Lower limb dieback in Australian orchards

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Introduction

International research has been unable to definitively determine the cause of LLD (Lampinen et al., 2009b, Michailides et al., 2010) and prior to this study, there was no research on LLD in Australia. These studies were aimed at determining the prevalence of the syndrome while monitoring symptom development and investigating the potential causes that have been implicated in Californian research.

Symptoms of LLD initially appear as chlorotic (yellow) leaves along the length of branches in the lower canopy (Figure 1A) in spring that fall after approximately 6 weeks. Defoliation is followed by shoot death and branch dieback (Figure 1B), particularly after hot weather events in late spring and summer (Trouillas et al., 2016, Doll, 2009, Doll, 2014, Doll and Brar, 2014). Symptoms can also include brown necrotic lesions under the bark, central staining (Figure 1C), and occasionally wedge-shaped cankers in cross-sections of symptomatic limbs (Figure 1D). Stained wood usually produces little to no gumming (Doll, 2009). Although staining is often present in the

Hort Innovation Strategic levy investment ALMOND FUND AGRICULTURE VICTORIA SOUTH AUSTRALIA Government of South Australia Primary Industries and Regions SA SARDI

symptomatic wood tissues, it is unclear whether fungi causing this staining are causal agents of LLD or opportunistic secondary invaders (Doll, 2009, Trouillas et al., 2016, Doll, 2014).

In California, LLD is predominantly observed in cultivars Butte and Padre but has been recorded in other cultivars, including Nonpareil, Fritz, Carmel, Mission and Wood Colony (Adaskaveg et al., 2017, Doll, 2014).

There are multiple hypotheses as to the causes of LLD. Factors such as scale infestations, shade out of lower limbs, water management, herbicide drift and fungal pathogens associated with the death of woody tissue have all been implicated as possible contributors to LLD (Doll, 2015, Doll and Brar, 2014, Lampinen et al., 2010, Lampinen et al., 2009b).

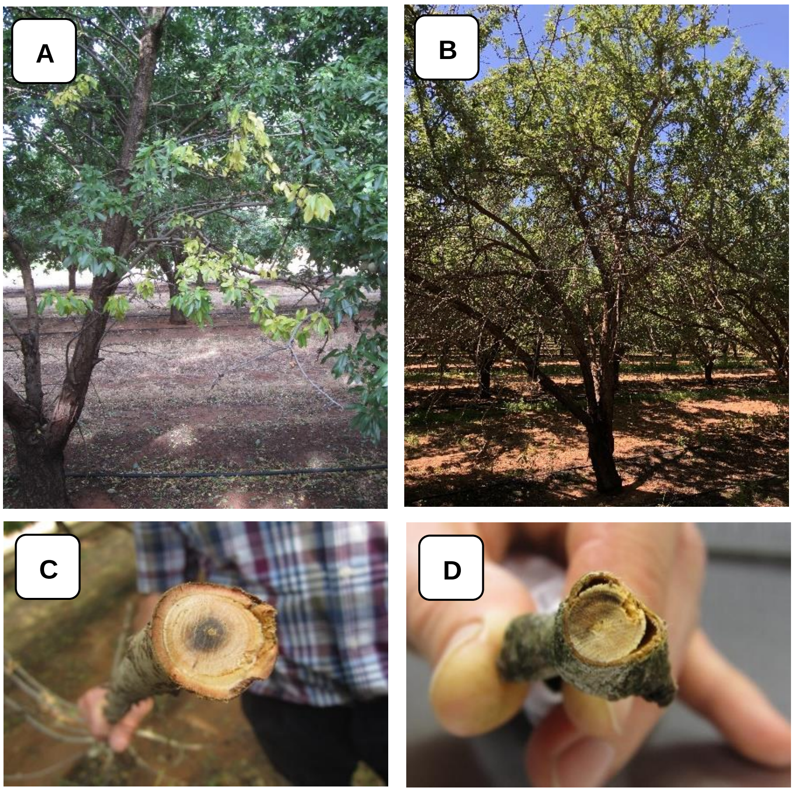

Figure 1. Typical LLD symptoms. **A.** Yellow leaves appearing in the lower canopy. **B.** Dead branches in the lower canopy. **C.** Central staining and **D.** wedge-shaped canker in symptomatic branches.

