, Zn, P, K, N (p<0.05)
		leaf Ca, Mg, Cu, Zn, P (p<0.05)



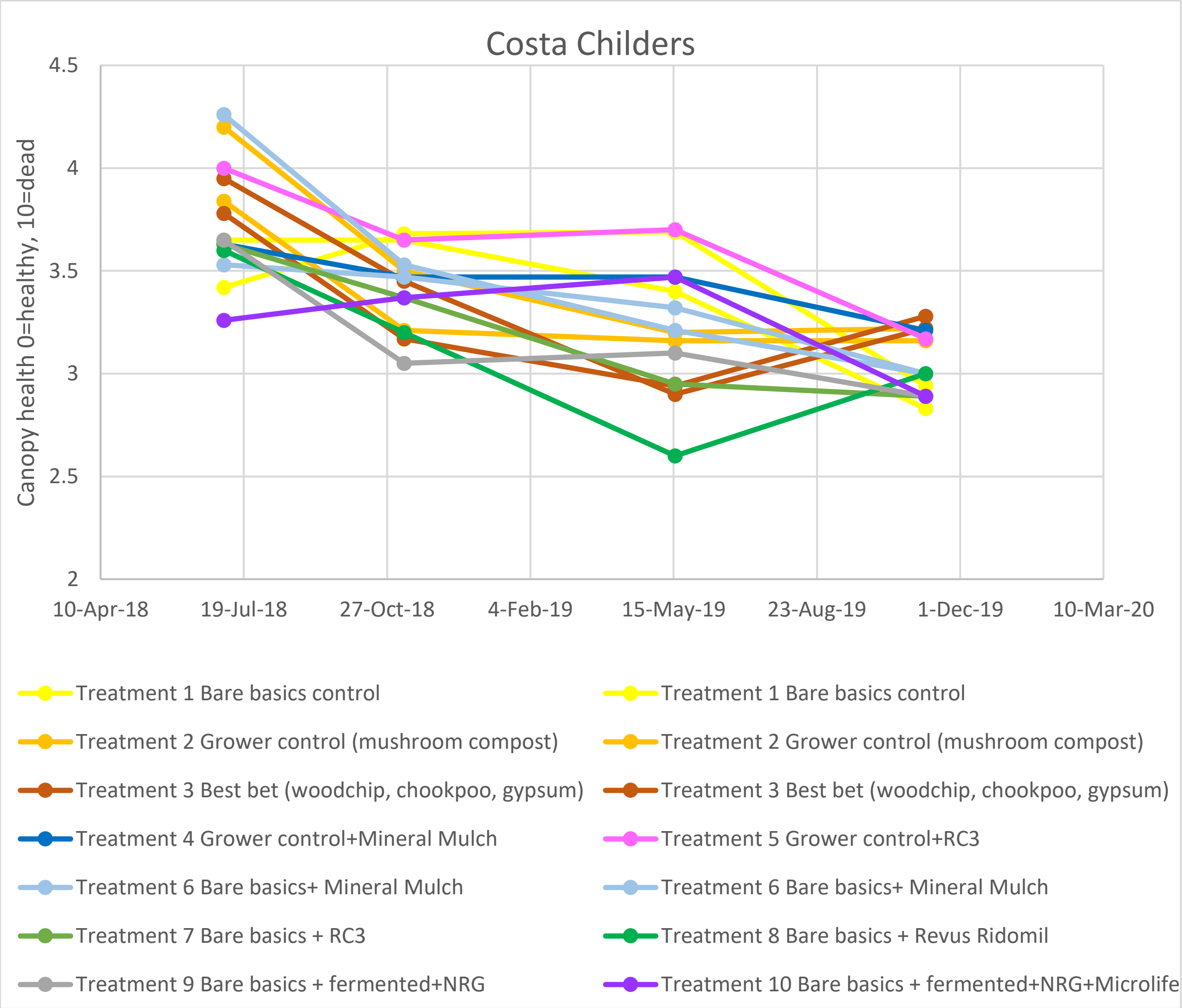


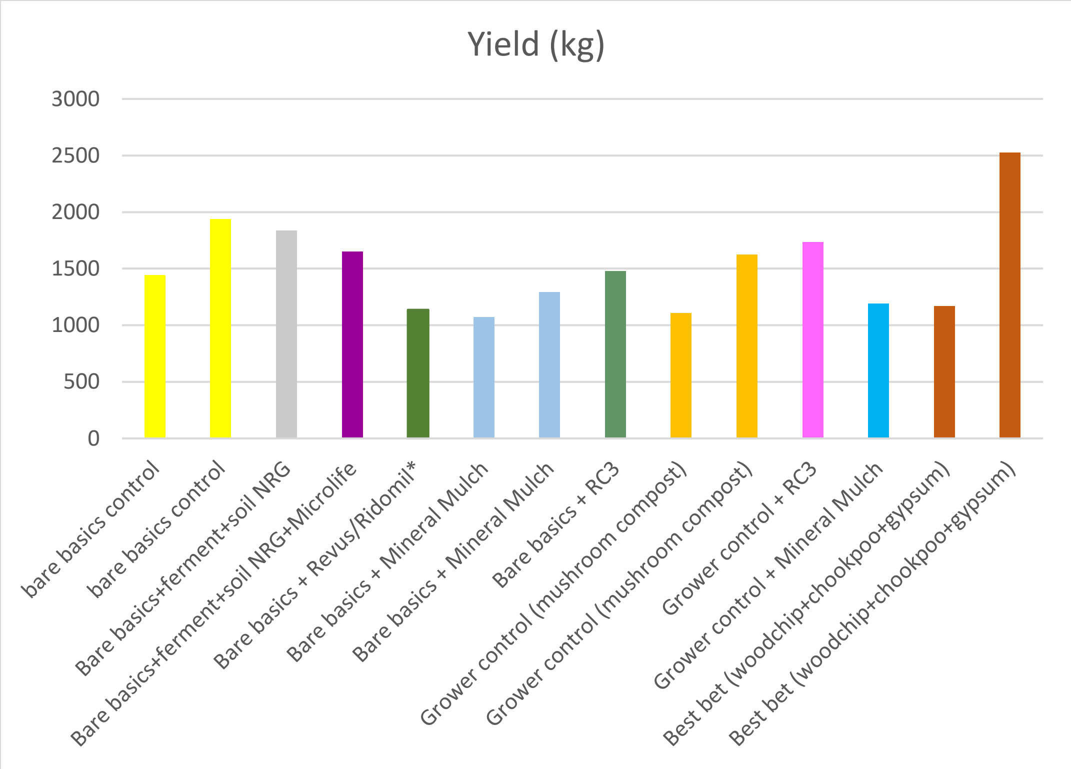
Comparison of pulp and leaf elements in 2019 and 2020

Element	Pulp		Leaf	
	2019	2020	2019	2020
Nitrogen (%)	1.30	1.56	2.53	2.62
Calcium (%)	0.014	0.019	1.94	1.85
N:Ca	109	83.4	1.32	1.42
Boron (ppm)	152	132	59.8	61.1
Magnesium (%)	0.059	0.061	0.381	0.476
Potassium (%)	2.10	2.13	1.03	1.01
Zinc (ppm)	13.3	15.4	31.1	48.5
Iron (ppm)*	19.2	17.9	108	342
Copper (ppm)*	1.99	1.88	222	322
Sulfur (%)	0.135	0.145	0.251	0.274
Phosphorus (%)	0.167	0.161	0.181	0.187
Silicon (ppm)	306	298	336	373

* Cu and Fe are strongly influenced by fungicide applications and dust on leaf surface which was not washed off prior to analyses
In 2019 – leaf samples collected 16 May, fruit harvested 22 July
In 2020 – leaf sampled collected the same day as fruit harvest, 13 May

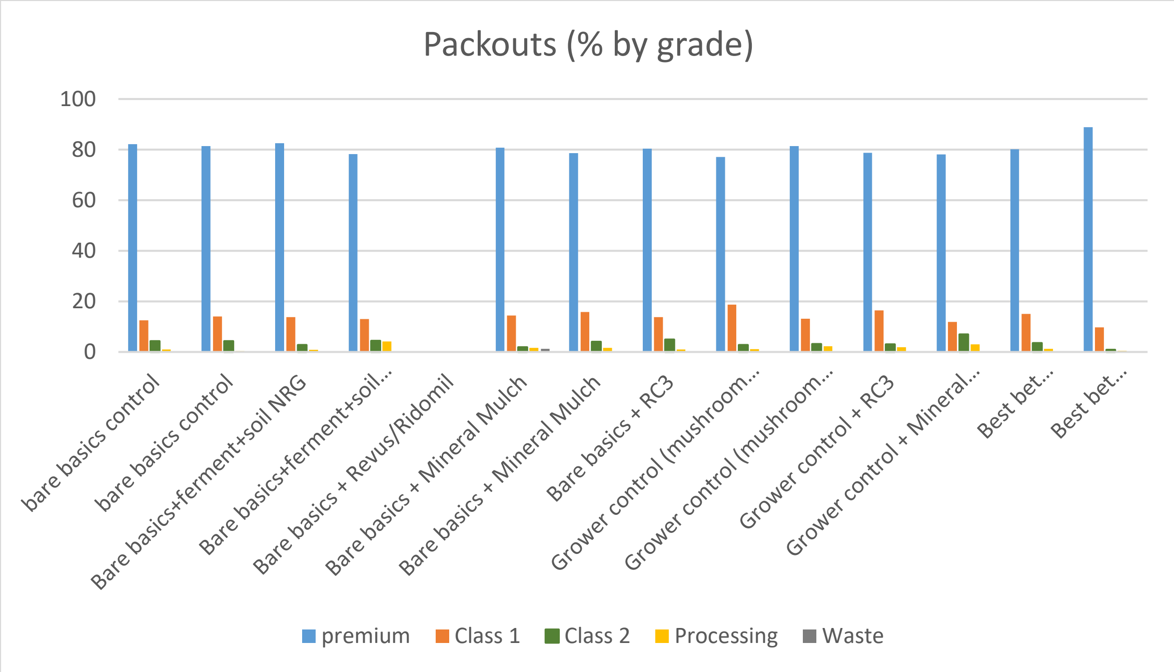
2019 Results Pages 16-20

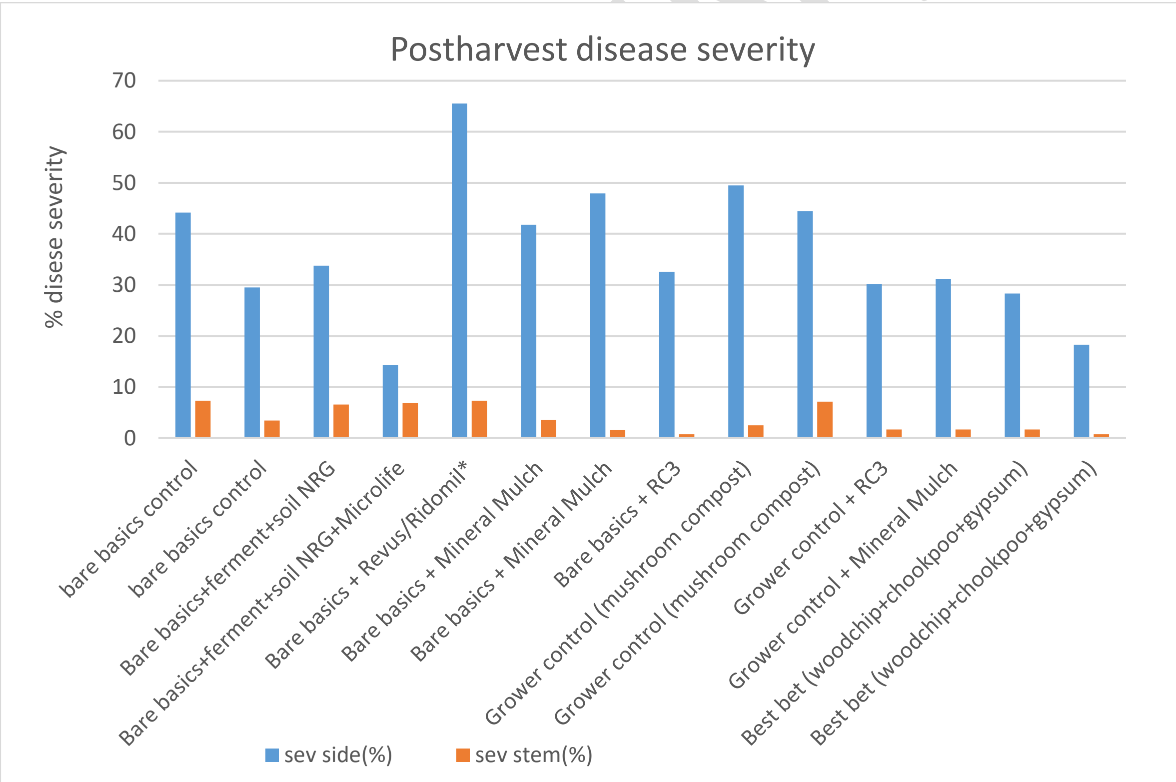




* This is an estimate for Revus/Ridomil as this fruit did not go through the packingline and exact weights not determined

NB. The southern end of the rows (section 2) yielded higher, in general, than the northern ends (section 1). Total yields across all rows in southern section was 11.48T, compared with 8.58 T for northern rows. This can be seen in the above plot, where yields for the second replicate for Bare Basics control, Grower control and Best bet are higher than for the first rep.





Revus/Ridomil – yield very low, only 2.5 bins.

Yield correlations

Across treatments, there were significant **positive** correlations between

Yield and % premium packout (p=0.002, R^2=55.2%)

Yield and Class 1 fruit (p=0.046, R^2=25.1%)

Across treatments, there were significant **negative** correlations between

yield and % anthracnose (side) (p=0.038, R^2=27.5%)

Disease correlations

Across treatments, there were significant **positive** correlations between

% anthracnose severity and Pulp N (p=0.049, R2=22.7%)

% anthracnose severity and Leaf N (p=0.096, R2=14.8%)

% SER severity and Pulp N (p=0.027, R2=29.3%)

% SER severity and Leaf N (p=0.049, R2=22.7%)

No significant correlations between leaf or pulp Ca or Si and disease

Leaf and pulp nutrients

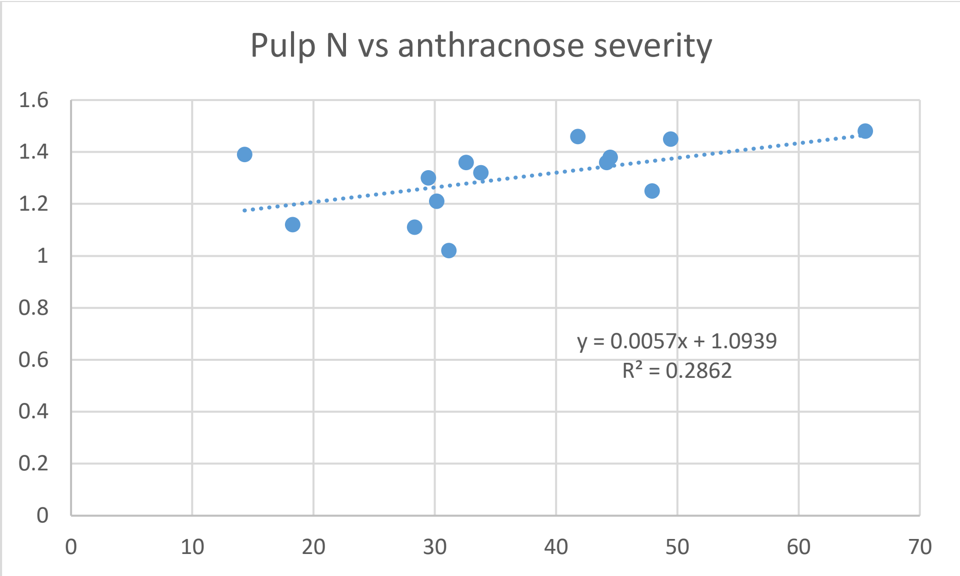
leaf N and Pulp N (p=0.039, R2=25.0%)

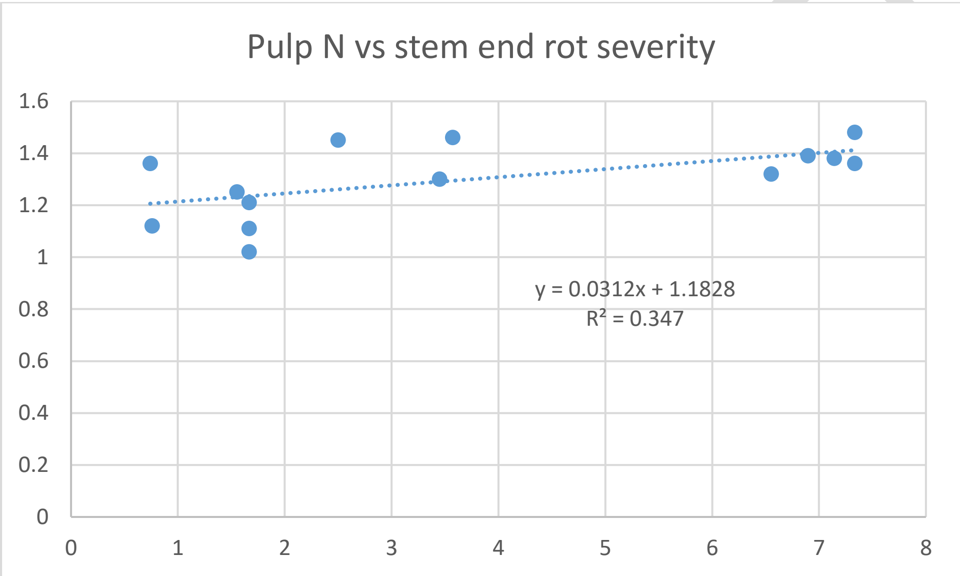
no correlations between leaf Ca and pulp Ca

No correlations between tree health and yield, % premium, anthracnose or stem end rot

Other correlations, updated Feb 2020

- *Yield vs leaf Mn (p=0.035); leaf K (p=0.038, -ve)*
- *Yield vs pulp – there were no significant correlations*
- *Leaf N vs leaf B (p=0.081); leaf Ca (p=0.069, -ve); leaf S (p=0.054); leaf K (p=0.098, +ve); no other significant correlations*
- *Pulp N vs pulp Ca (0.016, -ve); pulp S (p=0.0007); pulp K (p=0.0007, +ve); no other significant correlations*
- *Leaf N:Ca vs pulp N:Ca not significant*

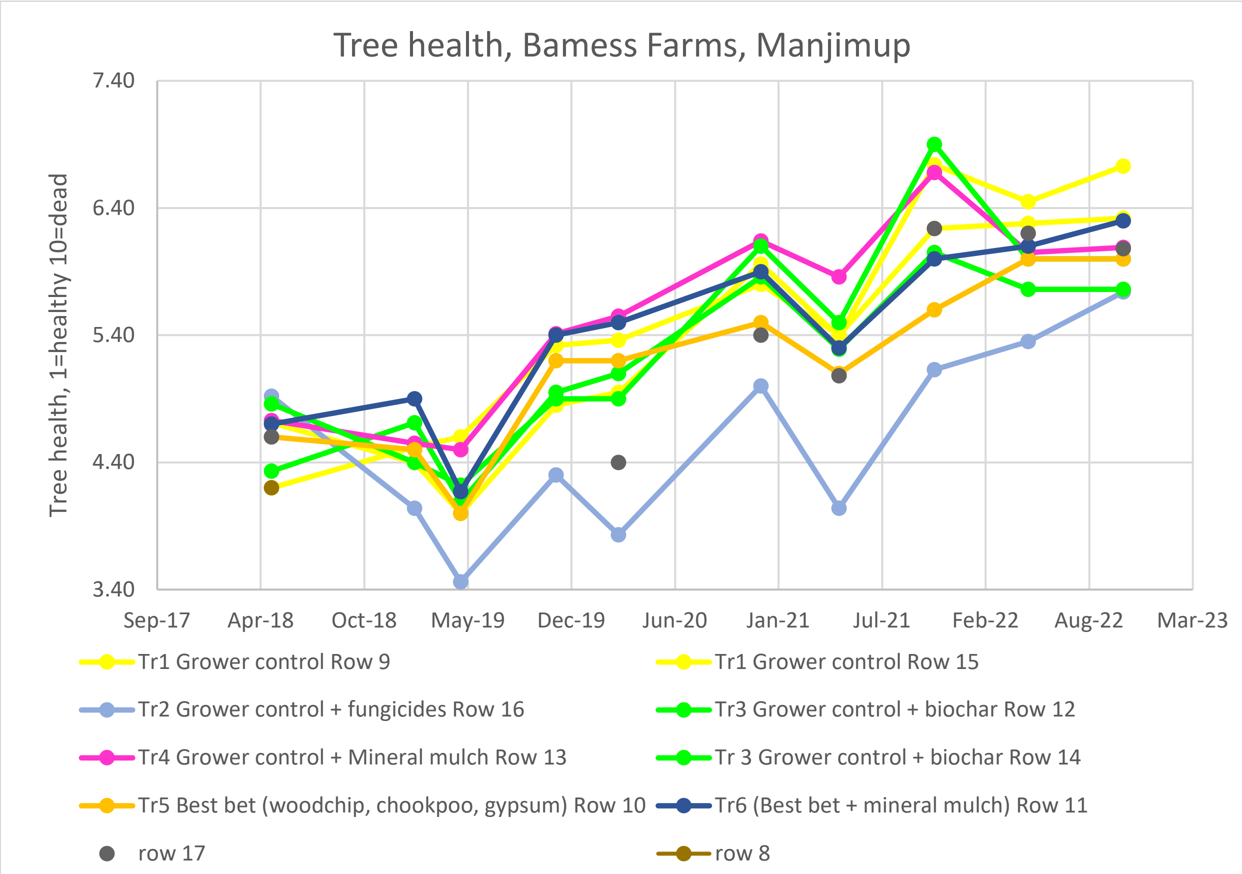

Pulp N vs anthracnose severity
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
y = 0.0057x + 1.0939
R² = 0.2862


Pulp N vs stem end rot severity
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
8
y = 0.0312x + 1.1828
R² = 0.347

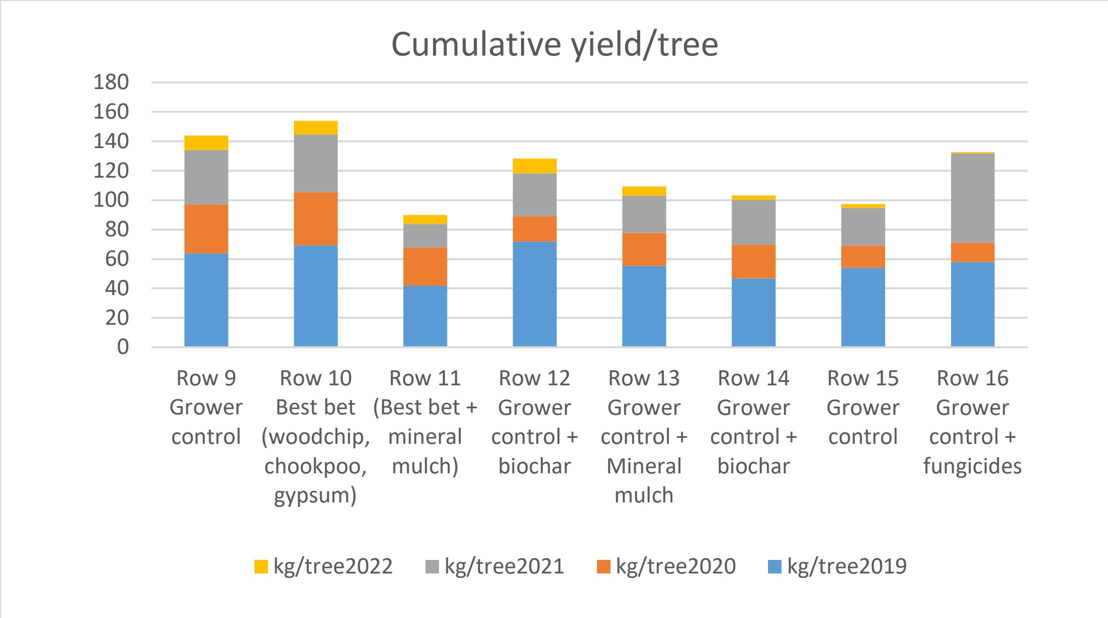
DRAFT AV16007 Bamess Farms soil amendment trial summary, updated June 2022

