Final Report

An Integrated Disease Management Programme for the Australian Almond Industry

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Delivery partner:

The Victorian Department of Economic Development, Jobs, Transport and Resources (DEDJTR)

Project code:

AL16005

Project:

An Integrated Disease Management Programme for the Australian Almond Industry (AL16005)

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Funding statement:

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

Publishing details:

ISBN 978-0-7341-4856-8

Published and distributed by: Hort Innovation

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Public summary

The $1.63 billion Australian almond industry has seen a dramatic sixteen-fold increase in plantings in the last 20 years. Area of plantings increased from 3,546 hectares in 2000 to 58,523 in 2020. This rapid growth means that a significant percentage of plantings are young, with 19% of orchards not yet crop-bearing, and 33% not yet mature. In 2020, Australian almond production made up 8% of the global production of almonds, following California and Spain. In response to the rapid growth and intensification of almond production in Australia, there has been a great deal of research directed at integrated pest and disease management to ensure the sustainability of production.

As part of this research, disease surveys were conducted in 2018-2020 in the major almond production regions and diseases of almonds were identified. The most important diseases were found to be hull rot, trunk diseases, *Phytophthora* and lower limb dieback (LLD). Significant correlations were found between these diseases, agronomic practices and weather conditions.

Extensive field trials were conducted investigating the factors affecting hull rot, caused in Australia by *Rhizopus* sp. Variety and rootstock had a significant effect, as well as water availability. Mummy nuts only had a weak association with hull rot. The Ceres Imaging predictive tool based on thermal and multispectral imaging showed promise for identifying locations with high disease risk. Pathogen produced fumaric acid, which contributes to hull rot strikes, was influenced by water and nitrogen treatments. Management trials found that currently used fungicides reduced hull rot but did not eliminate it. The product diKaP™ a nutrition and stress tolerance product, previously investigated in California, had a similar success rate.

It was found that LLD is unlikely to be caused by trunk disease pathogens alone. Other factors such as tree age, light interception, cultivar and management practices are also involved.

Pathogenicity trials were conducted using seven different trunk disease pathogens. All species caused disease, some more consistently than others. It is also likely that factors such as water, nutrition and temperature affect canker development. *Phytophthora* and *Phytopythium* species were also tested, with many found to be aggressive pathogens. No effect of cultivar was seen among the six cultivars tested.

Of 23 fungicides tested *in vitro* against truck diseases, nine were progressed to orchard trials and four were identified as potential wound protectants. *In vitro* and detached fruit assays identified the three fungicides that were most effective against *Rhizopus*, one biological agent also acted comparably to many chemical treatments.

Economic analysis showed that grower actions to combat disease were economically beneficial but varied with disease. Treatments for trunk diseases had a positive economic benefit whereas the benefit of treatment for hull rot depended on the cost of the fungicide.

This project provided accurate up-to-date knowledge of the cause and expression of economically important almond diseases. This information is key to developing effective management strategies. An updated disease guide was developed and will be made available on the ABA website along with other resources such as fact sheets and presentations.

Technical summary

The Australian almond industry is a significant sector in the Australian horticulture industry, with an estimated farm-gate value of $1.63 billion and earning $545 million in exports [1]. The industry has expanded rapidly over the last 20 years with plantings increased from 3,546 hectares in 2000 to 58,523 in 2020. Australian almonds are grown in five major producing regions: Adelaide Plains and Riverland, SA; Riverina, NSW; Sunraysia, Vic; and the Swan Region, WA. Sunraysia produces the largest volume of almonds followed by the Riverland and Riverina. New plantings in the Riverina will soon see that region be the second largest contributor to the national crop. The Australian almond industry is striving for yield improvements through the development of sustainable orchards across regions with different climates and growing conditions, which has been linked to varying incidence and severity of diseases. The costs of diseases to industry can be considerable, based on orchard management inputs, reduced tree longevity, yield loss, downgraded kernel quality and loss of market confidence in Australian product. A good understanding of disease prevalence, causes and management is required to develop a robust integrated disease management (IDM) system for almond production, adaptable to changes in weather.

The overall objective of the project was to improve on-farm management of economically important almond diseases. This objective was addressed by:

- Conducting disease surveys across major almond–producing regions in Vic, SA, NSW and WA to determine the prevalence and impact of endemic diseases and factors contributing to disease increase and effectiveness of current management practices
- Undertaking trials to determine the cause and development of hull rot, lower limb dieback and trunk disease
- Undertaking trials to identify management practices for hull rot, lower limb dieback and trunk disease, including cultural, biological and chemical options
- Updating IDM guidelines for the diseases of economic importance in almond production in Australia.

An industry-wide disease survey over two seasons from 2018-2020 found the most prevalent diseases were shot hole, hull rot, lower limb dieback (LLD) and trunk diseases (which included Phytophthora). While shot hole symptoms were consistently observed, they were not considered serious as the disease severity was generally low and managed with fungicide spray programs. Less frequently recorded diseases were bacterial spot, anthracnose, scab, rust and foamy canker syndrome. Despite industry perception of anthracnose as widespread, very little anthracnose was observed, likely due to hot and dry weather during these two seasons. Conclusions from the survey were that hull rot, lower limb dieback and trunk disease are the most serious and widespread diseases in the Australian almond industry. Regional differences were apparent based on regional climatic conditions. Significant correlations were found between disease incidence and some agronomic practices and weather parameters. Hull rot symptoms were influenced by water (both irrigation and rainfall), variety and rootstock and fungicide application. Both lower limb dieback and trunk disease were influenced by age of trees.

During field surveys more than 400 isolates of 56 genera were collected. Approximately 200 were further identified using DNA fingerprinting. The majority were Rhizopus spp. (67) and trunk disease pathogens (65) and *Phytophthora* spp (13). The causal organism of hull rot was always *Rhizopus spp*. Pathogens associated with trunk disease symptoms included *Diplodia seriata*, *Eutypa lata*, *Colletotrichum acutatum*, *Collophora hispanica*, *Collophora rubra*, *Pleurostoma richardsiae* and *Cytospora diatrypelloidea* and *Phytophthora* spp.

Field trials were conducted to investigate factors influencing hull rot, including variety and rootstock susceptibilities and management practices that impact on disease outcomes.

Hull rot assessment of four Australian breeding program plantings covering six commercial varieties (Capella, Carina, Maxima, Mira, Rhea and Vela) and 23 advanced breeding lines showed material had less hull rot than the industry standard Nonpareil. Evaluation of an established rootstock trial over two seasons showed that Nonpareil grafted onto Peach Almond *(P. persica x P. dulcis*) rootstocks particularly Monegro, Garnem, Hansen and Felinem developed more hull rot than other rootstock choices.

Investigating the influence of water and nitrogen on hull rot over two seasons showed high disease severity was reduced with a sustained deficit water schedule of 70%ETc (10.5 ML water/ha) while restricted nitrogen at 179 kgN/ha did not influence hull rot. Fumaric acid analysis of infected hulls of Nonpareil found high levels in 15 ML water/ha treatments and 320 kgN/ha nitrogen treatments compared to restricted water and nitrogen treatments.

Three seasons of validating the Ceres Imaging hull rot prediction tool based on thermal and multispectral imagery data shows promise. This technology will enable orchards to prioritize fungicide application in high-risk areas and follow up with a second spray after a significant rainfall event.

Management trials at hull split found that current chemistry can reduce hull rot but not eliminate the disease. diKaP imported from California shows similar efficacy to registered products. A timely application of a protectant product at hull split is beneficial in high-risk hull rot areas.

Mummy formation and orchard canopy hygiene were investigated in three field trials. The findings suggest hull rot is not the primary cause of mummy formation and that several factors are involved. Neither are retained canopy mummies the primary cause of hull rot in the following season. Mummy retention can be reduced by appropriate agronomy in the lead up to shaking. Hull rot strike development takes between 7 – 10 days after infection, resulting in spur death which reduces productivity in future seasons.

Both *In vitro* and detached fruit fungicide evaluation for hull rot identified Luna Experience (fluopyram + tebuconazole), Seguris Flexi (isopyrazam) and Blue Shield (copper hydroxide) as the most effective chemical products when applied prior to inoculation with Rhizopus. In detached fruit assays most products had little effect as curatives with the best being fluopyram + trifloxystrobin (Luna Sensation). The best biological control agents were products containing *Bacillus amyloliquefaciens* (Serenade Opti and Serifel) which performed comparably to many of the chemical treatments.

A PhD study demonstrated differences in hull composition of susceptible Nonpareil and resistant Carmel, and the changes that occur when grown under various nitrogen and water regimes which affect the growth of *Rhizopus* and its metabolite production. *Rhizopus* isolates from almonds produced both fumaric acid and lactic acid in infected Nonpareil hulls, and their production was greatest under the high-nitrogen and high-water treatment. Both fumaric and lactic acid were toxic to almond shoots *in vitro*.

Following the collection of isolates during the disease surveys, evaluations were conducted to determine pathogenicity, cultivar susceptibility and fungicide efficacy to better understand and manage trunk disease in Australia.

The pathogenicity of fungal species *Ca. rubra, Ca. hispanica, C. diatrypelloidea, Cm. acutatum, D. seriata, E. lata* and *P. richardsiae* were evaluated in potted tree experiments. They revealed that all species caused disease, some more consistently than others. It is likely that extrinsic factors also influence canker development, possibly including water stress, soil nutrients and temperature. It is important to investigate how these factors may influence common trunk disease pathogens to refine management recommendations.

Pathogenicity testing was also conducted on the oomycete species. Most of the *Phytophthora* and *Phytopythium* spp. were aggressive, causing lesions within 6 weeks similar to that caused by other trunk pathogens over 6 months. The *Pythium sp.* was not pathogenic. These results suggest that Phytophthora species are amongst the most aggressive pathogens affecting almonds in Australian orchards.

To investigate the susceptibility of cultivars, an experiment was conducted to evaluate six almond cultivars, commonly planted in Australia, against five pathogenic fungal species. Results suggest that the tested cultivars do not vary in susceptibility to fungal trunk disease pathogens.

Previous studies in almond have shown that application of fungicides and biocontrols as wound dressings are an effective method of preventing infection by trunk disease pathogens. A list of fungicides and biocontrols was compiled for screening against trunk disease pathogens. Twenty-three commercial products were screened in the laboratory and nine products found to be effective in the screenings were evaluated in the orchard. Merivon®, Emblem®, Custodia® and Luna Experience® were identified as potential wound protectants.

Intensive studies were conducted to investigate the factors influencing LLD, including pathogens, cultivar, tree age and light. It was found that trunk disease pathogens are unlikely to be the only causative factor of LLD. Symptom severity increased with tree age, except for some orchards that had significantly less severe dieback in older trees, which suggested that management had an impact on LLD symptoms. Light is likely to have a significant impact on dieback severity and almond cultivar varied in dieback severity, with Nonpareil and Price having the most dieback.

An economic analysis in the final years of the program found grower actions to combat disease were economically beneficial but varied with disease. Fungicide treatment for trunk disease (Phytophthora) provided a positive economic benefit to growers for all treatment options and cost levels. The expected net benefit ranged from $A114/ha to $A200/ha, the benefit cost ratio was upward of 1.8:1 and outcomes were positive 99% to 100% of times.

One spray at hull split for hull rot gave mixed outcomes depending on the cost of the fungicide used. A low-cost fungicide provided a net benefit of $134/ha with a benefit cost ration of 2.2 with outcomes positive 97% of the time. A high-cost fungicide resulted in a negative net benefit of -$21/ha with a benefit cost ration of 0.9 with costs of control exceeded the economic damages caused by the disease with outcomes being positive only 37% of the time.

The outcomes of this project provided accurate up-to-date knowledge of the cause and expression of the economically important almond diseases hull rot, lower limb dieback and trunk diseases. An updated disease guide was developed in collaboration with the almond industry extension and adoption projects AL16000 and AL19001. These outcomes contributed to Outcome 1 of the Almond Strategic Investment Plan 2017-2021: by 2022 serious almond defects caused by diseases will have been reduced.

Further research and development of cultural management methods and improved decision support systems to manage disease is required for the almond industry to maintain productivity.

Keywords

Almond; Integrated Disease Management; industry-wide disease survey; hull rot; trunk disease; lower limb dieback

Introduction

The Australian almond industry is a significant sector in the Australian horticulture industry, with an estimated farm-gate value of $1.63 billion and earning $545 million in exports [1]. Australian almonds are grown in five major producing regions: Adelaide Plains and Riverland, SA; Riverina, NSW; Sunraysia, Vic; and the Swan Region, WA. Sunraysia produces the largest volume of almonds followed by the Riverland and Riverina. New plantings in the Riverina will soon see that region be the second largest contributor to the national crop. The Australian almond industry is striving for yield improvements through the development of sustainable orchards across regions with different climates and growing conditions, which has been linked to varying incidence and severity of diseases. The costs of diseases to industry can be considerable, based on orchard management inputs, reduced tree longevity, loss of yield, reduced kernel quality and loss of market confidence in Australian product. A good understanding of disease prevalence, epidemiology and management is required to develop a robust integrated disease management (IDM) system for almond production, adaptable to changes in regulatory policy and new disease incursions that may impact market access.

