



Lower limb dieback research update

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Lower limb dieback (LLD) is an emerging issue in almond production regions around the world, causing concern amongst growers in California (Doll 2014) and now in Australia. In a recent almond grower census, 60% of respondents indicated that LLD is present in their orchard, and more than 50% of participants reported that LLD is having a medium to high impact on yield (Edwards 2018). The LLD syndrome is poorly understood and the cause of the syndrome is unknown.

To improve our understanding of LLD, in particular the role of trunk disease pathogens, researchers at SARDI are collaborating on the Hort Innovation project 'An Integrated Disease Management Program for the Australian Almond Industry' led by Agriculture Victoria. In order to determine the distribution and significance of LLD in Australia, industry wide disease surveys have been underway since 2018 and will be continued through to the year 2020. Overall, 1,169 ha of almond plantings within 10,179 ha of orchards selected from regions across New South Wales, South Australia, Victoria and Western Australia were assessed. Plant samples were collected in South Australia, and potential pathogens isolated and identified to help determine the causes of LLD. This research aims to help to develop management strategies for trunk diseases and LLD for the Australian industry.

LLD symptoms

LLD symptoms include chlorotic (yellowing) leaves in spring (Figures 1A-B), followed by dieback of lower scaffolds and branches after warm weather events in summer (Figure 1C) (Doll 2009, Duncan 2015). In severe cases, whole trees can die back (Figure 1D). Brown spots and wedge-shaped cankers are often observed, producing little to no gumming (Doll 2009, Trouillas, Michailides et al. 2016; Figure 1E-G). Internal staining of the wood appears to be caused by fungi colonising the symptomatic branch (Doll 2009, Doll 2014). However, it has not yet been established whether associated fungi are the cause of LLD or merely secondary invaders (Michailides, Morgan et al. 2010).



Figure 1. Chlorotic leaves (A-B) and dead branches (C-D) associated with lower limb dieback syndrome. E. Brown spots found under the bark of a branch with foliar LLD symptoms. F-G. Internal staining within an LLD symptomatic branch.

What are the possible causes of LLD?

Although the cause of LLD remains unexplained, there have been a few possible causal or exacerbating factors identified by Californian researchers. Scale infestations, trunk diseases, shading of lower limbs, herbicide drift, hull-rot infections and water management have all been implicated as possible contributors to LLD (Lampinen, Adaskaveg et al. 2009, Michailides, Morgan et al. 2010, Doll 2015). The primary cause or causes of LLD remain unresolved as the symptoms have not yet been replicated in trials.

Trunk disease pathogens have been of particular interest due to the extensive pruning practices used in the industry. Pathogens enter through wounds to infect the woody tissues. Hedge pruning may present a major risk due to the large number of wounds created (Carter 1991).

Disease surveys

The disease surveys have so far confirmed the grower reports that the issue is wide spread and occurs in all growing regions of Australia. Data from the South Australian growing regions revealed that LLD symptom expression occurred in blocks aged 8 years and above, which concurs with Californian research.

Figure 2 shows the rapid increase in incidence of LLD symptoms in orchards from 8 to 12 years of age. However, there was a notable decrease in LLD incidence in orchards at 22 years and then over the age of 30. The blocks with lower incidence of LLD were relatively small and smaller canopies were observed to have greater light interception to the lower limbs, than in high incidence blocks of similar age. It was also noted that these blocks were not regularly hedge pruned.