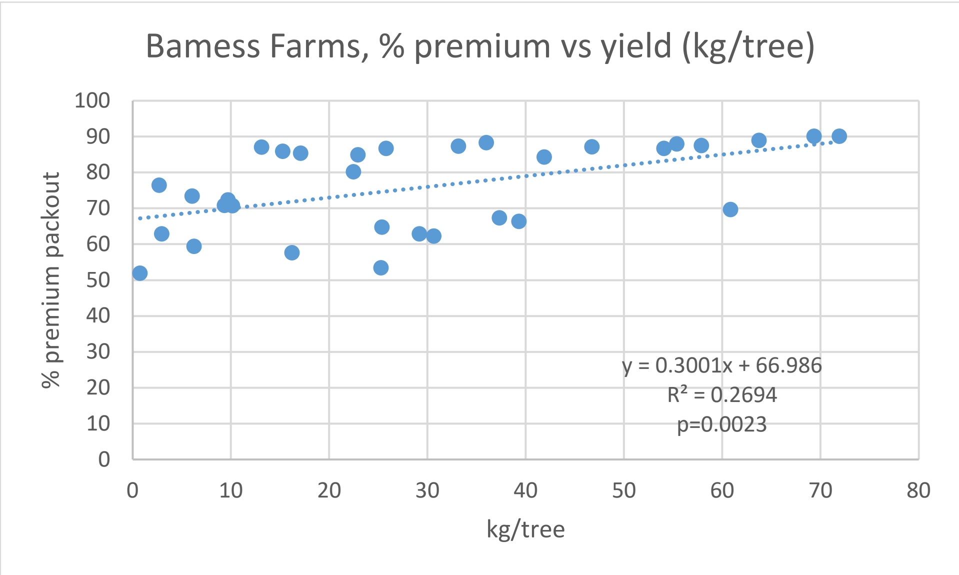
Liz Dann and team, University of Queensland

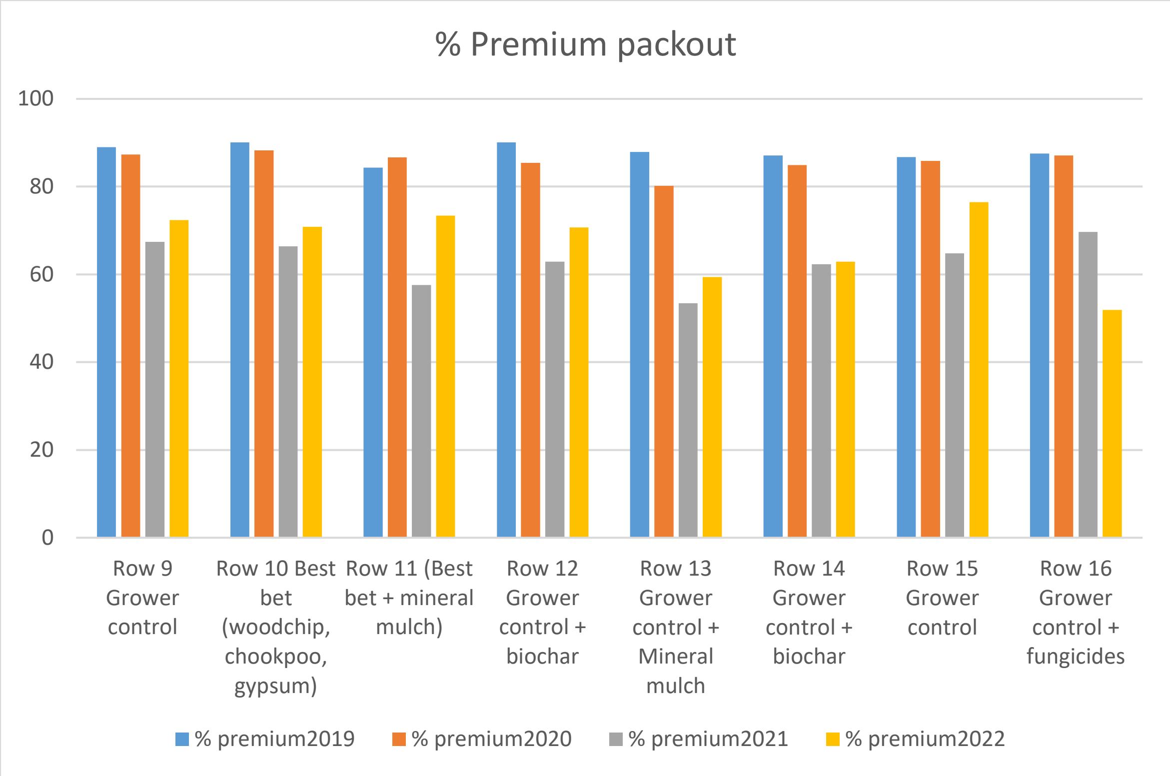
Figure 1. Canopy health over time



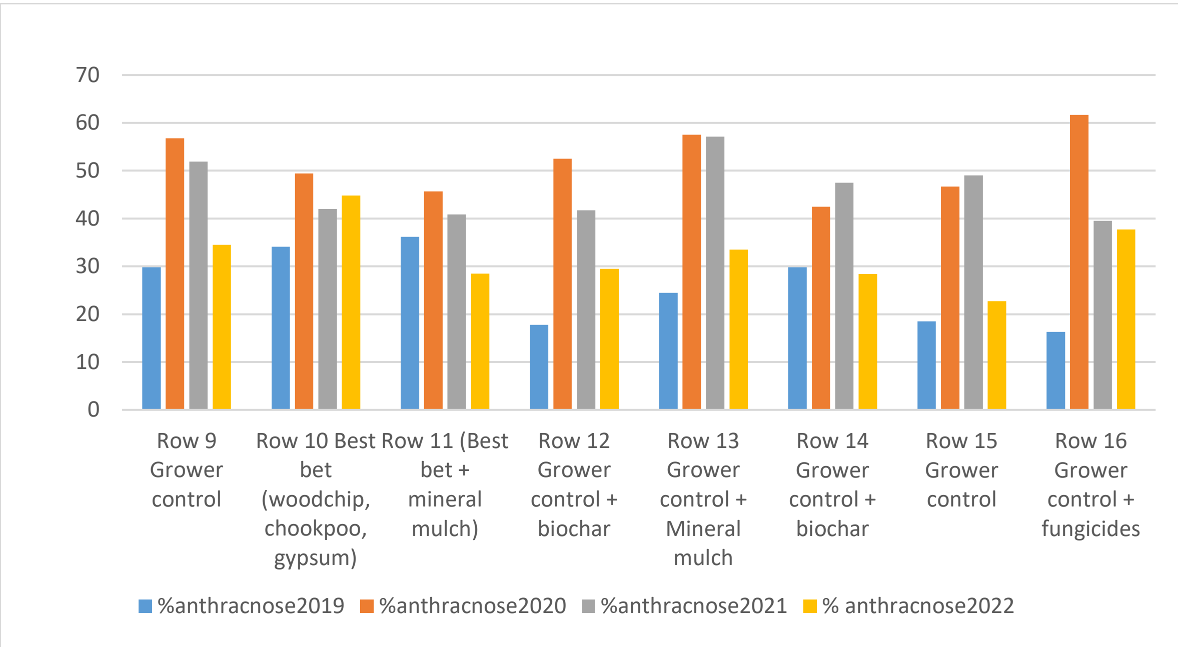
2022


Cumulative yield/tree
180
160
140
120
100
80
60
40
20
0
Row 9 Grower control
Row 10 Best bet (woodchip, chookpoo, gypsum)
Row 11 (Best bet + mineral mulch)
Row 12 Grower control + biochar
Row 13 Grower control + Mineral mulch
Row 14 Grower control + biochar
Row 15 Grower control
Row 16 Grower control + fungicides
kg/tree2022
kg/tree2021
kg/tree2020
kg/tree2019

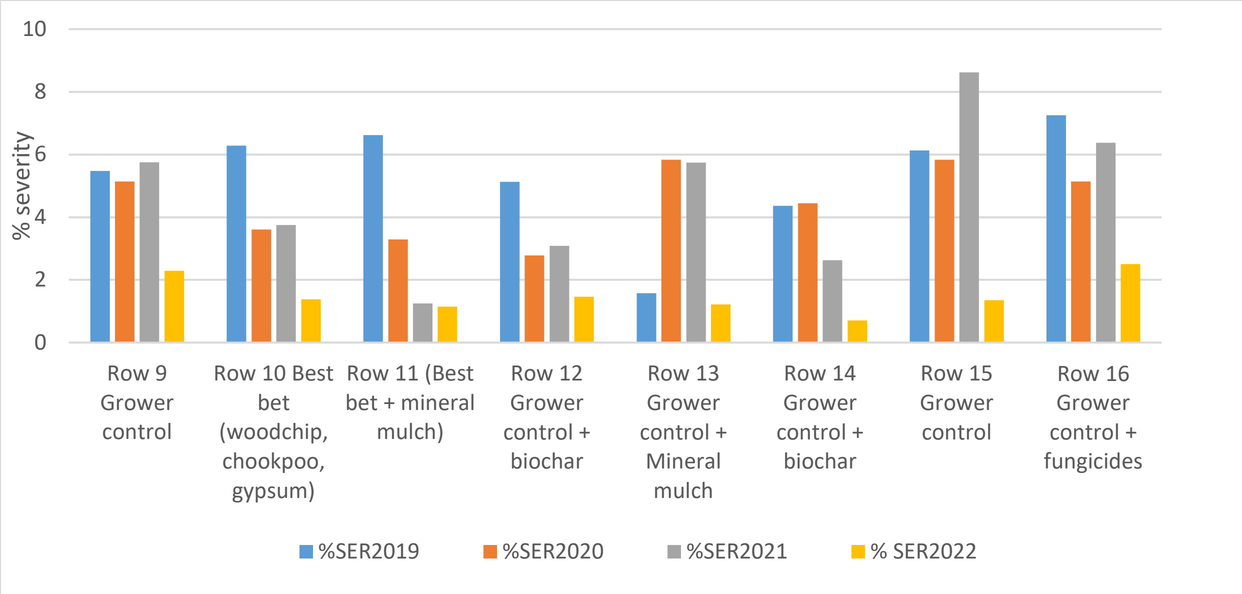

Bamess Farms, % premium vs yield (kg/tree)
% premium packout
100
90
80
70
60
50
40
30
20
10
0
y = 0.3001x + 66.986
R² = 0.2694
p=0.0023
0
10
20
30
40
50
60
70
80
kg/tree



% Premium packout
100
80
60
40
20
0
Row 9 Grower control
Row 10 Best bet (woodchip, chookpoo, gypsum)
Row 11 (Best bet + mineral mulch)
Row 12 Grower control + biochar
Row 13 Grower control + Mineral mulch
Row 14 Grower control + biochar
Row 15 Grower control
Row 16 Grower control + fungicides
% premium2019
% premium2020
% premium2021
% premium2022

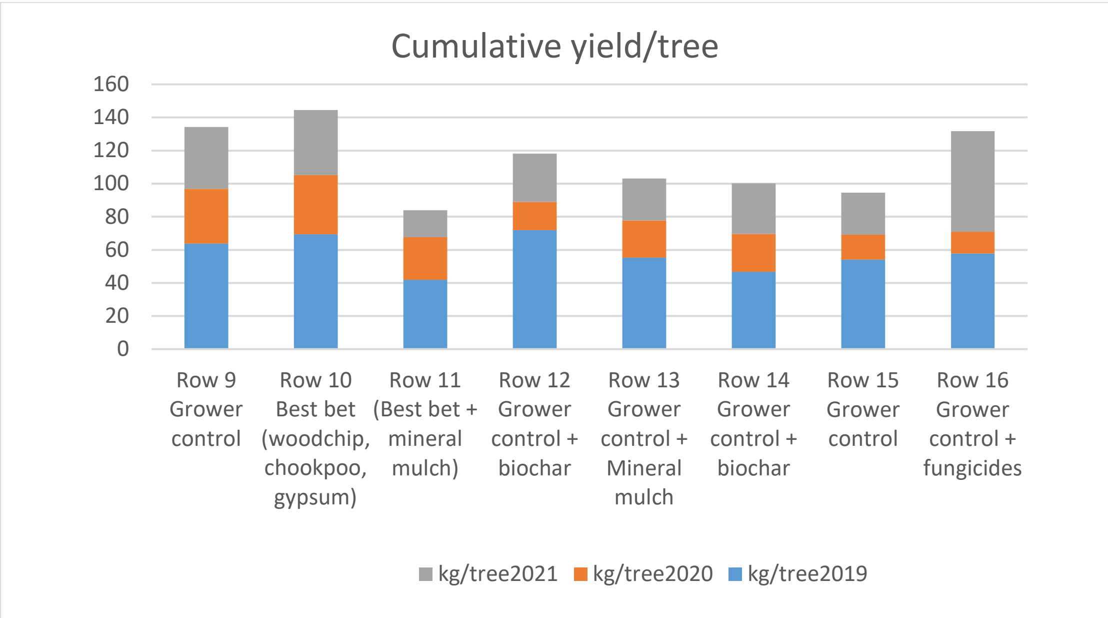


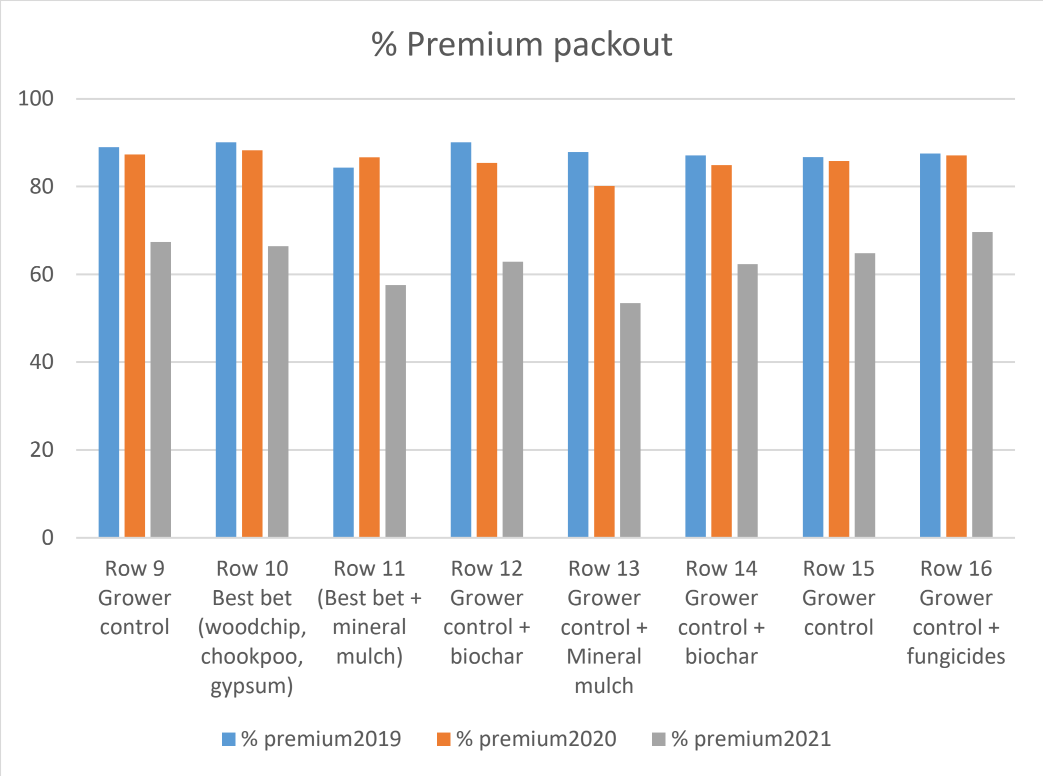
70
60
50
40
30
20
10
0
Row 9 Grower control
Row 10 Best bet (woodchip, chookpoo, gypsum)
Row 11 (Best bet + mineral mulch)
Row 12 Grower control + biochar
Row 13 Grower control + Mineral mulch
Row 14 Grower control + biochar
Row 15 Grower control
Row 16 Grower control + fungicides
%anthracnose2019
%anthracnose2020
%anthracnose2021
% anthracnose2022

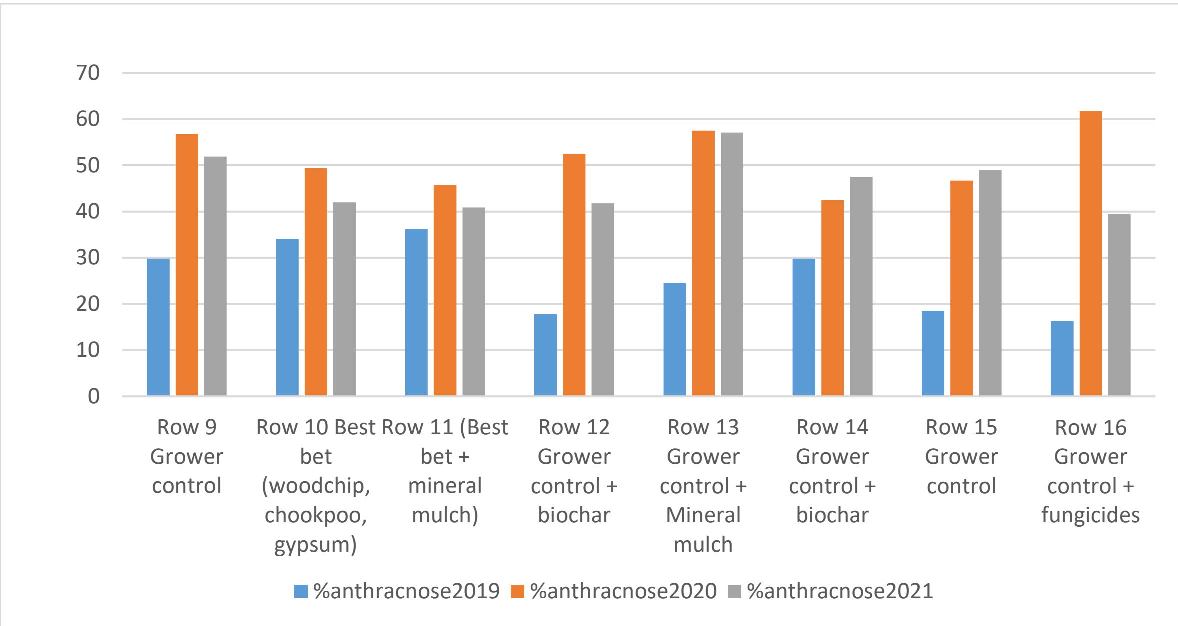


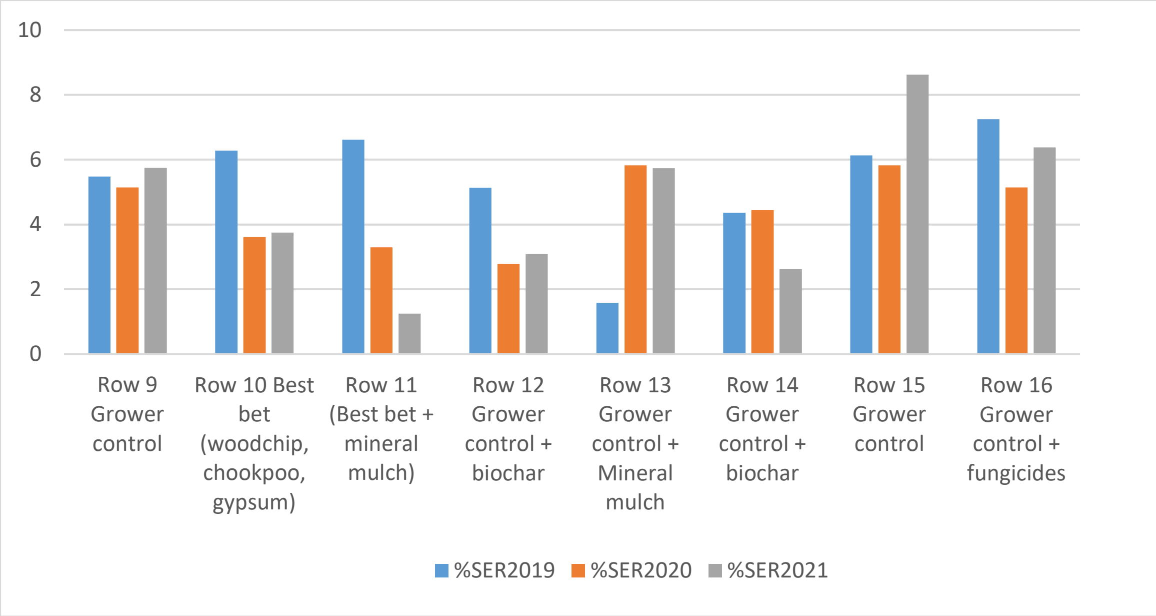
2021 Fruit yield, packout and disease severity

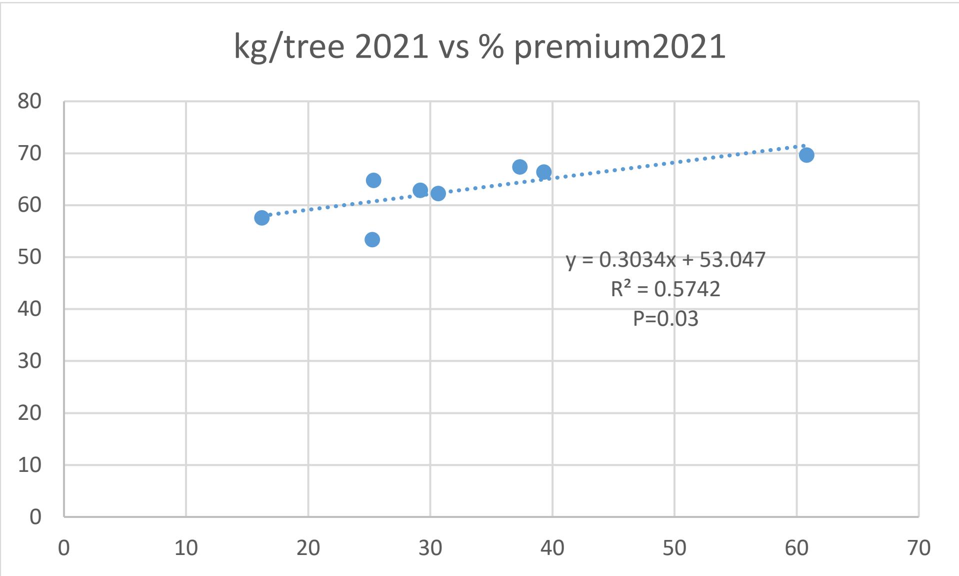
Yields across the district were generally light in 2020, due to cold weather, hail and potentially other factors at flowering in Spring 2019. Across the trial rows, the average yield per tree in 2020 was 23kg,

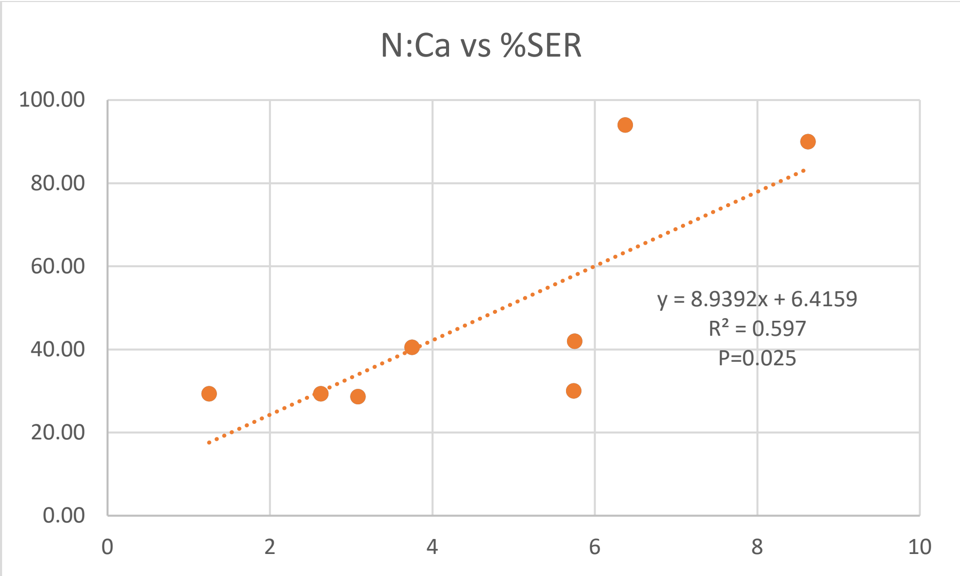



% Premium packout
100
80
60
40
20
0
Row 9 Grower control
Row 10 Best bet (woodchip, chookpoo, gypsum)
Row 11 (Best bet + mineral mulch)
Row 12 Grower control + biochar
Row 13 Grower control + Mineral mulch
Row 14 Grower control + biochar
Row 15 Grower control
Row 16 Grower control + fungicides
% premium2019
% premium2020
% premium2021


70
60
50
40
30
20
10
0
Row 9 Grower control
Row 10 Best bet (woodchip, chookpoo, gypsum)
Row 11 (Best bet + mineral mulch)
Row 12 Grower control + biochar
Row 13 Grower control + Mineral mulch
Row 14 Grower control + biochar
Row 15 Grower control
Row 16 Grower control + fungicides
%anthracnose2019
%anthracnose2020
%anthracnose2021


10
8
6
4
2
0
Row 9 Grower control
Row 10 Best bet (woodchip, chookpoo, gypsum)
Row 11 (Best bet + mineral mulch)
Row 12 Grower control + biochar
Row 13 Grower control + Mineral mulch
Row 14 Grower control + biochar
Row 15 Grower control
Row 16 Grower control + fungicides
%SER2019
%SER2020
%SER2021


kg/tree 2021 vs % premium2021
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
y = 0.3034x + 53.047
R² = 0.5742
P=0.03



2020 Tree health

Canopy health was assessed 2 Dec 2020, during early fruit set. Massive flowering and fruit set places a lot of physiological stress on trees, and it is not surprising that there was a decline in tree health since the March 2020 assessment (Figure 1) However, vegetative flush was a couple of weeks more advanced than in the Channybearup trial, and one of the factors included in tree health assessments was the strength of that flush, i.e. colour, size and number of leaves etc. Treatments and agronomic practices which minimise the effects of these phenological and environmental stresses will be most useful. The healthiest trees continue to be the row treated with the Revus+Ridomil anti-oomycete products, although the rate of decline from March to December 2020 was faster than all other treatments except one of the Biochar rows. The slowest (least) decline was in trees in the two Best bet treatment rows, and one of the Grower controls (Figure 1).