The Australian almond industry identified hull rot, trunk diseases lower limb dieback (LLD) and anthracnose as the high priority diseases increasingly reported in orchards. Hull rot and anthracnose result in increased numbers of mummies (‘stick-tights’ - mummified fruit remaining on the tree), creating breeding habitat for insect pests such as native *Carpophilus* beetles and the introduced carob moth, further reducing nut yield and quality. LLD reduces the productivity, and most likely longevity, of the orchards. There is currently little knowledge about the cause(s) of LLD. Anecdotally, orchard management practice may contribute and there have been several reports of trunk pathogens associated with almond dieback so extensive research is required to understand the aetiology so that effective management practices can be developed. Potential yield losses due to the various almond diseases is not well understood but will be driven by inoculum levels, climatic conditions and types of disease management practices used, and this information will be collected during this project.

Hull rot is a serious problem throughout all production areas in Australia [2], causing significant loss of productivity and yield [3]. *Rhizopus stolonifer* and *Monilinia fructicola* have both been implicated as the cause of hull rot overseas. In Australia, research conducted by Agriculture Victoria (AL11009) demonstrated that *R. stolonifer* was the main pathogen detected in Sunraysia.

Almond hulls are susceptible to infection from the beginning of hull split until the hulls dry [3, 4] and severely infected nuts become mummified and remain attached during harvest. Previous research demonstrated that mummies were the most likely source of *Rhizopus* inoculum for hull rot infections in the following season, and that rain during hull split increased the incidence of disease (AL11009). Cultivar differences were reported, with the highest incidence of hull rot in 2014 observed in Price. Research in California showed that hull rot strikes per tree can range from 200 to 500 nuts in susceptible varieties, with the number substantially decreasing in less susceptible varieties (Almond Board of California, 2009-2010 Annual Research Report). In addition to being a source of inoculum for hull rot pathogens, mummies provide breeding habitat for almond insect pests (AL15004). In California effective management of hull rot requires a combination of cultural measures including counting fruit mummies during the tree dormancy period, if present removing and destroying them to reduce disease pressure, and monitoring hull split timing and local environmental conditions to predict the risk of infection.

Understanding the aetiology and epidemiology of hull rot under Australian conditions is key to developing effective management. In California, management consists of four key elements; reduction of mummy numbers, deficit irrigation to induce hull splitting and reduce hull moisture levels, restricted nitrogen fertilization and well-timed fungicide application at hull split can reduce hull rot incidence by 60-70% [5]. However, fungicides recommended in California are not necessarily registered for use in almonds or for control of hull rot in Australia. Further research is required to evaluate Californian management practices under Australian production systems.

LLD has been increasingly reported in Australian almond orchards, causing serious concern to the industry. LLD is also an emerging problem in California [6] and Spain [7], and although the cause of LLD is yet to be fully understood, fungal wood pathogens have been implicated. Research is underway in California to understand the causative factors of LLD, with particular focus on diagnostics, aetiology and management and will provide a foundation for research and control strategies in Australia. Management strategies for limb and trunk diseases can include chemical or biological wound protection, remedial surgery and/or removal of trees, so accurate and timely diagnosis of these diseases is critical.

The fungus *Diplodia seriata* (syn. *Botryosphaeria obtusa*), the cause of band canker in California and Spain and dieback of other perennial tree crops [5, 7], was recently identified on almond trees suffering LLD in South Australia in early 2015 (Crop Health Services). In addition, *Botryosphaeria dothidea, D. seriata* and *Eutypa lata* have recently been isolated from branches showing dieback symptoms collected from almond orchards in South Australia and Victoria. Other pathogens are also likely to contribute to cankers and dieback [5].

Anecdotal reports in Australia suggest that LLD is likely to be associated with infection of pruning wounds, since dieback has been particularly apparent with the increased use of mechanical hedging, and cankers observed extending from wounds along twigs and branches. Poor light interception, particularly at the base of the canopy, toxin production by other fungi causing anthracnose, hull rot and brown rot as well as overwatering, have also been implicated. However, the extent, distribution and importance of LLD in the main almond growing regions is not known. It is important to establish the cause of LLD in almonds, before effective management strategies can be developed.

In the establishment phase of the Australian almond industry, anthracnose and rust were identified as serious diseases and funding allocated to address these (AL06007,[8]). Variable yield losses (infected fruit) to anthracnose were reported in major growing regions of Australia [8, 9]. In an epidemiological study in an almond orchard in South Australia over a three year period (2002-04), disease incidences were correlated with rainfall and daily temperatures, with greatest disease development observed in the coolest year with the greatest rainfall [8]. Anthracnose is also a serious problem in California, with symptoms observed on blossom, fruit, leaves and woody tissue. In severe cases with multiple infections, trees can have limb dieback [10].

Rust (*Tranzschelia discolour*), shot hole (*Wilsonomyces carpophilum*) and scab (*Venturia carpophila*) have been reported in Australia, requiring foliar disease control in some almond orchards [11]. Severe rust infections can cause premature defoliation of trees but the effect on crop yield is not well understood [12] and shot hole infections can cause crop losses of at least 36% [13]. Scab infections occur on both leaves and fruit, with severe leaf infections resulting in premature defoliation of trees [14]. Fungicide treatments, applied during flowering and early fruit stages, were necessary to control rust, shot hole, scab and blossom blight/brown rot (*Monilinia laxa*) in field trials in South Australia [11]. Control of multiple diseases increases on-farm management inputs.

The current situation with these and other diseases now that the industry has expanded considerably is unclear. Industry-wide disease surveys are required to provide clarity on the current prevalence and impact of diseases before strategies can be implemented to manage them.

Understanding how almond pathogens behave in the different Australian production regions is required in order to effectively tailor and implement IDM measures developed for California.

The overall objective of the project was to improve on-farm management of economically important almond diseases. This objective was addressed by:

- Conducting disease surveys across major almond–producing regions in Vic, SA and NSW to determine the prevalence and impact of endemic diseases and factors contributing to disease increase and effectiveness of current management practices

- Undertaking trials to determine the cause and development of hull rot, lower limb dieback and trunk disease
- Undertaking trials to identify management practices for hull rot, lower limb dieback and trunk disease, including cultural, biological and chemical options
- Updating IDM guidelines for the diseases of economic importance in almond production in Australia.

Methodology

Study tour to California and almond disease review

Two visits (July and September 2018) were made to UC Davis Kearney Research Center, California, to promote collaboration and to see first-hand the research conducted on hull rot and lower limb dieback in their production systems. While there are many fungal and bacterial diseases of almonds, the diseases of major concern are hull rot, lower limb dieback and trunk diseases, including *Phytophthora*. The causal organisms of these diseases are poorly understood as there are several putative pathogens for each disease. In California, several pathogens cause hull rot and more cause lower limb dieback and associated trunk diseases. Similarly, with *Phytophthora* diseases. There is little information about many of these diseases in Australia, which is a knowledge gap that will be addressed by an almond disease review. Almond production practices in Australia were also reviewed. More than 200 scientific and industry publications on almond diseases worldwide (excluding viruses) were sourced and reviewed.

Grower Census

A census was designed to determine growers' perception of prevalence and severity of diseases across the Australian almond industry. The primary objectives were to determine the proportion of growers with disease in their orchards and their estimated impacts. The census contained 17 questions in total, with four questions covering disease prevalence and severity, estimated impact on yield, and management options used; nine questions concerning orchard characteristics such as size, tree age, varieties, rootstocks, row and tree spacing, soil type and drainage and previous land use prior to almonds; and three questions concerning orchard practices (pruning type, irrigation method and winter defoliation). Growers were asked about orchard characteristics and practices to identify relationships between these features and disease prevalence. The questionnaire was distributed via Survey Monkey in July 2018 to all growers registered with the Almond Board of Australia.

Industry-wide disease survey

The survey design was a stratified method of orchard selection known as 2-stage cluster sampling where the sample size per orchard is determined based on orchard size. The design was chosen as it is regularly used in national health surveys and accounts for the nationwide distribution of the sample sites. It allows for two types of analyses: regional estimation of diseases - appropriate weightings are applied to derive estimates of disease per region and influence of variable - a logistic link function is applied to determine correlations between variables (orchard factors, weather) and disease.

The survey was designed to account for the different proportions of plantings in different regions and differing numbers of assessment trees in different regions. Disease incidences in regions were scaled based on the total area of the region. As Sunraysia accounts for the largest planted region, the disease incidences in this region have the largest effect on the overall disease incidences of the industry-wide survey. In contrast, Adelaide Plains and WA have the smallest plantings so have only a minor influence on the overall disease incidence for the industry.

The following regions and districts were included: Adelaide Plains – Angle Vale; Riverina – Hillston, Euroley;

Riverland – Loxton, Murtho, Waikerie, Walker Flat, Renmark, Lindsay Pt; Sunraysia – Carina, Cullulleraine, Euston, Lake Powell, Merbein, Nangiloc, Wemen, Wentworth and Carnamah WA.

Four surveys were undertaken over two seasons. The first round of surveying occurred in spring 2018 (Oct – Dec) and summer 2019 (Jan – March), whilst the second round of surveying occurred in spring 2019 (Oct – Dec) and summer 2020 (Jan – March) to investigate the prevalence of disease in the Australian almond industry. Trees were visually assessed and the presence of disease symptoms were recorded for every tree.

Collection and identification of isolates of almond diseases

During the course of disease surveys, field experiments and consultation with growers over the 2018-2022 seasons, many examples of the diseases affecting almonds were observed in the field. Many observations included the diseases of most concern to growers in particular hull rot and trunk diseases. A large number of fungal and bacterial isolates were obtained from symptomatic almond fruit, leaves, woody tissue and soil collected from all four almond growing states.

Almond samples were returned to the laboratory and processed to obtain fungal/bacterial cultures. Samples were generally either moist incubated to encourage sporulation or surface disinfested and sections plated onto appropriate media. Once pure cultures were obtained, they were stored for future reference and in many cases accessioned into the VPRI herbarium.

All isolates were initially identified by sequencing the ITS region for fungi (using ITS4 and ITS1F primers) or the 16S region for bacteria (using 27F and 1492R primers) and blasting against the NCBI database of sequences. All DNA regions were amplified using appropriate PCR conditions and sent to Macrogen Inc, Seoul, Korea for sequencing.

Those that were pathogenic organisms known to cause disease, especially those related to diseases of interest, (Hull rot and trunk diseases) were, if possible, identified more closely by sequencing with additional genes of interest and comparing against libraries of reliable published sequences using Geneious® bioinformatics software. Consensus trees were built for each isolate showing the closeness of association with reference sequences.

Economic analysis

In years 4 and 5 of the Integrated Pest (IPM) and Disease (IDM) Management Projects for the Almond Industry, Agriculture Victoria Research (AVR) conducted an economic analysis to provide baseline benefits and costs against which emerging IPM and IDM measures could be compared.

The economic analysis consisted of three key elements. First, close involvement with scientists on the project for advice on pest and disease impacts and the efficacy of control measures. Second, consultation with several technical/operations managers from large corporate or family-run orchards from the SA Riverland, Vic Sunraysia and NSW Riverina, who shared knowledge about their pest and disease control operations. Many inputs to the analysis are uncertain (pest and disease impacts) or risky (yields and prices); hence a third component of the analysis was stochastic budgeting incorporating the Monte Carlo simulation technology of @Risk [15].

Six industry-leading orchards were approached to provide data for the analysis and comprehensive data was obtained for three corporate or family-run orchards from the South Australian Riverland, Victorian Sunraysia and New South Wales Riverina. Semi-structured interviews based on a discussion guide were conducted over the phone and via email between July and August 2021.

Data included: information about a representative orchard block (orchard configuration, tree density, maturity, main cultivar grown) for a realistic context for the analysis; the fixed and variable cost of the pest and disease control operations (machinery, work rates, materials and labour); and both uncontrolled and controlled pest and disease impacts on kernel yield, kernel quality and tree longevity. AL16005 project research findings were used to confirm subjective evaluations used in this analysis. Other data used in the analysis were: historical almond prices and almond grading standards and pricing (Thomas Dobbin, AlmondCo, pers. comm.).

The data collected was used to populate a partial budget built in Excel using @Risk. The budget was used to quantify economic damages caused by diseases under three scenarios: in a disease-free environment; with disease present but uncontrolled, and with disease present and controlled.

Thousands of 'what if' Monte Carlo simulations using @Risk generated the following 'stochastic' outputs: distributions of net benefits from the control measure estimated as economic damages without control measure less damages with control measure less additional costs of control measure. Undertaking the control measure is a good investment for the grower if the additional grower profit is positive; distributions of benefit cost ratios estimated as the reduction in economic damages from implementing control measure divided by additional costs of control measure. Undertaking the control measure is a good investment for the grower if the benefit cost ratio exceeds 1.