As part of the Almond Integrated Disease Management project (AL16005), surveys were conducted in four states from 2018-2020 to investigate the prevalence of diseases in the Australian almond industry (Wiechel et al., 2022). During the surveys, a disease severity rating scale was developed, in which trees were assigned a percentage of the canopy affected (Figure 2). The surveys revealed that LLD appeared in trees aged 8 years and older as reported in Californian research (Doll, 2014, Michailides et al., 2010). LLD was widespread, occurring in all cultivars assessed in Australia, including Nonpareil, Carmel, Price, Peerless, Ne Plus, Wood Colony, Johnson, Carina and Monterey. Generally, as trees matured, the severity of dieback increased, and there appeared to be less light interception to the lower canopy compared to younger blocks. There were some exceptions where orchards with mature trees had a lower incidence and severity of LLD. These blocks were generally pruned with smaller canopies and appeared to receive ample light in the lower canopy (Figure 3). There also appeared to be variation in the severity of LLD between cultivars (Wiechel et al., 2022).

Intensive surveys were carried out in the Riverland of South Australia between 2019 and 2021 to examine the effects of cultivar, tree age, canopy light interception and trunk disease pathogens on LLD symptom expression and progression.

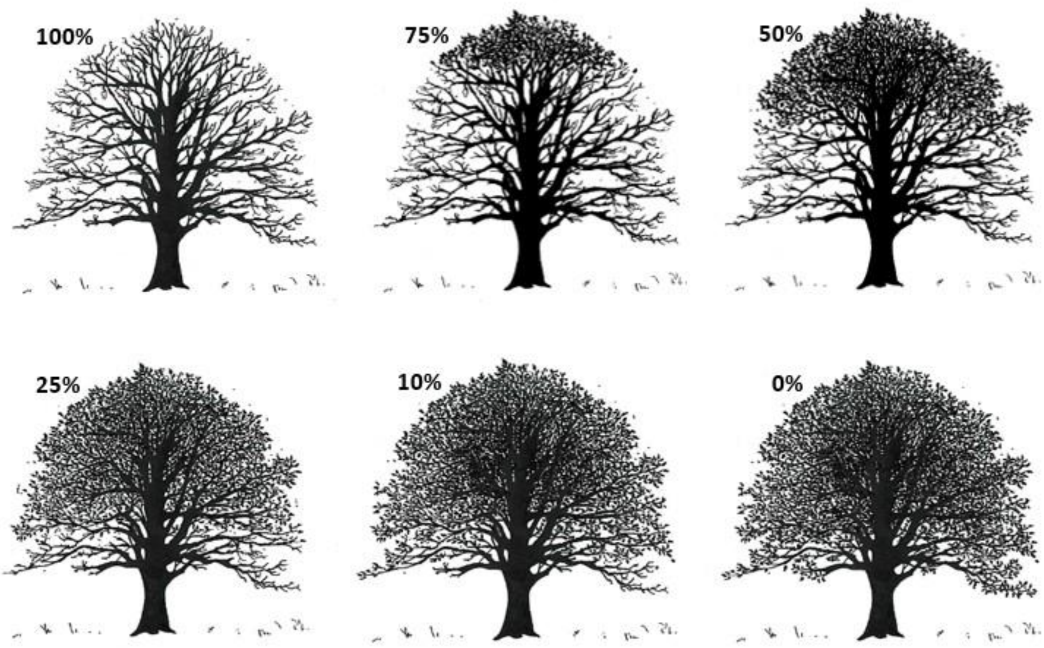

Figure 2. Lower limb dieback severity rating scale which is based on percentage of dead or unproductive limbs and branches.