The May 2020 leaf N analysis demonstrates the direct link of this element with tree health. There was a negative correlation between the March 2020 and December 2020 canopy health ratings and May 2020 leaf N content (significant for March 2020 at p=0.021, not significant for December p=0.068). That is, as leaf N increases then tree health also improves. This effect was also observed in 2019.

2020 Fruit yield, packout and disease severity

Yields across the district were generally light in 2020, due to cold weather, hail and potentially other factors at flowering in Spring 2019. Across the trial rows, the average yield per tree in 2020 was 23kg, compared with 58kg in 2019. The highest yielding trees were in Rows 9 and 10, and not obviously linked to particular soil amendment treatments (Figure 2). It is possible that this is due to a few very high yielding “super trees” in those rows, although this is purely speculative. The low yield (13kg per tree) in the Revus+Ridomil row could be linked to its healthier canopy, enabling enhanced vegetative growth at the expense of fruit production. When yields for both 2019 and 2020 are combined (Figure 3), rows 9 and 10 have the highest yield per tree at approximately 100 kg, followed by row 12 at 89 kg. All other rows had combined yields across both years of less than 80 kg per tree. There was no significant alternate bearing effect from 2019 to 2020. Individual fruit weights were considerably greater for Row 16 (Revus+Ridomil) than all other rows in both 2019 and 2020 (Figure 4), and is not surprising given the healthier trees and lower crop loads. Leaf N in May 2020 was significantly (p=0.007) correlated with fruit weight, so that bigger fruit at harvest is correlated with higher leaf N in May. This was also observed in 2019.

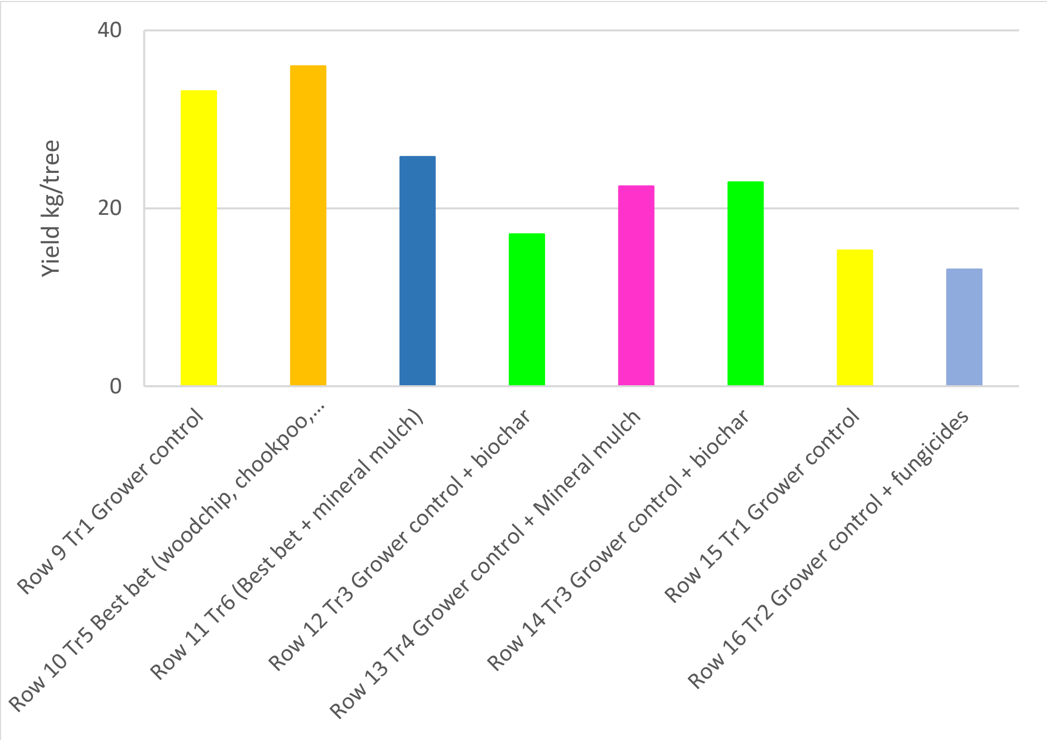


Figure 2. Average yield (kg) per tree in 2020

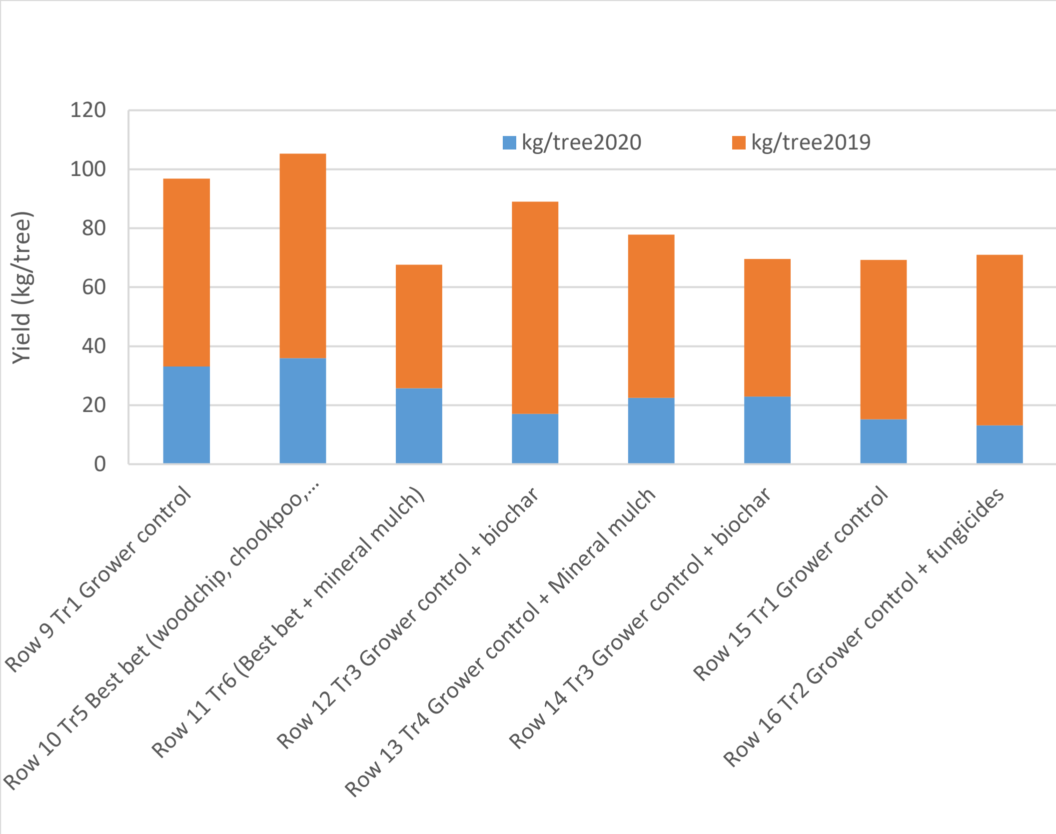


Figure 3. Cumulative yield (kg) per tree, 2019, 2020

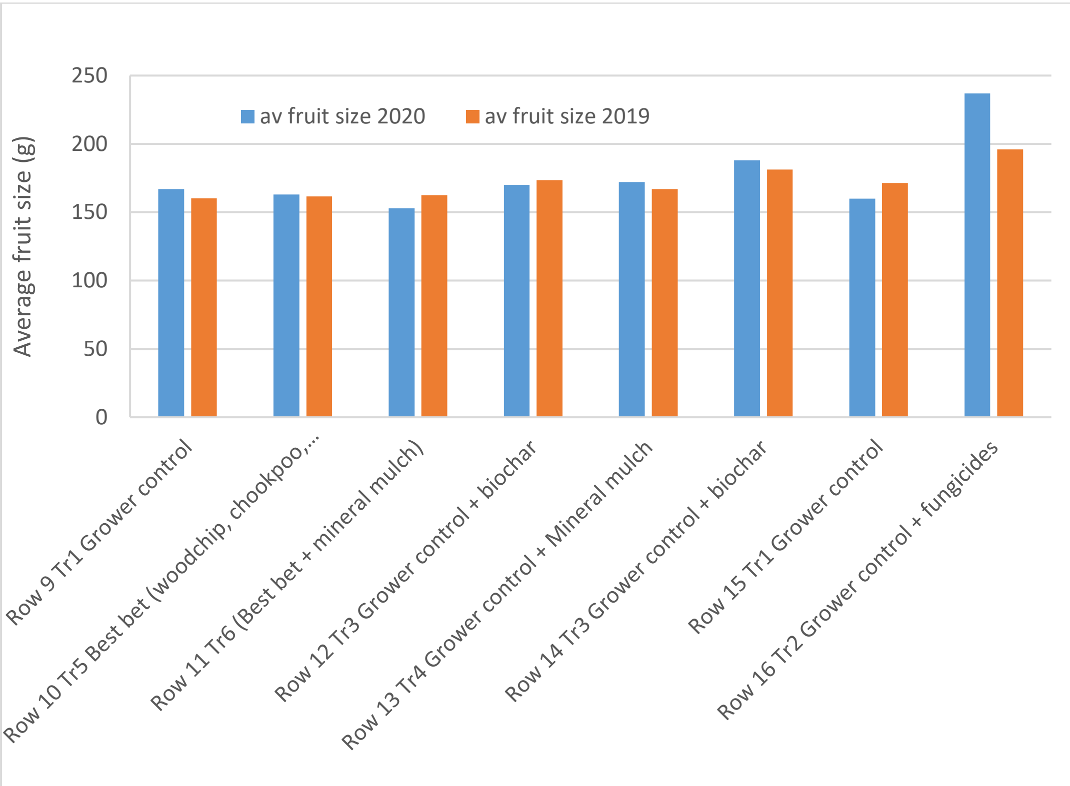


Figure 4. Average weight per piece of fruit (g)

There were no clear treatment effects on packout rates of fruit harvested in 2020 (Figure 5), with 80-88% of fruit in premium grade, which was similar to that recorded in 2019 (Figure 6).

In 2020, fruit took approximately 20 days to each "eating ripe" from the time they were picked (averaged across treatments), compared to 26 days in 2019. The corresponding dry matters were 32% DM in 2019 and 33% DM in 2020. The slightly higher DM in 2020 is unlikely to solely account for the considerably shorter ripening time. It is possibly due to differences in fruit physiology, picking conditions, storage and transport, ripening and disease assessments, and corresponds to more severe anthracnose disease in fruit in 2020 (Figure 7), which was higher across all treatment rows compared with 2019, without clear or consistent treatment effects (Figure 7). The fruit peel was perhaps a bit thicker and tougher in 2020, which made it more difficult to determine when fruit were "eating ripe", which is the stage that fruit are removed from the ripening room and assessed for anthracnose and stem end rot development. Consequently, fruit may have stayed in the ripening room (22-23°C and 65% relative humidity) for an extra day, allowing anthracnose to progress. A wetter year in 2020 may also have contributed to more severe infection and disease in fruit compared with 2019. Stem end rot was similar or lower in 2020 compared with 2019, except for Row 13 grower control + Mineral mulch, which had a very low SER symptom development in 2019. Fruit anthracnose severity was also higher in 2020 in the other two WA field trials – approx. 70% at one site, and 20-45% at the other site, with no clear treatment effects.

A summary of leaf and pulp nutrient analyses averaged across treatment rows is presented in Table 1. There were no clear treatment effects. High pulp calcium is desirable for improved fruit quality, presumably due to stronger cell walls. The pulp N:Ca ratio in mature fruit is tentatively linked to fruit quality and postharvest disease, with an optimum ratio (J. Bower, pers comm. 2018) of less than 20 (i.e. <1% N and >0.05% Ca). In this trial, ratios of 24-26 are quite good, and lower than those observed in the other WA trials (29 and >50) and >80 in QLD). Pulp Ca is negatively correlated with leaf N ($p=0.03$), so that higher leaf N (May) is associated with lower pulp Ca at harvest.

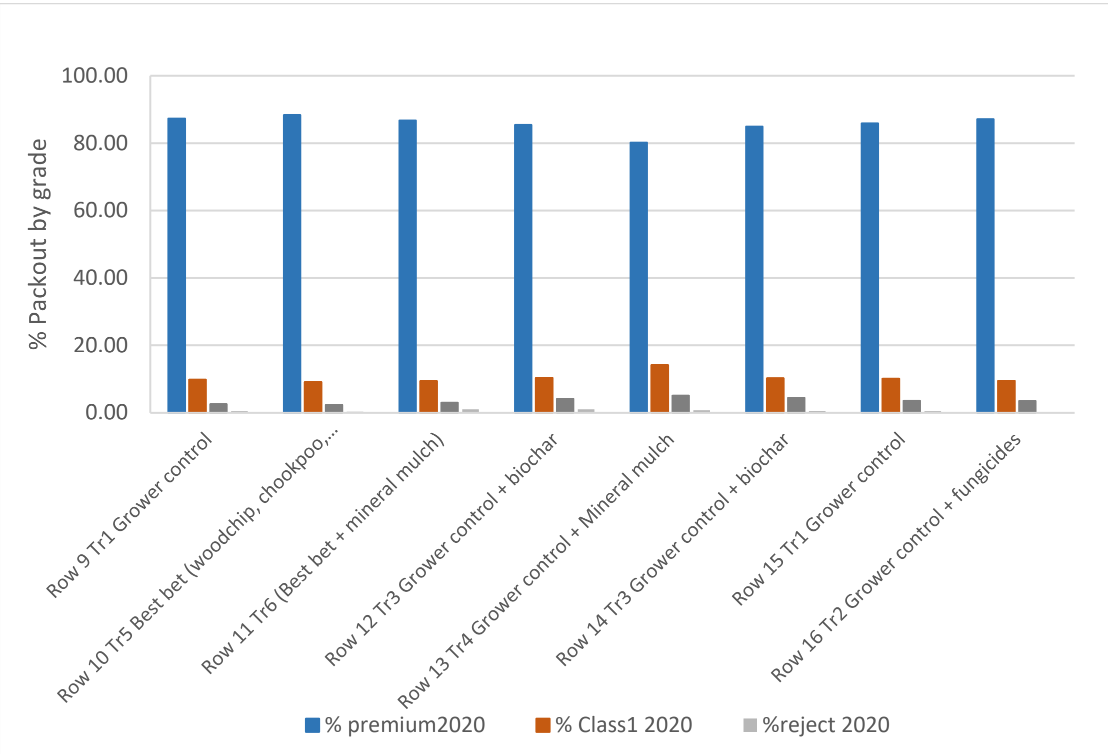


Figure 5. Packout % by fruit grade, 2020

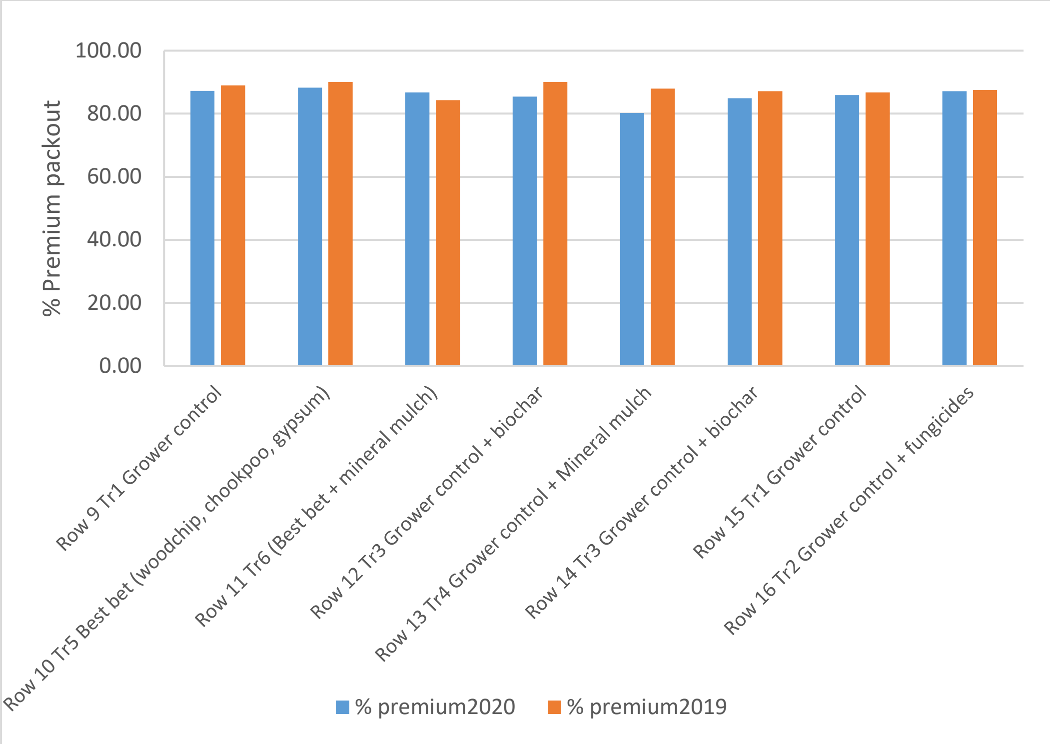


Figure 6. Premium fruit packout % 2019 and 2020

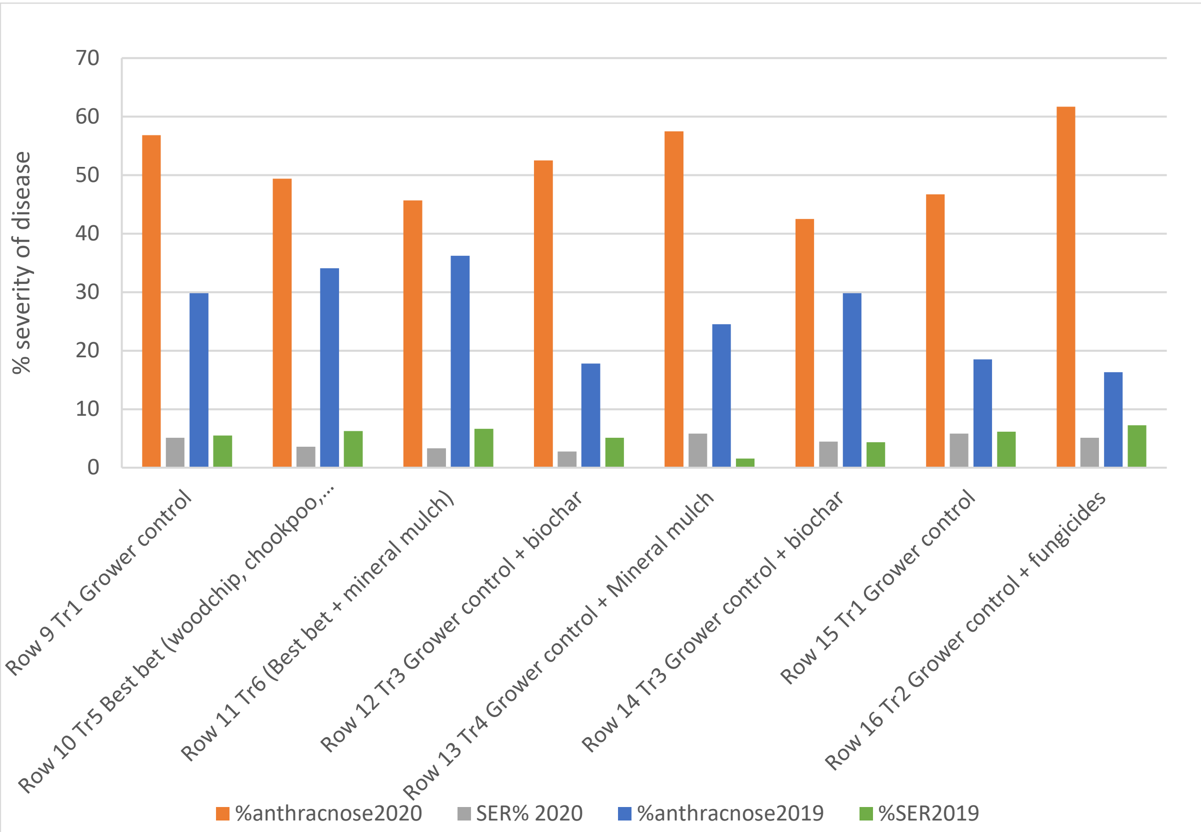


Figure 7. Fruit anthracnose and stem end rot (SER) severity in 2019 and 2020

Table 1. Comparison of pulp and leaf nutrients across all treatments in 2019 - 2022

Element	Pulp				Leaf		
	2019	2020	2021	2022	2019	2020	2021
Nitrogen (%)	0.771	0.909	0.876	0.907	1.97	2.20	2.61
Calcium (%)	0.034	0.035	0.023	0.029	1.23	0.953	1.28
N:Ca	23.7	26.4	48.0	32.3	1.60	2.31	2.09
Boron (ppm)	41.1	44.0	28.3	32	33.4	46.9	84.9
Magnesium (%)	0.075	0.076	0.045	0.074	0.296	0.407	0.496
Potassium (%)	1.42	1.69	1.26	1.46	1.27	1.67	1.40
Zinc (ppm)	11.5	14.1	9	21	36.3	36.8	38.1
Iron (ppm)*	27.4	31.1	5	11.9	58.3	74.1	77.3
Copper (ppm)*	2.66	3.23	1.29	11.6	8.14	9.06	10.3
Sulfur (%)	0.116	0.154	0.128	0.109	0.213	0.284	0.26
Phosphorus (%)	0.133	0.169	0.085	0.156	0.149	0.210	0.234
Silicon (ppm)	233	283	413		266	326	95