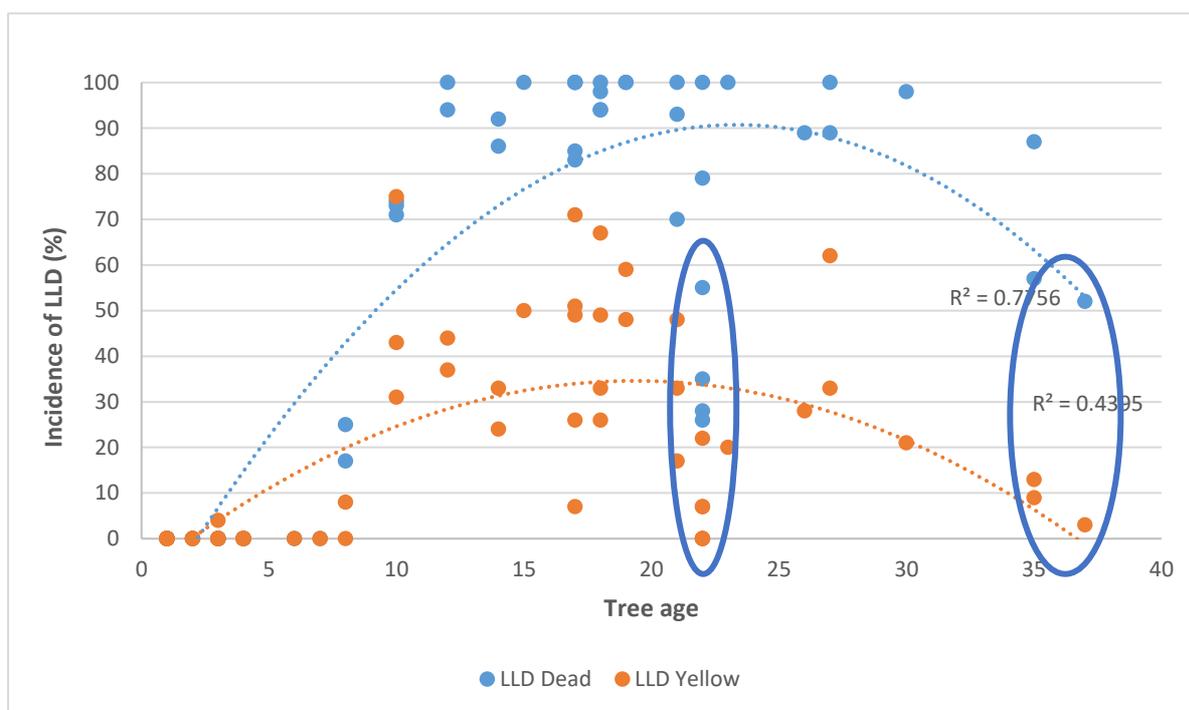


Figure 2. Incidence of lower limb dieback symptoms as trees age, from South Australian surveys in summer 2019. Each data point represents a block with either yellowing leaves (orange dots) or dead lower branches (blue dots). The blue ovals indicate mature blocks (>10 years) with lower incidence of LLD.

The correlation between tree age and LLD symptom expression may be partly explained by shading, as orchards younger than 8 years will experience very little shading to the lower canopy (Figure 3 A-B), whilst trees older than this tend to experience more shading and have a greater incidence of LLD symptoms

(Figure 3 C-D). Dying lower branches from shading is commonly observed and occurs in orchards with large, dense canopies (Doll 2015).

Light penetration can be increased by pruning, which traditionally has been done by strategically removing scaffolds and branches, leaving only a few large wounds which can be treated with a wound paint. However recently, mechanical hedging has been introduced as a more cost-effective and efficient method of managing canopies. This technique creates thousands of smaller wounds which may equate to a greater surface area than the traditional method, and there are currently no wound protection strategies available. This may facilitate infection by trunk disease pathogens which may have led to the emergence of LLD.



Figure 3. A. One-year-old trees near Waikerie with no LLD symptoms. B. Three-year-old trees near Waikerie with no LLD symptoms. C. Twelve-year-old trees in the Riverland with some dying back in the lower canopy. D. Twenty two-year-old trees at Lindsay Point with no live branches in the lower canopy.

All observations were made during the 2018-2019 survey period and are anecdotal. Further research is required to elucidate any possible relationship between LLD incidence and age, or whether there may be a complex interaction between the syndrome, light interception, pruning practice, trunk disease pathogens or other factors.

Trunk disease pathogens

In California, commonly identified fungal pathogens isolated from symptomatic LLD samples include *Botryosphaeria* and *Phomopsis* species, with *Eutypa lata* and *Cytospora* species being isolated less frequently (Doll 2009, Lampinen, Adaskaveg et al. 2009, Michailides, Morgan et al. 2009). In Australia, trees with trunk disease and dieback symptoms in the Riverland and the Adelaide Plains have been sampled extensively (Figure 4). Commonly occurring fungal organisms found in the diseased tissues have been identified as potential trunk disease pathogens, based on the literature. These include, *Botryosphaeriaceae* spp., *Cytospora* spp., *E. lata* and *Pleurostoma richardsiae*.

