SAR1701-1.1
Grapevine trunk disease management for vineyard longevity in diverse climates of Australia

Industry partner progress report
Limestone Coast Wine Industry Council

August 2019

SOUTH AUSTRALIA

Government of South Australia
Primary Industries and Regions SA

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SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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Project Summary

Eutypa and botryosphaeria dieback are fungal trunk diseases that reduce yield, cause vine decline and eventually death of grapevines. Australian vineyards are increasingly affected by grapevine trunk disease, threatening the sustainability of our wine industry, which contributes $40 billion to the Australian economy. Many advances have been made in understanding the extent and distribution of pathogens, along with efficient methods of pruning wound management and control of grapevine trunk diseases, in past projects supported by AGWA. However, due to the climatic diversity of Australian wine regions, gaps in the knowledge have been identified, such as inoculum dispersal and wound susceptibility, with implications for management of these diseases. Wound coverage is correlated to efficacy of control, so there is potential to maximise coverage with fungicide adjuvants. Remedial surgery is an effective method of controlling eutypa-affected vines, but this method is yet to be proven to provide control of botryosphaeria dieback, and methods to improve the rate of vine regrowth are lacking. Evaluating disease susceptibility of clones and rootstocks could lead to identification of tolerant planting material. Concerns with the role of grapevine propagation in the spread of trunk disease need to be addressed, particularly with understanding the threshold of infection and abiotic factors that lead to vine decline, using highly sensitive molecular diagnostic tools. The proposed project aims to address these gaps by;

(i) developing climate specific recommendations to minimise infection,
(ii) optimising wound protection and remedial surgery techniques,
(iii) assessing susceptibility of clones and rootstocks, and
(iv) investigating infection thresholds for grapevine propagation material.

Background

Eutypa dieback (ED) and botryosphaeria dieback (BD) are major grapevine trunk diseases (GTDs) worldwide, threatening the sustainability of Australian vineyards. Causal fungi infect pruning wounds and colonise wood, causing dieback and death. This project builds on previous AGWA-funded research, to develop practical strategies for GTD management with consideration for the diverse climates of Australian viticulture.

Pruning recommendations to avoid infection in Australia are based on 50-year-old data. Recent, conflicting information in other parts of the world highlights the need for regionally focussed research in Australia. Methods for quantifying pathogen DNA on spore trap tape have been developed, but a novel loop-mediated isothermal amplification (LAMP) assay has potential to detect DNA on spore tape and in propagation material in the field.

Pruning wound fungicides are being registered and can be applied with sprayers. However, the ability of fungicide adjuvants to maximise wound coverage and critical timing of biocontrol application are unknown. Remedial surgery is the only effective method for managing ED-affected vines and current research is providing short-term results for managing BD-affected vines, but data on medium-term effectiveness are lacking.

There is evidence of cultivar tolerance to trunk disease but observations that clones and rootstocks vary in susceptibility may offer additional means of disease management.

With development of highly sensitive diagnostic tools and concern about disease spread in nurseries, it is important to determine infection thresholds in propagation material that lead to subsequent disease symptoms.

The increasing prevalence of GTDs in Australia makes it vital that research continues to address these nationally significant constraints to the sustainability of the wine industry. This project is strongly supported by industry through regional grower associations, which have helped guide the direction of research.

Project team

Project Leader and Supervisor SA: Dr Mark Sosnowski (SARDI)

Project Supervisor NSW: Dr Sandra Savocchia (CSU/ NWGIC)

Principle Investigator SA: Mr Matthew Ayres (SARDI)
Principle Investigator NSW: Dr Regina Billones Baaijens (CSU/ NWGIC)

Objective 1: Ascertain inoculum dispersal throughout the pruning season

Determine presence and quantity of spores of GTD pathogens in relation to weather throughout the year in selected climatic regions of Australia and develop an inexpensive field-based diagnostic tool for spore tape analysis.

Six spore traps are currently operating in SA and NSW, with spore tapes collected monthly and stored. The qPCR analyses for the 2018 samples have been completed. Results showed 23-47% and 11-22% of the samples from the six locations were positive to Eutypa (ED) and Botryosphaeria dieback (BD) pathogens, respectively. Analysis of the spore trap sample and weather data was initially undertaken by plotting results on a graph. Preliminary results attributing rain as the primary factor for the release of Eutypa (ED) and Botryosphaeria dieback (BD) pathogen spores were confirmed, although spores were occasionally detected when no rain had fallen for the previous week, and sometimes spores were not detected during rainfall events. It may be that other factors such as dew, wind speed/direction and relative humidity have an effect. Preliminary computer modelling of spore trap data collected from 2013 to 2016 showed very weak correlation between other weather factors (temperature, relative humidity, dew point, wind speed) indicating that the spore release may be difficult to predict for these pathogens. Future studies may be required to understand the biology of these pathogens and the influence of environmental conditions on their development.

Data from the Coonawarra spore trap, deployed in August 2013 and located in the Wynns Coonawarra Vineyard, SA are shown in Figures 1 and 2. They show that, despite significant spore trap malfunctions in 2014, overall, ED pathogen spores were more frequently detected than BD pathogen spores and in greater quantities. In 2016, BD spores were more predominant than in other years of spore monitoring, and in 2018, no BD spores were detected at all, a pattern also observed in other regions. In general, spores were mostly detected from May to November, particularly in response to rainfall and during the winter pruning season. Spores were also detected, although less frequently and in lower quantities, from December to April in response to rainfall events. These data reinforce the need to protect wounds following pruning, and future research will focus on susceptibility of wounds made during shoot thinning in spring and summer, given the possible presence of GTD spores at these times.

Spore traps will continue to operate until June 2020, and all data will be presented in the next report.