* Cu and Fe are strongly influenced by fungicide applications and dust on leaf surface which was not washed off prior to analyses
In 2019 – leaf samples collected 1 May 2019, fruit harvested 18 November 2019
In 2020 – leaf sampled collected 4 May 2020, fruit harvested 29 October 2020
In 2021 – leaf sampled collected 17 May 2021, fruit harvested 10 November 2021
In 2022 – fruit harvested 14 November 2022

Statistically significant correlations 2020 data

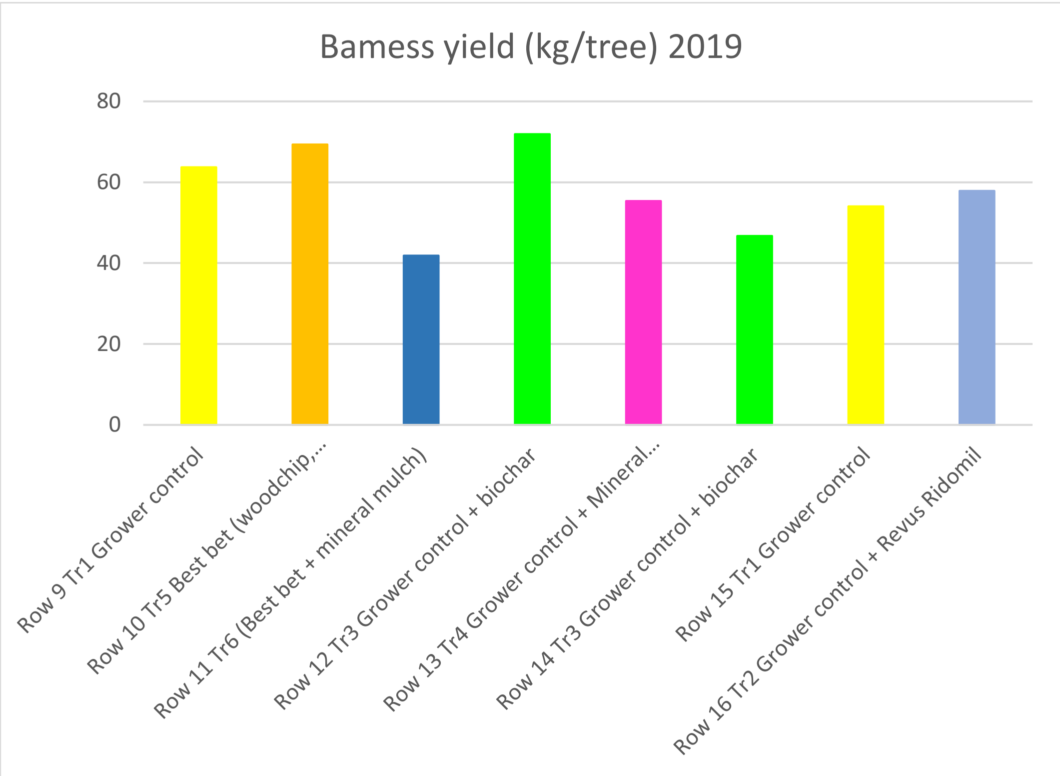
- No significant correlations between 2020 yield (kg/tree) and any parameter
- No significant correlations between % stem end rot severity in 2020 and any parameter
- % anthracnose severity vs pulp magnesium (+ve, p=0.017, more disease with higher pulp Mg)
  pulp phosphorus (+ve, p=0.036, more disease with higher pulp P)
- Leaf N (May 2020) vs canopy health rating March 2020 (-ve, p=0.0210)
  canopy health rating December 2020 (-ve, p=0.068)
  average fruit size Oct 2020 (+ve, p=0.007)
- Pulp N (Oct 2020) vs pulp K (+ve, p=0.027)
- Pulp Ca (Oct 2020) vs leaf N May 2020 (-ve, p=0.030), higher leaf N then lower pulp Ca
  pulp Si (+ve, p=0.021), higher pulp Ca then higher pulp Si
  tree health March 2020 (+ve, p=0.038), more pulp Ca in less healthy trees
- Pulp Si (Oct 2020) vs % premium packout (-ve, p=0.053), higher Si, lower premium packout

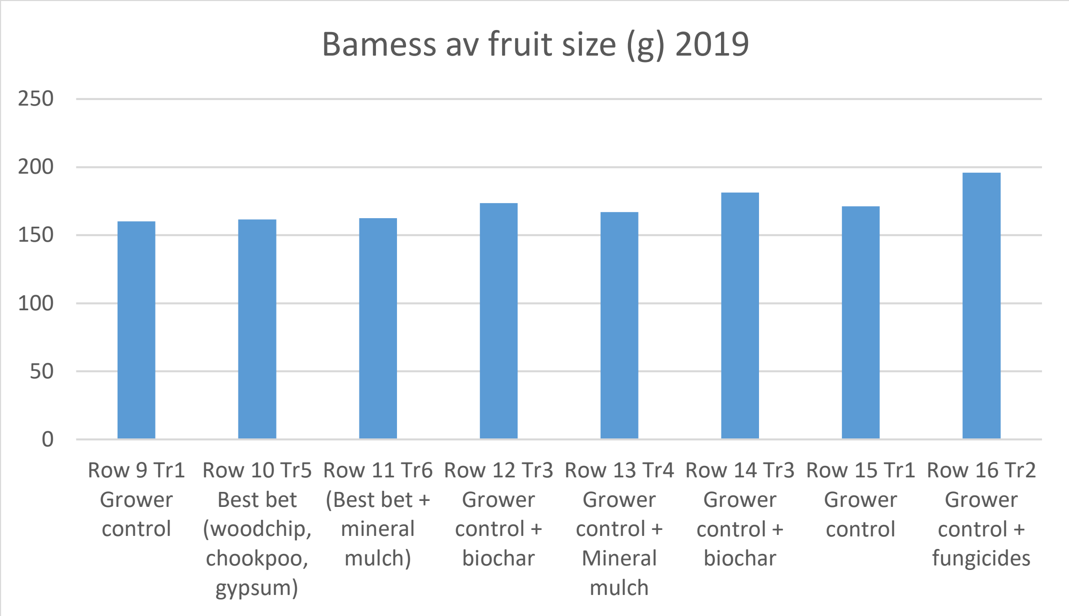
% Class 1 packout (+ve, p=0.024), higher Si, higher % class 1 packout

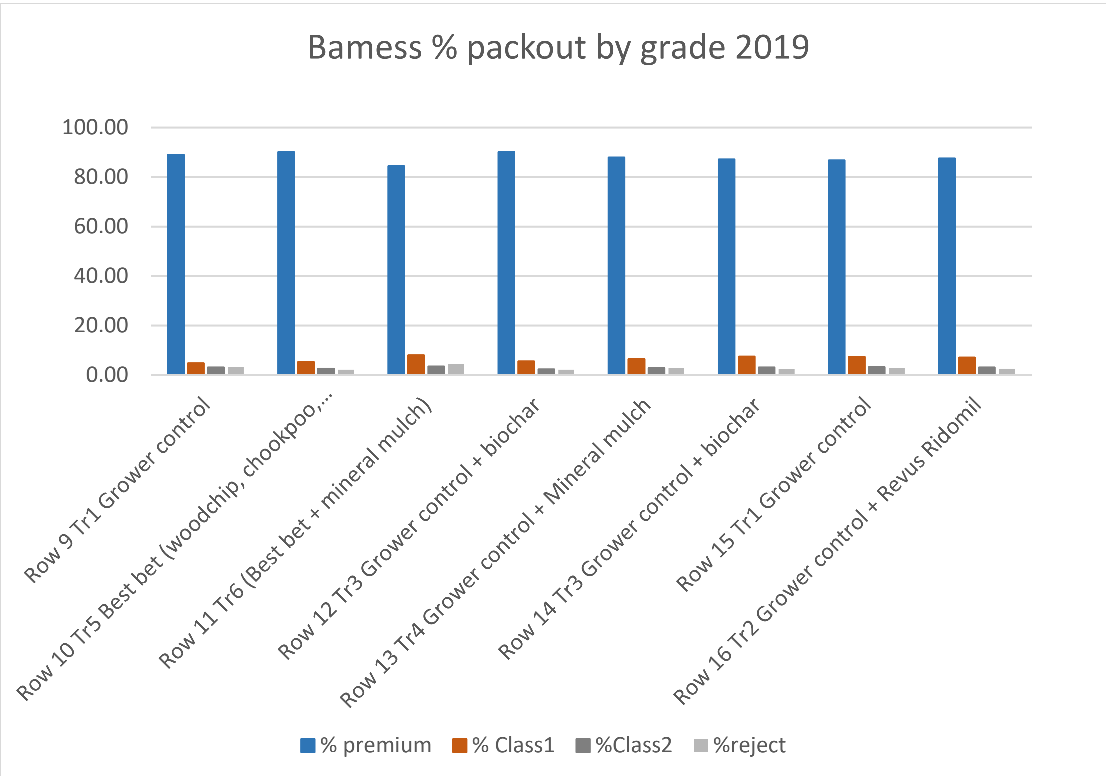
2020 Leaf nutrient data

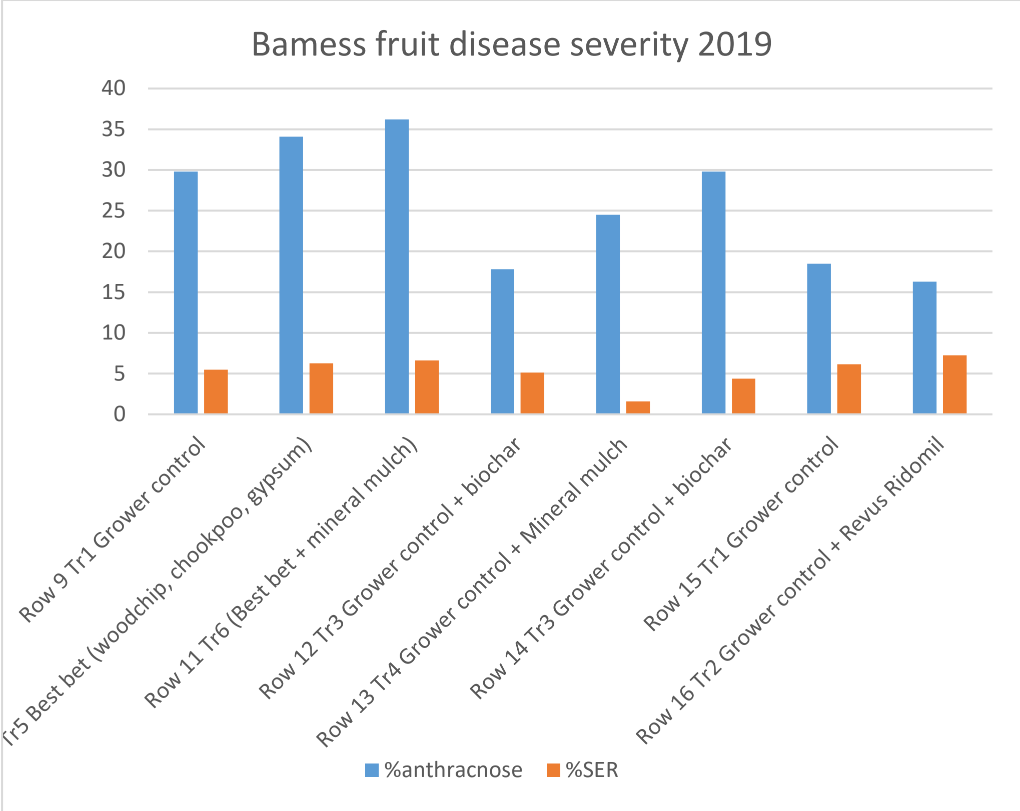
- Taking average across all treatments, there were increases over 2019 leaf nutrients in B, N, P, K and Si, however there was a decrease in Ca.
- Significant negative correlation between tree health assessed in March 2020, and leaf nitrogen (sampled May 2020). This is also what we found in 2019.
- No outstanding treatment effects.
    - Best bet + Mineral mulch has higher Ca, Mn, and Si compared with the average across all treatments
    - Revus/Ridomil has higher Mn, Zn and N compared with the average
    - Grower control + mineral mulch has higher Mo, B, P, K (and slightly higher Ca), and lower Mn compared with average across all treatments.

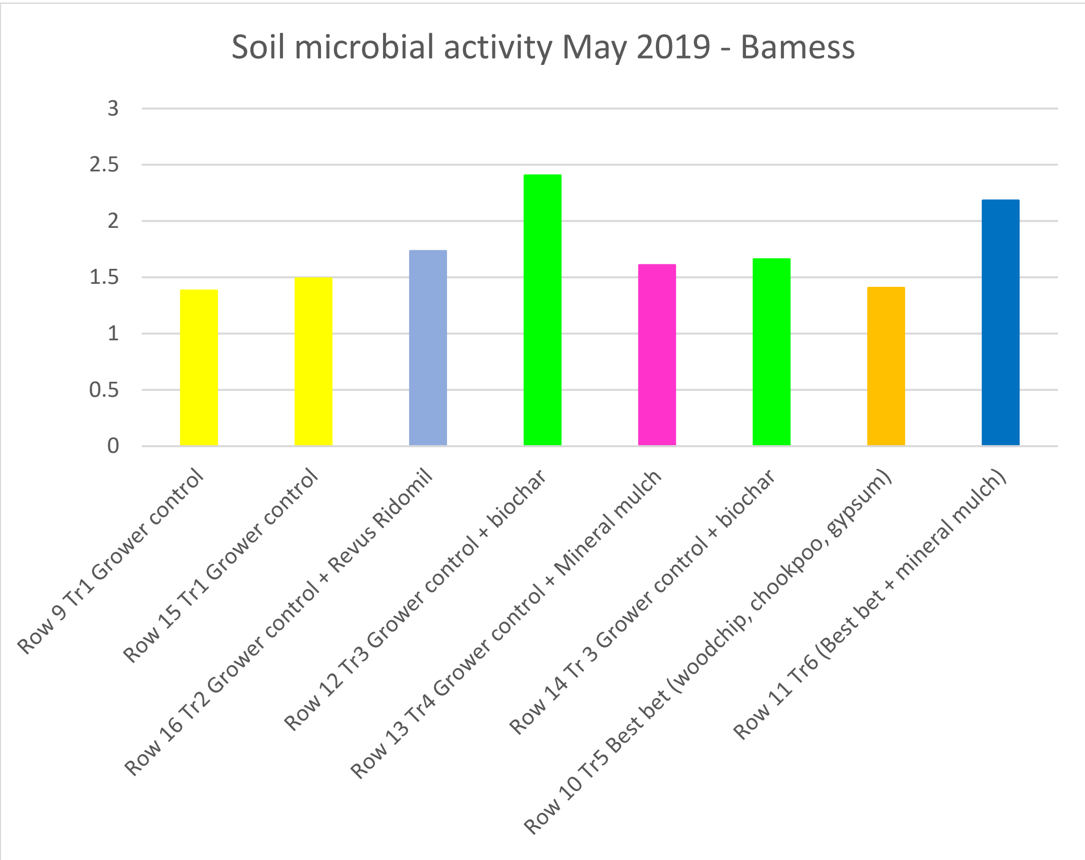
2019 Trial Data Summary




Bamess av fruit size (g) 2019
250
200
150
100
50
0
Row 9 Tr1 Grower control
Row 10 Tr5 Best bet (woodchip, chookpoo, gypsum)
Row 11 Tr6 (Best bet + mineral mulch)
Row 12 Tr3 Grower control + biochar
Row 13 Tr4 Grower control + Mineral mulch
Row 14 Tr3 Grower control + biochar
Row 15 Tr1 Grower control
Row 16 Tr2 Grower control + fungicides


Bamess % packout by grade 2019
100.00
80.00
60.00
40.00
20.00
0.00
Row 9 Tr1 Grower control
Row 10 Tr5 Best bet (woodchip, chookpoo,...
Row 11 Tr6 (Best bet + mineral mulch)
Row 12 Tr3 Grower control + biochar
Row 13 Tr4 Grower control + Mineral mulch
Row 14 Tr3 Grower control + biochar
Row 15 Tr1 Grower control
Row 16 Tr2 Grower control + Revus Ridomil
% premium
% Class1
%Class2
%reject


Bamess fruit disease severity 2019
40
35
30
25
20
15
10
5
0
Row 9 Tr1 Grower control
Tr5 Best bet (woodchip, chookpoo, gypsum)
Row 11 Tr6 (Best bet + mineral mulch)
Row 12 Tr3 Grower control + biochar
Row 13 Tr4 Grower control + Mineral mulch
Row 14 Tr3 Grower control + biochar
Row 15 Tr1 Grower control
Row 16 Tr2 Grower control + Revus Ridomil
%anthracnose
%SER


Soil microbial activity May 2019 - Bamess
3
2.5
2
1.5
1
0.5
0
Row 9 Tr1 Grower control
Row 15 Tr1 Grower control
Row 16 Tr2 Grower control + Revus Ridomil
Row 12 Tr3 Grower control + biochar
Row 13 Tr4 Grower control + Mineral mulch
Row 14 Tr 3 Grower control + biochar
Row 10 Tr5 Best bet (woodchip, chookpoo, gypsum)
Row 11 Tr6 (Best bet + mineral mulch)

Isolation results

Date		Tissue isolated from	*Colletotrichum fioriniae*	Botryosphaeria sp	Others
5/12/2019	S.B 1	Fruit tissue	*4*	0	0
6/12/2019	S.B 2	Fruit tissue	3	0	0
6/12/2019	S.B 3	Fruit tissue	4	0	0
7/12/2019	S. B 4	Fruit tissue	5	0	0
9/12/2019	S.B 5	Fruit tissue	3	0	3xAspergillus

Statistically significant correlations 2019 data

Yield vs % premium packout (+)

Leaf N vs canopy health ratings (-) (May 2019 and November 2019, ie. healthier trees with higher leaf N)

average fruit size (+)

anthracnose (-) (different to what we normally see, ie. as N goes up, anthracnose usually goes up too)

Leaf Ca vs leaf Mg (+)

leaf P (+)

leaf K (+)

Leaf Si vs % stem end rot (+) (would have expected stem end rot to decrease as Si increases)

pulp Si (+)

Pulp N vs canopy health rating (-) May 2019

stem end rot (+)

% stem end rot (+) This is more typical, ie, as N increases then disease also increases

leaf B, leaf K, pulp Mn, pulp S (all +)

leaf Cl (-)

ALL WA CORRELATIONS 2019 (data from Bamess, Doug Pow and West Pemberton combined for analyses)

- 20 data points
- Statistically significant correlations:

Yield vs Ca (+)

pulp B (-)

anthracnose, SER (-)

% Premium vs leaf N, pulp N, leaf B (-)

leaf N vs anthracnose, SER (-)

leaf B (+)

AV16007 West Pemberton Avocados soil amendment trial summary, updated May 2023

Canopy health (Figure 1 and Table 1)

- A final canopy health rating was done in November 2022, a year after the trial was completed. There were no residual effects apparent for any treatment. Trees which had been drenched with the oomycete fungicides twice a year for the duration of the trial declined most rapidly from May and November 2021 (when they were the healthiest compared to trees from other treatments), to November 2022, and this highlights the danger of applying a "fungicide only" approach to managing decline due to Phytophthora root rot.
- There was a very clear improvement in canopy health in the fungicide treated trees from Dec 2020 to May 2021. Other trees to improve over the Dec 20 to May 21 period were Grower control and Grower control + mineral mulch.
- November 2021 assessments were challenging as trees had been pruned or stag-horned, and there was limited canopy volume to assess. If the numbers of stumped trees are an indicator of tree health (sickest trees stumped), then fewer trees from the fungicide, Grower Control, Grower Control + mineral mulch 1 and Grower control + Ecogrowth/Switch treatments were stumped. These were the trees which were healthiest in May 2021 and Nov 2021.

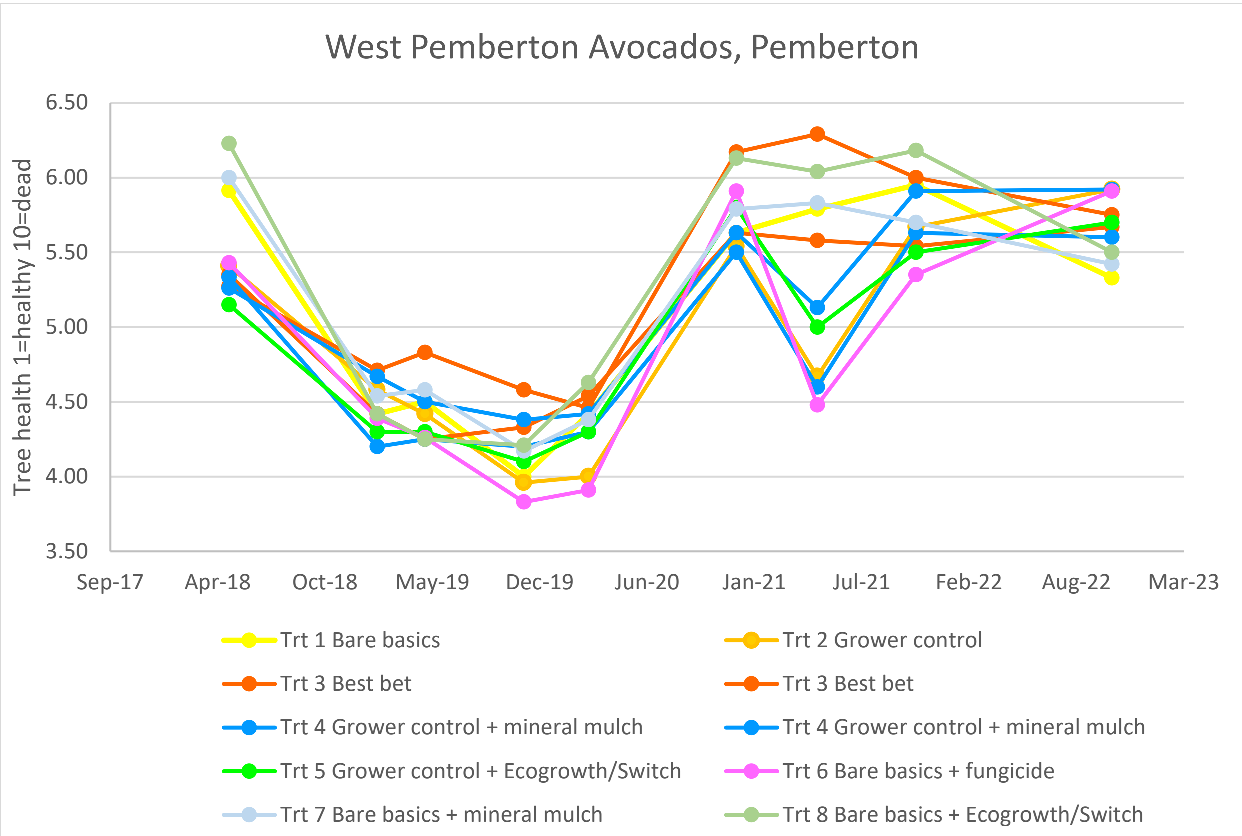


Figure 1. Canopy health over time. All trees included except those completely stumped (Nov 2021).