The budget caters for both annual in-crop benefits and costs and impacts over multiple years, where capital investments are involved, or if tree longevity is a relevant consideration. Future streams of benefits and costs (in real $A 2020/21 terms) were accounted for using discounting procedures; costs and benefits that extended beyond the current season were discounted to present values using a real interest rate of 5%.

Hull rot

Varietal resistance to hull rot

Over two consecutive seasons breeding lines from (AL17005, AL12015) led by the University of Adelaide planted at Lacton Farms, Lindsay Point were evaluated for hull rot susceptibility and compared to industry standard, Nonpareil. Four plantings 2013, 2016, 2010 and 2006 were assessed for hull rot symptoms in February/March. The plantings consisted of the following: 2013 – 19 breeding lines on Nemaguard; 2016 - five breeding lines on Nemaguard: 2010 - Vela and Nonpareil on GF677 and 2006 - Capella, Carina, Maxima, Mira, Rhea on Nemaguard.

Influence of rootstock on hull rot

Over two consecutive seasons an established replicated rootstock trial planted as part of AL11006 was evaluated for hull rot susceptibility and compared to industry standard, Nemaguard. The trial consisted of 12 rootstocks – (Nemaguard, GF55, GF677, GF749, Adafuel, Garnem, Felinem, Monegro, Bright's Hybrid, Hansen, Cornerstone, and Krymsk) with Nonpareil, which were planted in 2013. In 2021 the trees had reached their sixth harvest and percentage hull split for each rootstock was recorded weekly in January. By the first week of February in both years all trees had passed the susceptible hull split stage. Hull rot severity was determined by counting the number of strikes per tree in early February in 2021 and 2022.

Investigating the influence of nitrogen, water and cultivar on hull rot

As part of project AL14005 'Identifying factors that influence spur productivity in almonds', an experimental trial site was set up to investigate the effects of sustained deficit irrigation and reduced nitrogen on spur productivity. The opportunity was taken to utilise the trial to investigate hull rot disease development in relation to varying nitrogen and water management practices. Restricting water and nitrogen has been demonstrated to reduce the incidence and severity of hull rot in California. The treatments included: +W+N (15 ML water/ha, 320 kgN/ha); -W+N (10.5 ML water/ha, 320 kgN/ha); +W-N (15 ML water/ha, 179 kgN/ha); and -W-N (10.5 ML water/ha, 179 kgN/ha).

Hull rot severity was assessed by counting the number of hull rot strikes per tree prior to harvest for both Nonpareil and Carmel during the 2019 and 2020 seasons. Shoot dieback associated with hull rot disease development was investigated by tagging three healthy and three infected shoots on one tree from four of the six replicates, across the four treatments. Tagged shoots were assessed post-harvest to determine fruit retained and the following spring to determine degree of dieback carried over to the next season.

Two shoots, one healthy and one infected, were also collected from each tree in the 2019 season for pathogen isolation as well as hull tissue analysis. During the 2020 season an inoculation experiment was undertaken to further investigate treatment effect on hull metabolite production as part of the PhD project 'Investigating the infection process of *Rhizopus stolonifer* and symptom development in the almond disease, Hull rot (Details in Appendix G).

Imaging technology for identifying hull rot hot spots

A predictive tool based on thermal and multispectral imaging was developed by Ceres Imaging to determine areas of high hull rot susceptibility within the orchard. The Ceres imaging technology tool was evaluated over three seasons in Sunraysia.

In season 2019/20 the prediction algorithm identified zones at low and high risk of developing hull rot using data collected during the November fly over across an orchard block. November was chosen so growers could apply a preventive fungicide prior to the susceptible stage of hull split to high-risk areas. GPS mapping of the predicted zones was used to pinpoint specific areas within the orchard for assessment. Three low risk and three high risk replicates were selected.

In season 2020/21 it was evaluated across multiple blocks in a Lake Powell Orchard. The predictive algorithm identified zones at low and high risk of developing hull rot based on cumulative thermal units across all flights (Sept-Jan). Eighteen high risk and 18 low risk sites across six blocks (three each per block) were chosen for hull rot severity assessment.

In season 2021/22 we undertook to further evaluate the predictive algorithm across multiple blocks in a different orchard. The predictive algorithm identified twelve areas, comprising six zones with low-risk and six with high-risk of developing hull rot based on cumulative thermal units across all flights (Sept-Jan).

In each season five trees per replicate were assessed for hull rot disease severity at harvest (strikes per tree). The five trees consisted of every second tree in a row of ten trees.

Comparison of hull rot treatments

Two field trials were conducted in 2021 and 2022 in the Sunraysia region to assess treatments for the control of hull rot at hull split. In both seasons, treatments were applied at 10% hull split (mid-January) (Table 1). In 2021, treatments were: the standard orchard practice of Custodia® (200 g/L tebuconazole + 120 g/L azoxystrobin); Luna Sensation® (250 g/L fluopyram + 250 g/L trifloxystrobin); dipotassium phosphate; and Luna Sensation® + dipotassium phosphate. Treatments were applied across two blocks, both consisting of 17-year-old trees on Nemaguard rootstock and planted at 7.5 m between-row spacing and 5 m in-row spacing, established in 2003, with alternate plantings of Nonpareil and pollinisers Carmel and Price. The trial evaluated four hull rot treatments in four replicated blocks consisting of four tree rows with 38 trees along 185 m total length. Each experimental unit was approximately 0.5 ha in size.

In 2022, treatments were; Custodia® (200 g/L tebuconazole + 120 g/L azoxystrobin (the current practice); Luna Sensation® (250 g/L fluopyram + 250 g/L trifloxystrobin); and diKaP™ ((P2O5) 31%, (K2O) 50%) which is a nutritional and stress tolerance product promoted by Dr Jim Adaskaveg (University California - Riverside) and imported by AVR from Redox Pty Ltd. Treatments were applied across one block, consisting of eight-year-old trees on Nemaguard rootstock and planted at 7.5 m between-row and 5 m in-row spacing with alternate plantings of Nonpareil and Carmel. Trees were maintained according to commercial standards. This trial evaluated three hull rot treatments in four replicated blocks. Replications consisted of one tree row with 25 trees along 127 m total length. Each experimental unit was approximately 0.2 ha in size.

In both seasons treatments were applied with a Interlink Twin Fan Orchard Sprayer driving at 5.0 km/h to deliver 2000 L/ha and hull rot assessments were done in mid-February. In 2021 large trees were assessed using percent canopy affected by hull rot strikes and in 2022 smaller trees were used in the trial and which allowed the number of hull rot strikes per tree to be counted.

Table 1 Treatments applied during hull split for the management of hull rot in 2021-22.

			2021		2022	
Product name	Active ingredient	Application rate	Timing % hull split	Applied	Timing % hull split	Applied
Custodia®	200 g/L tebuconazole + 120 g/L azoxystrobin	1.2 kg/ha	1	6-Jan	10	19-Jan
Luna Sensation®	250 g/L fluopyram + 250 g/L trifloxystrobin	0.8 l/ha	10	18-Jan	10	19-Jan
Dipotassium phosphate	Dipotassium phosphate (food grade)	3.363 kg/ha	10	18-Jan		
Luna Sensation® + dipotassium phosphate	250 g/L fluopyram + 250 g/L trifloxystrobin + dipotassium phosphate	As above	10	18-Jan		
diKaP™	(P_2O_5) 31%, (K_2O) 50%	3.363 kg/ha			10	19-Jan

Orchard sanitation and hull rot

Mummy nuts are an important hygiene issue in almond orchards as they provide shelter and overwintering sites for insect pests such as carob moth and carpophilus beetle. Hull rot has often been reported to be a major cause of mummy nuts (stick tights) and these have been assumed to be one of the main sources of overwintering *Rhizopus* inoculum. However, no one has determined whether this is the case in Australian orchards.

A trial was conducted to ascertain the effect of mummy numbers on the occurrence of hull rot in the subsequent season. Twenty-nine plots were chosen within the Almas orchard at Bannerton Victoria with mummy numbers per tree falling into 5 categories, <4, 4-9, 10-19, 20-40 and >40 mummies/tree. During dormancy all mummies in each plot were counted and mummies were collected from each of three positions within the tree canopy, high, mid and low. The mummies were incubated and examined microscopically to determine the presence of hull rot pathogens. Hull rot occurrence was then assessed in the same plots just prior to harvest.

Spatial distribution of hull rot strikes and infected and infested mummy nuts

Often sampling takes place in orchards without an understanding of the natural distribution patterns of the pests and diseases being measured. A joint trial was designed between AL16005 and AL16009 to provide information on spatial distribution of infected and infested nuts and mummies within a block, including segregation of mummies from high and low in the trees. This allows for analysis of any association between patterns of infection of overwintering mummies and the subsequent new crop. The effect of mummies on the development of hull rot in the subsequent crop and of hull rot on the development of mummies was analysed over two seasons as well as the proportion of mummies harbouring *Rhizopus* inoculum and the location of infected mummies within the tree. The distribution patterns of hull rot strikes within topographical areas of the orchard block was also recorded.

The trial was established in a large block at the Lake Powell Orchard in Robinvale. Fourteen orchard rows were included in the trial, utilising every 6th row i.e. every 3rd row of Nonpareil. In the 1st row (518), assessments started at tree 5 and continued every 9th tree from there. Assessments in the 2nd row (512) began at tree 9 and continued every 9th tree, and so on. The design over the 14 rows created a staggered layout.

During dormancy, in winter 2018 and 2019, the number of mummies per trial tree were counted and recorded against location. Approximately 6-12 mummies were collected per tree at each of three positions, high and low in the canopy and from the ground. The mummies were moist incubated at room temperature for 7 days and were then examined for signs of newly sporulating *R. stolonifer*. Mummies without sporulation were examined for old fungal structures on the inside of the hull from the previous year.

During February 2019 and 2020, just prior to harvest, the trial trees were assessed for hull rot severity. Disease severity was assessed by visually estimating the % of fruiting spurs/tree that were affected by hull rot strikes. A rating scale published for diseased leaves was used as a visual aid. Any correlation between mummy numbers and hull rot strikes were then analysed.

Investigating the relationship between hull rot and mummy retention

Anecdotally, hull rot during nut maturation has been linked to the retention of fruit on spurs. Fruit remain attached after harvest and can persist into dormancy, resulting in mummy nuts. However, determining the relationship between hull rot disease expression and mummy formation is difficult due to confounding factors such as multiple generations of mummies retained on trees, fruit retention caused by poor shaker force in relation to tree architecture, variable maturation across the tree at harvest and other diseases.

In collaboration with AL16009 a trial was conducted on a commercial orchard at Lake Powell. Trees with a history of high mummy incidence were stripped of all retained fruit in 2019 and again in 2020. Hull rot strike assessment was carried out pre-harvest and twenty shoots with hull rot strikes (infected) and 20 shoots without hull rot strikes (healthy) were tagged. Visual assessment of tagged shoots was carried out post-harvest, the presence and number of retained fruit recorded and any evident gumming noted. A digital force gauge (Starr instruments model FGD-100) was used to determine whether there was a difference in the force, newtons (N), required for removal of retained fruit on infected shoots, in comparison to those on healthy ones.

Dormancy assessments of total mummy numbers for the five trees was conducted during winter of 2020 and 2021. Data collected allowed the relationship between hull rot incidence and mummy formation to be analysed without the confounding effect of multiple generations of mummies.

Rhizopus stolonifer *– hull rot disease expression study*

There are very few studies exploring the progression of hull rot disease expression post infection, or the pre-harvest factors that may influence this and there are none based in an Australian orchard context. To facilitate a better understanding of the epidemiology of *R. stolonifer*, a pilot study was conducted in the 2020-2021 season to investigate the process of disease expression *in-planta* and the effect that variations in canopy microclimate may have on this.

The study was established across two blocks within a small privately owned orchard at Merbein in the Sunraysia area. Four shoots on five trees were selected, two in shaded and two in exposed positions within each tree, to examine the effects of potential microclimate differences on disease expression. A single fruit at each location was marked and inoculated with *R. stolonifer* and fruit and leaf symptom expression monitored over the following days. Data loggers were installed in both locations on each of the five trees and microclimate data - temperature, humidity and leaf wetness collected from time of inoculation to harvest. Data was used to determine relationships between disease symptom development and variations in microclimate.

In vitro *fungicide assays*

In collaboration with Hort Innovation Regulatory Affairs – Crop Protection Manager a list of fungicides and biological control agents was compiled for efficacy against the hull rot pathogen *R. stolonifer*.

Small samples of eighteen fungicides and biological agents were obtained from chemical companies covering groups M1, M3, M4, 3, 7, 9, 11 and combinations of these (Table 2). The products were assessed at their label rate for efficacy in controlling the *in vitro* growth of *R. stolonifer*. In initial trials of the chemical fungicides, potato dextrose agar was amended with each fungicide so that the media contained the label rate per litre. For the biological control agents, a suspension containing the label rate was made in sterile water and a 100 µl aliquot evenly spread over the surface of each plate. Plates were inoculated with *R. stolonifer* isolates and its growth over time measured against that of an untreated control. *R. stolonifer* isolates used were collected during the 2018-19 disease surveys. An isolate from each of the 3 main growing regions, Riverina NSW, Sunraysia (Robinvale area) VIC, and Riverland SA were tested.