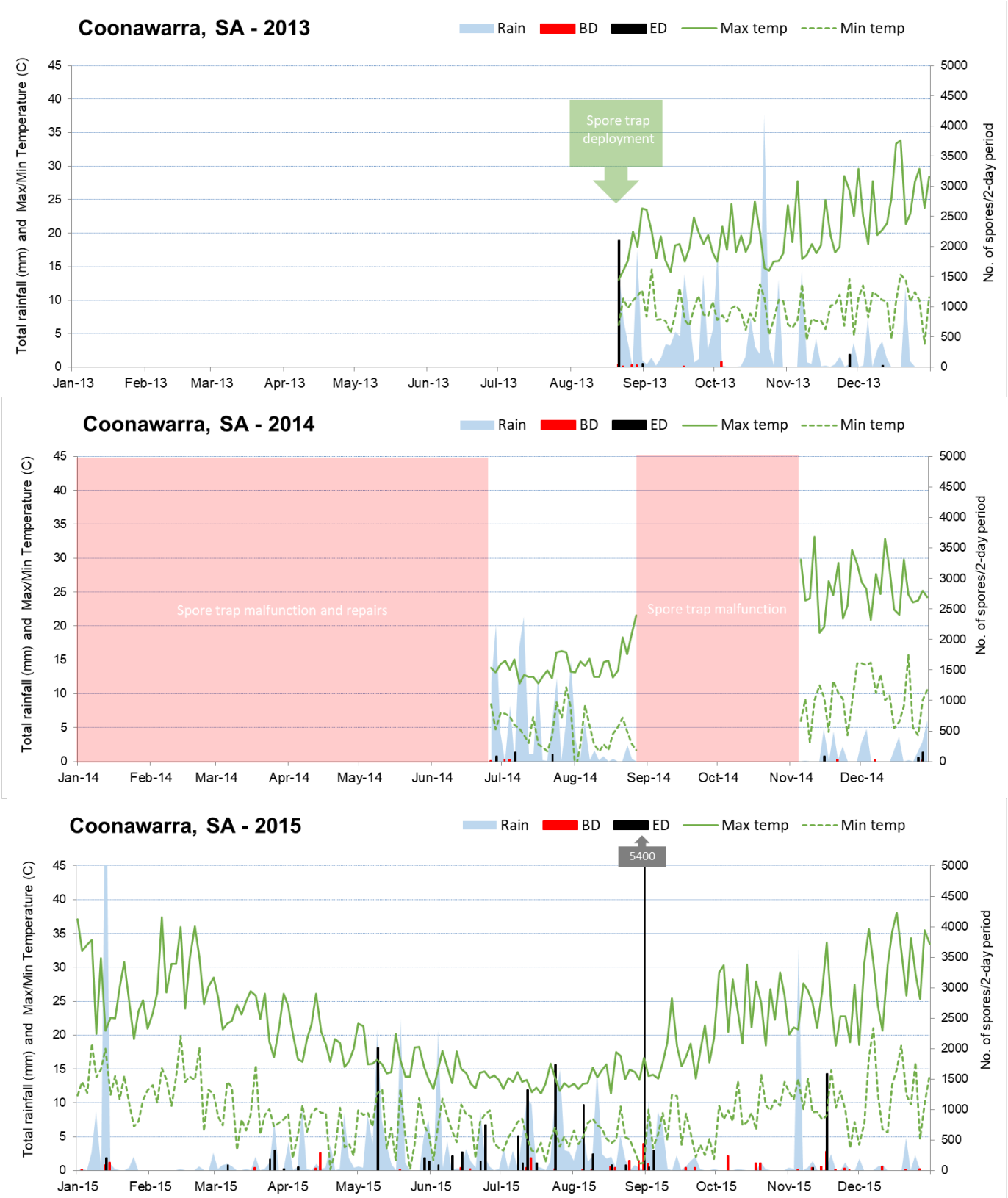

Figure 1. Data collected between August 2013 and December 2015 from the Coonawarra spore trap located in the Wynns Coonawarra Estate Vineyard, SA. Number of pathogen spores detected per 2-day period of Eutypa dieback (ED; black bars) and Botryosphaeria dieback (BD; red bars) are plotted with total rainfall (mm; blue area) and temperature (°C; green lines; maximum-solid, minimum-dashed).

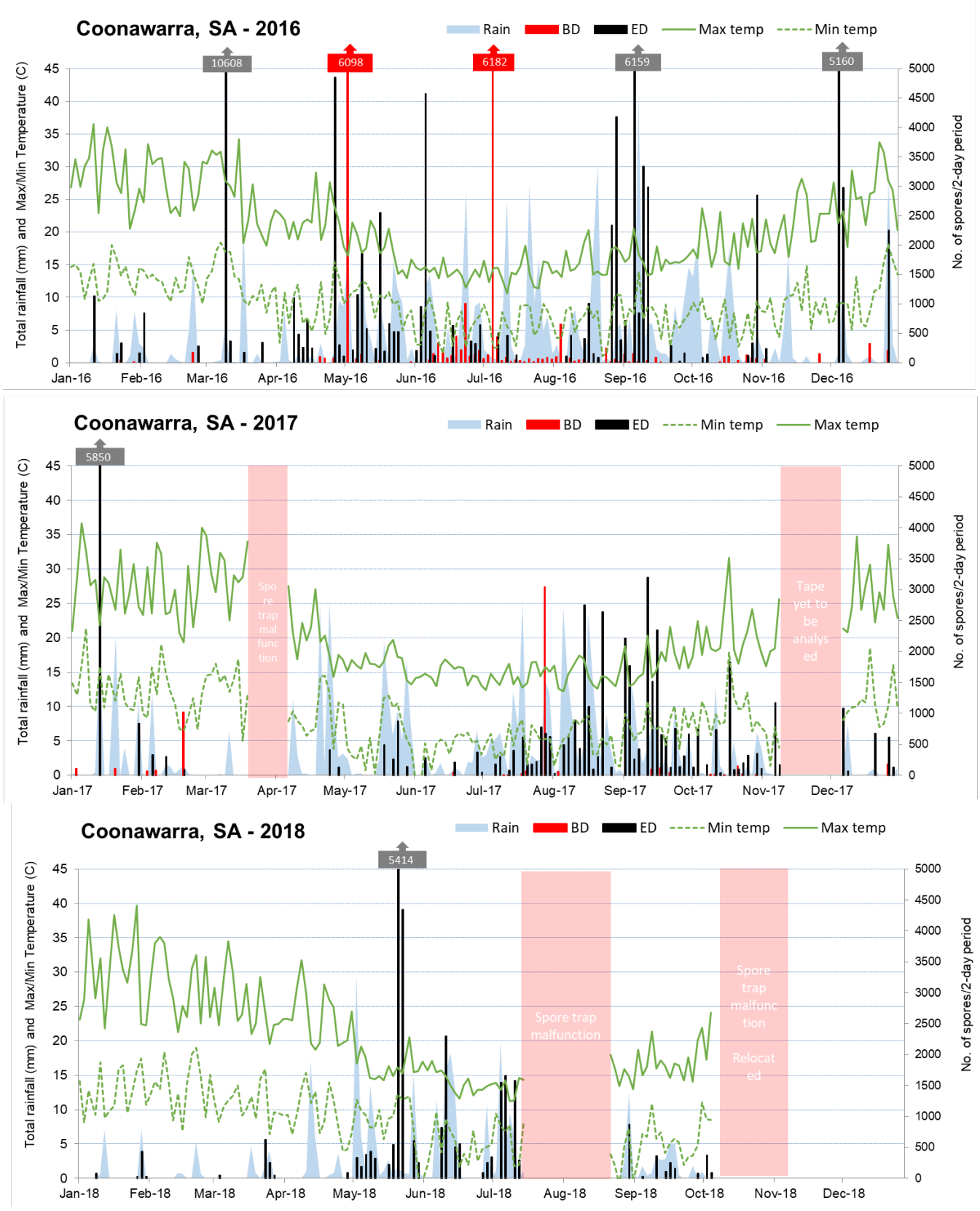

Figure 2. Data collected between January 2016 and October 2018 from the Coonawarra spore trap located in the Wynns Coonawarra Estate Vineyard, SA. Number of pathogen spores detected per 2-day period of Eutypa dieback (ED; black bars) and Botryosphaeria dieback (BD; red bars) are plotted with total rainfall (mm; blue area) and temperature (°C; green lines; maximum-solid, minimum-dashed).