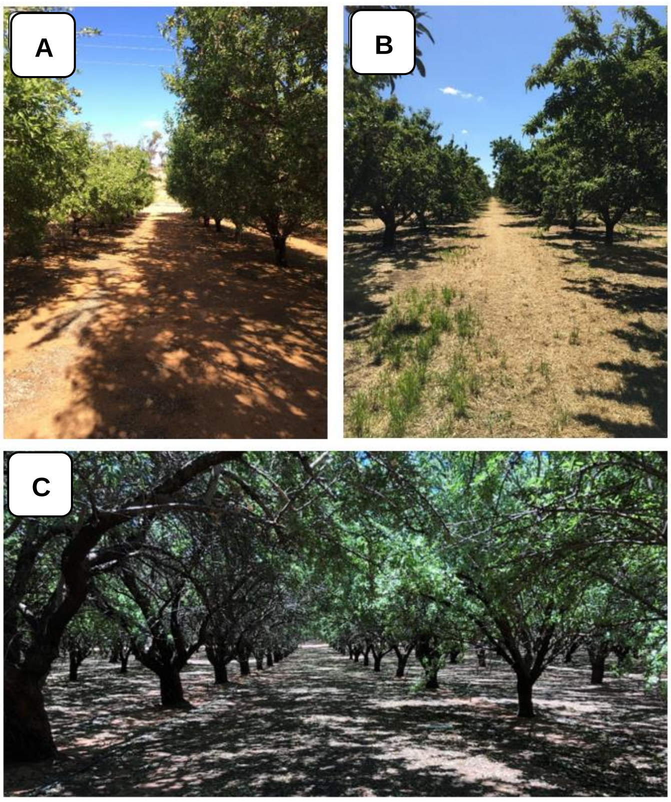

Figure 3. A. Twenty-three-year-old trees in the Riverland with low severity of lower limb dieback (LLD) and little shading. **B.** Thirty-eight-year-old trees in the Adelaide Plains with low severity LLD symptoms and little shading**. C.** 22-year-old trees in the Riverland with high severity of LLD symptoms and heavy shading, with canopies meeting between rows and trees.

Methods

Intensive monitoring

Lower limb dieback severity ratings

On several properties in the Riverland, South Australia, the LLD symptom expression and progression were intensively monitored using the dieback severity scale previously developed for LLD (Figure 2) for each spring from 2019 to 2021. Monitoring was conducted on various blocks with a range of tree ages and cultivars within each property.

Orchard 1

In Orchard 1, a 2.8 ha block of 24-year-old trees (cvs. Price, Carmel, and Nonpareil) was intensively monitored in October 2019. Every second tree within each row of the block was assessed for LLD severity. Heat maps were produced to show the spatial distribution of LLD severity across the block. In the second and third years of monitoring, LLD assessments were repeated during November 2020 and 2021 to monitor disease progression and seasonal differences in aging trees.

Orchard 2

At Orchard 2 assessments were conducted in two blocks in 2020 and 2021. Every second tree within each row of the blocks were assessed for LLD severity. The blocks consisted of 12- 13-year-old trees (cvs. Nonpareil, Carmel and Monterey) and 25- to 26-year-old trees (cvs. Nonpareil, Carmel, Price and Peerless).

Extended monitoring

Orchard 1, 2 and 3 Three additional blocks in Orchard 2 and a further five blocks from Orchard 3 were added in 2021. Surveys in these additional blocks were limited, where only every second tree within one row of each cultivar was assessed per block, rather than the whole block. This allowed for more tree ages (7, 10, 14, 21 and 33 years) and cultivars (Nonpareil, Carmel, NePlus, Peerless, Price and Monterey) to be included in the analysis. These additional assessments were conducted to better understand the relationships between LLD severity and cultivar, age, and location. Data collected from extended monitoring activities were combined with data collected from intensive monitoring in 2021.