Table 1. Canopy health assessment and numbers of stumped trees

		Number stumped Nov 2021	Canopy health May 21	Canopy health Nov 21[a]	Canopy health Nov 21[b]
WPA Trt 1 30 nth	Trt 3 Best bet 1	20	5.58	5.50 (24)	5.25 (4)
WPA Trt 2 30 sth	Trt 2 Grower control	6	4.67	5.67 (24)	5.61 (18)
WPA Trt 3 31 nth	Trt 5 Grower control + Ecogrowth/Switch	9	5.00	5.50 (23)	5.30 (15)
WPA Trt 4 31 sth	Trt 4 Grower control + mineral mulch 1	5	4.60	5.60 (24)	5.30 (19)
WPA Trt 5 32 nth	Trt 4 Grower control + mineral mulch 2	14	5.13	5.91 (23)	5.56 (9)
WPA Trt 6 32 sth	Trt 3 Best bet 2	13	6.29	6.00 (23)	5.80 (10)
WPA Trt 7 33 nth	Trt 6 Bare basics + Revus Ridomil *	5	4.48	5.35 (23)	5.28 (18)
WPA Trt 8 33 sth	Trt 8 Bare basics + Ecogrowth/Switch	13	6.04	6.18 (22)	5.90 (10)
WPA Trt 9 34 nth	Trt 1 Bare basics	18	5.79	5.95 (21)	5.67 (6)
WPA Trt 10 34 sth	Trt 7 Bare basics + mineral mulch	13	5.83	5.70 (23)	5.30 (10)
Row 35 South	Revus only		5.36	6.05 (22)	

* One dead/missing tree in Revus Ridomil treatment from commencement of the trial in Sept 2018

[a] All trees included except those completely stumped (numbers of trees in brackets)

[b] Only trees that had not been severely pruned or stumped included

Fruit yield, packout, disease severity (Figures 2-6, Table 2)

- 2021 yields ranged from 460 kg to 2056 kg for the 24 trees in treatment area. (although 460 kg for best Bet 1 is somewhat anomalous given that there were 3 bins tipped. Perhaps the yield on packout run reports does not include reject fruit??)
- Highest yields from Grower control + mineral mulch 1 and 2, Best bet 2 and Grower control
- All of the 4 Bare basics treatment had the lowest yields. The best of these was Bare basics + fungicides.
- Across the 3 years, highest cumulative yields are from Grower control + mineral mulch (both reps), Grower control + Ecogrowth/Switch, Best bet 2 and Bare basics + fungicides.
- Largest fruit fruit (31 – 36) in 2021 were from the Grower control, Grower control + Ecogrowth/Switch, Mineral mulch and fungicide treatments, which loosely corresponds to higher yielding and healthier treatments.
- Very little difference amongst treatments in premium packout %, with all above 70%, except the low-yielding Best bet 1 which had 53% premium (although the yield was also reported as very low compared with all other treatments, and is possibly incorrect).
- As in 2020, disease and pulp nutrient results must be viewed with caution as fruit were not selected uniformly from trees across each treatment.
- Anthracnose was less severe in 2021 than 2020, and there was a broad range in % severity across treatments. Not surprising to see less disease with higher crop load, and in 2021 fruit were picked about 2 weeks earlier with 27.2% DM, than in 2020 (30.7%DM).
- As for anthracnose there was a broad range in % stem end rot severity across treatments. Most severe in the Best bet treatments, one of the Grower control + mineral mulch treatments and Bare basics control. Least severe in the fungicide treatment.

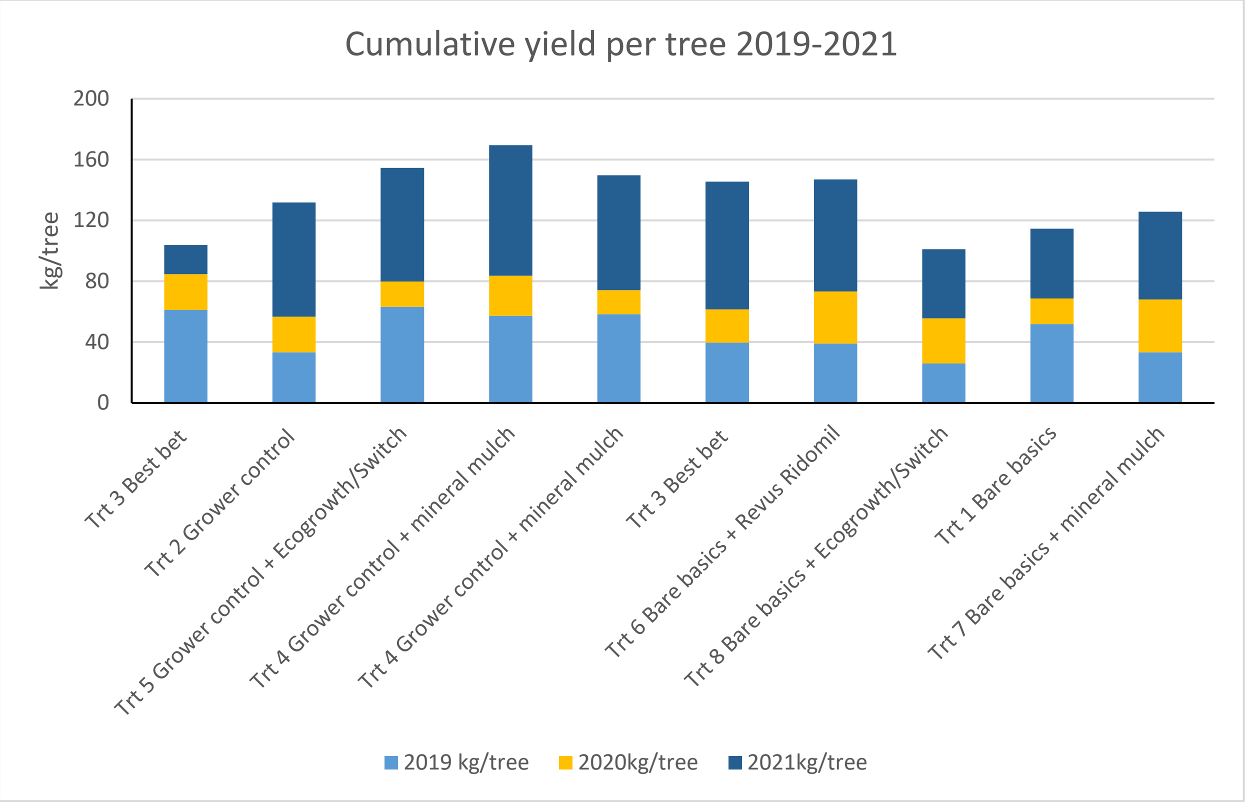


Figure 2. Cumulative yield 2019-2021, for treatment area (24 trees in each, except for Revus Ridomil which has 23 trees). Note: yield in 2021 for Trt 3 Best Bet (first column) was reported very low compared with all other treatments, and is possibly incorrect

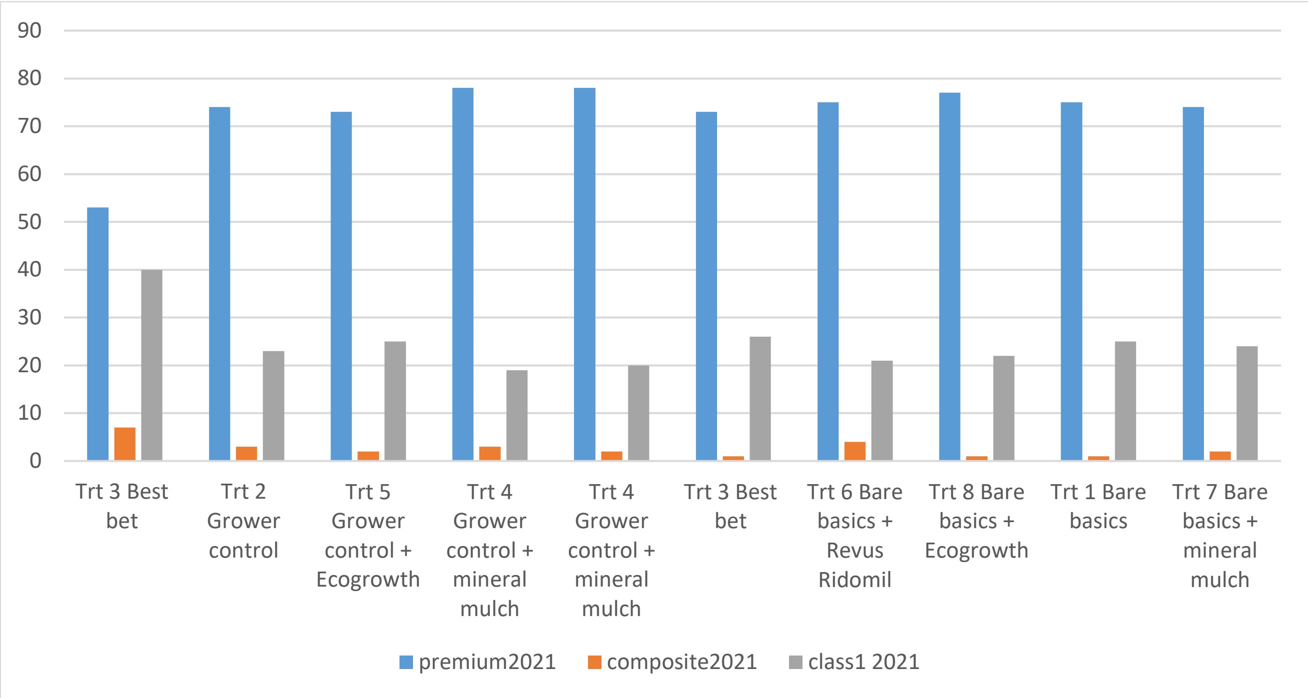


Figure 3. 2021 % packout by grade

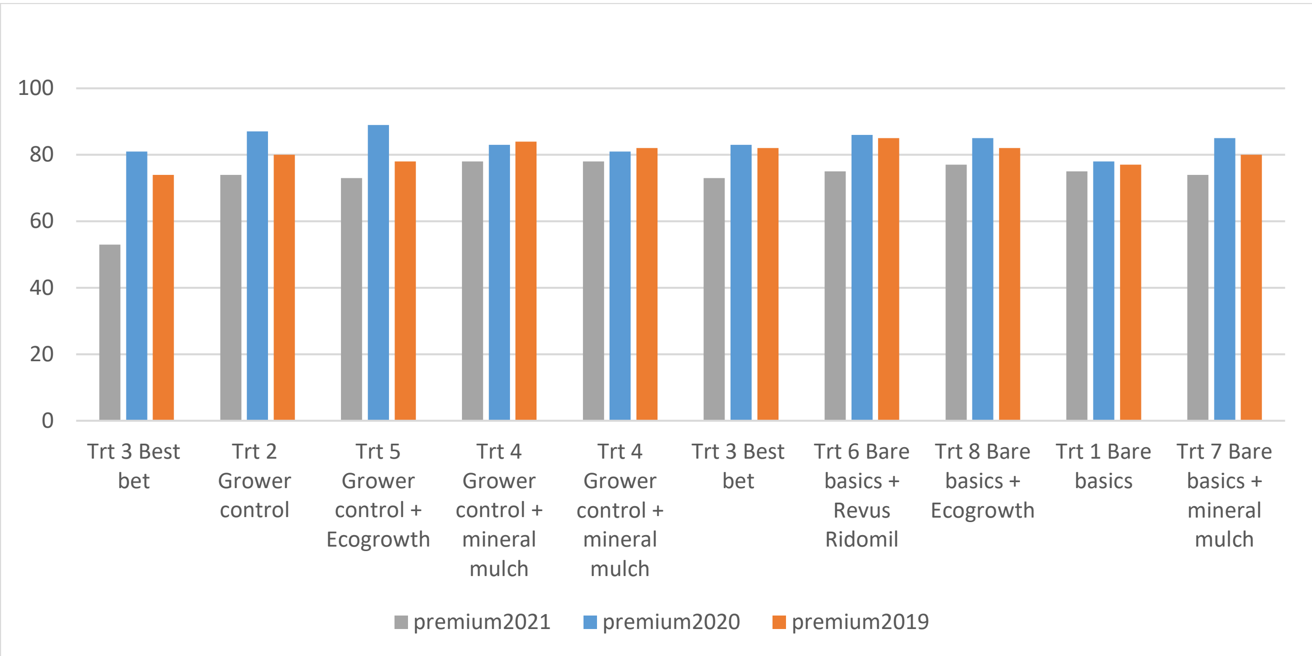


Figure 4. Premium packout % 2019-2021

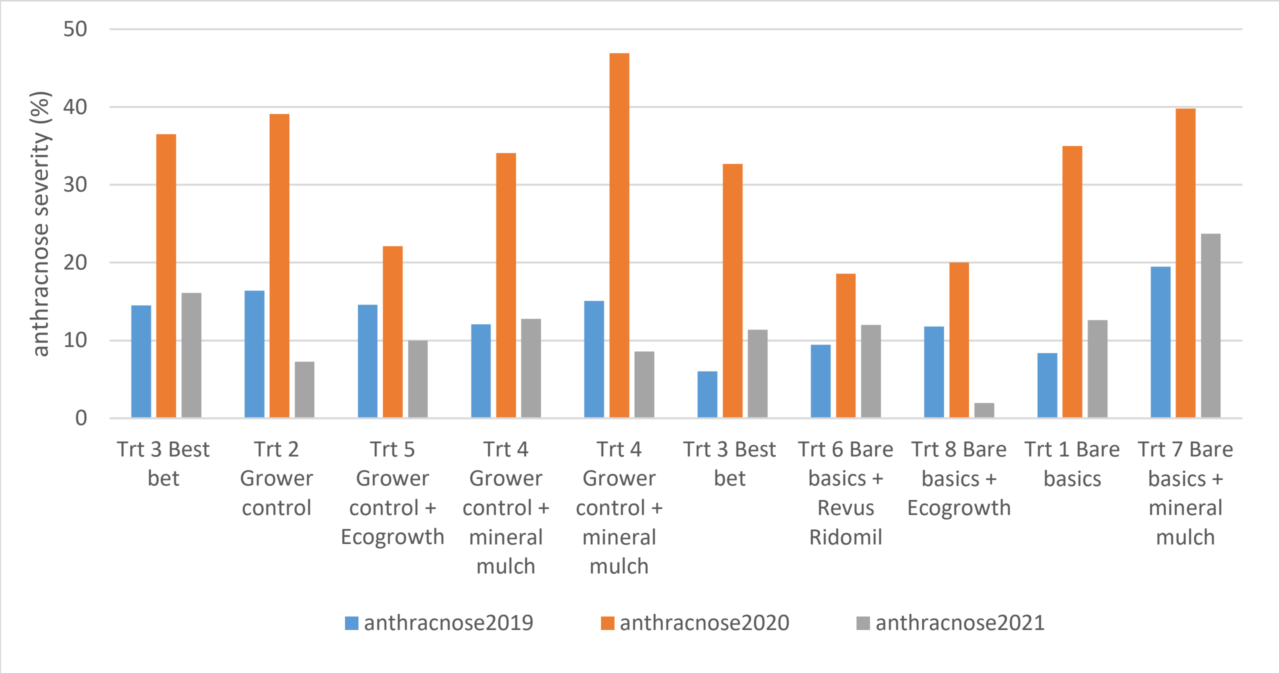


Figure 5. Anthracnose severity 2019-2021

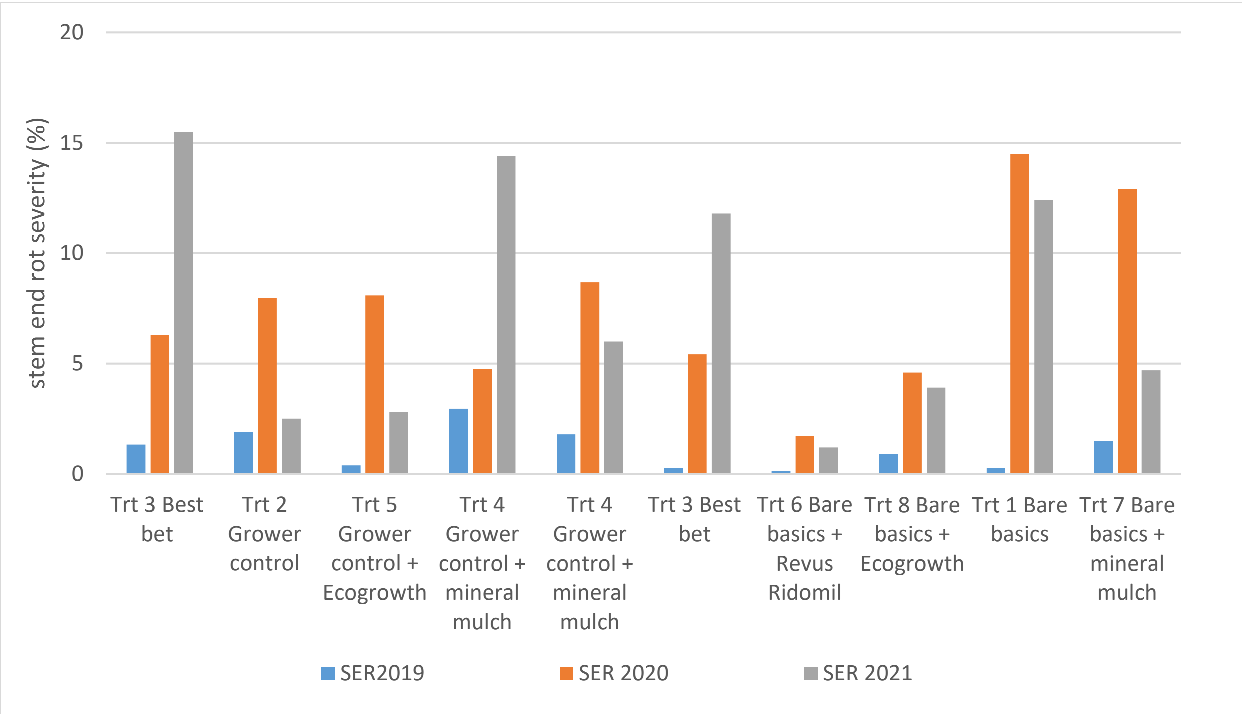


Figure 6. Stem end rot severity 2019-2021

Table 2. 2021 Dry matter, fruit size and days to ripe

		%DM	Fruit size	DES
WPA Trt 1 30 nth	Trt 3 Best bet	27.47	39	30.3 (22)
WPA Trt 2 30 sth	Trt 2 Grower control	26.44	32	29.5 (24)
WPA Trt 3 31 nth	Trt 5 Grower control + Ecogrowth/Switch	27.18	34	28.0 (25)
WPA Trt 4 31 sth	Trt 4 Grower control + mineral mulch	27.28	31	28.6 (25)
WPA Trt 5 32 nth	Trt 4 Grower control + mineral mulch	26.22	36	29.6 (25)
WPA Trt 6 32 sth	Trt 3 Best bet	27.59	38	28.2 (25)
WPA Trt 7 33 nth	Trt 6 Bare basics + Revus Ridomil *	27.53	33	27.6 (25)
WPA Trt 8 33 sth	Trt 8 Bare basics + Ecogrowth/Switch	29.20	39	29.5 (23)
WPA Trt 9 34 nth	Trt 1 Bare basics	25.75	43	30.2 (25)
WPA Trt 10 34 sth	Trt 7 Bare basics + mineral mulch	27.34	39	28.7 (23)

%DM was determined from approximately 6 pieces of fruit per treatment group
DES (days from harvest to eating soft/ripe), % anthracnose and % stem end rot was assessed from the best 22-25 pieces of fruit received

Leaf and pulp nutrients (Table 3)

Leaf (sampled May 2021)

N range 2.45-3.1 across treatments

Ca range 1.71 – 2.31 (highest was Revus/ridomil)

Pulp (harvested September 2021)

N range from 0.69 to 0.97 across treatments

Ca range from 0.01 to 0.05 (0.05 for Grower control + Ecogrowth/Switch, and Revus/Ridomil)

N:Ca range 16-78 - lowest (16-18) for Grower control + Ecogrowth/Switch, and Revus/Ridomil and Bare Basics + mineral mulch; highest (71-78) for Grower control , Best Bet2 and Bare Basics + Ecogrowth/Switch)

Table 3. Comparison of average pulp and leaf elements across all treatments 2019-2021

Element	Pulp			Leaf		
	2019	2020	2021	2019	2020	2021
Nitrogen (%)	0.739	0.843	0.797	2.765	2.332	2.662
Calcium (%)	0.027	0.029	0.027	1.392	1.857	1.994
N:Ca	29.4	29.1	41.6	1.99	1.256	1.353
Boron (ppm)	74.3	57	59.9	74.1	38.4	39.7
Magnesium (%)	0.071	0.066	0.038	0.296	0.407	0.453
Potassium (%)	1.218	1.397	1.10	1.062	1.031	1.119
Zinc (ppm)	12.8	11.1	11.0	36.5	89.4	68.6
Iron (ppm)*	38.8	23.8	6.7	55.5	205	83.9
Copper (ppm)*	2.41	1.45	1.91	194.3	417	664
Sulfur (%)	0.103	0.172	0.12	0.227	0.232	0.242
Phosphorus (%)	0.132	0.148	0.078	0.185	0.168	0.217
Silicon (ppm)	344	293	486	248	354	121

* Cu and Fe are strongly influenced by fungicide applications and dust on leaf surface which was not washed off prior to analyses

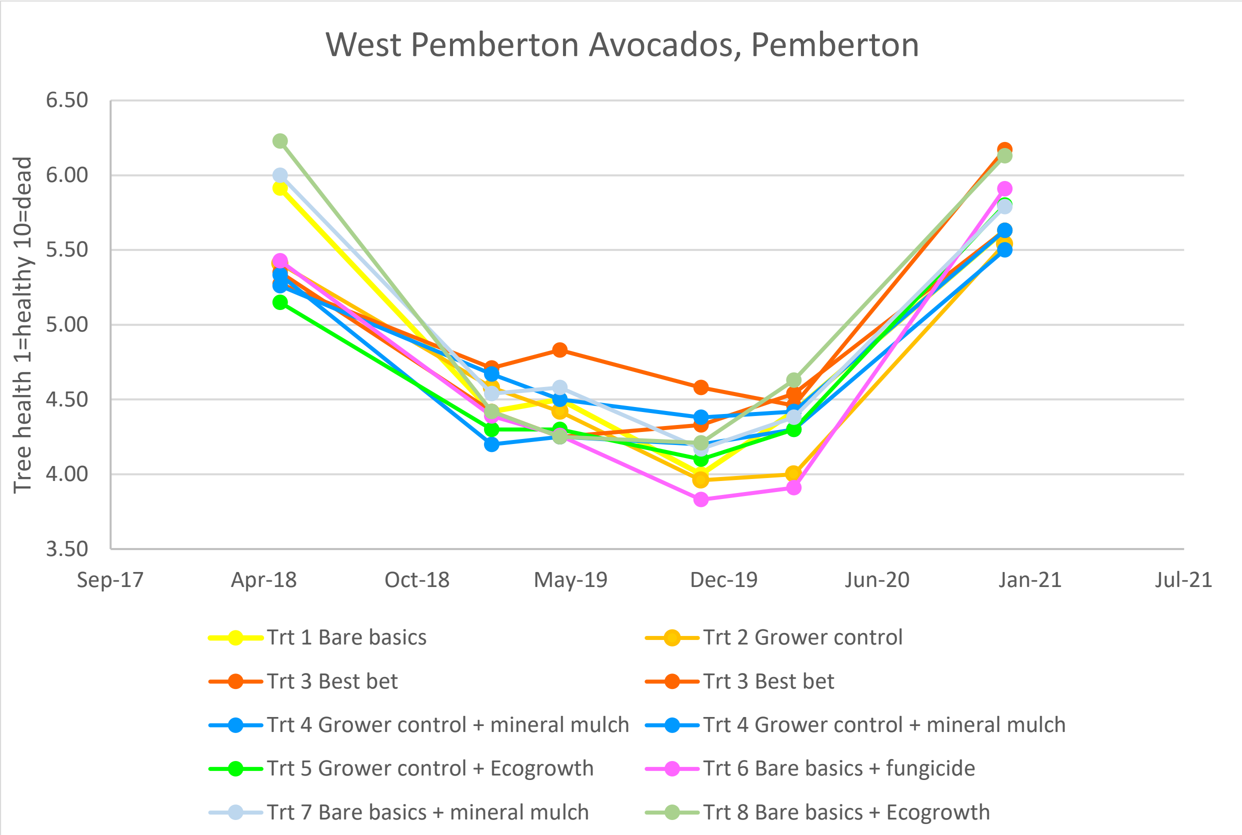
In 2019 – leaf samples collected 1 May 2019, fruit harvested 18 November 2019

In 2020 – leaf sampled collected, 11 May 2020, fruit harvested 7 October 2020

In 2021 – leaf sampled collected, 18 May 2021, fruit harvested 24 September 2021

Pre-2021

Figure 1. Canopy health over time



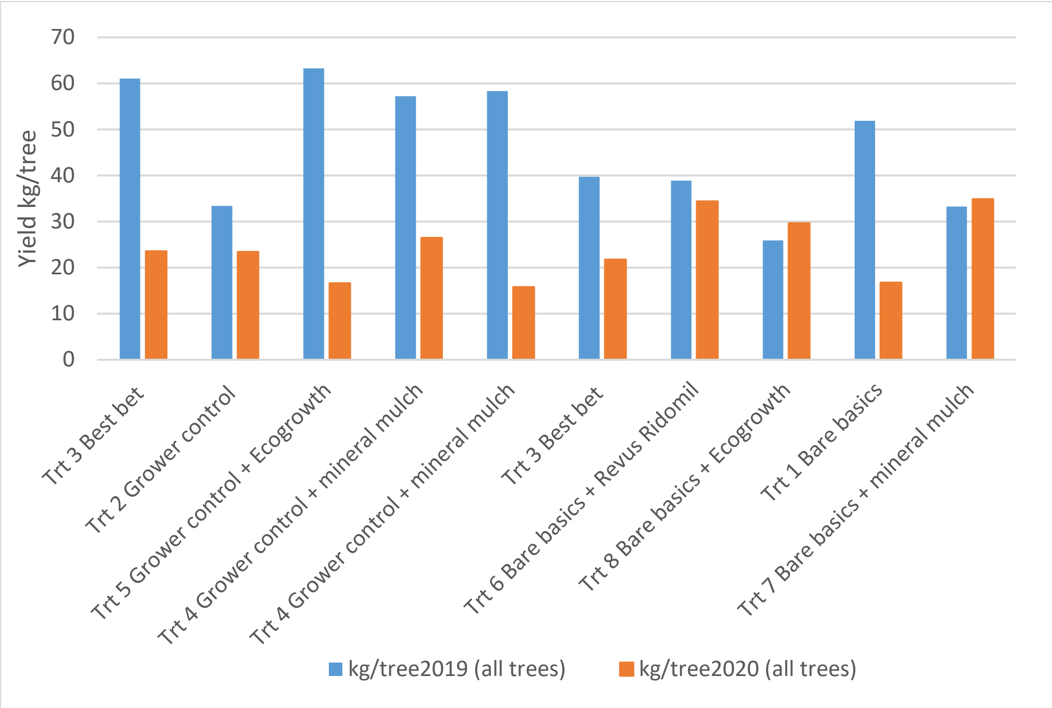
Tree health

Canopy health was assessed 1 Dec 2020, during early fruit set. Massive flowering and fruit set places a lot of physiological stress on trees, and it is not surprising that trees look poor prior to dropping a lot of fruit and putting out a vegetative flush, thus accounting for the sharp decline in tree health since the March 2020 assessment (Figure 1). Treatments which minimise the effects of these phenological and environmental stresses will be most useful. There was very little difference amongst treatments in canopy health. The Switch Innovation program replaced the Ecogrowth treatments from early November 2020.