Objective 2: Determine pruning wound susceptibility under different climatic conditions

Determine the length of time for which grapevine wounds are susceptible to infection by spores of GTD pathogens at key times during the pruning season in different climatic conditions.

A vineyard wound susceptibility trial was established in 2017 on 420 Shiraz vines near Hahndorf in the Adelaide Hills, SA. Pruning treatments were undertaken early (7 June), mid (19 July) and late (30 August), with inoculations of both ED and BD pathogen spores undertaken on selected dates 1-84 days after each pruning time. The trial consists of 21 treatments, with one treatment applied per vine (10 treated canes per vine) and 10 replications. Canes were removed in May 2018 and assessed in the laboratory for recovery of pathogens.

ED pathogen recovery was greatest for inoculations made up to 14 days post-pruning, after which recovery was negligible, similar to that of non-inoculated controls (Figure 3). Recovery was lower in the late pruning compared with the earlier pruning times. BD pathogen recovery was greatest for inoculations up to 7 days post-pruning, with negligible levels recovered thereafter. Recovery was greater for the early pruning compared with the later pruning times.

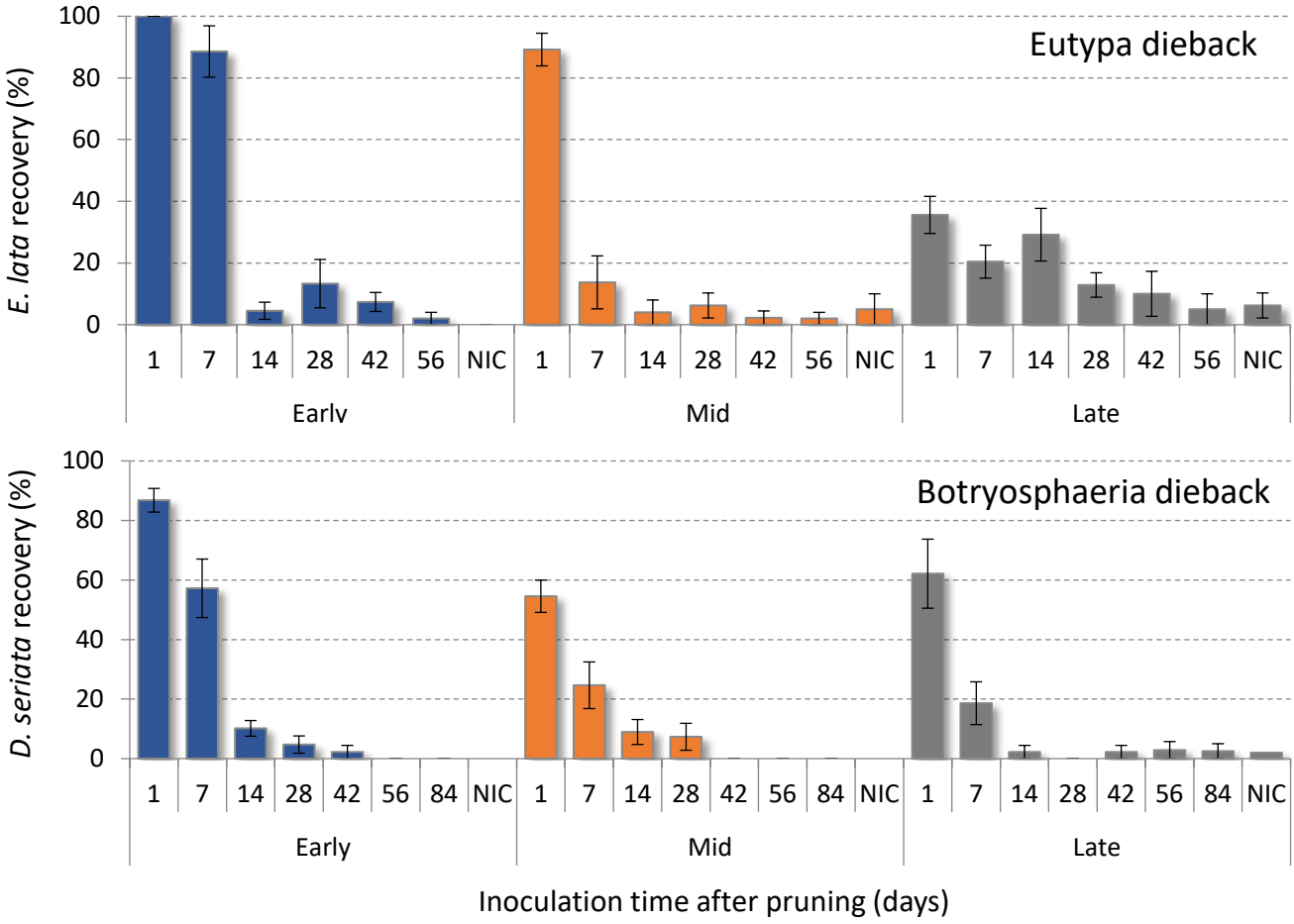

Figure 3. Incidence of recovery of the ED (*Eutypa lata*) and BD (*Diplodia seriata*) pathogens at the Adelaide Hills site from canes pruned on 7 June (Early), 19 July (Mid) and 30 August (Late) in 2017, and inoculated with 200 spores at 1, 7, 14, 28, 42, 56 and 84 days after pruning. NIC = non-inoculated control. Bars represent standard error of the mean.

The trial was repeated in 2018. Pruning treatments were undertaken early (6 June), mid (18 July) and late (29 August), with inoculations of ED and BD pathogen spores undertaken on selected dates 1-84 days after each pruning. Canes were removed in May 2019 and are being assessed for pathogen recovery, with results available in the next report.

In winter 2019, the trial was further repeated and canes will be removed for laboratory assessment in May 2020.

Susceptibility trial site in the Adelaide Hills, SA

Objective 3: Maximise wound coverage by fungicide and evaluate efficacy of organic alternatives for pruning wound protection

Evaluate the addition of adjuvants to fungicide to improve spray coverage on wounds and efficacy of fungicide, and evaluate efficacy of biocontrol and natural alternatives for sustainable control of GTDs.

A wound coverage trial was established on Shiraz vines at the McLaren Vale & Fleurieu Visitor Information Centre vineyard in SA during July 2017 and repeated in winters of 2018 and 2019. A recycle sprayer was used to apply two fungicides (Emblem® and Gelseal™) at an output rate of 200 L/ha, with or without surfactants; wetter (DuWett®) and sticker (Flextend®), to assess wound coverage and efficacy against the ED pathogen. Fluorescent dye was added to the treatments to assess wound coverage, and compared with coverage on water sensitive paper (WSP).