Light investigation

Light was investigated as a possible contributor to LLD during the second year of the survey. Light interception readings were measured using a Model MQ-200 Quantum meter (Apogee© Instruments) in three survey blocks that were intensively monitored in Orchards 1 and 2. Light was measured as photosynthetically active radiation (PAR) in the wavelength range of 400-700 nanometers, which is available to plants for photosynthesis. The methods used were adapted from Lampinen et al. (2009a) and Zarate-Valdez et al. (2015). Light readings were recorded underneath every second tree from one row for each cultivar in each block. The light sensor was placed at the end of a pole, and readings were taken approximately 30 cm from the ground at 20 positions around the circumference of the trunk at a distance of approximately 50 cm.

Trunk disease pathogen investigation

Fifteen chlorotic branches were tagged for each cultivar in Orchard 1 in 2019, and photos of each tagged branch were taken. Six weeks later, almost all tagged branches were completely defoliated on a return visit to the orchard. For each variety, five out of 15 tagged branches were removed and returned to the laboratory for diagnostic analysis.

Statistical analysis

Dieback severity data were analysed using RStudio (R Core Team 2021, Nielsen et al. 2021). Data from the extended and intensive monitoring in 2021 were combined and analysed using ANOVA and generalised mixed effects linear models. Tukey's multiple comparison test was used to determine whether the results were significantly different from one another.

The correlation coefficients between light readings below the canopy and LLD severity ratings were calculated.

Results

Intensive monitoring

Orchard 1

Heat maps were created to show the spatial distribution of LLD severity for 2019, 2020 and 2021 assessments (Figure 4). Dieback severity ranged from 0-90% across the block. Lower limb dieback was less severe on the outer rows and tended to be more severe toward the southern end of the block in both years of assessments. There were 23 dead or missing trees (3.9%) in 2019, 27 (4.6%) in 2020 and 32 in 2021 (5.39%). From observations and previous diagnostic results, the missing trees were likely lost to infection by Phytophthora disease.

Lower limb dieback severity was significantly lower on Carmel than on Price and Nonpareil in both years. Dieback severity ratings increased by 9 and 16% from 2019 to 2020 for all three cultivars.