The biological control agents (BCAs) were retested using two different methods to confirm our previous results. The spread plate method described above was repeated but the products were allowed to grow before adding the *R. stolonifer*. In addition, a dual culture technique was investigated to look at the effect of fungal metabolites rather than physical inhibition.

Of the 18 fungicides and biological agents tested in *in vitro* assays nine products were chosen for further evaluation in split green almond fruit bioassays (Table 2). Unsprayed nuts at split stage B3 were collected from a commercial orchard and spray inoculated with *R. stolonifer* at 10^5 spores/ml. Fungicide treatments were applied at the label rate either before or after inoculation to evaluate preventative and curative effects. Nuts were assessed microscopically for signs of sporulation 5 days after treatment.

Table 2. Products used in each assay for *in vitro* fungicide testing.

®Product name	Active ingredient	Initial plate assay	Repeat assay of BCAs	Green fruit assay
Luna Experience	fluopyram + tebuconazole	✓		✓
Blue Shield	copper hydroxide	✓		✓
ProBLAD plus #2	BLAD poly peptide	✓		✓
Seguris Flexi	isopyrazam	✓		✓
Custodia	tebuconazole + azoxystrobin	✓		✓
Solaris	cyprodinil	✓		✓
Serenade Opti	*Bacillus amyloliquefaciens* 1, strain QST 713	✓	✓	✓
Fontelis	penthiopyrad	✓		
Serifel	*Bacillus amyloliquefaciens* 2	✓	✓	✓
Luna Sensation	fluopyram + trifloxystrobin	✓		✓
Merivon	pyraclostrobin + fluxapyroxad	✓		
Captan	captan	✓		
Dithane Rainshield	mancozeb	✓		
Amistar	azoxystrobin	✓		
Botector	*Aureobasidium pullulans*	✓	✓	
Superzyme	*B. subtilis*, *P. putida*, *T. koningii* and *T. harzianum*	✓	✓	
Fulzyme Plus	*Bacillus subtilis*	✓	✓	
Belanty	mefentrifluconazole	✓		

Tri-D25	*Trichoderma koningii* and *T. harzianum*	✓	✓	

Trunk disease

Sampling and culture collection

From 2018-2021, samples were collected from almond orchards in South Australia and Victoria. In the laboratory, fungal species and oomycetes (*Phytophthora, Phytopythium* and *Pythium* spp.*)* were obtained from wood, soil and root samples. From wood, isolates were obtained by removing bark taking large chips of wood from the margin of necrotic and healthy woody tissue. Wood chips were surface disinfested and small pieces were cut and placed onto amended selective media, and pure cultures obtained by subculturing hyphal tips. Oomycetes were obtained from soil and root samples by using baiting techniques with selective agar blocks, Eucalyptus cotyledons, green apples and pears.

Foamy canker

Foamy canker was reported during two of the hottest summers on record. Foamy canker was not reported in 2020-2021, when relatively cooler weather and higher rainfall occurred. Samples were taken from trees with foamy canker in January 2019 and February 2020 and returned to the laboratory for diagnosis of causal organisms.

Pathogenicity testing of fungal species

Seven fungal species; *Diplodia seriata*, *Eutypa lata*, *Colletotrichum acutatum*, *Collophora hispanica*, *Collophora rubra*, *Pleurostoma richardsiae* and *Cytospora diatrypelloidea,* were tested in three pathogenicity experiments on potted almond trees. One-year-old branches were surface disinfested in ethanol. Wound made in branches were inoculated with the organisms. Six months after inoculation, branches were removed and returned to the laboratory for assessment. The bark was removed and lesions measured. Sequential isolations were made to determine the distance the fungus had colonised the wood.

Further pathogenicity testing was conducted for other potential trunk disease pathogens of almond. Detached branch assays (DBA) were evaluated as a rapid method of pathogenicity testing.

Green shoots were randomly selected and removed from 5-year-old potted trees, cv. Price on Nemaguard rootstock. Shoots were surface disinfested in bleach solution and were cut into approximately 10-cm-long sections. Shoots were then inoculated with fungal species and were sealed for a 5-week incubation period. The length of necrosis from the point of inoculation was measured and recorded.

One-year-old branches were inoculated through wounds made with a sterilised drill bit. Branches were incubated for 6 months before being removed and length of necrosis from the point of inoculation was measured and recorded.

Further experiments were conducted on potted almond trees as described above to confirm results of DBA experiments, and pathogenicity of the species tested.

Pathogenicity testing of oomycetes

Phytophthora species were highly prevalent during surveying and sampling. Phytophthora root and crown rot are considered to be of economic importance, with sudden tree losses in orchards of any age often reported. The aim of this research was to investigate the symptoms associated with *Phytophthora* spp. and to evaluate pathogenicity of oomycete isolates that were isolated from symptomatic trees.

Two repeated experiments were conducted on cvs. Price and Carmel. One-year-old branches were inoculated with *Phytophthora acerina*, *P. cactorum*, *P. hedraiandra*, *P. multivora*, *P. niederhauserii* and a

Phytophthora isolate identified to be closest to *P. rosacearum*. A *Pythium* sp., *Phytopythium littorale* and a *Phytopythium* isolate not identified to species level were also included in experiments. Experiments were incubated for 4 and 6 weeks.

Cultivar susceptibility experiments

Selecting a resistant cultivar is an important part of an integrated disease management program. To investigate the susceptibility of cultivars commonly planted in Australia, an experiment was conducted to evaluate six almond cultivars, commonly planted in Australia, against five pathogenic fungal species collected from Australian orchards. Experiments were conducted on potted 2-year-old trees of cvs. Nonpareil, Carmel, Wood Colony, Maxima, Carina and Price, all grafted on Rootpak rootstock. Cultivars were inoculated with *Cm. acutatum, Ca rubra, D. seriata, E. lata* and *P. richardsiae*. Pathogenicity of species on each cultivar was analysed, and then the susceptibility of cultivars compared for each species. A non-inoculated control was included. The experiment was repeated

Fungicide evaluations

Previous studies on grapevine and in almond have shown that application of wound protectants are an effective method of controlling trunk disease pathogens. However, there are currently no products registered as wound protectants against trunk disease pathogens in Australia.

A list of fungicides and biocontrols was compiled for screening against trunk disease pathogens. Products were selected based on registration status for other diseases of almonds in Australia, efficacy against trunk disease pathogens in almonds or other hosts and commitment by suppliers to apply for registration or label extension if proven effective. Some products that are soon to be released into the almond industry were also included.

In vitro experiments in 2020 evaluated a total of 17 fungicides and six biocontrol products. Five pathogenic fungal species were included *D. seriata, Cm. acutatum, P. richardsiae, E. lata* and *Ca. rubra.*

Based on results from the laboratory evaluations, nine treatments were selected for field evaluations. Field experiments were established to evaluate potential products as wound protectants. These products were evaluated against the most common and pathogenic species *D. seriata,* which causes Botryosphaeria dieback and canker in Australia. Two field experiments were established orchards in South Australia. Branches (1-2 years old) were pruned using secateurs to create a wound. That same day, fungicide and biological treatments were applied using a paintbrush. Both controls, inoculated and non-inoculated were included. The following day, all wounds other than the NI controls were inoculated with a *D. seriata* spore suspension. After 3 months, branches were returned to the laboratory and were assessed for pathogen recovery.

Lower limb dieback investigations

During the general disease surveys trees were assessed for all disease, including the presence or absence of LLD dieback symptoms including shoots with chlorotic (yellow) leaves. In the second year of surveying, trees were also assigned with a disease severity rating to better discern if there were differences in symptom severity between trees of different ages.

On several different properties in South Australia, LLD symptom progression and expression were intensively monitored each spring from 2019 to 2021. To find out what role the implicated factors may play in LLD, monitoring was carried out on various properties with a range of tree ages and cultivars.

Results and discussion

Study tour to California and almond disease review

Two visits (July and September 2018) were made to UC Davis Kearney Research Center, California, to promote collaboration and to see first-hand the research conducted on hull rot and lower limb dieback in their production systems.

In July 2018, Dr J Edwards spent a week with Dr Themis Michialides discussing potential collaborative research opportunities. She visited Madera, Hanford and Bakersfield Counties with Dr Michialides. In Madera County, Dr Edwards visited 3 almond orchards to see hull rot development first-hand and discuss aetiology, epidemiology, disease management with farm advisors in the region. In Hanford and Bakersfield Counties she assisted with field sampling and laboratory assessment of almond diseases. Information obtained has been incorporated into the review.

In September 2018, B Oswald spent 21 days in and around the UC Davis Kearney Research Center. Her visit was more focused towards training and began with meeting laboratory members Dr Florent Trouillas, Leslie Holland and Mohamed Nouri as well as an introduction to the Kearney Research Center and staff. Activities and visits were planned for the following 3 weeks.

The trip included multiple visits to orchards in different counties within a 400 km radius of Kearney Research Center. In these orchards, symptoms were observed, recorded and photos were taken of different gumming and canker symptoms. Samples were taken from symptomatic trunks and branches before being returned to the laboratory for diagnosis of the causal agents.

All sampling protocols and isolation techniques were documented, including photos and videos so the information could easily be communicated to other researchers on the AL16005 project. Protocols to isolate fungal pathogens were carried out on samples collected from the field and adjusted where necessary to optimise results whilst minimising contamination from fungi commonly found in woody tissue such as *R. stolonifer* and *Penicillium* spp.

Sampling techniques differed depending on the target pathogens and the location of symptoms. The consistent factors for successful isolation are that samples should always be taken from the margin of dead and live wood at the top leading edge of the canker, particularly from underneath fresh gumming. Bark should usually be removed, except when attempting to isolate *Ceratocystis fimbriata.*

Whilst at Kearney Agricultural Research and Extension Center, Miss Oswald worked collaboratively with Drs Florent Trouillas, Themis Michailides, Mohamed Taieb Nouri and graduate student Leslie Holland. She worked in the field with farm advisory and extension specialists DrsMae Culumber, Phoebe Gordon and Brent Holtz, who work in various counties throughout almond growing regions in California. Miss Oswald also met with Dr Daniel Lawrence and Dr Renaud Travadon, research experts in plant pathology and canker pathogens.

Several cultural management practices for these diseases in the USA have been recommended but are not widely adopted here in Australia. Chemical control for *Phytophthora* diseases is currently available, but for the others, it is either unknown, of limited effect or poorly adopted. The review has highlighted that there is poor understanding of the epidemiology of these major diseases, including sources of inoculum, environmental conditions required for infection and disease development, the basis of varietal differences, and orchard practices that influence disease. This information is essential for developing effective disease management practices that can be widely adopted.

The almond disease review can be found in Appendix A.

Grower Census

The number of growers who responded to the census represented 6,922 ha of almond orchards (27% of the industry total) across the major growing regions. Sixty-two percent of the plantings were less than 15 years old. Nonpareil was the major cultivar grown and Nemaguard was the most common rootstock. Grapes, grain, stone fruit and citrus were the most common crops prior to planting almonds. Irrigation was predominantly by drippers (64%), then by sprinklers (28%). Orchard drainage was rated as excellent (50%), fair (35%) and poor (7%). The main soil type was sandy loam (71%). Flooding resulting in tree dieback had been experienced by 57% of respondents.

In the census, growers reported that hull rot, LLD, rust, anthracnose, Phytophthora and shothole were the most commonly occurring diseases in their orchards. The census also indicated that hull rot, LLD, bacterial spot, Phytophthora and anthracnose were perceived by growers as having the highest impact on yield, followed by the various wood cankers. All respondents actively managed disease with a mixture of nutrition, irrigation, soil treatment, canopy management, orchard hygiene, fungicide applications and alternative treatments. For full details refer to Appendix B.

Industry-wide disease survey

Two years of orchard surveying, beginning in spring 2018 and concluding in summer 2020, found the most prevalent diseases were lower limb dieback (LLD), hull rot, shot hole and trunk diseases (which included Phytophthora) (Figure 1). After appropriate weighting was applied, in 2018/19, the major diseases identified across the almond industry were shot hole (74%), hull rot (69%), LLD (63%) and trunk disease (26%). Minor diseases recorded were rust (2%), bacterial spot (1%), anthracnose (<1%), scab (<0.1%) and blossom blight (<0.1%). In 2019/20, the major diseases identified were LLD (74%), hull rot (69%), shot hole (45%) and trunk disease (27%). Minor diseases recorded were bacterial spot (8%), rust (2%) and anthracnose (<1%). In general, shot hole was more prevalent in year 1 than year 2 and bacterial spot was more prevalent in year 2 than year 1. While shot hole symptoms were consistently observed, they were not considered serious as the disease severity was generally low and managed with fungicide spray programs. Despite industry perception of anthracnose as widespread, very little anthracnose was observed in both seasons.