Immediately following applications, a selection of treated canes was removed from vines and returned to the laboratory for assessment of spray coverage, using UV light. Digital images of wound surfaces exposed to dye, were captured and spray coverage of these and of WSPs was quantified using Image J software. Analysis revealed little effect of treatment on coverage over the first two years (Figure 4). There was a large difference between coverage assessed using fluorescent dye and water sensitive paper, however there was similarity in trends between treatments with correlation (R^2 = 0.58 and 0.28 in 2017 and 2018, respectively) between the two methods. The large difference between methods of assessing coverage reveals that WSP may not accurately reflect coverage on wounds, since they are designed to mimic grape leaves. The use of fluorescent dye appeared to be more informative, with regards to coverage of pruning wounds.

Treated canes were collected in May 2018 and assessed for efficacy of treatments, by fungal isolation in the laboratory. *E. lata* was recovered from 79% of inoculated controls and between 58 and 76% for all treatments (Figure 5). Gelseal without adjuvant and Emblem with wetter significantly reduced recovery of *E. lata* compared with the control. Most notable was the high level of recovery from wounds treated with fungicides (lack of disease control) when applying wound sprays at a low water rate of 200 L/ha. In this trial, the low rate was used to simulate poor coverage, and provided an opportunity to evaluate the ability of surfactants to increase the coverage and, hence, efficacy. Previous research recommended at least 600 L/ha for effective wound protection.

Treated canes from the repeated trial were collected in May 2019 and are being assessed for pathogen recovery, with results available in the next report.

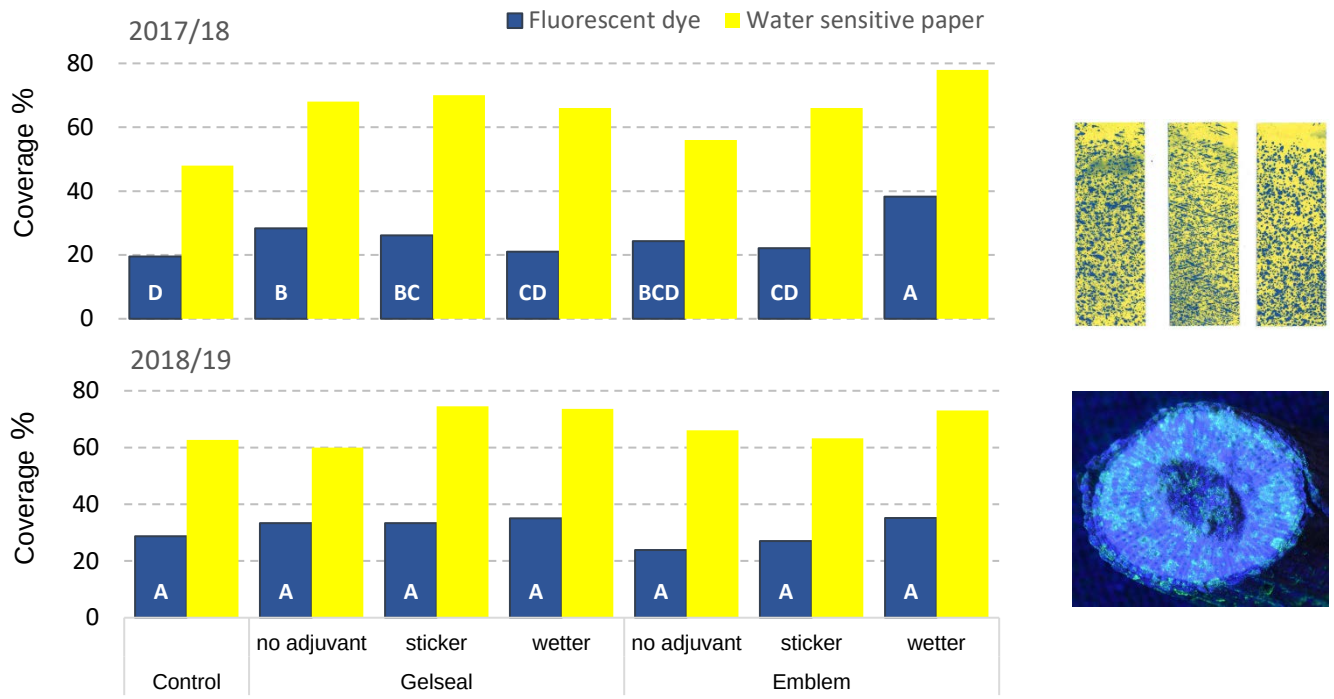

Figure 4. Spray coverage on wounds as indicated by fluorescent dye (blue bars) and water-sensitive papers (yellow bars) for trials in McLaren Vale during 2017/18 (top) and 2018/19 (bottom). Control wounds were sprayed with fluorescent dye in water. Bars with the same letter are not significantly different from one another (P = 0.05).

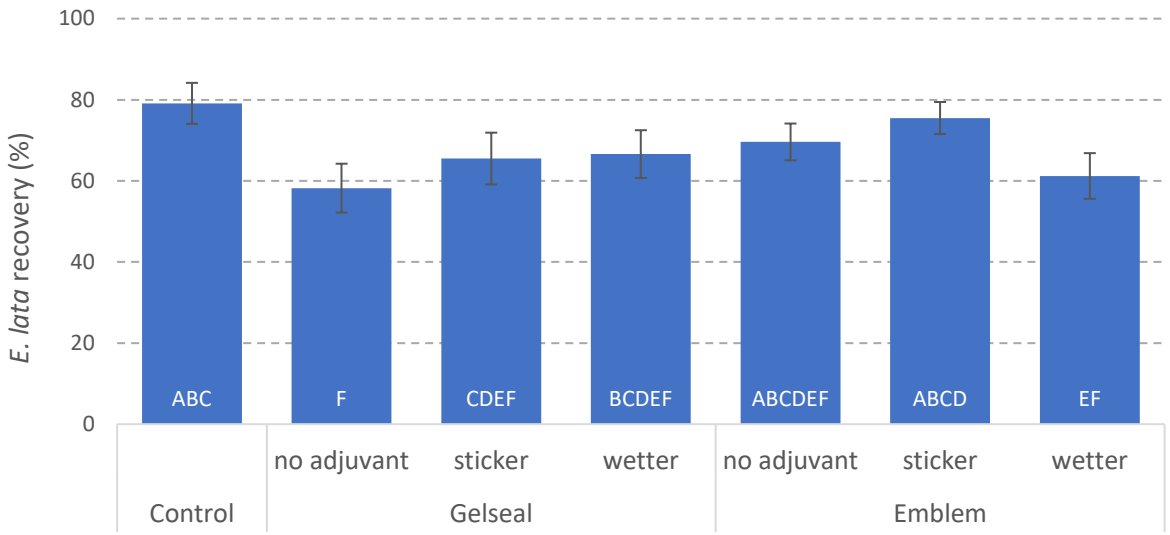

Figure 5. Incidence of the ED pathogen (*Eutypa lata*) recovery from Shiraz canes following harvest, 12 months after pruning and inoculation for the trial in McLaren Vale during 2017/18. Control (positive) wounds were inoculated but received no treatment. Bars with the same letters are not significantly different from one another (P = 0.05).