2020 Fruit yield, packout, disease severity

- Fruit were harvested into separate bins for each treatment group (24 trees) on 7 October 2020. All fruit went through the packingline, so had Graduate A+ fungicide treatment. For each treatment group (=APMS Run number), two packed boxes were selected at random and transported with commercial consignment to Brisbane. Fruit were collected from Brisbane Markets on 15 October and plugged for DM% and pulp nutrient analyses, then maintained in the ripening room at 22-23°C and 65% RH, to optimise postharvest disease expression. Therefore, there is not uniform or consistent representation of fruit from across the 24 trees in each treatment group, so disease severity, pulp nutrients, DM results for each treatment should be treated with caution.
- (In contrast, in 2019, fruit were hand-selected from trees immediately prior to commercial harvest. The 2 trays were selected across the 24 trees, one tray each side of the tree, were uniform in size (approx. count 23), and free of significant damage. This fruit did not go through postharvest fungicide in the packingline).

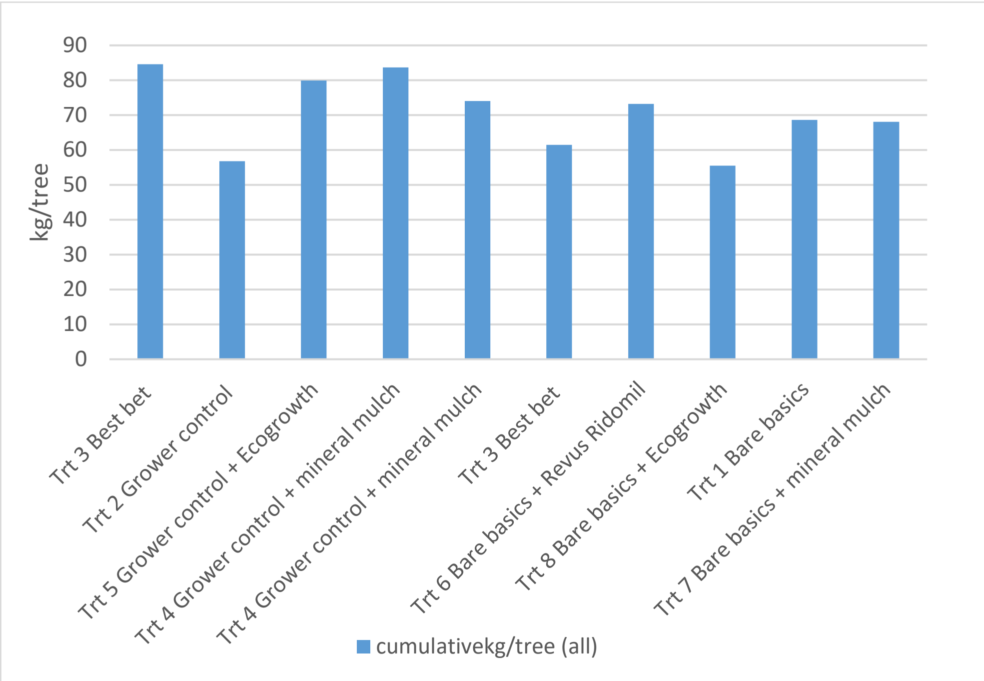
Figure 2. Fruit yield per tree in 2019 and 2020



- Yields across SW WA were generally low in 2020. Total kg of fruit picked from the trial in 2020 was about half that in 2019 (not adjusted for staghorned trees, Figure 2). Despite this, there was a significant (p=0.04) alternate bearing effect, i.e. trees which had lower yields in 2019 had higher yields in 2020.
- Yield per tree was greatest for trees in the Revus+Ridomil and Bare basics+MM treatments at about 35 kg/tree, and lowest yields from Bare basics and Grower control +MM at approx. 17 kg/tree.
- There was a significant positive correlation (p=0.04) between yield 2020 (October) and tree health score in December 2020, so that the higher yielding trees were still recovering when assessed a couple of months later.

- There were no significant correlations between yield 2020 and canopy health assessed in March 2020, or November 2019, and no other significant correlations between yield 2020 and other parameters, including pulp nutrients.
- Cumulative yield per tree (2019+2020) is presented in Figure 3. There are no consistent treatment effects, with a large variation, about 23 kg, between yields of the two Best bet treatments. The difference between the two replicate Grower control+MM treatments is about 10 kg.

Figure 3. Cumulative yield per tree for each treatment group (2019 + 2020)



- Packout by grade for 2020, and premium packout for 2019 and 2020 are presented in Figure 4 and Figure 5. All treatments had fruit packing above 80% premium except Bare basics, which had the highest percentage (20%) of lower grade Class 1 fruit compared with other treatments (Figure 4). There were slight improvements in 2020 in % premium fruit for the Ecogrowth treatments, Best bet, Grower control and Bare basics+MM (Figure 5) over 2019.

- Disease and pulp nutrient results must be viewed with caution as fruit were not selected uniformly from trees across each treatment. Table 1 presents disease severity data for each of the trays selected at random after packing, and shows that there was huge tray to tray variability even within treatments. However, across treatments anthracnose and stem end rot were both considerably more severe in 2020 than in 2019 (Figure 6 and Figure 7). While disease is often worse in low crop years, the 2020 disease level was somewhat surprising given that fruit were picked earlier (31% DM in 2019 compared with 35% DM in 2020), and went through postharvest fungicide treatment. Anthracnose severity ranged from 14% up to 53% of fruit surface area affected (Table 1). Fruit with the lowest severity of anthracnose were from Ecogrowth and Revus/Ridomil treatments (Figure 6). Stem end rot was also highly variable amongst treatments, ranging in severity from less than 1% to 18% of fruit volume affected (Table 1, Figure 7).
- Anthracnose severity was higher in all WA trials in 2020 compared with 2019.

- Correlation analyses shows that the severity of anthracnose is positively correlated with pulp Mg (p=0.036), P (p=0.023) and K (p=0.009), so that as the concentration of these elements increases, then disease is worse. This trend for Mg and P was also observed in the Bamess Farms trial in 2020.
- Results and photographs from extensive fruit disease work, including isolation, culturing and identification via DNA sequencing work are on page 7-8.

Figure 4. Packout % by grade, 2020

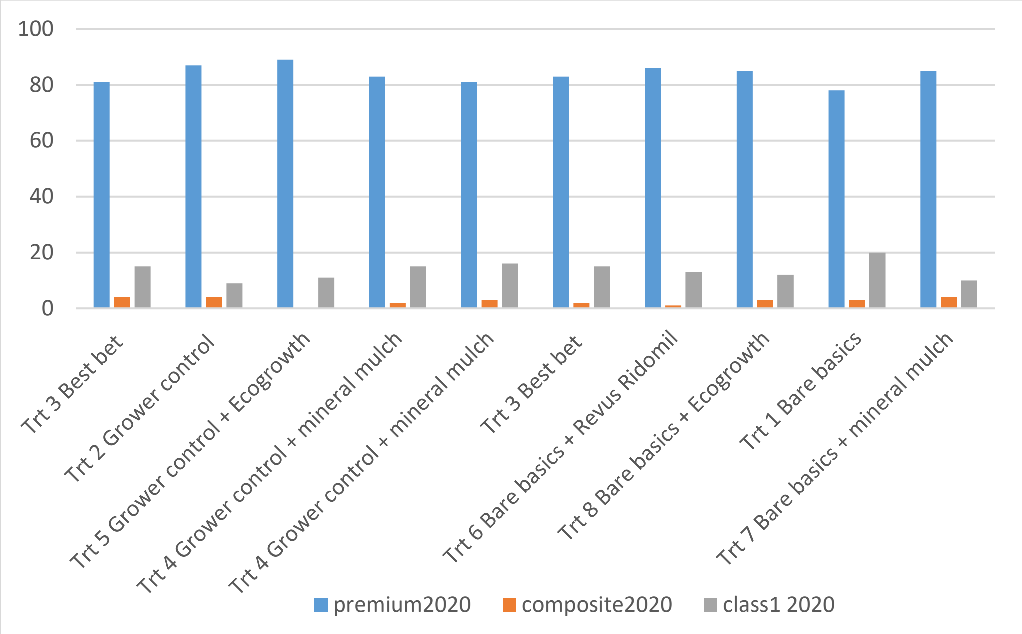


Figure 5. Premium packout %, 2019 and 2020

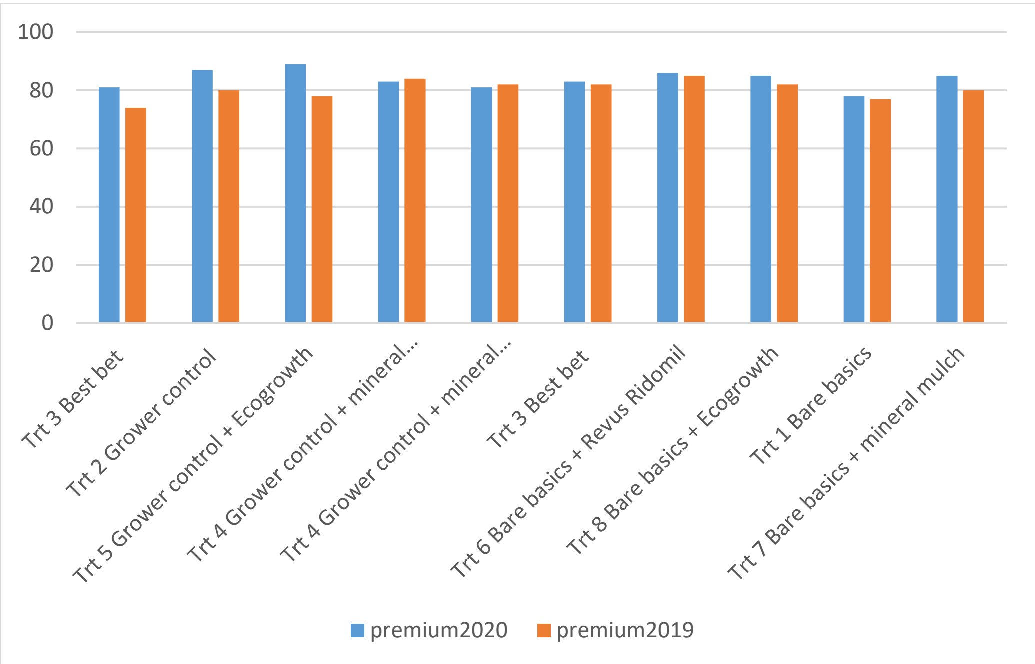


Figure 6. Fruit anthracnose severity at eating ripe stage 2019 and 2020

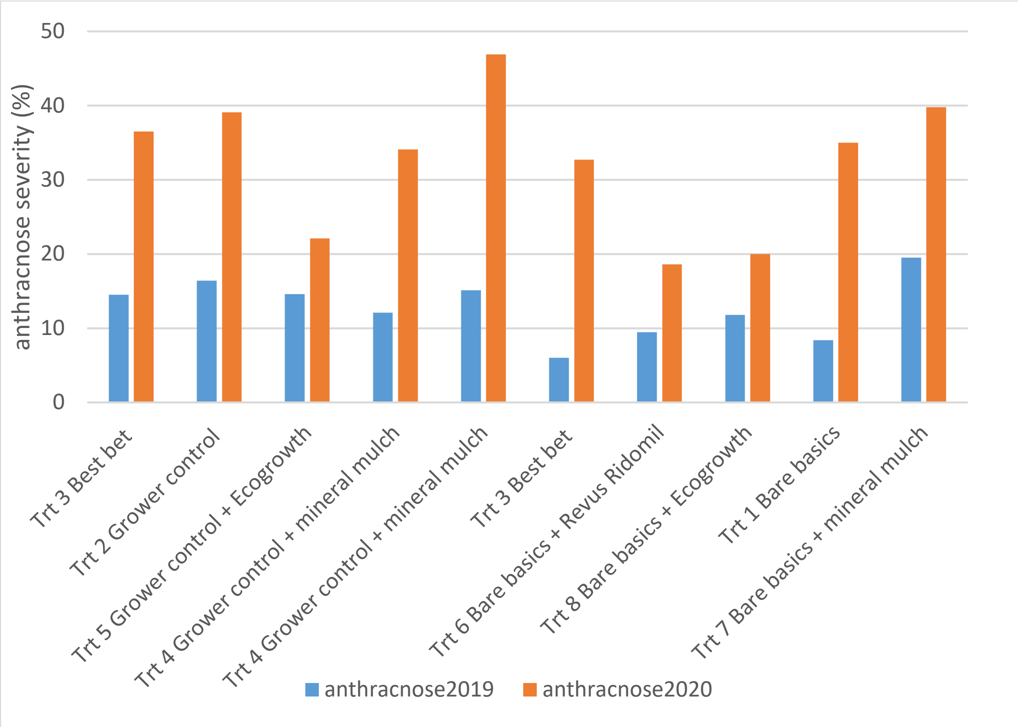


Figure 7. Fruit stem end rot (SER) severity at eating ripe stage 2019 and 2020

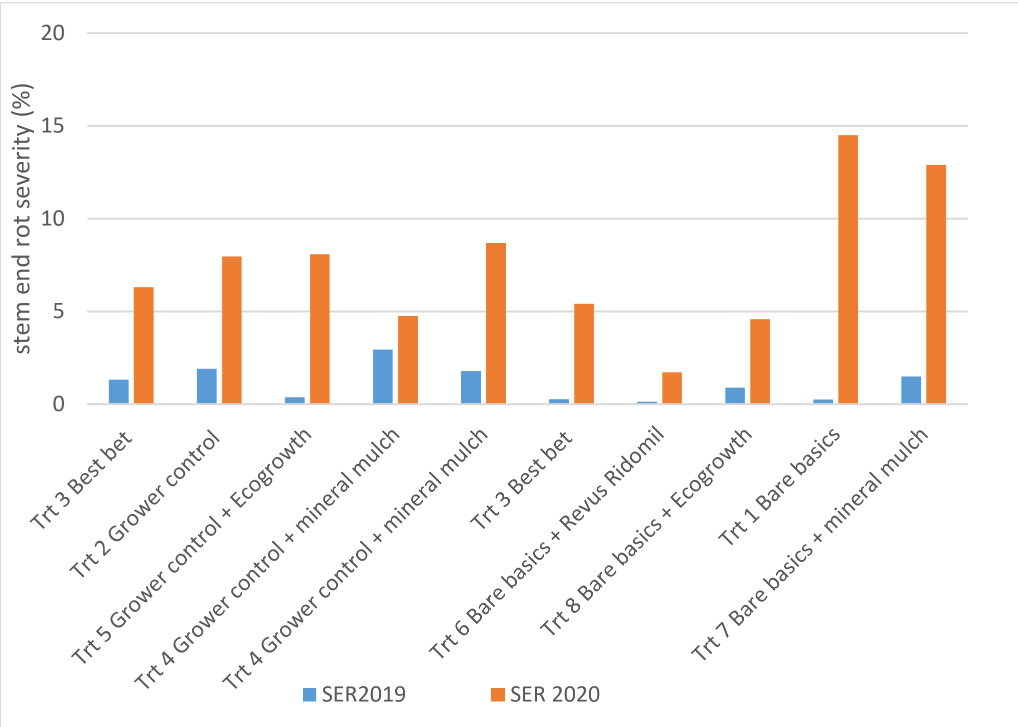


Table 1. Yield, packout and disease severity for fruit harvested from AV16007 soil amendment trial, West Pemberton Avocados, 7 October 2020

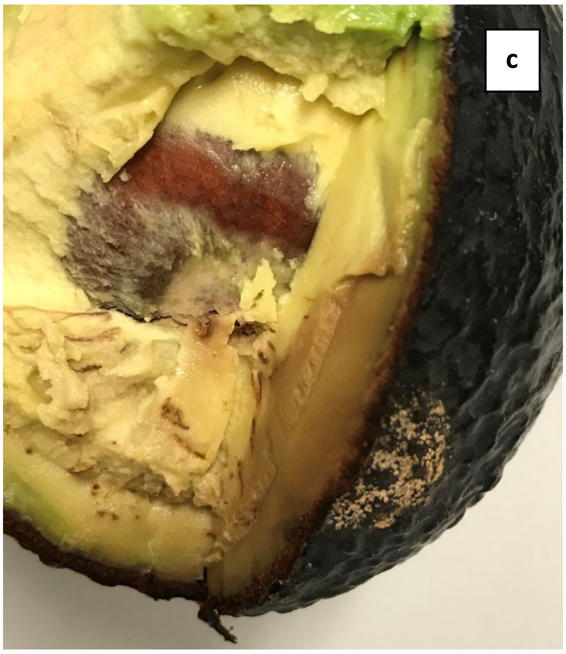
	WPA harvest No.	APMS Run no	Box size	DES	anth sev	SER sev	DM%	Total yield (kg)	Premium %	Comp'site %	Class 1 %	Av size
Best Bet	1	1029	23	20.6	40.3	5.5						
			25	20.3	33.2	7.05						
Average				**20.5**	**36.5**	**6.3**	**29.9**	**565**	**81**	**4**	**15**	**24**
Best Bet	6	1028	20	19.5	37.1	0.88						
			23	19.6	29	9.25						
Average				**19.5**	**32.7**	**5.41**	**30.4**	**522**	**83**	**2**	**15**	**23**
Grower control	2	1030	18	20.5	45	10						
			20	20.4	33.8	6.18						
Average				**20.4**	**39.1**	**7.97**	**29.6**	**561**	**87**	**4**	**9**	**23**
Bare Basics	9	1035	18	20.2	25.7	14.7						
			20	20.6	43.2	14.4						
Average				**20.4**	**35**	**14.5**	**32.7**	**402**	**78**	**3**	**20**	**26**
Grower+Ecogrowth	3	1034	18	19.5	14.3	3						
			28	19.9	25.8	10.8						
Average				**19.9**	**22.1**	**8.08**	**30.9**	**398**	**89**	**0**	**11**	**22**
Grower+MineralMulch	4	1032	20	20.3	30	0.29						
			25	20.2	37.2	8.04						
Average				**20.2**	**34.1**	**4.75**	**30.2**	**635**	**83**	**2**	**15**	**23**
Grower+MineralMulch	5	1031	16	20.7	52.1	5.71						
			23	20.9	43.3	10.8						
Average				**20.8**	**46.9**	**8.68**	**31.3**	**378**	**81**	**3**	**16**	**21**
Bare Basics+Ecogrowth	8	1026	20	19.8	26.8	5						
			23	19.6	14.3	4.25						
Average				**19.6**	**20**	**4.59**	**31.9**	**712**	**85**	**3**	**12**	**25**
Bare Basics+MineralMulch	10	1033	16	20.9	50.8	17.7						
			18	19.9	30.3	8.67						
Average				**20.4**	**39.8**	**12.9**	**32.3**	**836**	**85**	**4**	**10**	**22**
BareBasics+Revus/Ridomil	7	1027	18	20	17	0.33						
			20	20	20	2.94						
Average				**20**	**18.6**	**1.72**	**28.0**	**790**	**86**	**1**	**13**	**24**

DES= Number of days from harvest to eating soft; anth sev = anthracnose severity; SER sev = stem end rot severity; DM = pulp dry matter

Fruit disease symptoms and fungal culturing work, 2020

- An unusual symptom type was observed in a high proportion of fruit ripened at 23C and 65% RH. Large, tan-brown spongy lesions developed on the base of fruit (Figure 8 a-c). These were not typical anthracnose lesions, although there were also plenty of those. *Colletotrichum* sp. was most consistently isolated onto selective media from these lesions. The characteristic pink spores are evident on fruit surface in Figure 8c.
- A diffuse grey/brown rot was also common at the base and also on sides of the fruit eg. Figure 9. *Colletotrichum* sp. and *Neofusicoccum parvum* were consistently isolated.
- *Neofusicoccum parvum*, *Colletotrichum*, *Fusarium* and *Diaporthe* were commonly isolated from typical stem end rot lesions. These are all known to be associated with fruit SER. The *Fusarium* caused a very dark red flesh colour at the stem end, similar to that caused by *C. fiorinae* (the "cool climate" Colletotrichum).
- There was no *C. fiorinae* or *Lasiodiplodia* sp. isolated from any symptom type in 2020.

Figure 8 a-c. Spongy brown lesions present at the base of many ripe fruit in 2020.