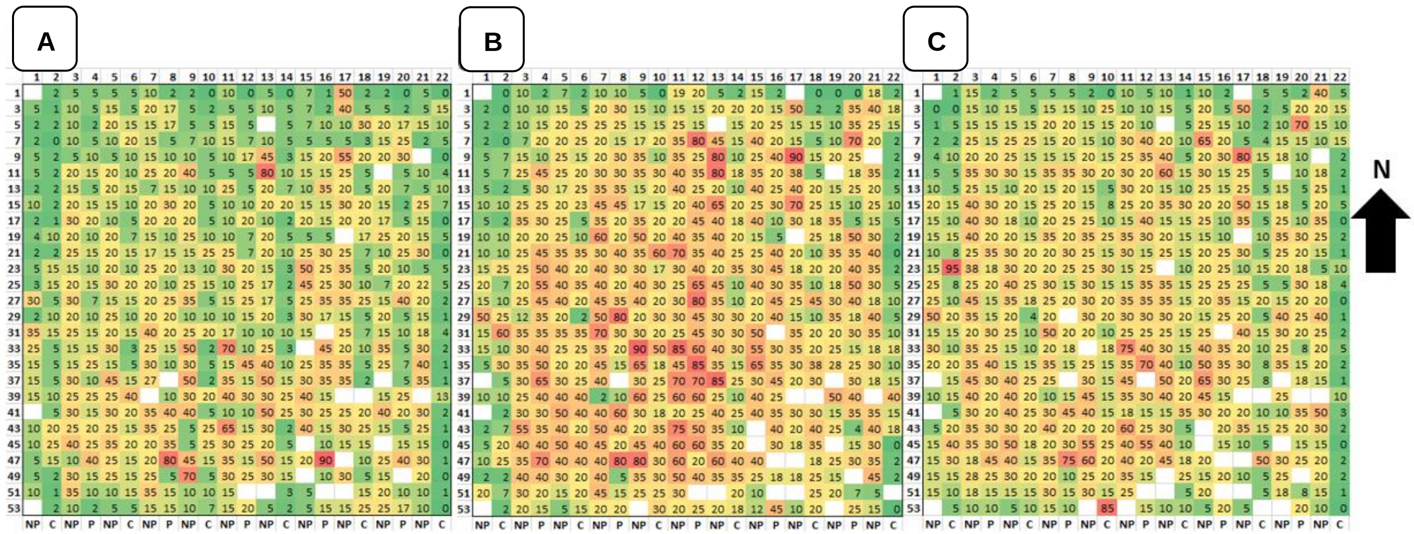

Figure 4. Lower limb dieback (LLD) severity ratings in an almond block (planted in 1996) assessed on **A**. 21 October 2019, **B.** 17 November 2020 and **C.** 18 November 2021 in Orchard 1, Riverland, South Australia. Every second tree in each row of the block was assessed and each square represents a single tree that was assessed. The block consisted of three cultivars, Price (P), Carmel (C) and Nonpareil (NP). The LLD severity rating assigned to each tree is denoted in each of the cells, with colours assigned from green (0% - no dead limbs) to red (100% - all limbs dead or unproductive). Blank white squares denote trees that are dead or missing.

Orchard 2

Heat maps were created to show the spatial distribution of LLD severity in 2020 and 2021 for the 12-13 and 24-25-year-old blocks (Figure 5). Dieback was more severe in the older block (5-90%) than in the younger block (0-25%). Dieback was less severe in trees at the ends of rows in the older block.

For the 2020 assessments, mean LLD severity differed among cultivars in the 12-y-o block, with significantly less LLD dieback in Carmel (0.3%) than in Nonpareil (2.3%) and Monterey (2.6%). Mean LLD severity did not differ among cultivars in the 24-y-o block, ranging between 45 and 53%.