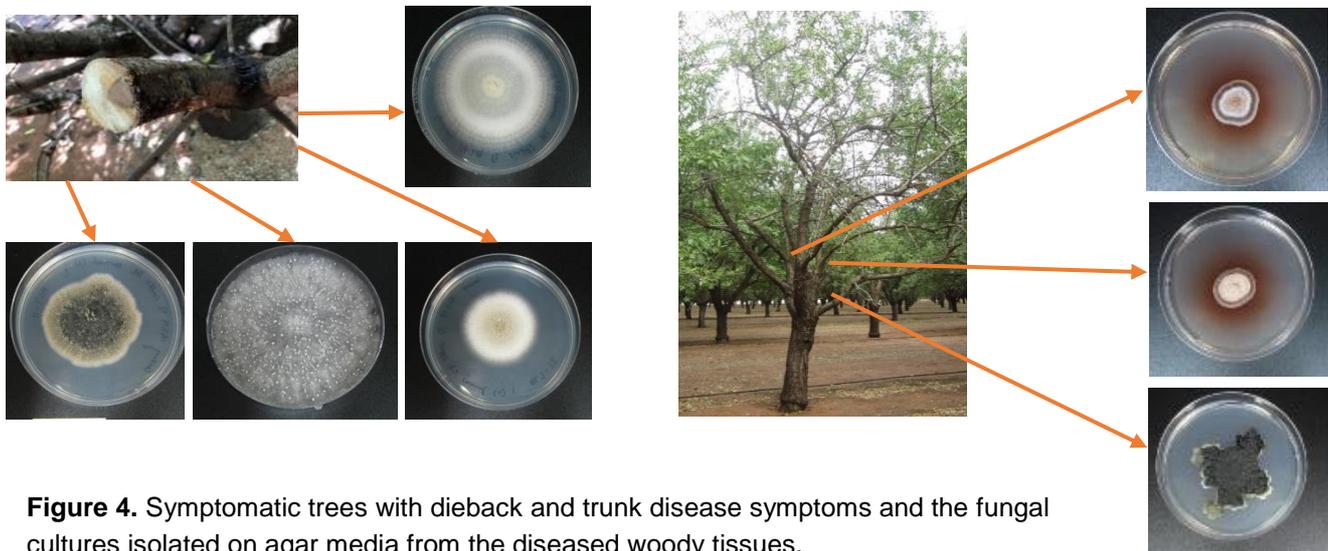


Figure 4. Symptomatic trees with dieback and trunk disease symptoms and the fungal cultures isolated on agar media from the diseased woody tissues.

Pathogenicity testing

The most commonly isolated and potentially pathogenic fungal species were tested for pathogenicity in 3-year-old, potted cv. Carmel trees. The branches of the trees were wounded with drills and inoculated with the potential pathogens. After 6 months, the inoculated branches were removed and assessed in the laboratory, where the lesion length caused by each fungal species was measured (Figure 5.).

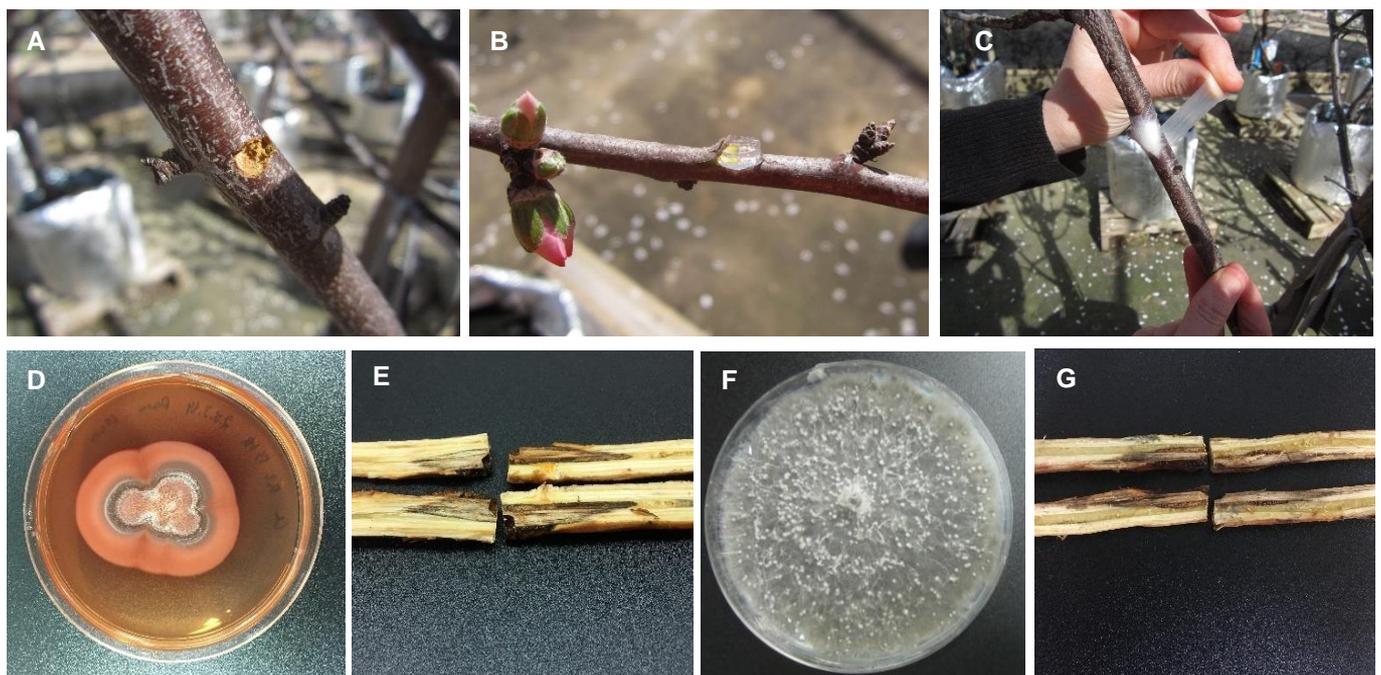


Figure 5. A. 1-year-old branch with a drill wound. B. Small mycelial plug inoculated into a wounded branch. C. Wound being sealed with plastic. D. *Collophora rubra* culture growing on agar. E. Necrotic lesion caused by *C. rubra*. F. *Diplodia seriata* culture growing on agar. G. Necrotic lesion caused by *D. seriata*.