Detached cane assays (DCAs) were established to assess efficacy of Vinevax™ (*Trichoderma*) in reducing infection of pruning wounds by trunk disease pathogens. The agar plates used to assess the Vinevax treated canes were rapidly overgrown with *Trichoderma*, raising concerns that relatively slow growing ED and BD pathogens may just be outcompeted on the agar, leading to an incorrect assessment of control.

In response, a longer-term field trial was established on Shiraz vines near Hahndorf in the Adelaide Hills, SA during July 2017. To simulate varying infection periods, pruning wounds were inoculated with trunk disease pathogens at different intervals, from the day of pruning and treatment up until 28 days later. Canes were harvested in May 2018 and were assessed in the laboratory for recovery of ED and BD pathogens. Preliminary data showed that Gelseal™ was effective at reducing infection of both pathogens at most inoculation times compared with inoculated controls, but Vinevax only reduced infection when the BD pathogen was inoculated 3 and 7 days after application, after which recovery from controls was minimal, suggesting natural wound healing had reduced susceptibility. The trial was repeated in July 2018 and canes were harvested in May 2019 and are being assessed for pathogen recovery in the laboratory, with results available in the next report.

DCAs were conducted in 2017 and 2018 to assess efficacy of alternative products; vanillin, chitosan and Botector® (*Aureobasidium pullulans*) as wound protectants, compared to the synthetic fungicide control Gelseal. There were significant reductions of the ED pathogen with Gelseal, *Aureobasidium* and chitosan but not vanillin (Figure 6). For BD, results from one DCA showed that Gelseal, Botector and the highest rate of chitosan reduced recovery of the pathogen (Figure 7). The disease pressure in this experiment was extremely high as reflected by the 98% recovery from inoculated controls. The assay will be repeated with a lower inoculum dose of the BD pathogen and results presented in the next report.

The results of these experiments indicate that Botector and chitosan have potential as pruning wound protectants against infection by trunk disease pathogens. Botector, registered for control of botrytis bunch rot in Australia, is now being evaluated in vineyard trials to control ED and BD pathogens. There are no chitosan products available in Australia for horticultural use, so enquiries are being made to locate an overseas manufacturer before field evaluation is considered. The use of natural alternative products as wound protectants would provide more management options for organic growers or those wishing to reduce their use of synthetic wound protectants.

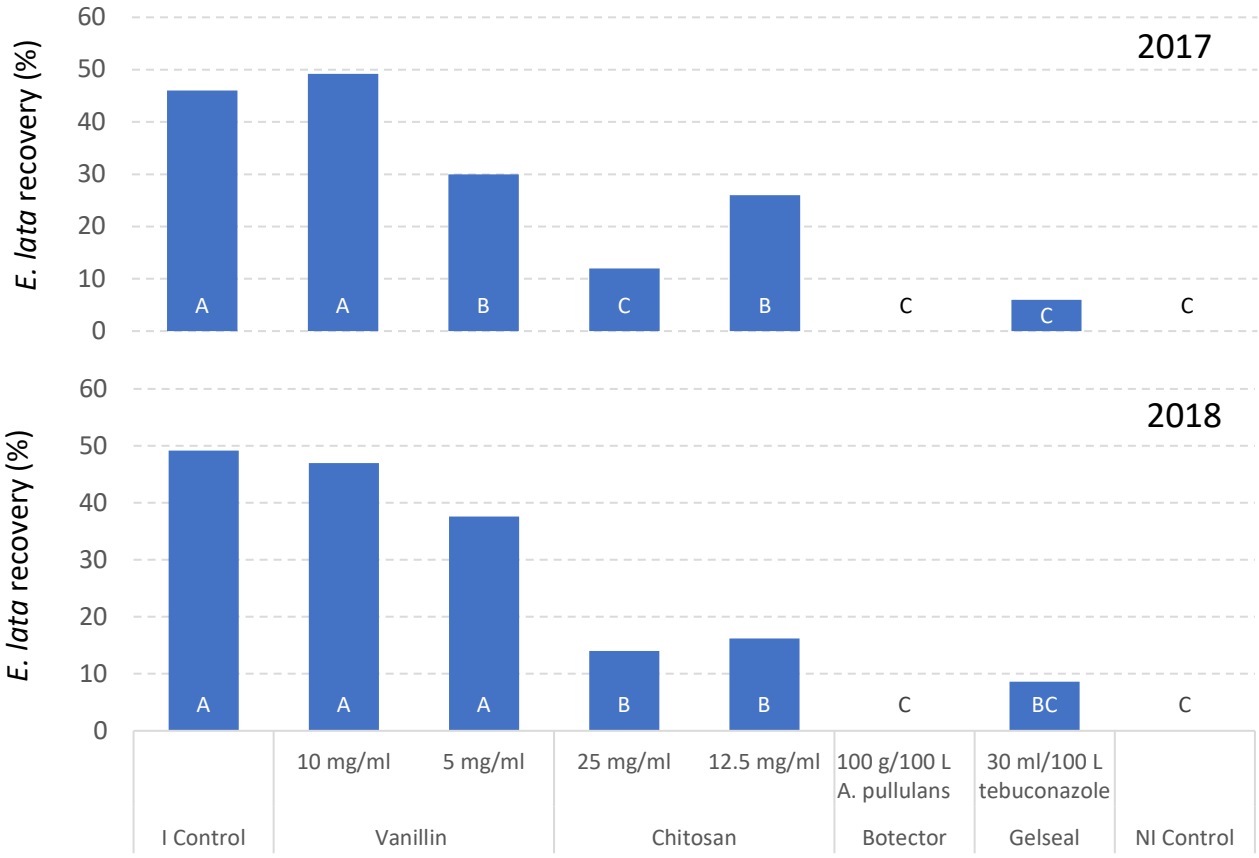

Figure 6. Incidence of the ED pathogen (*Eutypa lata*) recovery from treated single-node Shiraz canes in detached cane assays conducted in 2017 (top) and 2018 (bottom). I control = inoculated control, NI control = non-inoculated control. Bars with the same letter are not significantly different from one another (P = 0.05).