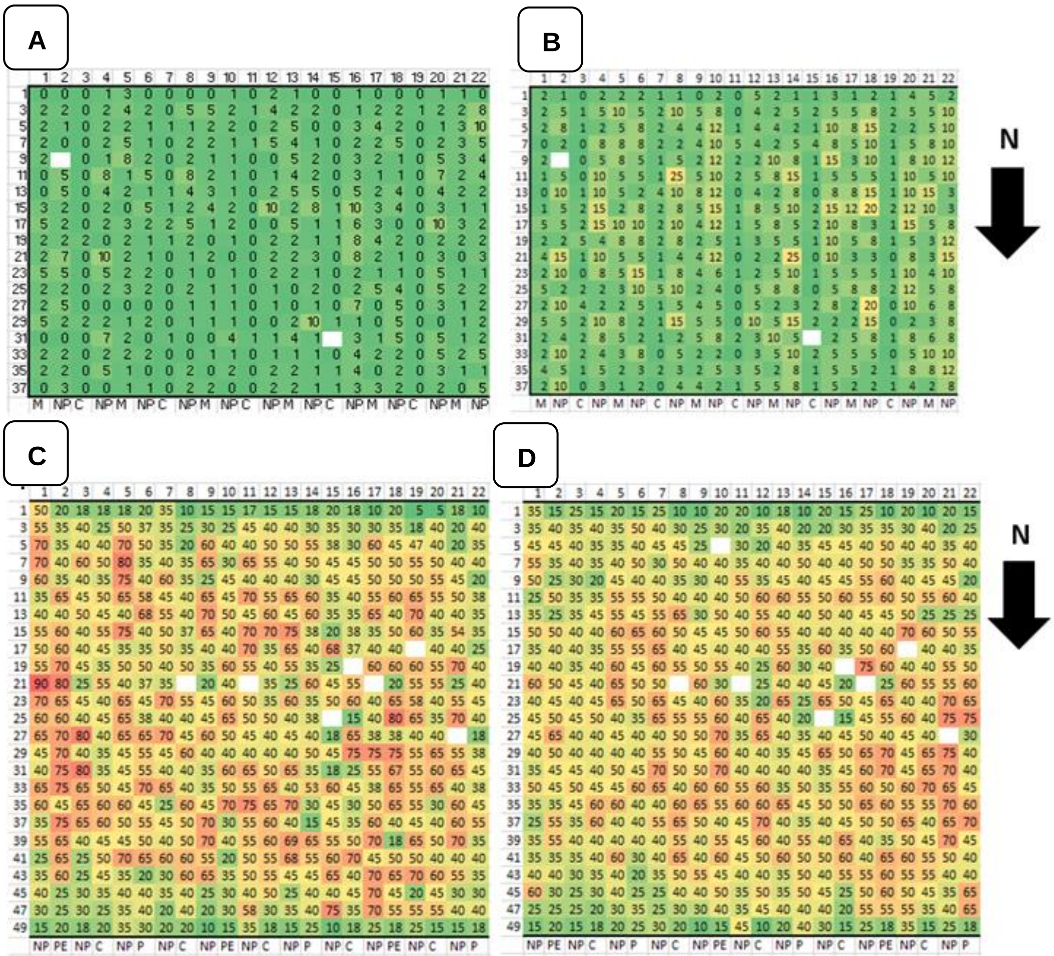

Figure 5. Lower limb dieback (LLD) severity ratings were recorded in two blocks of almond trees in Orchard 2, Riverland, South Australia. Trees were assessed in November 2020 (**A&C**) and November 2021 (**B&D)**. Trees were planted in 2008 (**A-B**) and 1996 (**C-D**). Every second tree in each row of the block was assessed, and each square represents a single tree that was assessed. The lower limb dieback (LLD) severity rating assigned to each tree is denoted in each of the cells, with colours assigned from green (0% - no dead limbs) to red (100% - all limbs dead or unproductive). Blank white squares denote trees that are dead or missing. The younger block (**A-B**) consists of cvs. Monterey (M), Carmel (C), and Nonpareil (NP), and the older block (**C-D**) consists of cvs. Carmel (C), Nonpareil (NP), Peerless (PE), and Price (P).

Extended monitoring

Orchard 1, 2 and 3

There was a statistically significant difference in LLD severity among the three different properties and varieties. Orchard 1 had significantly less LLD than Orchards 2 and 3. All varieties were significantly different from one another, except for cvs. Price and Nonpareil, which had the same LLD severity. As the blocks aged, the dieback severity increased (Figure 6). There was little difference in LLD severity between blocks of the same age and cultivar within an orchard, but trees of the same cultivar and age differed between orchards.