Regional differences were evident for the major diseases. Hull rot was most prevalent in Adelaide Plains, Riverina and Sunraysia with less in Riverland and WA. Hull rot is weather-driven, so difference in rainfall between the regions will have influenced the amount observed.

Shot hole was prevalent in all regions. In the first year of the survey, Adelaide Plains had less shot hole than the other regions. This may be due to smaller orchards allowing more effective spray coverage. However, in the second year, this observation was reversed with shot hole having increased in the Adelaide Plains but less prevalent elsewhere.

Lower limb dieback (LLD) was prevalent in all regions except WA. More LLD was observed in the second season. Trunk diseases were more prevalent in Adelaide Plains, Riverina and Western Australia compared to Riverland and Sunraysia. Bacterial spot, when present, was mostly found in Sunraysia. Rust was prevalent in Adelaide Plains and WA but rare in the other regions.

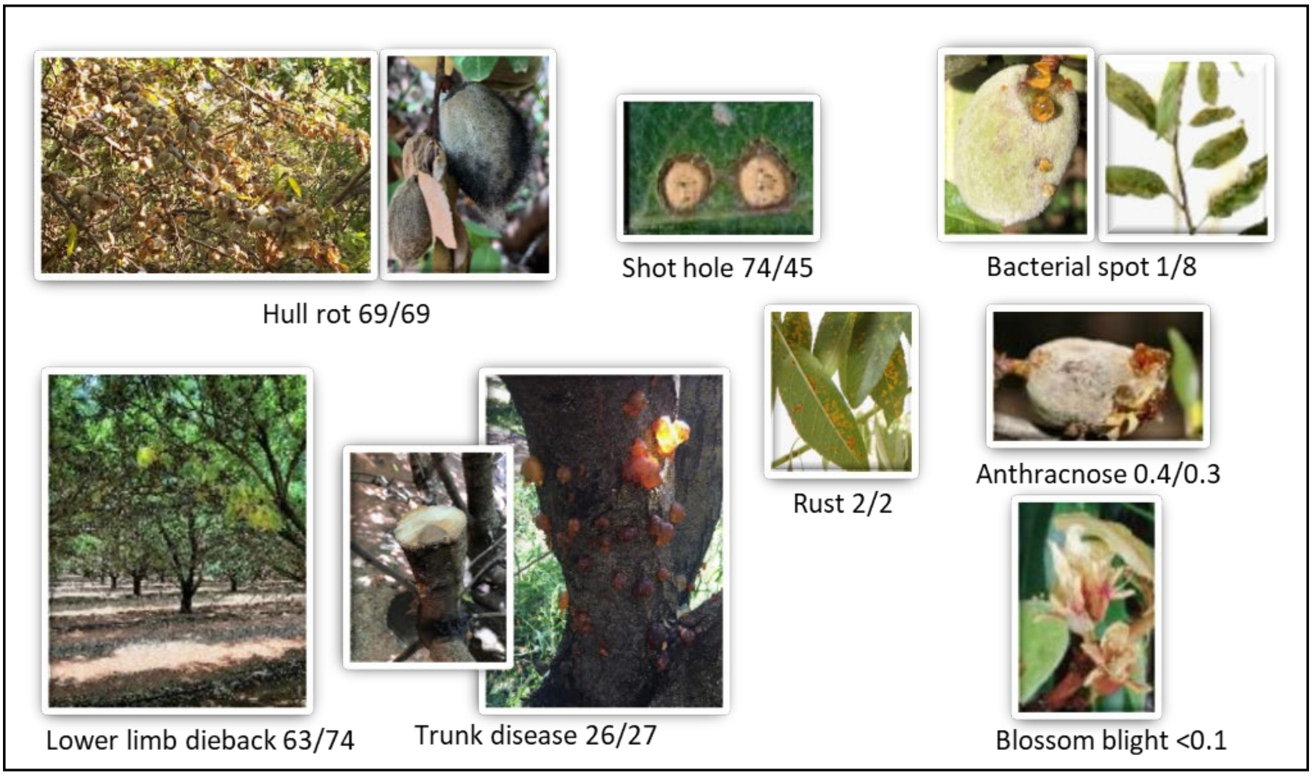

Figure 1 Incidence of major almond diseases observed over two seasons. Percent of surveyed trees with disease observed in Year 1 (2018-19)/ Year 2 (2019-20). Hull rot incidence was determined on split Nonpareil trees.

The causal organism of hull rot was always *Rhizopus spp.*, despite other pathogens reported to cause hull rot in California. Several potential pathogens were associated with trunk disease symptoms and these are being investigated further in pathogenicity trials.

Significant correlations were found between disease incidence and some agronomic practices and weather parameters, varying with disease. Hull rot symptoms were influenced by water (both irrigation and rainfall), cultivar (including rootstock) and fungicide used. Hull rot incidence was higher under drip irrigation and on Nonpareil but reduced on Garnem rootstock and with the applications of Luna Sensation® (Fluopyram/Trifloxystrobin), Merivon® (Pyraclostrobin/Fluxapyroxad) and to a lesser extent Tilt® (propiconazole). Both lower limb dieback and trunk disease were influenced by age of trees, spacing, cultivar and deficit irrigation, and trunk disease was also associated with January rain. Both lower limb dieback and trunk disease were observed more often on older trees. For full details refer to Appendix B.

Collection and identification of isolates of almond diseases

Greater than 400 individual isolates of at least 56 genera were collected. Of these a number were likely to be saprophytic organisms, ubiquitous environmental fungi or otherwise not of interest, these were not identified further.

Approximately 210 isolates have been identified more closely using multiple primers were necessary, these are detailed in Appendix B.

Economic analysis

Grower actions to combat priority pests and diseases were found to be generally economically desirable.

Impacts and hence economic damages without and with the various control measures are assumed to be similar for each control option. Costs varied between respondents; they included both the fixed and variable costs of machinery operations, and the costs of materials used.

The desirability of spraying for Hull rot was mixed. Higher costs reduced the net benefit from $A134/ha to -$A22/ha; and the benefit cost ratio declined from a positive 2.2:1 to an undesirable 0.9:1. Outcomes were positive just 33% of times for the higher-cost option.

Spraying and/ or chemigation for trunk disease (Phytophthora) provided a positive economic benefit to growers for all treatment options and cost levels. The expected net benefit ranged from $A114/ha to $A200/ha, the benefit cost ratio was upward of 1.8:1 and positive 99 - 100% of times.

As there was not one number for the net benefit or the benefit cost ratio, probabilistic results and sensitivity analysis are presented in the body of the report. For brevity, Table 3 simply shows the times the control measure was the best option for the grower, i.e., the times the net benefit > 0 or benefit cost ratio >1:1.

Table 3 Average annual net benefits accruing to individual growers of spraying for hull rot ($/ha[a]).

Damages without control	Damages with control	Material costs[b]	Machinery ownership costs[c]	Machinery operating costs[d]	Total costs	Net benefits (NB)	Benefit cost ratio (BCR)	Times control is the best option (%)
Hull rot								
Option 1: One spray at hull split; lower operation costs and a less expensive fungicide								
610	366	52	13	45	110	134	2.2	96
Option 2: One spray at hull split, higher operation costs and a more expensive fungicide								
610	366	180	30	55	265	-21	0.9	37
Trunk disease /Phytophthora								
Option 1: Two chemigations, one in September and another in late November								
405	155	50			50	200	5.0	100
Option 2: One single spray every year								
405	155	25	13	45	83	167	3.0	100
Option 3: One spray followed by a chemigation after hull split								
405	155	50	31	55	136	114	1.8	99

[a] Units are $/ha unless otherwise stated, [b] Material costs are for sprays at label rate and wetters (if required), [c] Machinery ownership costs are the annualised capital recovery cost plus repairs and maintenance plus insurance and housing, [d] Machinery operating costs include labour, fuel and lubricants.

There was not one single economic baseline for each disease control measure, as grower actions and the resources available to them varied. Furthermore, economic damages were very sensitive to uncertainty about the impacts of the various pests and diseases. Because of this uncertainty, research into emerging IDM measures should include not only the incidence and severity of disease impacts, but also the impact on marketable yields.

There is scope for emerging IDM measures to be cost advantageous compared to current practice due, in part, to high fixed and variable machinery costs. The report demonstrates a way of thinking about how to evaluate the farm-level benefits and costs of emerging IDM measures. The data collected and the risk budget built for this analysis will be useful for economic evaluations of our project endeavours going forward. Full details are in Appendix C.

Hull rot

Varietal resistance to hull rot

In assessments of the 2013 planting, hull rot disease severity was significantly different between seasons with 2019 having 35 strikes and 2020 having 68 strikes due considerable rainfall in early March 2020 (15.5 mm total) 3 weeks prior to assessment which would have favoured expression of disease, compared to 1 mm total in 2019. Due to the significant difference between seasons each year was analysed separately. In 2019, hull rot severity varied across breeding lines, from 1 strike to 192 strikes per tree. Nonpareil was most severely affected with the remaining lines having significantly fewer hull rot strikes than Nonpareil. In 2020, hull rot severity again varied considerable between lines, ranging from a high of 300 strikes per tree on Nonpareil to two strikes per tree on lines 19 and 35. Nonpareil was significantly more affected than any of the breeding lines, with the next highest being line 30 and line 33. Line 33 also had the highest disease severity of all the lines in 2019. As expected, Nonpareil had the highest severity and Carmel had low severity in both seasons, and lines 18, 23, 25, 26, 35 and 36 also had consistently low severity.

Assessment of the 2016 planting over two consecutive seasons found Nonpareil was significantly more affected than any of the breeding lines, followed by R27aT35 with 125 strikes per tree and the remaining lines (R33T145, R14bT162, R9bT153, R1bT129) with less than 20 strikes per tree.

The 2010 planting was assessed in 2021, Vela was the only commercial cultivar in this trial and had 35 strikes per tree, significantly less than Nonpareil. In 2022, Vela was not sufficiently split to assess for hull rot symptoms. Nonpareil trees were fully split and had only 35 hull rot strikes per tree.

Assessment of the 2006 planting in 2021, hull rot severity varied considerably between varieties, ranging from 206 strikes per tree on Nonpareil to < 20 strikes per tree on Maxima. Nonpareil was significantly more affected than any of the commercial varieties, with the next highest being Mira with 180 strikes per tree and the remaining varieties (Carina, Capella, Rhea) with < 70 strikes per tree.

In general, the almond breeding program lines have less hull rot than industry standard Nonpareil.

Influence of rootstock on hull rot

Hull rot severity varied considerably between seasons and rootstocks. In 2021, hull rot severity ranged from 90 strikes per tree on GF557 to < 30 strikes per tree on GF749 and Bright's Hybrid. Garnem, Monegro and GF557 had significantly more strikes than the industry standard Nemaguard. Adafuel, GF677, Krymsk, Felinem, Cornerstone and Hansen were not significantly different to Nemaguard. In 2022, hull rot severity ranged from 129 strikes per tree on Monegro to < 30 strikes per tree on GF749, Krymsk and GF677. Monegro, Garnem, Hansen and Felinem had significantly more strikes than the industry standard Nemaguard. GF749, Krymsk, GF677, GF557, Cornerstone, Adafuel and Bright's Hybrid were not significantly different to Nemaguard.

Analysis of two seasons data of hull rot incidence on Nonpareil scions across 12 rootstocks found GF749 Peach Almond (*P. kansuensis* x *P. dulcis*), Krymsk Peach Plum (*P. persica* x *P. cerasifera*) and Nemaguard Peach (*P. persica x P. davidiana*) generally had lower hull rot than Peach Almond *(P. persica x P. dulcis*) rootstocks particularly Monegro, Garnem, Hansen and Felinem.

Investigating the influence of water and nitrogen on hull rot

In 2019, water and nitrogen did not influence disease in Nonpareil, but disease was significantly lower *($P<0.001$)* in the reduced water treatment for Carmel. In 2020, Nonpareil showed a significant decrease ($P<0.001$) in disease severity in the low water treatments compared to the control. In Carmel, the number of hull rot strikes followed a similar trend to 2019, however, these differences were not significant. No significant differences were found between disease severity and nitrogen treatments.

Statistical analysis between years showed that there was a significant difference (*P*<0.001) between the degree of disease severity in 2019 compared to 2020. These results indicate that although irrigation management can influence disease expression, seasonal variation also plays a part. However, when disease severity was high for Carmel in 2019 and Nonpareil in 2020, the sustained deficit treatment resulted in significantly less disease, indicating that under conditions conducive for disease, restricted irrigation does reduce hull rot.

Degree of shoot dieback due to infection was not influenced by water, nitrogen or cultivar across both seasons. However, the diameter of the shoot at the point of infected fruit attachment had an apparent threshold for which significant dieback occurred in both cvs Nonpareil and Carmel. This suggests that there may be a relationship between infection and toxicity, and the physiological tendency of the tree to "abort" a limb or there may be a dilution effect occurring, driven by the ratio of metabolite production to the size of surrounding tissue.