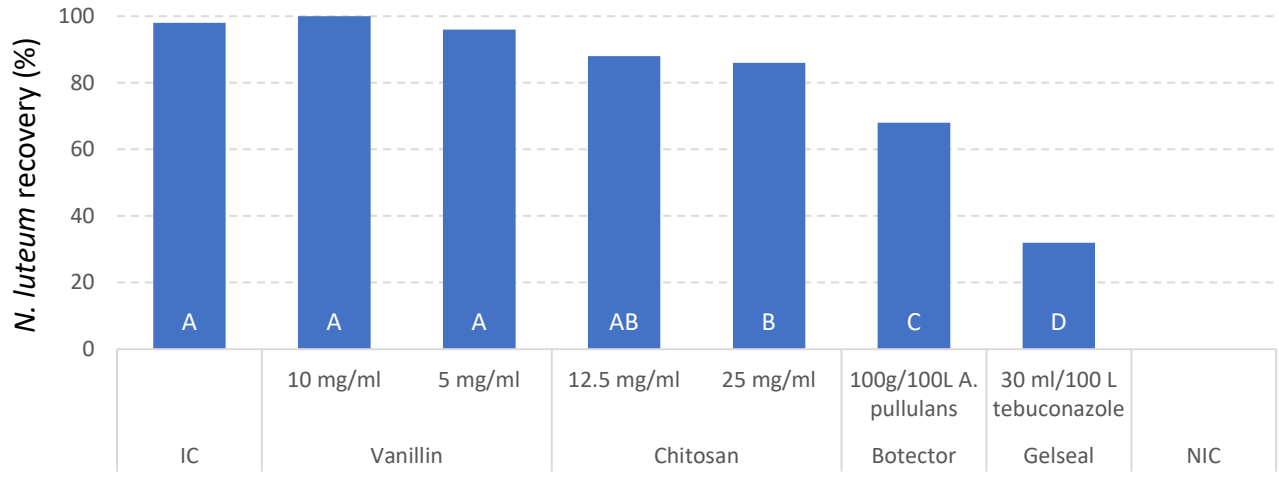

Figure 7. Incidence of the BD pathogen (*Neofusicoccum luteum*) recovery from treated single-node Shiraz canes in a detached cane assay conducted in 2018. IC = inoculated control, NIC = non-inoculated control. Bars with the same letter are not significantly different from one another (P = 0.05).

Objective 4: Optimise remedial surgery techniques to control GTDs

Monitor established and new remedial surgery trials to manage BD-affected vines and evaluate novel methods of water shoot induction to improve success of the technique.

A new remedial surgery trial for grafted plants was established on a Shiraz vineyard in Milawa, Victoria in October 2017. Mean severity of dieback symptoms in vines pre-surgery was 9-15% while wedge and central staining in trunk cross-sections was 5-14%. Assessment in October 2018 revealed that watershoot production was 57%, 58% and 76% for low, mid and high cut vines, respectively. Mean severity of dieback symptoms for uncut vines was 16% which was higher than the pre-surgery severity of 9%. No symptoms were recorded in any treated vines.

Assessment 5 years after remedial surgery at the Harden trial site in NSW occurred in November 2018. The severity of dieback in the uncut vines was 30% which was lower than in 2017. The difference in severity may be due to the assessment being conducted later in the season than for the 2017 assessment, when canopy growth was greater and most likely concealed some symptoms. Dieback symptoms were observed in 40%, 15% and 5% of the high, mid and low cut vines, respectively, although the severity was relatively low ranging from 0.3 to 3%.

A watershoot induction trial was established in 2017 in a Cabernet Sauvignon vineyard planted in 1995 near Auburn in the Clare Valley. Vines were assessed with 100% incidence of GTD symptoms, prior to a mid-winter remedial treatment on 21 June. Cross-sectional cuts of trunks with a chainsaw revealed disease staining at the top of the trunk (87%) and just above the irrigation wire (28%). Treatments aimed at inducing watershoot production were undertaken in an area of the trunks predesignated by painting a white circle. The treatments included hitting with a hammer, making an X shaped cut with a tomahawk, rubbing bark with a wire brush and application of a hormone (Dormex®). On 27 September, a spring remedial treatment was undertaken as described for the winter treatment. Grafting treatments were conducted on 28 October.

Watershoot and graft success was assessed on 16 February 2018. Watershoots were readily produced on 80-92% of all trunks, but rarely (<18%) originated within the white circle where treatments were applied, suggesting little influence of most treatments. No significant differences were observed between winter and spring remedial cuts, or treatments, other than grafting, which increased reworking success from 85 to 98% (Figure 8).

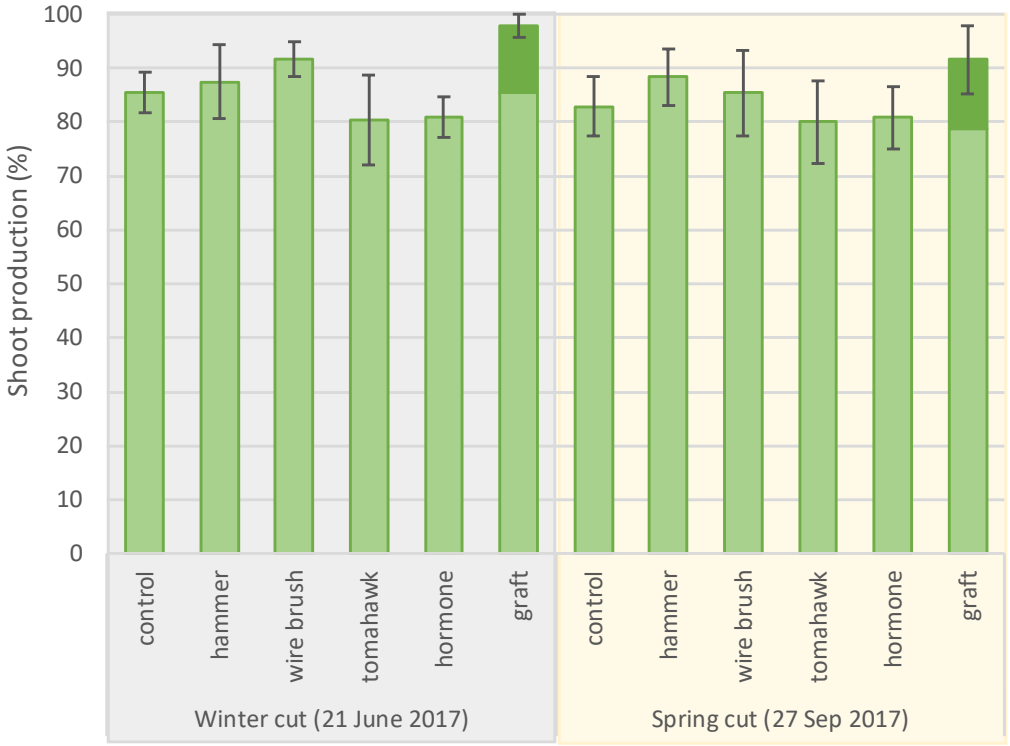

Figure 8. Watershoot induction trial in Cabernet Sauvignon vines planted in 1995 near Auburn in the Clare Valley, SA. Mean shoot production (light green bars) did not significantly differ between treatments, but grafting (dark green bars) increased rework success from 85 to 98%.

In 2018, a further four rows (583 vines) were reworked on 3 October, and stumps that failed to produce a watershoot (31%) were grafted on 23 November. Summer assessment revealed a small increase in rework success from 71 to 78% in response to grafting. The limited success of grafting was attributed to hot, dry conditions, with poor grafting results reported elsewhere in the region at that time.