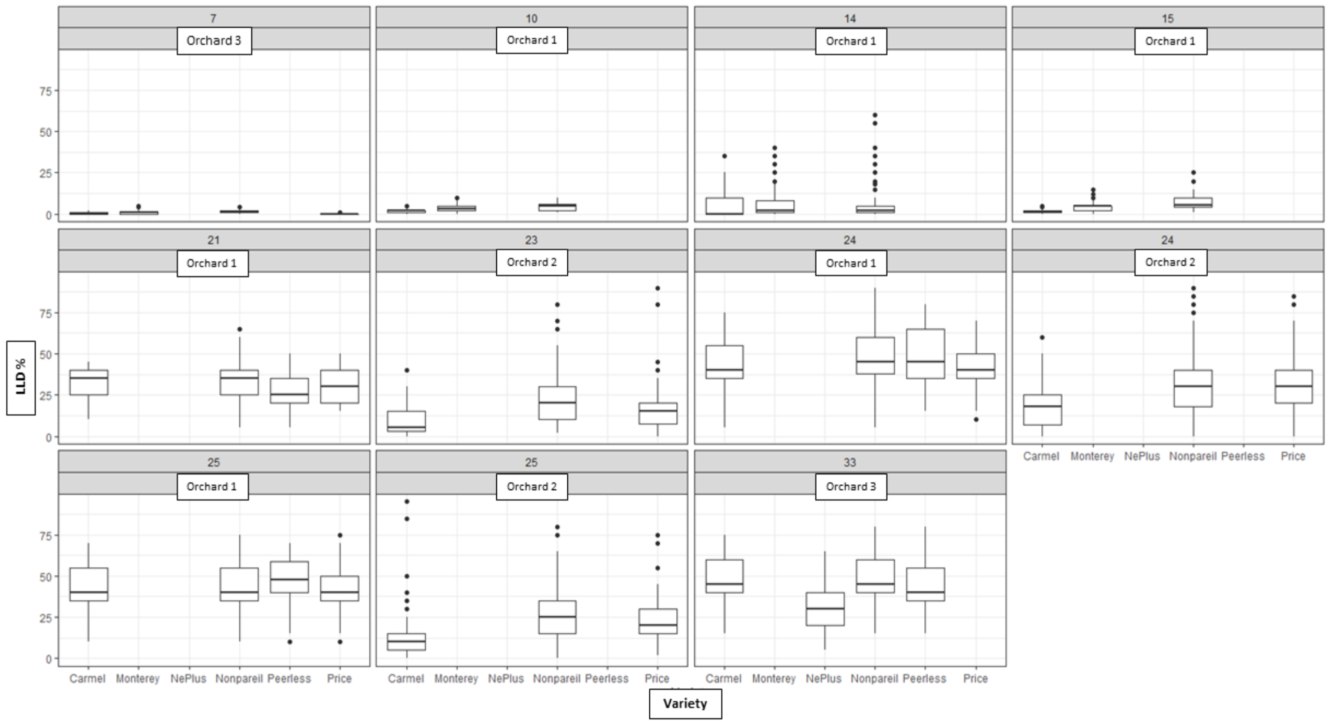

Figure 6. Differences in dieback severity among blocks of different ages on three different orchards in the Riverland in South Australia. Age of trees shown at the top of each plot.

Light investigation

In the 1995 planted block in Orchard 1, the correlation between PAR and LLD severity was weakly negative for Carmel (r = -0.42), but no correlations were evident for Nonpareil (r = -0.05) and Price (r = 0.19). In Orchard 2, there was no correlation between PAR and LLD severity for any cultivars in the 2008 planted block (r < 0.18), and for the 1996 planted block, there was a weak negative correlation for Peerless (r = -0.46) and Nonpareil (r = -0.36), but not for Carmel (r = -0.14).

Trunk disease pathogen investigation

Isolations from the Orchard 1 revealed that some pathogenic fungal species were present, including *Botryosphaeria* spp., *Collophora* sp. and several other fungal isolates yet to be identified. However, no single species was consistently isolated from the 15 samples and seven samples (50%) yielded no microorganisms at all. This suggests that trunk disease pathogens are unlikely to be the main cause of LLD in this orchard.

Discussion

Cultivars

Cultivars varied in LLD severity; Nonpareil and Price generally had more severe dieback than Carmel in older trees (>23 years). In younger trees (<15 years), there was little variation between cultivars, which had very low disease severity. Researchers in California have reported that LLD appears most severe in cultivars Padre and Aldrich, which are not commonly planted in Australia (Adaskaveg et al., 2017, Doll, 2014). These differences in symptom expression among cultivars could be because there are differences between spur mortality with lower light levels for different cultivars (Coetzee, 2019). Almond cultivars also differ in canopy structure (Thorpe, 2020, Montesinos et al., 2021), and some may allow more light penetration to lower branches than others.

Tree age

Orchards were observed with LLD from 8 years of age, with severity increasing as orchard age increased, consistent with Californian research (Doll, 2015, Lampinen et al., 2009a, Michailides et al., 2009). There were significant differences between properties with blocks of similar age and cultivar, and severity was less in some older blocks than others, suggesting that there are likely management or environmental differences that impact LLD severity.