Metabolite analysis in both seasons showed fumaric acid present in all infected hull samples. In 2019 Nonpareil hull samples were significantly higher (*P*<0.001) in fumaric acid for 100%ETc water treatments (10.5 ML water/ha). In 2020, however, no differences were observed among water treatments, while significantly higher levels of fumaric acid were detected in standard nitrogen treatments (320 kgN/ha).

Imaging technology for identifying hull rot hot spots

In 2020, areas designated as high risk resulted in significantly more hull rot (15%) than low risk areas (6%) (*P*<0.001). This orchard only received 10 mm of rain from the 1st of January until hull rot assessment. Had the rainfall been greater we may have observed more hull rot in the high-risk areas.

In 2021, the 18 areas designated high risk resulted in significantly (*P<0.001*) more hull rot (48%) than the 18 low risk areas (9%). High risk areas tended to be in low-lying parts of the orchard while low risk areas were generally in elevated or flat parts. Trees in high-risk areas were phenologically slower, with hull split of fruit being two weeks behind trees in low-risk areas. The disease assessments took this into account and were conducted at the same phenological age.

In 2022 the hull rot prediction model of Ceres Imaging was tested over a larger orchard scale. Again, high-risk areas tended to be in low lying parts of the orchard while low-risk areas were generally in elevated or flat parts. Trees in high-risk areas were phenologically slower, with hull split of fruit being 2 weeks behind trees in low-risk areas. Visual assessments found fruit was not adequately split in high-risk areas at assessment so incidence of hull rot symptoms in high hull rot zones may be underestimated this season. There was a significant difference (*P<0.05*) in disease severity between areas of the blocks predicted to be high-risk and those predicted to be low-risk by Ceres Imaging software. The mean hull rot severity in the high-risk zones was 26 strikes per tree and 52 strikes per tree in the low-risk zones. This difference to previous seasons is that hull rot assessments were conducted when only low-risk zones were fully split.

The mid-February hull rot assessment of partially split fruit in high-risk zones has shown significantly fewer hull rot strikes than in the drier low-risk zones. Due to Covid restrictions it was not possible to assess the high-risk zones later in the season. Based on last season's phenological stage, fruit split of the high-risk zones would have occurred 2 weeks later and would have coincided with 17 mm of rain on the 1st March.

Over the last 2 season's low-risk areas have trees with 5% of the canopy affected by hull rot while high-risk areas in 2019/20 had 15% and in 2020/21 had 47%. Based on the rainfall for both season's we can hypothesize that high-risk areas this season would have more than 50% of the canopy affected by hull rot strikes. The orchard received 58.4 mm of rain in January with an additional 7 mm rainfall in February after hull rot assessment occurred in the third week.

The use of Ceres Imaging as a tool for hull rot prediction shows promise and can predict hull rot susceptibility from thermal and multispectral imagery data collected over the season. This would enable orchards to prioritize fungicide application in high-risk areas and follow up with a second spray after a significant rainfall event.

Late hull rot infection may not allow sufficient time for strike development before harvest resulting in less yield loss in the following season by reducing the death of next seasons spurs.

Comparison of hull rot treatments

In 2021, hull rot symptoms developed in all trees and severity ranged from 30 – 39%. The application of dipotassium phosphate at 5-10% hull split resulted in significantly (P<0.001) more hull rot (39%) than the other treatments. In 2022, hull rot symptoms developed in all trees and severity ranged from 1 – 118 hull rot strikes per tree. The application of diKaP™ at 5-10% hull split did not significantly reduce the number of hull rot strikes per tree compared to the other treatments although fewer hull rot strikes were observed with the diKaP™ treatment.

While Custodia® and Luna Sensation® both gave similar protection, neither of them satisfactorily controlled hull rot, given the average severity of 45 hull rot strikes per tree. diKaP™ reduced hull rot by 10 hull rot strikes per tree (20%) compared to other treatments (average of 35 hull rot strikes per tree). diKaP™ gives similar protection against hull rot strikes on Nonpareil than either the current orchard practice or Luna Sensation®.

Orchard sanitation and hull rot

The only hull rot pathogen detected in these plots was *Rhizopus* sp. Overall less than 20% of the mummies collected from each of 3 canopy positions showed evidence of *Rhizopus* infection, and only 2.5-7% had viable *Rhizopus* inoculum. There appeared to be slightly more infection on nuts from the middle of the canopy.

When looking at the different density categories, mummies collected from the 20-40 mummies/tree category were the most infected, with 33% having visible *Rhizopus* and 14% with viable *Rhizopus*. All other categories had low visible infection (<15%) and <5% viable infection suggesting that while hull rot may be responsible for the formation of some mummies it is not the major cause, and the majority of infective spores do not survive between seasons. Overall, the presence of *Rhizopus* did not appear the be related to higher mummy numbers.

Little hull rot was observed in the trial during the following harvest with on average less than one strike per tree due to the hot and dry conditions leading up to harvest in 2019. So, no conclusions could be drawn regarding the effect of mummy density on hull rot development.

Spatial distribution of hull rot strikes and infected and infested mummy nuts

During the 2018 and 2019 seasons an orchard block was examined for the distribution of mummy nuts and hull rot strikes. The number of mummies per tree during dormancy varied from 0 to 300. There were more mummies observed in 2019 compared to 2018, with 45% of trees having at least 200 mummies. There was considerable variation in the distribution of mummies over the block with higher numbers occurring in a band from the southwest corner to the centre of the block which corresponds to areas of low-water stress within the block. This observation provides future evidence that restricting water is a useful strategy for reducing mummy retention and potentially hull rot development.

In both years all the trees were affected with at least one hull rot strike when assessed just prior to harvest. Disease severity varied greatly between years; hull rot expression was high in 2019, with a third of the trees affected by >30% spur deaths caused by hull rot strikes. There was much less hull rot in 2020 even though the mummy numbers were greater preceding the 2020 assessment. It may be that weather conditions, in particular rain events leading up to harvest played a part in disease development. There were a few rain events post hull split in each year. Both years had some rain in late January and early February, but there was more rain overall and more February rain in 2019 which may have led to increased disease expression before harvest.

It was shown that a small proportion of mummies do provide *Rhizopus* inoculum for the next season requiring only the addition of humidity, such as provided by rainfall events, to become active. Once active, *Rhizopus* spores can be easily distributed around the orchard by wind, water splash or animals/insects. However, further work needs to be done to determine what proportion of overwintering mummies are due to hull rot.

When mummies were examined for Rhizopus inoculum, there was no significant difference due to height in the canopy. In 2018 on average, 34% of canopy mummies produced viable *Rhizopus* inoculum, in 2019 there were more mummies overall but only 20% produced sporulating *Rhizopus*. Sporulation on mummies from the ground was 34% in 2018 and only 3% in 2019.

While there was no significant difference in the amount of *Rhizopus* sporulation due to height in the canopy, in 2019 there was a significant difference (P=0.02) in the percentage of mummies that had signs of previous infection, with the mummies from high in the canopy having significantly more evidence of prior infection. This indicates that fruit high in the tree may be more susceptible to *Rhizopus* infection but that not all infected fruit are able to produce inoculum the following season if left on the tree.

While it is generally believed that there is an association between overwintering mummies and hull rot, and between hull rot strikes and mummy formation, this had proven difficult to verify. After examining 2 seasons of data only weak correlations have been found between mummy numbers and hull rot. The correlation between the number of mummies per tree and % hull rot was (R^2 = 0.1043 and 0.1268, respectively for each season) and between % hull rot and mummy formation (R^2 = 0.2611).

This may in part be due to uncertainty on the origin of the retained mummies. Some mummies may be due to hull rot and harbour *Rhizopus* inoculum, but they may also be due to other diseases, physiological effects, tree architecture, poor shaking or mummies remaining on the tree into subsequent years. Further work has been undertaken with stripped trees to determine what proportion of mummies may be due to hull rot.

All mummies may act as overwintering sites and food sources for insect pests. There was clear correlation between mummy numbers and overwintering *Carpophilus* beetle in ground and low canopy mummies (R^2 0.50 and R^2 0.47, respectively). Reducing mummy numbers remains important for insect control but controlling hull rot is unlikely to be part of a solution.

Investigating the relationship between hull rot and mummy formation

For both seasons the number of hull rot strikes per tree was high, ranging from 124 to 207 in 2020 and 105 to 182 in 2021. Mummy numbers observed a greater spread, ranging from 21 to 361 in 2020 and 39 to 301 in 2021. The correlation between hull rot strikes pre-harvest and mummy number during dormancy was low for both seasons individually and combined (R^2 0.0016, R^2 0.126 and R^2 0.026 respectively).

Visual assessment of fruit retained on the 20 healthy and infected tagged shoots per tree was conducted post-harvest. Across both seasons no significant difference was found between the mean percentage of shoots that retained fruit on infected shoots, compared to those on healthy shoots (P=0.417). Visible gumming was only observed on one retained fruit in the 2020 season, attached to an infected shoot, but was minimal.

While there was a trend toward a higher force required to remove fruit retained on shoots which had hull rot strikes compared to that on healthy shoots there was no significant difference between shoot categories ($P<0.05$). Large numbers of mummies present on the trees were easily removed with little force, indicating that they were a result of poor shaker force in relation to tree architecture and/or variable maturation across the tree at harvest. While it cannot be concluded that hull rot doesn't contribute to mummy formation it is certainly not the main factor driving mummy retention in almonds within Australia.

Rhizopus stolonifer – *hull rot disease expression study*

Eighteen of the 20 inoculated fruit showed signs of infection, with an average expression time of 5.8 days for hull symptoms to occur. However, only 6 of the 18 infected fruit showed signs of associated leaf death with a slightly longer average expression time of 6.3 days. Temperature varied spatially within the tree canopy with exposed positions experiencing higher maximum temperatures during the afternoon, up to 11 degrees warmer than shaded positions. Conversely, night-time temperatures were up to 4 degrees cooler than those observed in shaded positions. Relative humidity followed a similar diurnal pattern in differences between shaded and exposed positions as temperature, with exposed positions experiencing up to 23% less humidity than shaded during the afternoon. Surprisingly, night-time relative humidity readings in exposed positions were up to 8% more humid than those in shaded positions.

Despite observed spatial variations in microclimate there was no apparent relationship between the position within the canopy and the expression of both hull and leaf disease symptoms. Half of the infected fruit that went on to have associated leaf symptoms were, however, situated on the same tree. This could indicate that the status of the tree, both water and nutrient, has a greater effect on the severity of disease expression than canopy relative humidity and temperature. *Rhizopus stolonifer* grows on the internal surface of the fruit and therefore it is likely that the environment inside the split has the greatest effect on disease expression. The internal environment will be more affected by the water and nutrient status of the tree, rather than the microclimate of the canopy surrounding the fruit.

This preliminary study was restricted in its replication due to the limited number of data loggers able to be deployed so definitive conclusions cannot be drawn. A more comprehensive and robust study with greater replication, across multiple growing regions will give a more definitive indication as to the main factors driving disease expression within the canopy environment.

In vitro *fungicide assays*

Most of the fungicides tested restricted the growth of *R. stolonifer* to some extent and could be categorised as fungicidal, fungistatic or ineffective. The most effective were fluopyram + tebuconazole, isopyrazam, copper hydroxide, tebuconazole + azoxystrobin and BLAD polypeptide, which inhibited the growth of *R. stolonifer* by >95% for at least 8 days. Group 3 + 11 and 7 + 11 combination fungicides were more efficacious than Group 11 fungicides alone, but the Group 7 fungicides, isopyrazam and penthiopyrad, applied singly performed better than the Group 7 + 11 combinations over 8 days.

Six biological control agents (BCAs) were tested. Two based on the bacterium *Bacillus amyloliquefaciens* (Serenade Opti and Serifel) were the most effective. Even though *R. stolonifer* is a fast-growing fungus, the bacterium inhibited fungal growth by 60-70%. The four other products containing a combination of bacteria and fungi were less effective with 0-20% inhibition. When the BCAs were retested after allowing time to establish Serenade and Serifel (*B. amyloliquefaciens*) were still the best performing products. In the dual inoculation assay Serenade performed best.

On split green fruit all products tested provided some protection against infection by *R. stolonifer*. This was generally better when fruit were treated with fungicide before inoculation with the fungus, suggesting that they are more effective as protectants than curatives. When assessed after 6 - 8 days, the most effective chemical products applied prior to infection were isopyrazam, copper hydroxide and fluopyram + tebuconazole (64-82% less infection). These were also among the most effective in the *in vitro* testing. Tebuconazole + azoxystrobin was highly effective *in vitro* and led to 54% less infection on detached fruit. The other product that was effective *in vitro*, BLAD protein, only reduced infection by 18% on fruit. Fluopyram + trifloxystrobin which is widely used in the industry had 50% less infection than the control and cyprodinil 36%. The two BCAs provided similar levels of protection (50-65% less infection) to most of the chemical products.