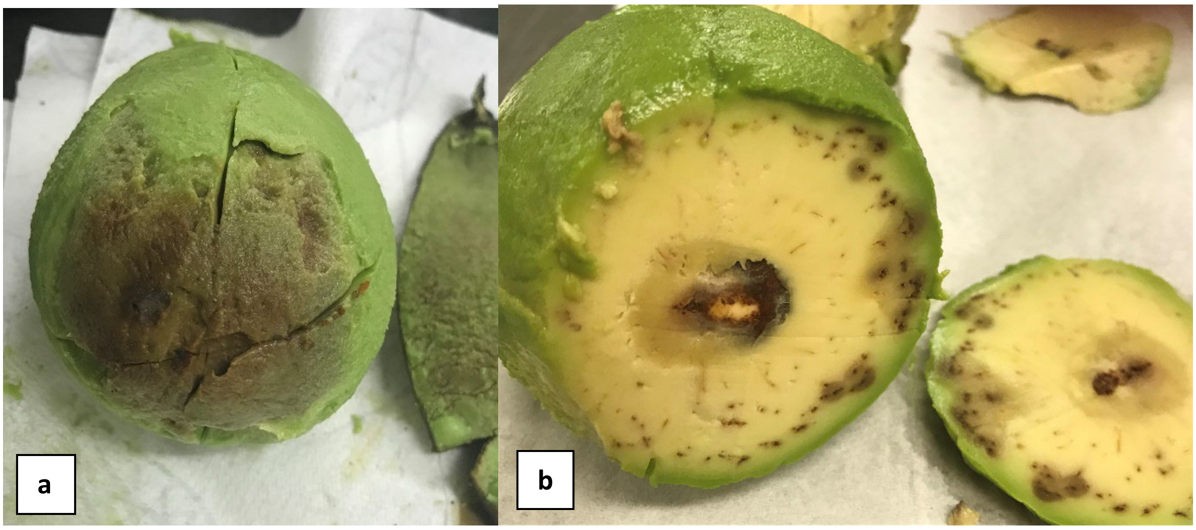


Figure 9 a,b. Diffuse grey-brown lesion originating at the basal end and extending to the seed and through the vascular tissue.

2020 pulp nutrient data (October 2020)

- There were no obvious trends or treatment differences in pulp nutrients in 2020 fruit, although after 2 years of regular application, a higher Si content in pulp of fruit from Mineral Mulch treatments was expected. Si in fruit from the three MM treatments was less than the average of 293 ppm. Highest Si content, 365ppm was in fruit from Bare basics treatment (nutrient results for each treatment are available but not presented here).
- 2019 and 2020 leaf and pulp nutrient data averaged across treatments is presented in Table 2.

2020 leaf nutrient data (May 2020)

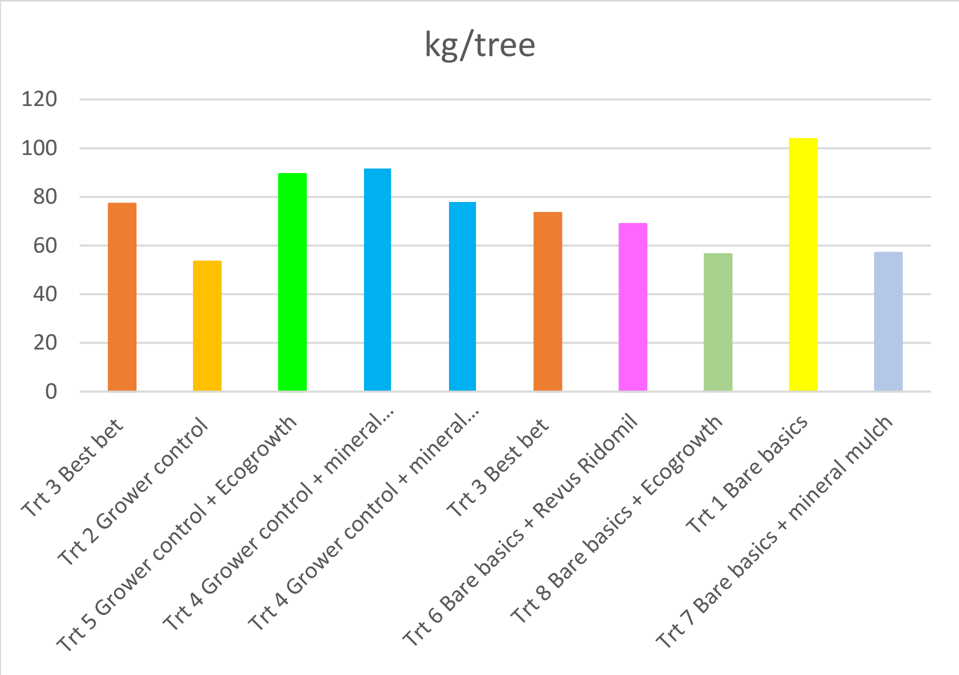
- Taking average across all treatments, there were increases over 2019 leaf nutrients in Ca (33%), Mg (38%), Mn (37%, but still within the OK range according to Phosyn) Zn (45%), and Si (43%)
- N decreased (by 15%)
- Leaf B is 38.4 ppm, on the low side (down by 50% on 2019)…. but maybe better being low in leaf now when it can be corrected, than last year when it was 74 ppm, and likely too high.
- Zn was highly variable across treatments in 2020 (range 34-132ppm), compared with more consistent range in 2019 (29-44ppm)
- Mn also highly variable across treatments in both 2019 and 2020
- No outstanding or consistent treatment effects (2020 data)
- No significant correlations between tree health assessed in March 2020 and leaf nutrients from samples collected May 2020

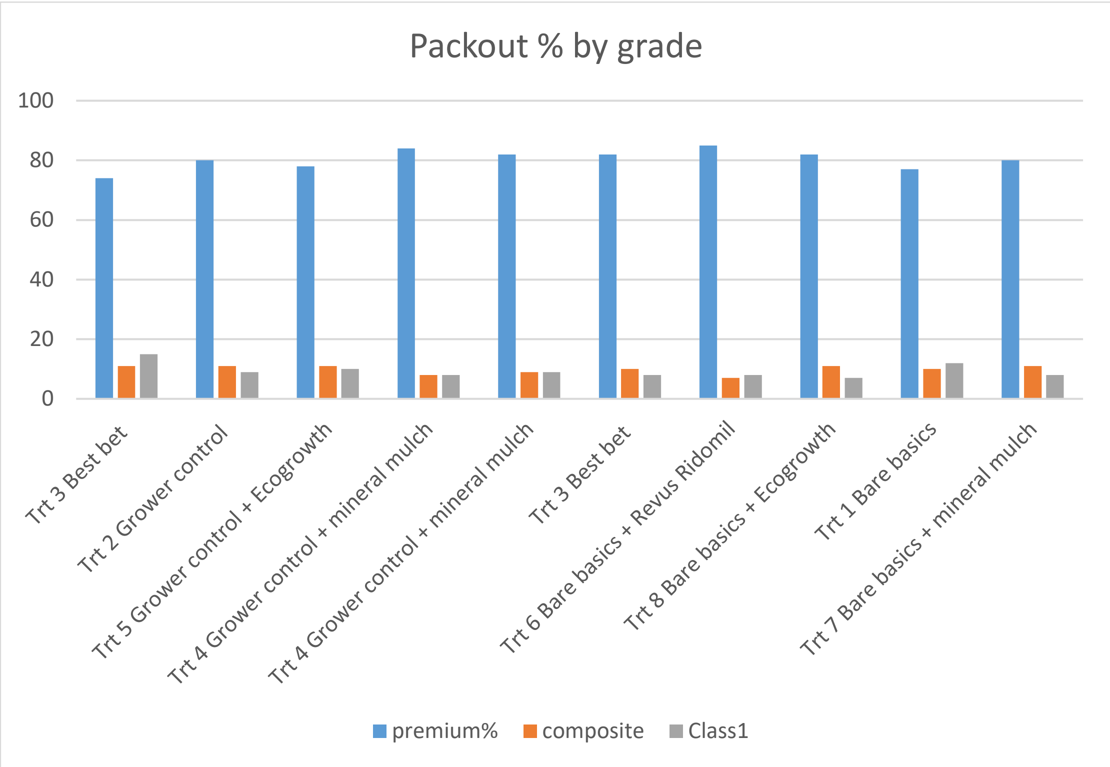
Table 2. Comparison of pulp and leaf elements across all treatments in 2019 and 2020

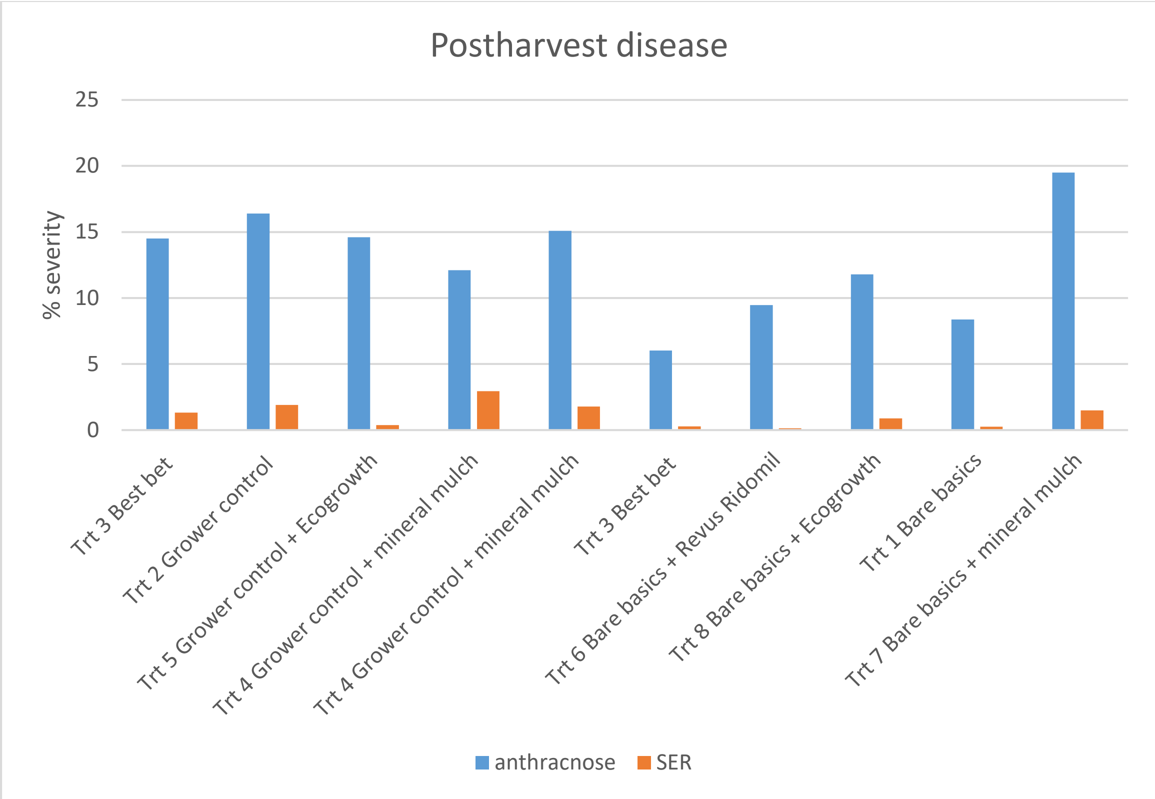
Element	Pulp		Leaf	
	2019	2020	2019	2020
Nitrogen (%)	0.739	0.843	2.765	2.332
Calcium (%)	0.027	0.029	1.392	1.857
N:Ca	29.4	29.1	1.99	1.256
Boron (ppm)	74.3	57	74.1	38.4
Magnesium (%)	0.071	0.066	0.296	0.407
Potassium (%)	1.218	1.397	1.062	1.031
Zinc (ppm)	12.8	11.1	36.5	89.4
Iron (ppm)*	38.8	23.8	55.5	205
Copper (ppm)*	2.41	1.45	194.3	417
Sulfur (%)	0.103	0.172	0.227	0.232
Phosphorus (%)	0.132	0.148	0.185	0.168
Silicon (ppm)	344	293	248	354

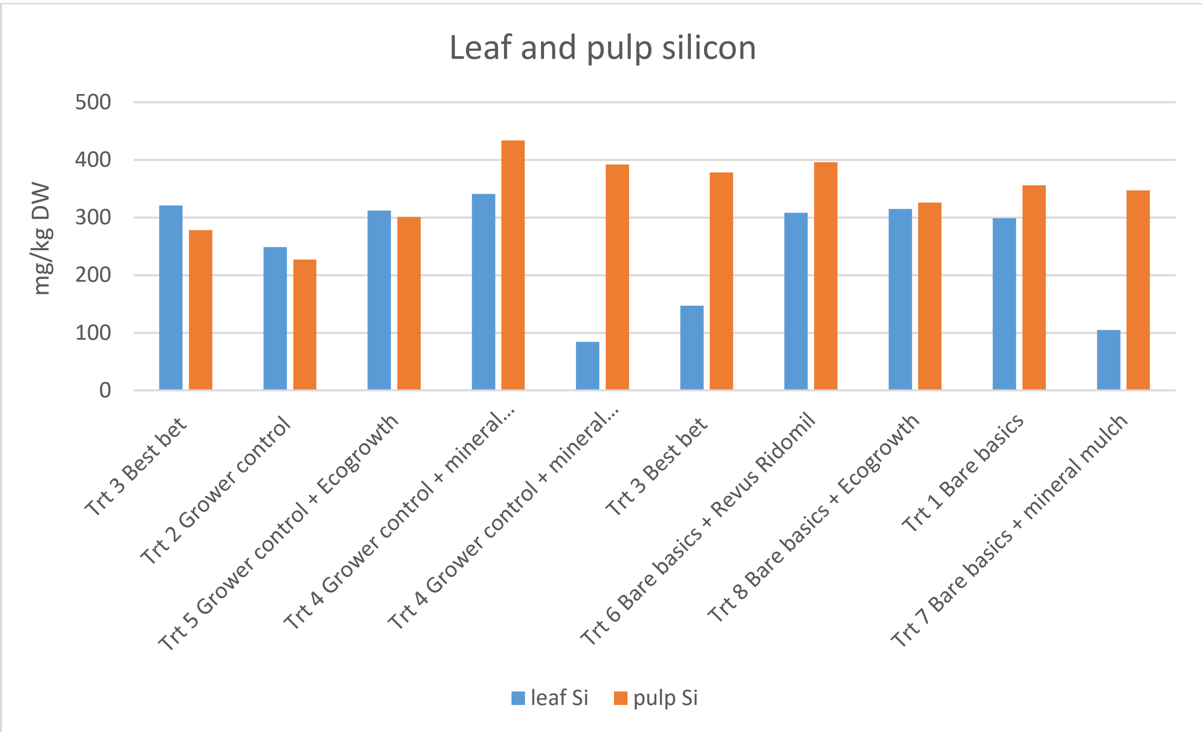
* Cu and Fe are strongly influenced by fungicide applications and dust on leaf surface which was not washed off prior to analyses
In 2019 – leaf samples collected 1 May 2019, fruit harvested 18 November 2019
In 2020 – leaf sampled collected, 11 May 2020, fruit harvested 7 October 2020

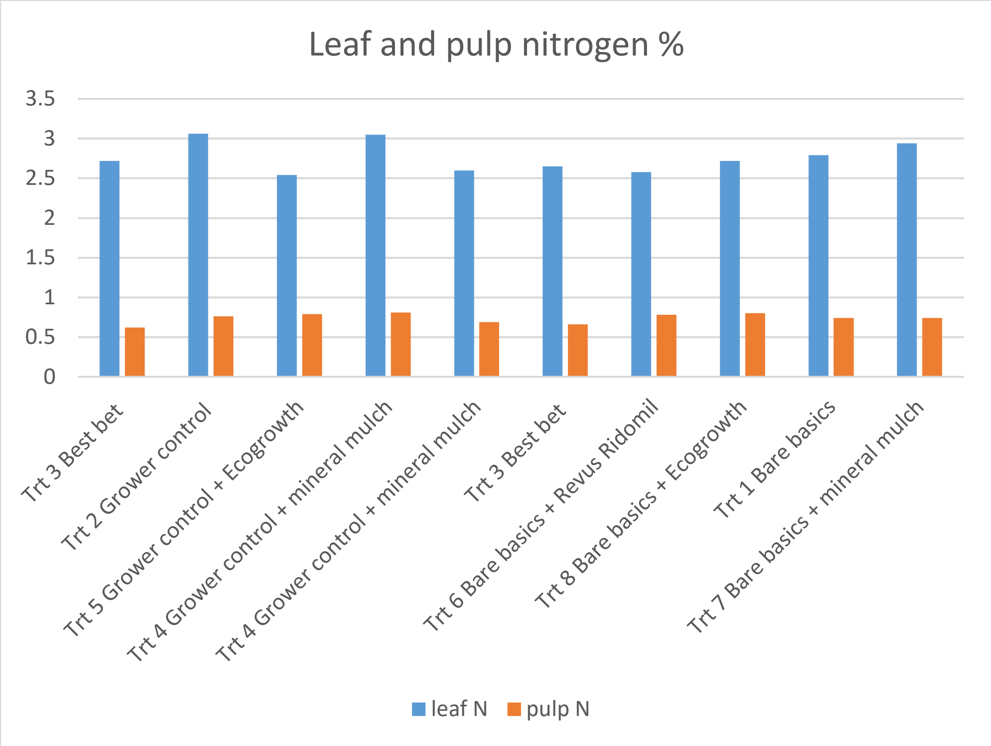
2019 results

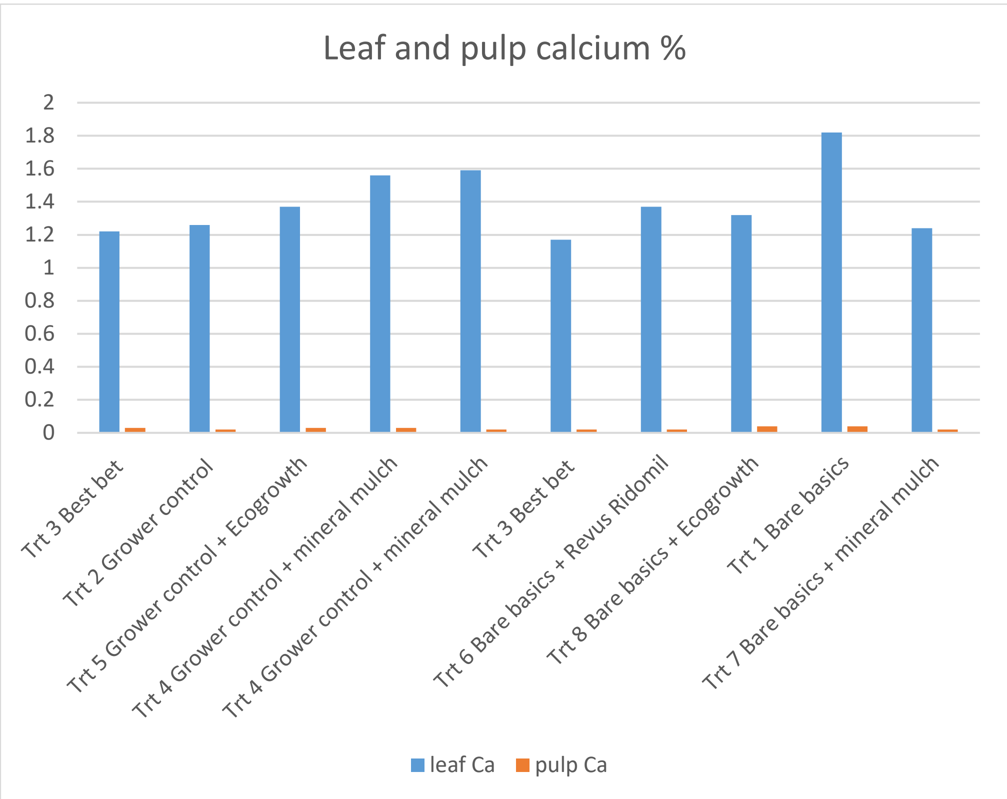





Postharvest disease
% severity
25
20
15
10
5
0
Trt 3 Best bet
Trt 2 Grower control
Trt 5 Grower control + Ecogrowth
Trt 4 Grower control + mineral mulch
Trt 4 Grower control + mineral mulch
Trt 3 Best bet
Trt 6 Bare basics + Revus Ridomil
Trt 8 Bare basics + Ecogrowth
Trt 1 Bare basics
Trt 7 Bare basics + mineral mulch
anthracnose
SER

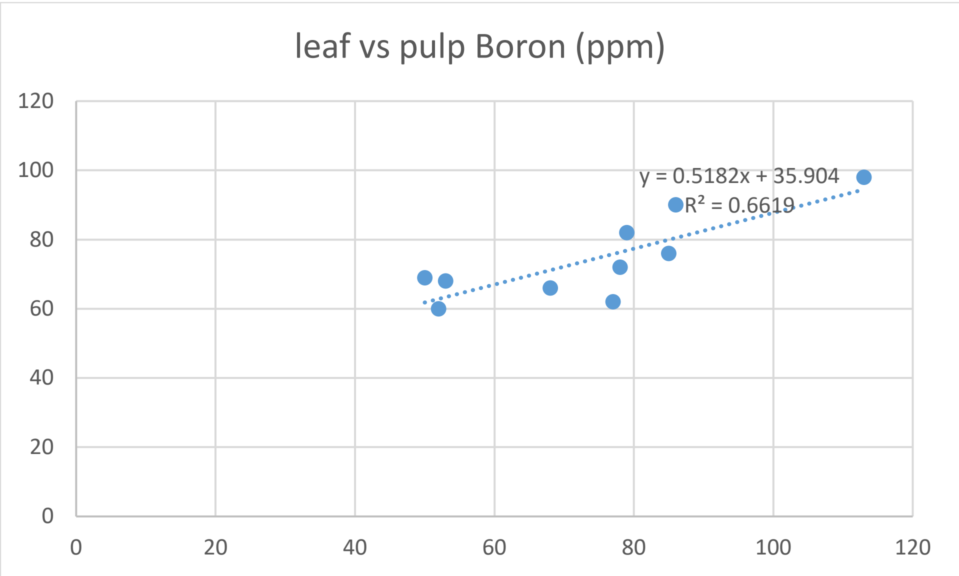

Leaf and pulp silicon
mg/kg DW
500
400
300
200
100
0
Trt 3 Best bet
Trt 2 Grower control
Trt 5 Grower control + Ecogrowth
Trt 4 Grower control + mineral...
Trt 4 Grower control + mineral...
Trt 3 Best bet
Trt 6 Bare basics + Revus Ridomil
Trt 8 Bare basics + Ecogrowth
Trt 1 Bare basics
Trt 7 Bare basics + mineral mulch
leaf Si
pulp Si

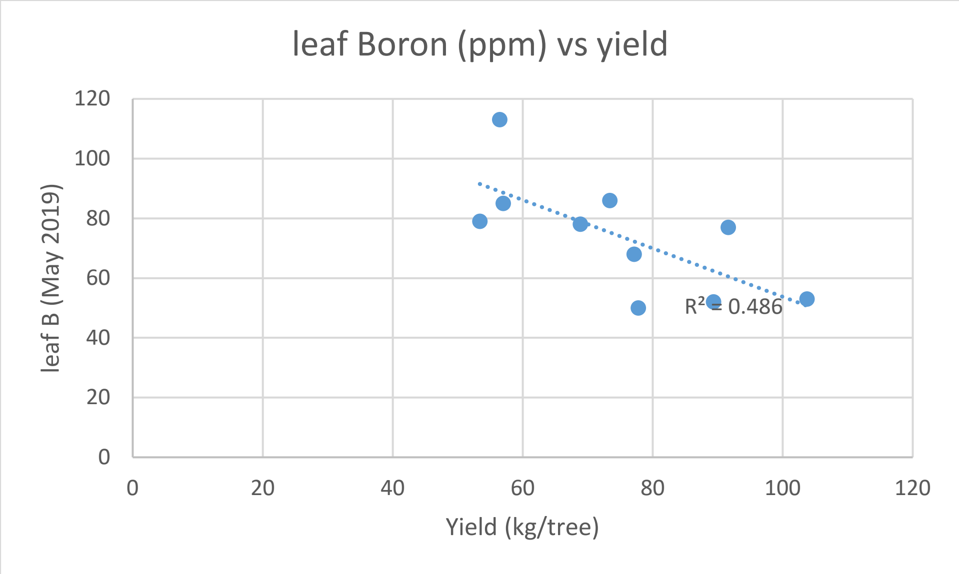


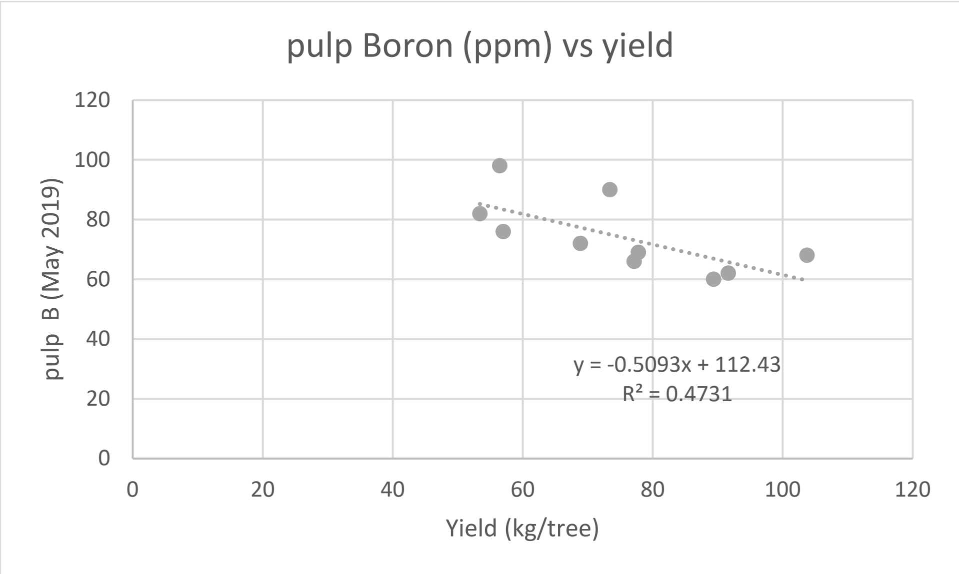


Isolation results from 6x WPA ripe fruit, December 2019

Date		Tissue isolated from	*Colletotrichum fioriniae*	Botryosphaeria sp	Others	Comments
6/12/2019	W.P 1	Fruit peel	0	0	0	No growth
7/12/2019	W.P 3	Fruit tissue	4	0	4x Aspergillus	
7/12/2019	W.P 4	Fruit tissue	0	0	4 *Fusarium sp* (Orange fluffy growth)	
9/12/2019	W.P 5	Fruit tissue (stem end rot)	0	4	0	
9/12/2019	W.P 6	Fruit tissue (stem end rot)	0	1	3(Aspergillus)	







Significant negative correlation between leaf (May) and pulp (Nov) boron and yield. Ie. higher boron negatively impacts yield.