The pathogenicity test confirmed that five out of the seven species tested were pathogenic to cv. Carmel. *Diplodia seriata* (part of the Botryosphaeriaceae family) was the most pathogenic, consistently causing the longest lesions (Figure 6). All other species tested, apart from two, caused lesions significantly longer than on control branches.

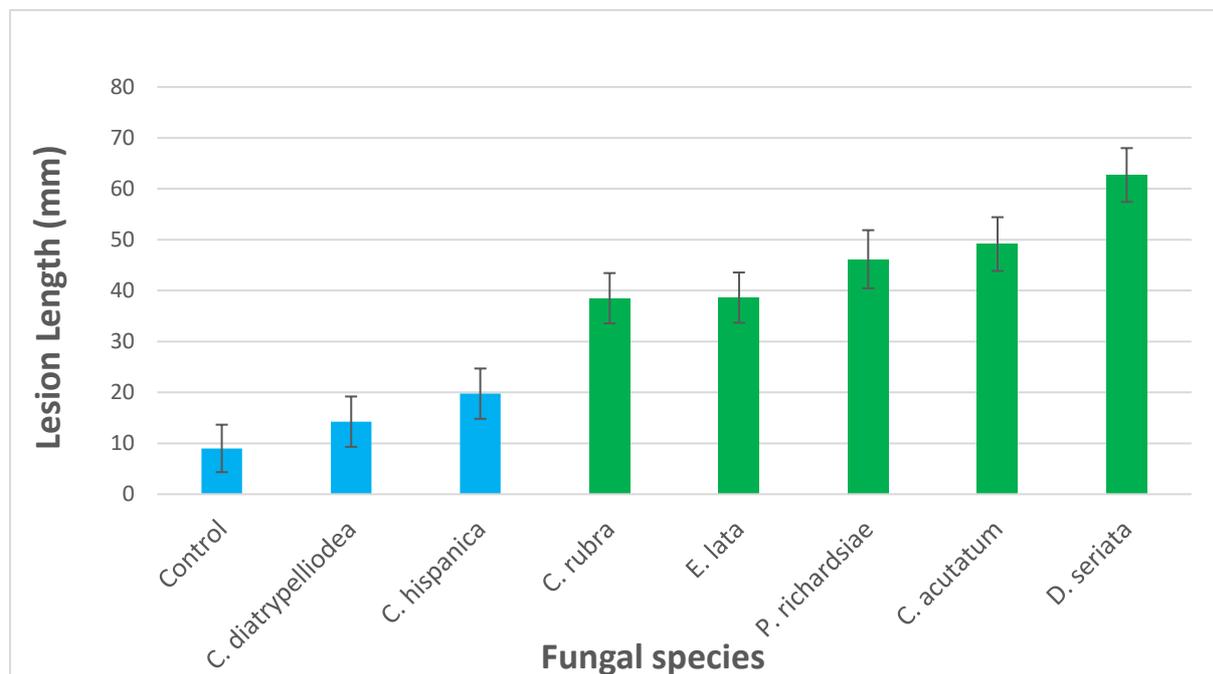


Figure 6. The average lesion length caused by *Cytospora diatrypellioidea*, *Collophora hispanica*, *Collophora rubra*, *Eutypa lata*, *Pleurostoma richardsiae*, *Colletotrichum acutatum*, *Diplodia seriata* and a non-inoculated control. The fungal species that produced lesions significantly greater than controls are indicated by green bars.

What's next?

The pathogenicity test will be repeated to confirm the results. The susceptibility of other common almond varieties to the most aggressive pathogens will also be evaluated.

Furthermore, in consultation with industry and agrochemical companies, a list of fungicides and biocontrol products is being compiled for evaluation against the range of trunk disease pathogens identified in this research.

Surveys and sampling will continue throughout the 2019-2020 season, as well as an intensive LLD observational study. This study will involve tagging symptomatic, chlorotic LLD branches in spring 2019, and observing how these symptoms progress throughout the season. Tagged branches will be removed from the orchard, and the presence of fungal organisms determined by isolation of cultures on agar in the laboratory.

This research aims to improve our understanding of the complex LLD syndrome and trunk disease, and develop strategies for management in the future.

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