When applied post infection as a curative, fluopyram + trifloxystrobin was the most effective with 45%

fewer infected fruit than the control, followed by tebucoanazole + azoxystrobin (33%). All other products resulted in <20% fewer to slightly more infections than the control.

Overall, there were some promising chemical and biological control agents identified in the *in vitro* screening. When considering both the *in vitro* and detached fruit assays, the most effective chemical products applied prior to inoculation with the pathogen were Luna Experience (fluopyram + tebuconazole), Seguris Flexi (isopyrazam) and Blue Shield (copper hydroxide). Two of these, Luna Experience (fluopyram + tebuconazole) and Seguris Flexi (isopyrazam), are new chemistries which warrant further investigation in orchard trials. The only product tested that showed any efficacy as a curative was fluopyram + trifloxystrobin (Luna Sensation).

The *in vitro* methods used to test the BCAs measured both the ability of the BCA to restrict growth by physical competition, as well as through metabolite production. Most products, except for Botector (*A. pullulans*), were effective using both methods. Our data suggests that the products containing *B. amyloliquefaciens* are the most effective and would be useful to test in orchard trials. Serenade Opti (*B. amyloliquefaciens* 1) resulted in the greatest reduction in pathogen growth and in the green fruit assay performed comparably to the best chemical products (65% fewer infection).

These results demonstrate the importance of applying protectant sprays in a timely manner. As the fruit is most susceptible to the hull rot pathogen during a narrow window of time during early hull split (B3 stage) it is crucial to have the crop protected before the onset of infection.

Trunk disease

Sampling and culture collection

The most commonly isolated pathogenic fungal organism associated with disease symptoms was *D. seriata*, recovered from 28 samples. Also isolated were *Phytophthora* (7), *Phytopythium* spp. (5), *Cytospora* spp. (12), *Neofusicoccum australe* (4), *Collophora* spp. (5), *Eutypa lata* (4), *Pleurostoma richardsiae* (3), *Leptosillia* spp. (2), *Colletotrichum acutatum* (2), *Ganoderma* spp. (2), *Phaeoacremonium* spp. (2), *Botryosphaeria viticola* (1), *Verticillium dahliae* (1) and *Pythium* sp. (1). All of these fungal organisms have been reported to cause disease in almond or in other plant species in Australia or other parts of the world.

These findings are largely consistent with recent findings from California that found Botryosphaeria cankers, caused by Botryosphaeriaceae species (*D. seriata* and *N. australe*) to be among the most common. Phytophthora diseases have been reported in Australian almonds historically, and are commonly documented in California, so the prevalence of these species in Australia was not unexpected. *Phytopythium* spp. have been reported in Spain, however, they have not been reported to be pathogens to almond in Australia. The abundance of these species in soil samples was unexpected. *Cytospora* spp. have been previously reported in South Australia on cherries, and on almonds in California, so it was not unexpected that they were found on almonds in Australia

Collophora spp. were isolated infrequently from trunk and scaffold cankers, although they have been reported on almonds in Spain and Germany. *Eutypa lata* was isolated sporadically during the project, mostly from branch cankers. The scarcity of this pathogen during sampling was unexpected, as it is a common pathogen of grapevine in Australia. *Eutypa lata* was also reported to be rare in Californian almonds. Several other species less commonly isolated during sampling are reported to be pathogenic to almond or other woody hosts.

Most of the fungal and oomycete species that have been isolated in this study have a broad host range of native and agricultural crops. It is likely that there are many inoculum sources near almond orchards, making it difficult to avoid risk of infection. Botryosphaeriaceae and oomycetes have been identified as the most prevalent in the industry and were typically found in trees that were rapidly declining.

Foamy canker

Foamy canker only appeared during the warmer months of spring and summer from late October to February, particularly in very hot years, in the Riverland and Adelaide Plains. Foamy canker mostly appeared in young trees, but in one instance also appeared in 38-year-old trees. Cases were sporadic in Australia, but losses of up to 10% were reported in a block of 2-year-old trees in the Riverland. Trees that had foamy canker symptoms often coincided with other stressors such as trunk disease cankers, shaker injury, and spray drift damage, as was also reported in California. Organisms that have previously been linked to foamy canker elsewhere are *Geosmithia pallida* in oak and *Zymomonas* sp. in almond.

From Australian samples, fungal and bacterial organisms were isolated, and identified by DNA sequencing. Species included *Aureobasidium* spp., *Bacillus* spp., *Brevibacillus* spp., *D. seriata, Fusarium* spp., *Phaeoacremonium* sp., *Pichia* sp., *Saccharomycopsis fibuligera* and *Xenoacremonium falcatum*, none of which were consistent with reports from abroad. Most of these species were reported to be endophytes, however, pathogens such as *D. seriata* and *Phaeoacremonium* sp. were also isolated, although not known to cause foamy canker symptoms. *Saccharomycopsis fibuligera* is reported to be involved with fermentation, producing ethanol from starch. However, pathogenic or fermentative species were not consistently isolated from different samples.

Pathogenicity testing of fungal species

Of the seven fungal species tested, *P. richardsiae*, *E. lata* and *Cm. acutatum* were consistently pathogenic. *Collophora rubra*, *D. seriata*, *P. richardsiae*, *E. lata* and *Cm. acutatum* and *Ca. hispanica* were pathogenic in two of three experiments, and *C. diatrypelloidea* only pathogenic in one experiment.

There are several possible contributing factors for the varying results between experiments. The experiments were carried out during different times of the year which may have influenced fungal growth since rate of colonisation is known to be favoured by warmer temperatures. Furthermore, although cv. Carmel was used for all experiments, trees varied in age and rootstock, with experiments conducted in various locations and conditions, in different sized pots and inoculation methods were altered. These differences in methodology may have also influenced the rate of colonisation.

The fungi were not regularly isolated in advance of lesions except for *P. richardsiae*, which was reisolated four times the distance of the lesion from the point of inoculation. These results were unexpected, as it has previously been reported in grapevines that trunk disease pathogens *D. seriata* and *E. lata* consistently colonise unstained wood well past the necrotic margin. These findings are important, as it could mean that less healthy wood may need to be removed during remedial surgery to control disease.

The pathogenicity experiments revealed that all species tested could cause disease, although some more consistently than others. It is likely that extrinsic factors influence canker development, possibly including water stress, soil nutrients and temperature. It is important to further investigate how these factors may influence common trunk disease pathogens to refine management recommendations.

DBAs were conducted to test the pathogenicity of other fungal species isolated from almonds in Australia, and results verified using potted plant experiments. *Neofusicoccum australe* was consistently pathogenic on detached branches, and *E. lata* was only pathogenic in one of two DBA experiments. To validate the results of the DBA, a potted tree experiment was conducted, and *Neofusicoccum australe* was shown to be pathogenic. The potted tree experiment needs to be repeated to validate results, however, DBAs may be suitable to rapidly evaluate the pathogenicity of fungal isolates. The potted tree experiment has been repeated and will be assessed as part of the next project.

Pathogenicity testing of oomycetes

In repeated experiments using potted trees, all *Phytophthora* spp. (except for *P. rosacearum*) and one *Phytopythium* sp. were pathogenic.

Phytophthora spp. were relatively aggressive pathogens causing significant lesions in stems within 4 weeks, compared to at least 3 months for trunk pathogens. These results suggest that *Phytophthora* species are the most aggressive trunk and root pathogens affecting almonds in Australian orchards. Further research is required to better understand the shifts in the range of species detected in Australia since the late 1980's and the rise in detection of *Phytopythium* species. This could be achieved by more extensive sampling across the major growing regions and further pathogenicity testing of scion trunks and rootstocks.

Cultivar susceptibility experiments

Experiments were conducted on potted trees to determine the susceptibility of almond cultivars to the most common trunk pathogen *D. seriata*. There was no difference between cultivars for susceptibility to *Cm. acutatum*, *Ca. rubra* or *E. lata*, and in only one experiment, cv. Carmel was less susceptible than Price for both *D. seriata* and *P. richardsiae*. These results suggest that the cultivars tested do not vary in susceptibility to trunk disease pathogens.

Research from Spain reported that all almond cultivars tested were susceptible to the trunk disease pathogen *Diaporthe amygdali.* In California, there was no difference in susceptibility between almond cultivars to trunk disease pathogen *N. parvum*. However, these recent publications are at odds with older research conducted in almond, other *Prunus* spp., and in grapevine, that indicate that cultivars vary significantly in their susceptibility to trunk disease pathogens.

Future research should expand the evaluation to a wider range of cultivars grown in Australia. It will also be important to test these pathogens in older trunk tissue, using a longer incubation time to give the pathogens greater opportunity to colonise and produce lesions.

Fungicide evaluations

The majority of the treatments tested *in vitro* reduced mycelial growth of most pathogens by over 50% with the most effective treatments reducing colony diameter by over 90%. Overall, Custodia®, Gelseal®, Luna Experience®, Topas®, Serifel®, Serenade Opti®, Merivon®, Dithane®, Solaris® and Belanty® were the most effective products at reducing mycelial growth.

In both field experiments, four of the nine products evaluated reduced recovery of *D. seriata* in the field. These included Luna® Experience (fluopyram & tebuconazole), Custodia® (azoxystrobin & tebuconazole), Emblem® (fluazinam) and Merivon® (pyraclostrobin + fluxapyroxad).

Preliminary results from the field experiments have identified fungicide products with potential to control trunk disease. More investigation is required to determine whether trunk disease is primarily occurring through hedge-pruning wounds, primary pruning wounds, shaker damage, or other physical damage so that appropriate methods for wound protection can be devised. It will also be important to evaluate these wound treatments against other trunk disease pathogens, develop practical methods of application with sprayers and determine wound susceptibility and critical timing for application. It will also be important to increase the number of products to be evaluated to include those that have been proven successful in Californian research, as well as other products that may be effective based on the *in vitro* screenings.

Lower limb dieback investigations

Lower limb dieback studies were conducted to observe how symptoms progress and to better understand the cause of the potential disease. Several possible causative or contributing factors to the dieback syndrome were investigated, including light, tree age, cultivar, and trunk disease pathogens.

It is unlikely that pathogens are the only cause of LLD. No single species was consistently isolated from the samples and 50% of samples yielded no microorganisms at all. Scale infestations were not reported during surveys, eliminating them as a potential cause of LLD in the assessed orchards.

Heat maps produced from orchards in Paringa and Loxton showed that there was less dieback in trees on the margins of the block in all three years. This "edge effect", suggests that light could be a contributing factor to LLD in these blocks. However, LLD branches with chlorotic leaves were sometimes located higher up in the canopy with ample light, indicating that there are likely to be other factors contributing to symptom expression.

The final data analysis also confirmed that numbers of yellow branches and dieback ratings increased with tree age. There were significant differences between properties among blocks that were of similar age and had the same cultivars, but there was very little difference between blocks of similar ages with the same cultivars on the same property. Differences between properties with blocks that were the same age were also highlighted in the general disease surveys, where some older blocks would have less severe LLD than blocks of the same age on different properties. This shows that there are likely management or environmental differences that impact LLD severity.

It was observed that almond trees that appeared taller than the rows were wide had more severe LLD than blocks with shorter trees, particularly when the canopies of those trees grew into one another across the rows. It has been indicated by international research that LLD appears to be more of a problem in orchards with high inputs and intensive production systems, which was reflected in our own observations. The "rise" of LLD in the Australian almond industry over the last decade could be attributed to the intensification of the industry and the increase of the mean age of trees. Greater nutrient and water inputs would likely increase the size of the trees, resulting in the lower canopies of trees getting shaded out be adjacent trees.

The syndrome is likely to be caused by a number of factors. In future research, canopy management, row spacing and the influence that these factors have on light interception and LLD severity could be investigated further in long-term studies.