Light interception

Heat maps produced from two intensively monitored orchards showed less dieback in trees on the margins of the block, with the highest severity ratings occurring towards the centre of the block. This “edge effect” suggests that light may contribute to LLD in these blocks. Trees at the edge of the block receive more light to the lower branches than trees within the blocks, that adjacent rows can shade out. Previous research conducted in Australia has indicated that spur mortality increases with decreased light interception (Coetzee, 2019). However, LLD branches with chlorotic leaves were sometimes located higher up in the canopy with ample light, indicating that there may be other contributing factors. Trees on the edge of the orchard have less root competition for water and nutrients, which may also affect canopy growth.

Weak negative correlations were observed between PAR and LLD severity for some cultivars in the older blocks. The data suggest that decreased light penetration to the lower branches may contribute to greater LLD severity. However, spurs along branches often receive different amounts of light, but all spurs on branches tend to die simultaneously (Lampinen et al., 2009a). It has been reported that light is not the only contributing factor to LLD. Although LLD symptoms are similar to shading, some minor differences exist. A key characteristic of an LLD-affected branch is the simultaneous dieback of spurs, which occurs within 6 weeks, whilst limbs dying back from shading usually take more than one season to defoliate and die (Lampinen et al., 2009b, Adaskaveg et al., 2017).

Trunk disease pathogens

It is unlikely that pathogens are the only cause of LLD. Branches with chlorotic leaves from several cultivars in Orchard 1 were removed and returned to the laboratory for diagnostic analysis. Isolations revealed that pathogenic fungal species were present in some samples, including *Botryosphaeria* spp., *Collophora* sp., and several other saprophytic organisms. However, no single species was consistently isolated from the samples, and half of the samples yielded no microorganisms at all. Our findings are consistent with Californian research, as they also could not isolate trunk disease pathogens consistently, reporting that symptomatic branches yielded trunk disease pathogens at a similar rate as asymptomatic branches (Michailides et al., 2010, Trouillas et al., 2016). Scale infestations were not reported during surveys or monitoring, eliminating them as potential causes of LLD in the assessed orchards.

Conclusion

Based on this study's results, LLD may result from weaker, lower branches receiving less light and ceasing to be productive and dying. This defoliation is more likely to occur as the tree ages and the canopy grows. The defoliation of LLD branches occurring within six weeks, rather than over multiple seasons, as is the case with shading, may be caused by other stress factors. These stress factors may include water stress, nutrient deficiency, and toxicity. International reports state that LLD is complex and is likely caused by multiple factors (Adaskaveg et al., 2017, Trouillas et al., 2016).

Fluctuations in LLD were recorded over the 3-year study, and dieback ratings even decreased in some cases. Activities such as hedging may ameliorate the effects of LLD, as hedging stimulates new growth in previously shaded parts of the canopy (Doll, 2016, Bright et al., 2016).

Management guidelines adopted by the Californian almond industry and Australian macadamia industry in Australia suggest tree rows be established far enough apart so that the foliage can intercept 80-85% of light. These canopy management practices ensure adequate light reaches the lower canopy, reducing dieback (Doll, 2016, Bright et al., 2016). Peak performance in macadamia orchards is said to be achieved when the canopy height is less than or equal to the row width (Bright et al., 2016). 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 that grow into one another across the rows. It has also been reported that LLD appears to be more of a problem in orchards with high inputs and intensive production systems (Doll, 2015). The "rise" of LLD in the Australian almond industry over the last decade may be attributed to the intensification of orchards, increased inputs, and the greater mean age of trees.

Future research should focus on quantifying the economic impacts of LLD as well as better understanding the influence of canopy management, row spacing, water and nutrient inputs on light interception and LLD severity.

This research was part of AL16005 *An integrated disease management program for the Australian almond industry – Phase 1* (2017-2022)

Hort Innovation ALMOND FUND

This project has been funded by Hort Innovation using the almond research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Acknowledgement

Thank you to the growers for allowing access to orchards for this study, and to Cathryn Todd and Ian Bogisch for their support. The authors acknowledge statistical support from Sam Rogers (Biometry Hub, University of Adelaide, School of Agriculture, Food & Wine) and Jessica Jolley (South Australian Research and Development Institute).

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