In 2019 a further 4 rows will be reworked, half in winter and half in early spring at budburst, with grafting treatments planned again for late spring. This will provide additional data towards determining the annual variability of watershoot production, and confirm the use of grafting to maximize success of remedial surgery.

Objective 5: Assess susceptibility of clones and rootstocks to GTD

Assess clones of common cultivars and scion/rootstock combinations for symptoms of GTD.

Twelve rootstock and clonal trials planted in Adelaide Hills, Barossa Valley, Clare, Limestone Coast and McLaren Vale, South Australia between 1980 and 2000, have been located and assessed for GTD symptoms between 24 October and 24 November 2017.

Data have been analysed, and showed some differences in disease severity between different clones (Figure 9) and rootstocks (Figure 10).

For the more susceptible varieties; Cabernet Sauvignon, Shiraz and Sauvignon Blanc, the majority of clones were highly susceptible, with only a few clones observed with reduced severity of symptoms. For the moderate and less susceptible varieties; Chardonnay, Semillon, Pinot Noir and Riesling, clones varied greatly in symptom severity, with many clones identified with potential tolerance or resistance to trunk disease. These results should be treated with caution as clone plots were not replicated and the ages of vines varied considerably.

Rootstocks appeared to influence the severity of symptoms on the scion, with own-roots most susceptible for Shiraz, and least susceptible for Chardonnay.

Based on these results recommendations will be made to further evaluate prospective clone and rootstock material in coming years.

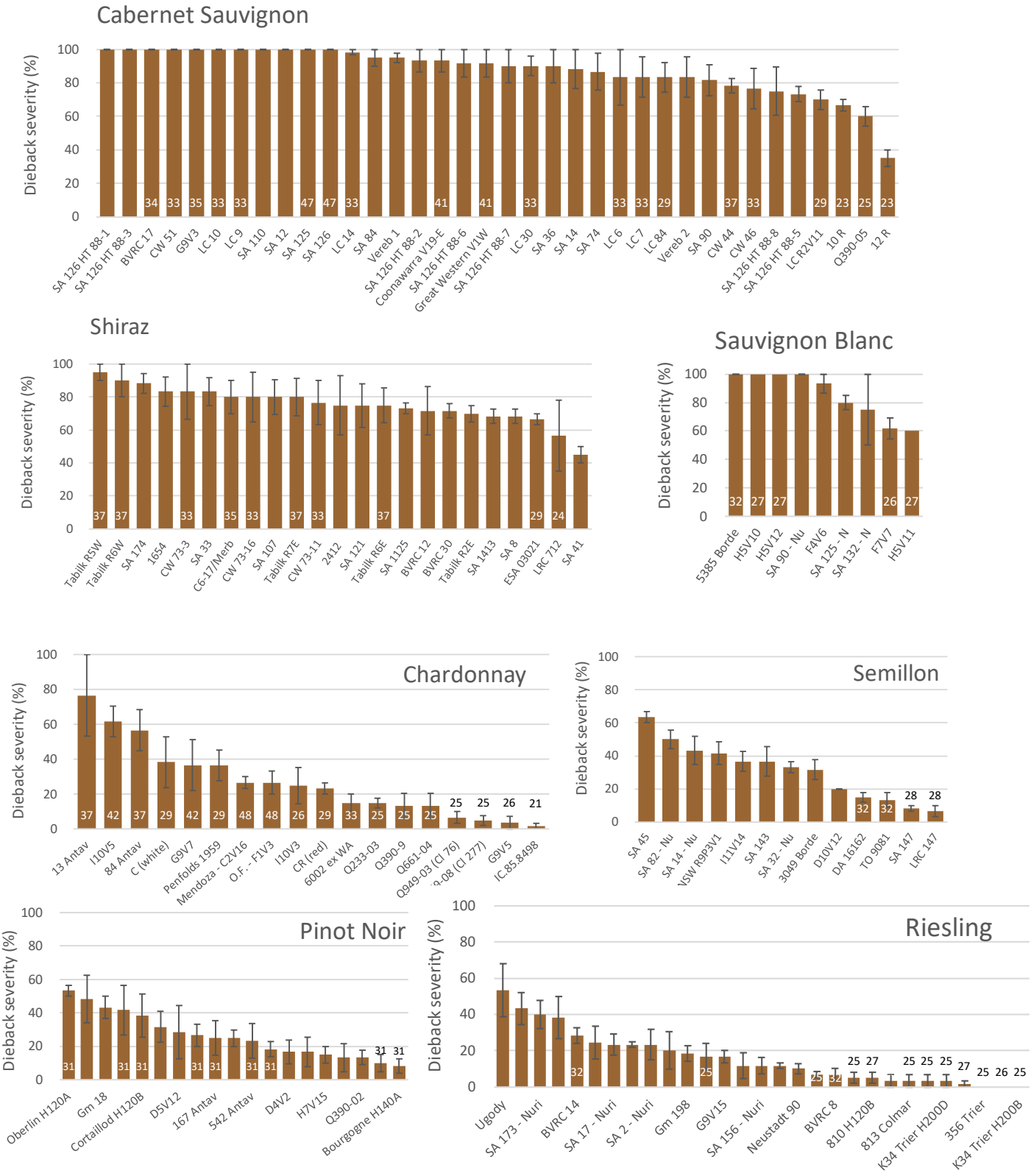

Figure 9. Mean dieback severity on clones of various varieties planted at the Nuriootpa Research Centre germplasm collection. Age of vines, where known, are indicated at the base of bars for each clone. Figures are based on three vine plots which are not replicated.