Outputs

Output summary

Output	Description	Detail
Publication in industry magazines	9 articles in In a Nutshell	Appendix H
Reports to industry	1 Technical report 10 Milestone reports to Hort Innovation 4 reports to ABA board 10 reports to SRG	Appendix C: Economic analysis Appendix H Communication outputs
Farm management tool	Updated disease guide	Appendix F
Scientific publications	3 Journal articles 12 Conference proceedings 1 PhD Thesis	Appendix H Appendix G
Industry meetings, events and	10 SRG meetings 4 webinars	List of Industry meetings and events is available in Appendix H

workshops	3 Invited speakers 2 ABA conferences 1 ABA field day	

Outcomes

Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Practices identified for management of hull rot	Outcome 1, Strategy 2, KPI 4	Data from grower surveys used to develop adaptive management guidelines. Potential risk factors identified and evaluated (varieties, rootstocks, nitrogen and water). Disease risk prediction guidelines for hull rot developed.	Risk guidelines drafted, fact sheets measures of effectiveness incorporated into feedback from growers and SRG
Improved awareness of hull rot, LLD, and trunk disease by industry	Outcome 1, Strategy 2, KPI 4	Disease guide updated Fact sheets developed, webinar had	Updated disease guide Growers able to recognise symptoms of hull rot, LLD & trunk disease
Status of *in vitro* efficacy of registered and new chemistries established	Outcome 1, Strategy 2, KPI 2	Field trials evaluated and recommendations developed.	Chemistries tested and recommendations made to Hort Innovation
Enhanced management practices for disease control including planting and pruning	Outcome 1, Strategy 2, KPI 4	Factors contributing to diseases analysed, adaptive guidelines for disease management (hull rot and LLD focus), management practices formulated and provided to industry through fact sheets, extension project (AL16001) and other forums	Fact sheets, presentations, publications. Webinar attendance records and feedback
Strengthening of breeding program by understanding varietal susceptibility of new selections	Outcome 1, Strategy 2, KPI 2	Susceptibility of main varieties and breeding lines to hull rot and LLD assessed and information provided to Australian almond breeding programme	Information shared with breeding programme and industry
Increased knowledge of the causes of major diseases of almonds, including hull rot, LLD and trunk disease	Outcome 1, Strategy 2, KPI 4	Causal organisms identified, factors contributing to diseases analysed, regional differences analysed.	Specimens deposited with VPRI. Knowledge of causes shared via fact sheets.
Up to date knowledge of the economically important diseases of almonds in Australia	Outcome 1, Strategy 2, KPI 4	Completed report on trials, monitoring guidelines, risk guidelines, benchmarking	Final Report

Enhanced almond disease diagnostic capabilities in Vic, SA and NSW	Outcome 1, Strategy 2, KPI 4	Staff at AVR and SARDI able to identify symptoms and causal organisms of prevalent diseases.	Record of training

Monitoring and evaluation

Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
1. To what extent has the project achieved its expected outcomes?		
To what extent has the project contributed to increased knowledge of the causes and management of: hull rot, LLD.	Updated Disease Guide, Risk guidelines drafted, webinars, fact sheets, records of publications Appendix F and H	Increase collaboration with US researchers by regular travel to California and hosting them in Australia.
Have disease diagnostic capabilities been improved through project activities?	Disease diagnostic capabilities have been improved with team members increasing their knowledge and experience on symptom identification in the field. Numerous field trips with all team members to different almond growing regions has enabled the team to see diseases of prevalence in the different regions. AVR and SARDI both have diagnostic laboratories with which the information has shared. Growers have benefited from the updated disease guide available through ABA.	Masterclass on disease symptoms should be developed and held in almond growing regions to teach industry personal about diseases.
2. How relevant was the project to the needs of intended beneficiaries?		
How has the project contributed to improved management of diseases of economic importance for almond growers?	**Hull rot** Studies have identified the main causal organism of hull rot in Australia. Rootstocks were identified that result in less hull rot on Nonpareil scions. Australian breeding lines were shown to have more tolerance to hull rot than Nonpareil. *In vitro* fungicide evaluation found x promising chemicals and biologicals that could be incorporated into spray schedules. This new knowledge is important for disease management strategies. **Economic analysis** found application of fungicides for hull rot and trunk disease has a net monetary benefit to growers.	
3. How well have intended beneficiaries been engaged in the project?		

Have sufficient numbers of growers and/or areas of production been engaged in the disease surveys conducted by the project?	Industry-wide disease surveys covered all almond growing regions and included 26 orchards. Specific field trials were also conducted across Sunraysia and Riverland.	
Have all major producing areas been adequately covered?	Yes	Find grower champions in each region to host trials.
What level of engagement (Outreach) has occurred beyond the direct trial sites of the project?	Team members have attended numerous orchards with disease issues that were not included in the survey. Monterey decline was identified as an emerging problem in the Riverina based on these visits.	More systematic study on Monterey decline to identify abiotic or biotic causes.
Have the agreed levels of communication (presentations, articles fact sheets etc) been delivered.	Extensive communication activities were undertaken during the project exceeding the number proposed at the beginning of the project. These are summarized in Outputs table and detailed in Appendix H.	
Were industry extension and communication programmes utilised for this (AL16000, AL16001)?	Yes, these activities were delivered in collaboration with AL16001 and 19001. AL19001 facilitated 3 webinars for team members to present results and produced a video on diagnosing trunk disease in the orchard.	Develop an annual extension and communication plan in collaboration with AL19001.
Were corporate growers engaged to field test early adoption of improved management techniques, and how was their feedback incorporated into further development of the techniques?	**Hull rot** Field evaluation of several products were done on corporate orchards. The following season the corporate changed their spray schedule based on our results. Validation of the Ceres Imaging algorithm was done across 2 corporate farms. A small epidemiology study was done on a family farm. **LLD** A commercial orchard in Paringa was intensively monitored over three seasons for LLD symptoms. Results were discussed with the orchard manager and owner, helping to elucidate irrigation and canopy management activities that impacted symptoms.	In the future identify grower champions who are willing to host a demonstration site on their property and allow field walks for other industry personnel.
4. To what extent were engagement processes appropriate to the target audience/s of the project?		

Were engagement events undertaken in all major production regions at appropriate times to allow growers to test and implement project information?	Regular webinars were held during the project. This allows industry actors to get information while still maintaining their operations. Orchard walks were attended early in the project. COVID prevented more regular face to face activities.	In future, need to provide exit survey to attendees to capture future issues.
How well were the extension materials (e.g. fact sheets) adopted by participants, and was the information considered appropriate by the growers?	During AL16005, 7 factsheets were generated and made available on the ABA website resulting in 466 unique views and 4 webinars/videos received 111 unique views.	Further collaboration with extension specialist to determine what medium the industry prefers its information.
Has engagement with the Stakeholder Reference Group been utilised to extend engagement to industry?	Yes. Twice yearly SRG meetings were held to provide research activity updates and receive feedback on future planned research activities.	Determine if SRG members are providing 'talking points' to other industry participants. SRG should include more crop agronomists that service large parts of the industry. SRG is dominated by corporate members.
5. What efforts did the project make to improve efficiency?		
Did the planned collaboration with AL16009 (Almond IPM) take place?	The two projects shared a SRG and alternated the management and reporting responsibilities when the independent coordinator withdrew from the role. Joint IPM/IDM discussions were held to share planned work programs and discuss areas suitable for collaborative work. There was close collaboration between the two projects during the following studies: •Spatial distribution (IPM), Lake Powell (2018/19). Joint fieldwork involving extensive mummy nut surveys, nut sample collection and sharing for pest infestation and hull rot assessments. •Mummy nut thresholds (IPM), Bannerton (2018/19). Joint fieldwork as above. This study was later abandoned due to almost total mummy removal by birds. •Mummy nut development (IPM), Lake Powell (2019/20). Joint fieldwork involving	

	mummy nut counts and complete pre-harvest removal of mummy nuts from trees, assessment of new mummy nut formation after subsequent harvest, assessment of pest infestation levels, hull rot strikes and strength of mummy nut attachment.The two projects shared information on Phase 2 research areas of common IPM/IDM interest.	
What (if any) efficiency improvements were evident in using combined trial sites with AL16009? How was this assessed?	Combining IPM and IDM field teams to perform mummy nut counts and sample collections required for the spatial distribution and threshold studies reduced the duration of fieldwork for those studies improved our understanding of each other's research and allowed for a level of analysis of links between mummy nuts and hull rot strikes on the new crop, by the IDM team, that would not have otherwise been possible.	
What efficiency improvements were achieved by the off-season collaboration with California researchers?	Hull rot management practices were suggested by Californian researchers during the study tour. LLD and trunk disease experimental methodologies were designed with advice from Californian researchers, fast-tracking outputs without the need for pilot experiments and delays from trial and error.	Regular engagement with Californian researchers should take place in future projects. Hand-on experience in California will enable emerging diseases to be better understood prior to them becoming issues in Australia.
Did this add to the project team's ability to test ideas and innovations?	Yes, diKap™ a product available in the US that is promising for hull rot management was procured from US for trials in Sunraysia. This was facilitated by Redox Australia.	
Were trials undertaken with industry actors (growers, processors) effective at improving uptake of information?	Trials on industry sites do facilitate the uptake of information by those industry actors involved. Depending on the industry actor there may have limited dissemination to the wider industry if a field walk is not conducted. However, results are presented at industry forums for a wider audience.	Demonstration sites should be included in future projects.

Recommendations

Almond growers should adopt integrated disease management practices.

Application of cultural practices that contribute to reduction in pathogen inoculum should be encouraged. This reduces the risk of poor disease control when environmental conditions prevent fungicide applications.

Knowledge of the varietal susceptibility is important consideration when choosing varieties for new orchard establishment or replanting.

New knowledge on the importance of *Phytophthora* spp. in the trunk disease complex have led to the proposal to further investigate the prevalence and impact of these aggressive pathogens and to better understand epidemiology of all important trunk pathogens to improve recommendations for management of almond trunk disease.

Efficacy data for fungicide and biocontrol products on almond diseases provides a foundation for further evaluation which could lead to registration of products, making them accessible to industry for managing disease.

Resistance status of current fungicides in use in the almond industry requires reviewing.

Monterey decline systemic study to identify abiotic and biotic causes to develop preventative strategies.

Further studies are required on water and nutrient management to reduce the incidence of hull rot.

Further studies are required to understand the influence of orchard design and canopy management on hull rot severity.

Refereed scientific publications

Journal article

Zaveri, A.; Edwards, J.; Rochfort, S., 2022**.** Production of primary metabolites by *Rhizopus stolonifer*, causal agent of almond Hull rot disease. Molecules 27, 7199-7223.

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Manuscripts in preparation

Wiechel T, Oswald B, Kreidl S, Faulkner P, Giri K, Zaveri A, Tesoriero L, McKay S, Sosnowski M, Edwards J., 2023. Survey of diseases in Australian almond orchards. For submission to Australasian Plant Pathology.

Oswald, B, McKay S, Kreidl S, Dinh Q, Edwards J, Sosnowski M., 2023. Preliminary evaluation of fungicides and biological control products as pruning wound protectants for trunk disease pathogens of almond in Australia. For submission to Australasian Plant Pathology.

Oswald, B, Tesoriero L, McKay S, Kreidl S, Dinh Q, Edwards J, Sosnowski M., 2023. Pathogenicity testing of *Phytophthora* species in Australian almonds. For submission to Australasian Plant Pathology.

Paper in conference proceedings

Oswald, B.J., McKay, S.F., Tesoriero, L.A., Kreidl, S.L., Dinh, Q., Edwards, J. and Sosnowski, M.R., 2022 The current status of *Phytophthora* spp. in Australian almond orchards. 11th Australasian Soilborne Disease Symposium, 1-5 August 2022, Cairns.

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Intellectual property

No project IP to report

Acknowledgements

This project has been funded by Hort Innovation using the almond research and development levy and funds from the Australian Government with in-kind contributions from Agriculture Victoria and SARDI. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Select Harvests
Central – Ben Brown, Upul Gunawardena, Tim Kennedy, Josh Moss, Vili Ale, James Connolly, Aaron Bailey, Peter Jealous, Gemma Nunn, Jane Fisher, David Smith,
Northern – Eryn Lehmann
Southern – Michael Ward, Peter Smith

Agriculture Victoria – Dolf de Boer, David Madge, Cathy Taylor, Blair Grossman, Zelmari Coetzee, Michael Treeby, Quang Dinh, Mofakar Hossain, Chin Gouk

SARDI - Ian Bogisch, Matt Ayres, Anna Child, Cathryn Todd, Jessica Jolley, Tim Pitt

University of Adelaide – Michelle Wirthensohn, Samuel Rogers

UC Davis - Florent Trouillas, Leslie Holland, Mohamed Nouri, Themis Michailides

Almond Board of Australia - Josh Fielke, Deidre Jaensch

Ceres Imaging – Scott Gillett

Lacton Farms – Tony Spiers

CMV Farms – Tim Preusker, Callum McNaughton

Almas Almonds – Abby White

Century Orchards – Scott McKenzie, Gemma Nunn, Brendan Sidhu

Woolenook Fruits – Ben Haslett, Ashley Sleep

Ruggiero Almonds – Vince Ruggiero

Walker Flat Almonds – Peter Cavallaro, Melina Mueller

Cowanna – Neale Bennett

Manna Farms – Brendan

Outback Almonds Tom and Sholto Douglas

Aroona Farms – Brian Slater

Indian Ocean Farms – Stephen Beckworth

Nutwood – Peter Rohrlach

Big River Almonds - Simon Vause

Domenic Cavallaro

Brent Wickham

Nick Pezzaniti

Gary Thorpe

Peter Freeman

Appendices

Appendix A: Almond disease review

Appendix B: Grower Census and Industry-wide disease survey

Appendix C: Economic analysis

Appendix D: Hull rot

Appendix E: Trunk disease

Appendix F: Updated Disease Guide

Appendix G: PhD Thesis 'Investigating the Infection Process of *Rhizopus stolonifer* and Symptom Development in the Almond Disease Hull Rot' by Anjali Zaveri School of Applied Systems Biology, La Trobe University, Bundoora

Appendix H: Communication outputs

Appendix I: Project related outputs (program logic)