- Statistically significant correlations:

Yield vs	leaf B (-)
-	pulp B (-) ie. Higher B, lower yield
-	leaf Ca (+)
-	leaf Mn (-)
Tree health May 2019	leaf Si (-)
Tree health Nov 2019	pulp N (-) P=0.05 leaf N:Ca (+) P=0.0528

% premium pulp Si (+) P=0.0456
Class 1 (-)
composite (-) makes sense, as more fruit in premium, then less in Class1 and composite

% SER leaf N (+)

% anthracnose – no significant correlations

ALL WA correlations

- 20 data points
- Statistically significant correlations:

Yield vs	Ca (+)
	pulp B (-)
	anthracnose, SER (-)
% Premium vs	leaf N, pulp N, leaf B (-)
leaf N vs	anthracnose, SER (-)
	leaf B (+)

Investigating the microbial activity in the rhizosphere and bulk soil from declining and healthy avocado trees

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2 Queensland Alliance for Agriculture and Food Innovation (QAAFI), University of Queensland, Australia

Introduction

Tree decline due to Phytophthora root rot (PRR) is one of the major constraints to orchard productivity in the avocado industry worldwide. Well-mulched orchards with high soil organic matter promote feeder root growth and stimulate the population and diversity of microorganisms which likely suppress *Phytophthora cinnamomi* and other soilborne pathogens. The interactions between tree health, *P. cinnamomi* and microbial activity in different seasons are likely to be complex and have not been well researched.

Aim

- **To investigate the microbial activity in rhizosphere and bulk soil from healthy and declining avocado trees in two seasons**
- **To compare two methods for measuring soil microbial activity (cotton decomposition and MicroResp^{TM})**

Methods

- Several healthy and declining mature avocado trees (Figure 1) were selected from a commercial orchard at Glasshouse Mountains, in the Sunshine Coast hinterland of Queensland
- Rhizosphere (Rhizo) soil and roots were collected from the top ~5-8 cm after removing mulch, and bulk (Bulk) soil was collected from 10-20 cm depth in June and December 2021
- **Direct root plating** on 3P and sPDA selective media was undertaken to isolate *P. cinnamomi* and a common black root rot pathogen, *Dactylonectria* sp.
- **Lupin baiting** was done to confirm the presence of, and approximate the abundance of, *P. cinnamomi* inoculum in soil
- **Decomposition of cotton** was determined by sandwiching uniform-sized cotton tea towel squares in soil samples and maintaining in moist condition in the glasshouse. After 12 weeks the squares were recovered, dried and weighed
- **Soil microbial activity** was quantified by substrate induced respiration (SIR), using the MicroResp^{TM} assay

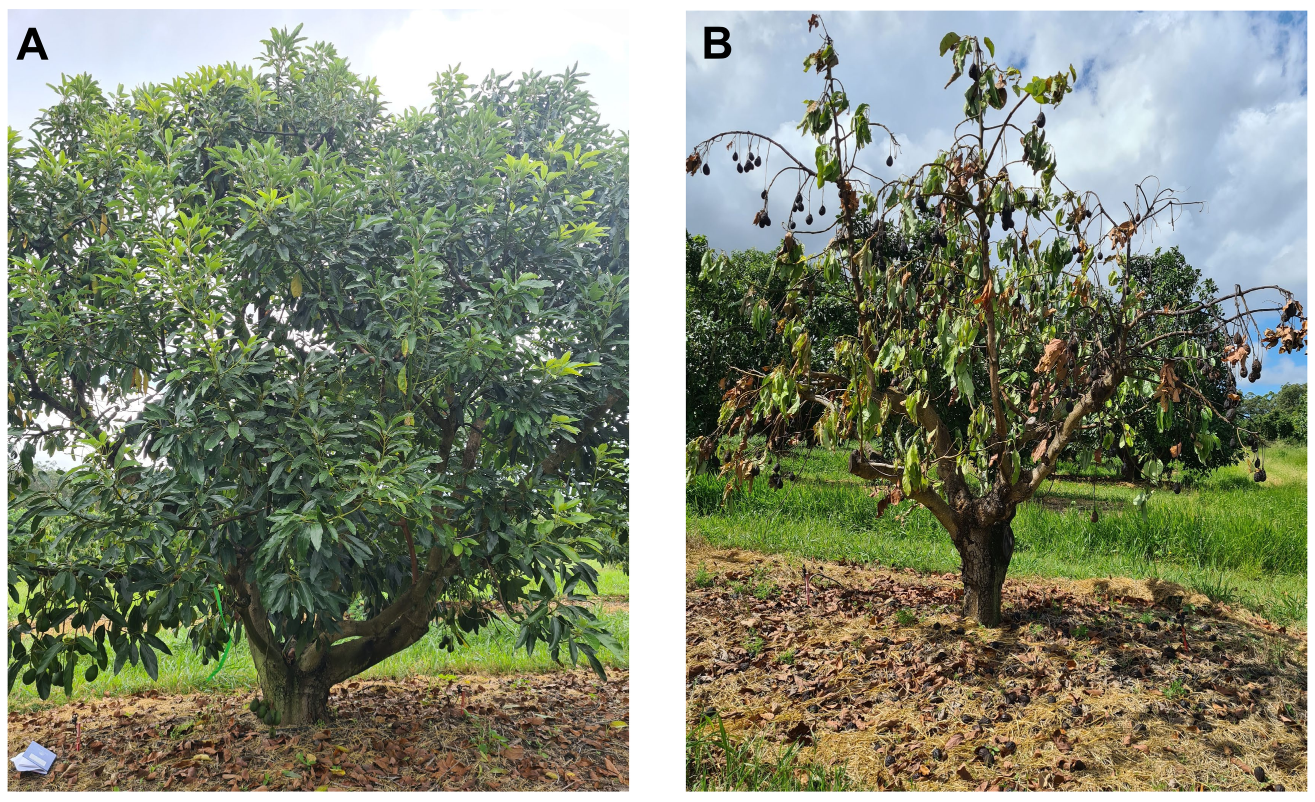
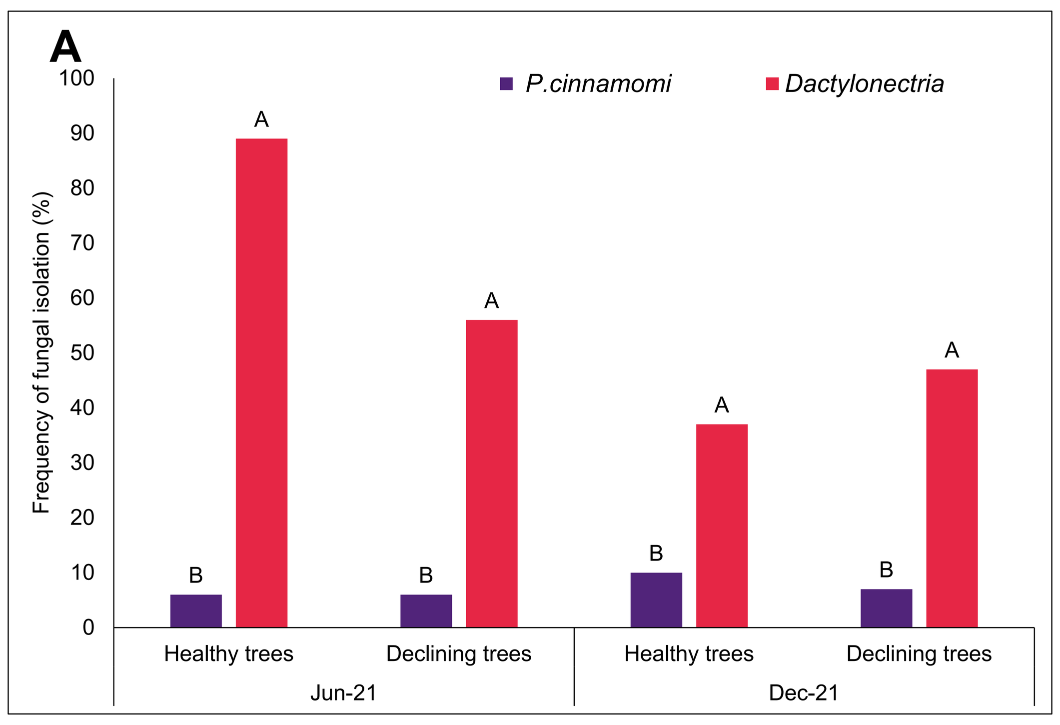


Figure 1. Representative pictures of healthy (A) and declining (B) avocado trees at Glasshouse Mountains, QLD in Dec 2021

Results

- High frequencies of *Dactylonectria* sp. isolated directly from avocado roots across all treatments at both sampling times with extremely low frequencies of Pc recovered (Figure 2A)
- Significantly higher percentage of wilted lupin seedlings observed in soils collected in June 2021, compared with soils collected in December 2021 (Figure 2B).
- Significantly greater ($P<0.001$) decomposition of cotton squares (Figures 3A & 4), and SIR (Figure 3B), observed in rhizosphere soil compared with bulk soil across sampling times
- No significant changes observed in abundance of *P. cinnamomi* (soil and root), rate of decomposition and SIR between soil collected from the declining and healthy trees
- Strong negative correlation (r = 0.66) between cotton decomposition and substrate induced respiration determined by the MicroResp^{TM} assay (Figure 5)



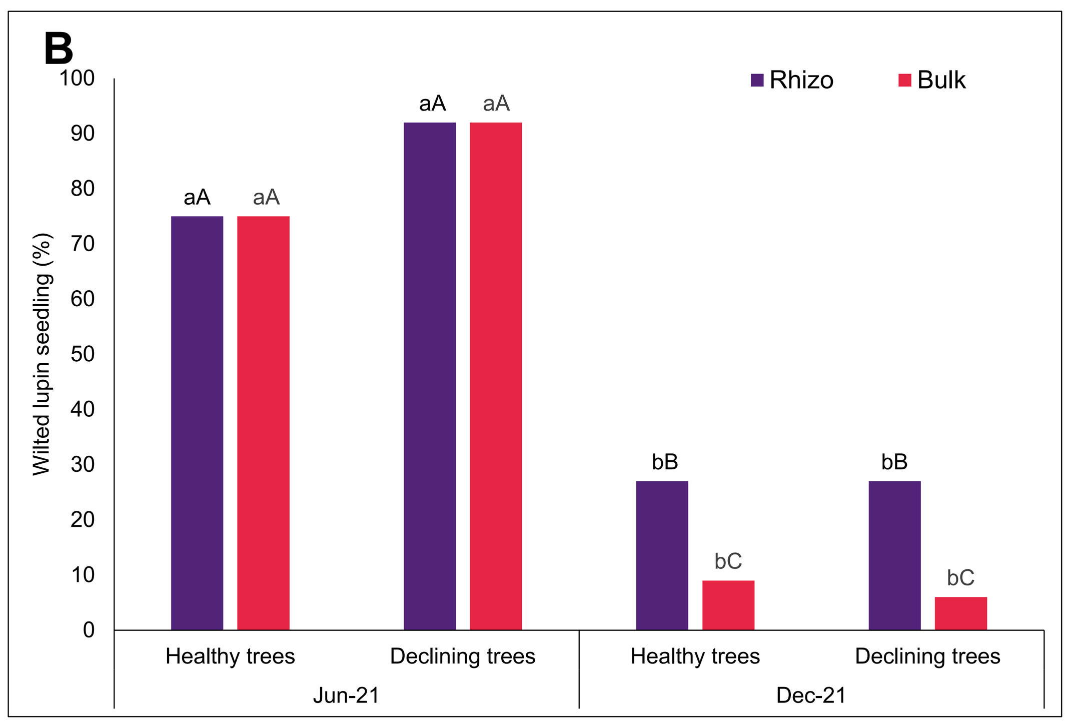


Figure 2A. Isolation of *P. cinnamomi* and *Dactylonectria* sp. from roots collected in Rhizo soil
Figure 2B. Wilted lupin seedlings as a proxy for *P. cinnamomi* propagules in soil samples. Bars with same letter indicate no significant differences ($P<0.001$); lowercase = sampling time, uppercase = soil type

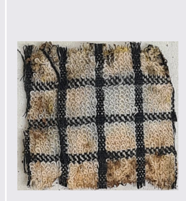
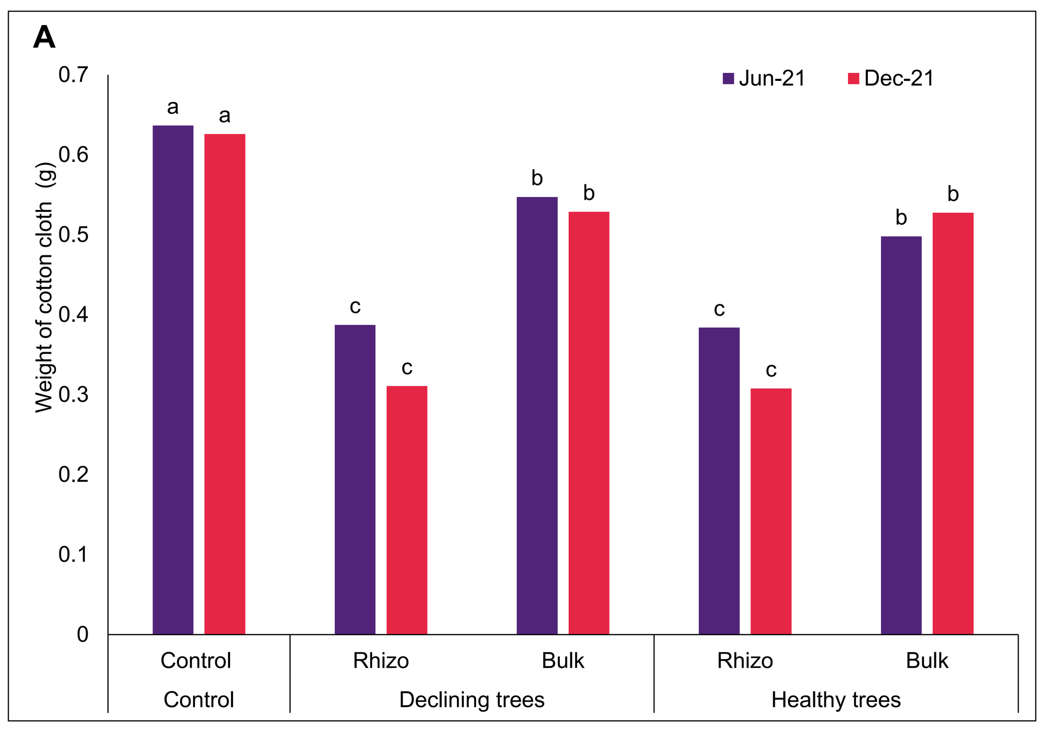
Sampling time	Control	Declining trees + Rhizo soil	Declining trees + Bulk soil	Healthy trees + Rhizo soil	Healthy trees + Bulk soil
June 2021					
Dec 2021					

Figure 4. Representative pictures of decomposition of cotton squares after 12 weeks incubation in each soil sample



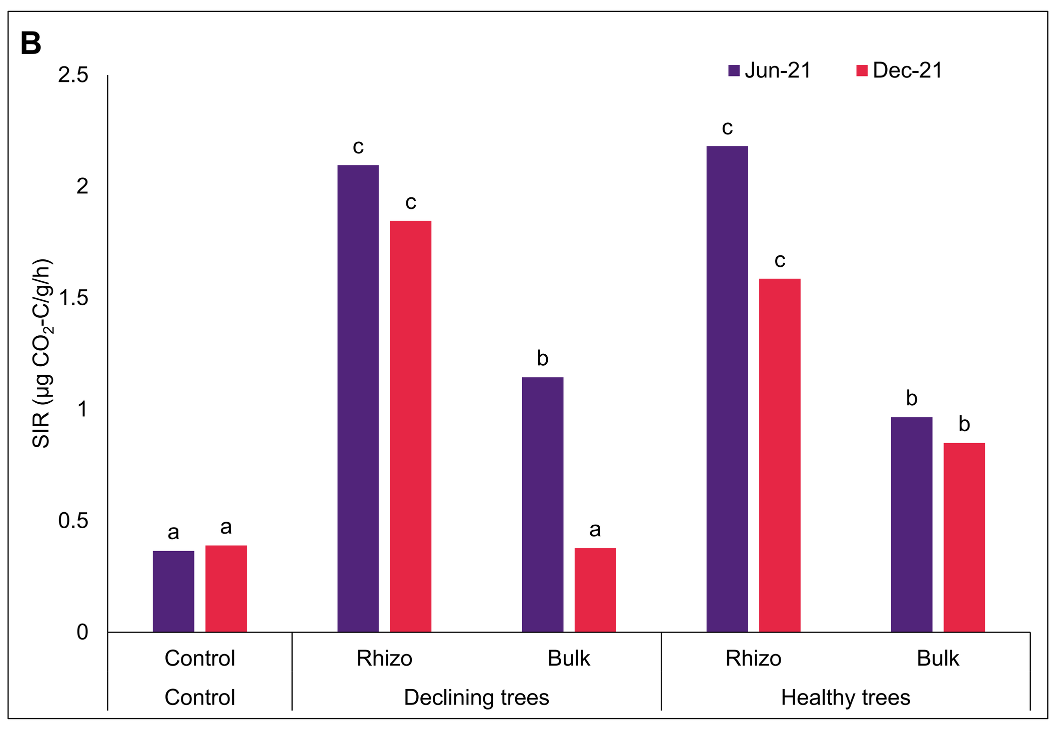


Figure 3. Weight of cotton cloth squares (A) after 12 weeks incubation, and substrate induced respiration (B) from Rhizo and Bulk soils. Bars with same letter indicate no significant differences ($P<0.001$) for soil type

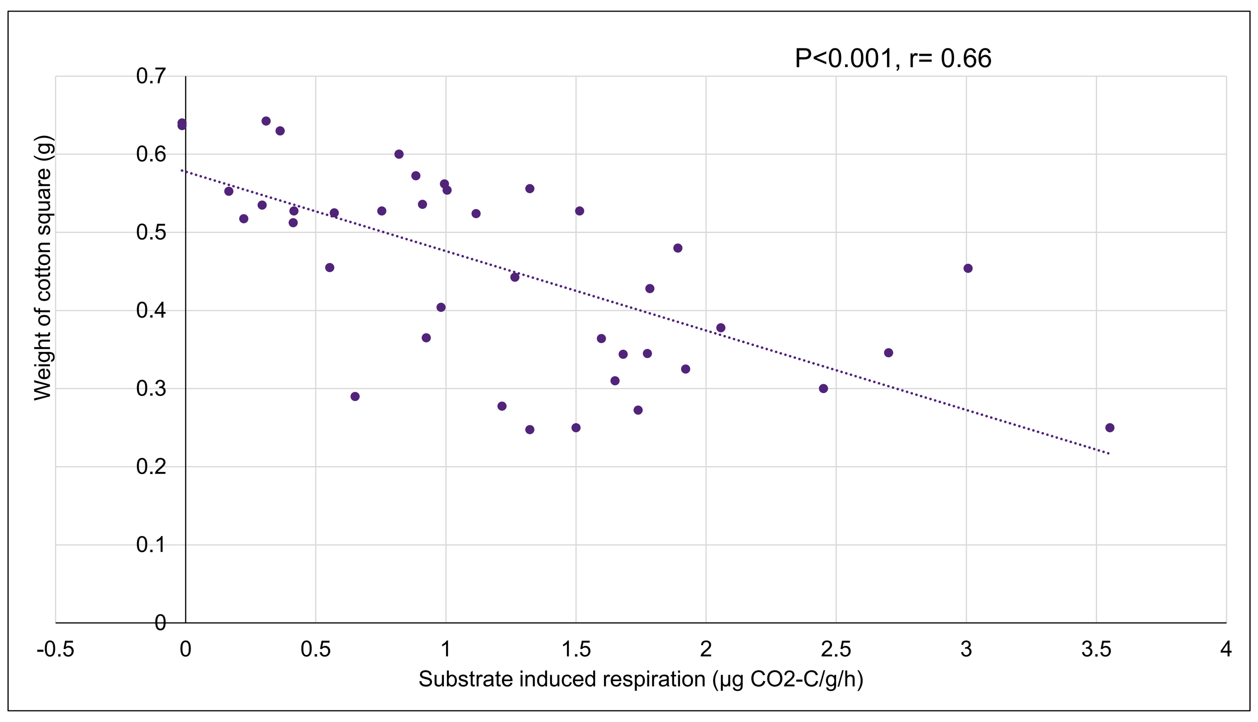


Figure 5. Correlation between cotton square weights (g) and the substrate induced respiration

Conclusions

- Surprisingly, *Dactylonectria* sp. was more frequently isolated from roots than *P. cinnamomi* and its possible contribution to mature tree decline requires further investigation
- Microbial activity was consistently greater in the rhizosphere soil compared to bulk soil
- There were no differences in microbial activity in rhizosphere soil between declining and healthy trees
- The cotton decomposition assay could be a useful on-farm tool for testing microbial activity in avocado orchards

Acknowledgements

- Dr Hazel Gaza (DAF) and Dr Vivian Rincon-Florez (QAAFI) for assistance with MicroResp^{TM} assay methodology
- Norm Pringle, Jahade Farms, Glasshouse Mountains
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Hort Innovation Strategic levy investment | AVOCADO FUND | THE UNIVERSITY OF QUEENSLAND AUSTRALIA | QAAFI Queensland Alliance for Agriculture and Food Innovation | Queensland Government

Queensland Alliance for Agriculture and Food Innovation

AV16007 Claytons Block 3 panicle dieback pruning trial – 2020-2022

Liz Dann, University of Queensland, updated 3 May 2023

Nov 2020 -selected 6 consecutive trees in rows either side of main trial. Trees were injected with phos acid, and pruned to approx. 2 – 3m and painted.

28 Jan 2021

11 Feb 2021 new shoots, sunburn, anthracnose


3 June 2021 – regrowth

<u>18 August 2021</u> – pre flowering, monolepta damage, early flower removal




<u>20 Sept 2021</u> - flowering

<u>8 October 2021</u> fruitset. Not every tree is flowering/fruiting

<u>February 2022</u>

Approx 1 tray per tree was harvested in June 2022.

<u>July 2022</u>