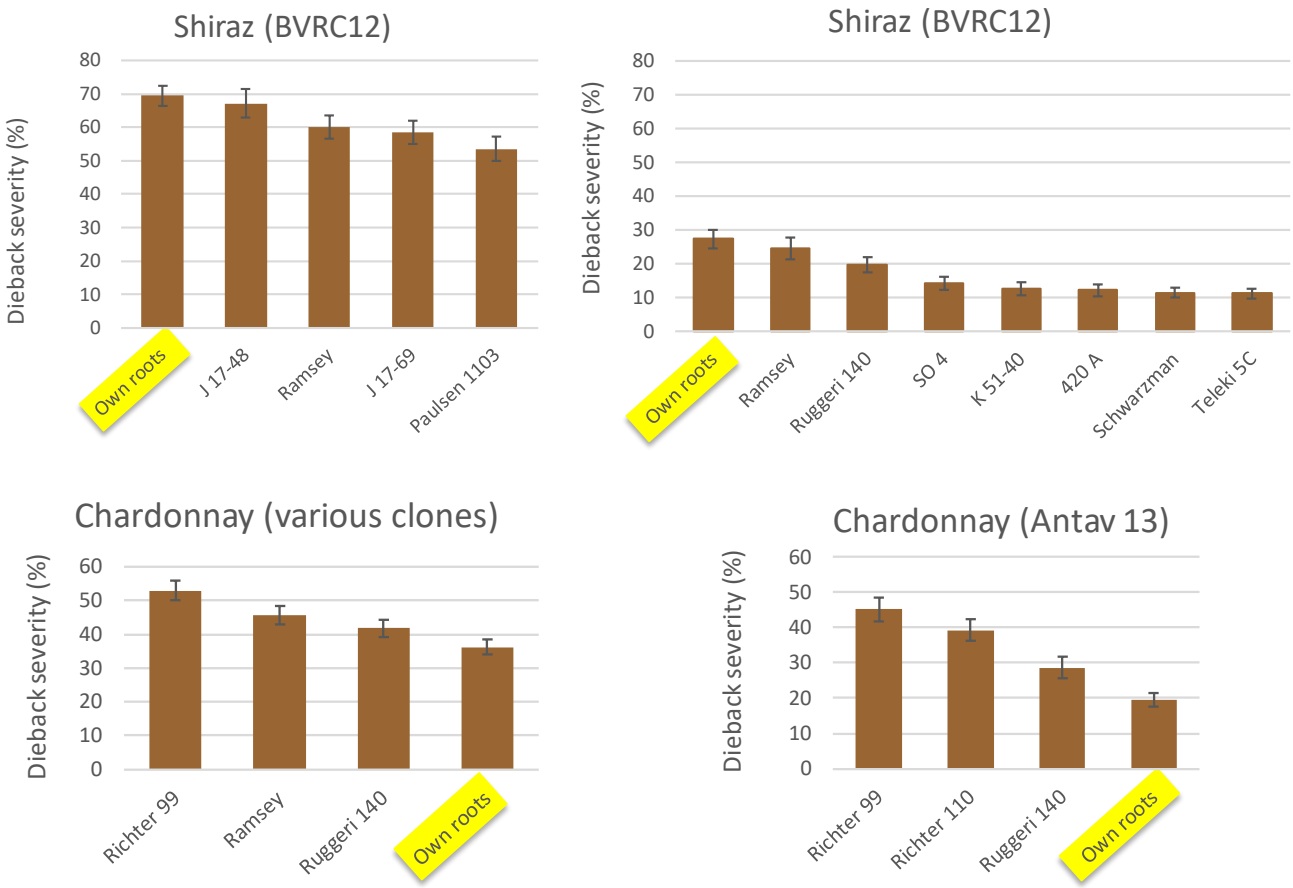

Figure 10. Mean dieback severity on Shiraz and Chardonnay scions on varying rootstocks planted as replicated trials in various commercial vineyards around South Australia.

Objective 6: Investigate infection thresholds of BD in propagation material

Quantify amount of GTD pathogens in nursery propagation material followed by planting under various stress conditions to monitor for disease expression.

Two commercial DNA extraction protocols with modifications were tested in May 2017, and one protocol was found to be highly suitable for extracting DNA from wood. The BD qPCR assay developed in SAR 2015 was shown to be highly suitable for detecting BD pathogens in wood samples.

LAMP species-specific primers designed for the ED pathogens, *E. lata*, *E. leptoplaca*, *Eu. citricola*, *Eu. microtheca* and *C. ampelina* were tested using 8-20 randomly selected DNA extracted spore tapes collected from the Barossa Valley and Coonawarra, SA, and Hunter Valley and Griffith, NSW. The DNA were pre-amplified prior to LAMP to increase sensitivity and the amount of DNA required for the assays. All primers were able to detect their corresponding target pathogen from spore tapes with a high level of spores. *C. ampelina*, *Eu. citricola* and *Eu. microtheca* were the most common species detected while *E. lata* was only detected in the Barossa Valley tapes. The genus-specific primers for BD will be tested using spore tape samples by September 2019. The LAMP primers for ED species will be further tested using inoculated wood samples in December 2019.

Wood samples were collected from the vineyard in June 2017 to represent propagation material, but isolations and DNA analysis revealed very low (1%) natural infections by BD pathogens, which were insufficient to establish a pot trial. To simulate natural infection, a vacuum inoculation method was evaluated for its suitability to inoculate different concentrations of BD spores in grapevine cuttings. Preliminary results from re-isolations and molecular analysis showed the method was suitable for inoculating spores, and this was confirmed by the uptake of fluorescent dye in single-node and 4-node cuttings. This experiment was repeated using a greater number of samples, and showed that the artificial inoculation method was successful.

Dormant Shiraz cuttings were vacuum-inoculated with 300 (low), 3,000 (moderate) and 30,000 (high) *N. luteum* spores in August 2018. Negative control vines were vacuum-inoculated with saline solution. The qPCR was able to differentiate the low, moderate and high infections from the sub-sampled vines (10) with the highest infection detected from those inoculated with 30,000 spores and lowest from those inoculated with 300 spores. Rooted cuttings were also sub sampled and analysed, revealing similar infection levels according to spore inoculum dose, and distribution throughout cutting (Figure 11).

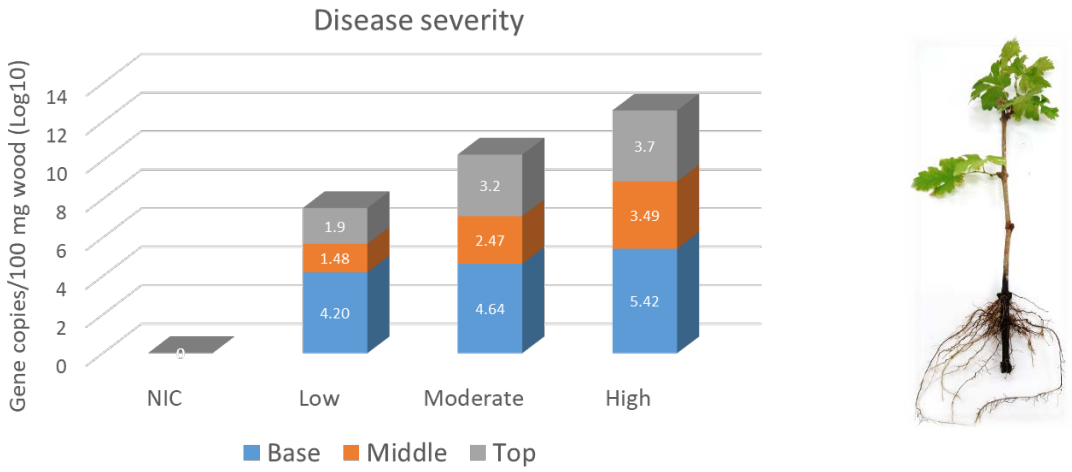

Figure 11. Quantity of BD pathogens detected at the base, middle or top of the cuttings post-rooting, following vacuum inoculation with low (300 spores), moderate (3,000) or high (30,000).

The vacuum-inoculated cuttings were rooted and used to establish pot trials in September 2018. Half of the vines for each inoculation treatment were subjected to water-stress while the remaining half were given sufficient water. No symptoms associated with BD infections has been observed for all treatments to date and observations will continue for another 2 years.


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