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SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

11th International Workshop on Grapevine Trunk Diseases, Canada, July 2019



REPORT TO WINE AUSTRALIA FOR TRAVEL

Project Number: **WAT 1901**

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1. ABSTRACT

Dr Mark Sosnowski attended the 11th International Workshop on Grapevine Trunk Diseases (IWGTD) in Canada with support from Wine Australia. The primary objective was to present results from Wine Australia research and to hear about the latest international developments in grapevine trunk disease (GTD) research. As regional councillor for Australasia on the International Council on Grapevine Trunk Disease, he attended the biennial council meeting and was a member of the Scientific Committee. Whilst at the workshop, Sosnowski built on international collaborative links and progressed research activities with Canada and Europe. He was an invited keynote speaker at the 11th IWGTD "Industry Day" presenting a summary on managing GTDs in the vineyard. Following the workshop, he visited Dr José Ramon Úrbez Torres (Agriculture and Agri-Food Canada), who convened the 11th IWGTD and leads a GTD research program based at the Summerland Research and Development Centre in the Okanagan Valley in British Columbia, to discuss collaborative research. Research highlights from the workshop included; non-destructive detection methods, inoculum dispersal patterns from diverse environments, interaction between pathogens and disease severity, xylem morphology and disease susceptibility, biocontrols with promise for management in the nursery, success of preventative and curative management in vineyards and profitability of early intervention and regular management of GTDs. Recommendations for future research in Australia include; continuing the search for alternative natural compounds and biocontrols for wound protection, better understanding the risk of wound infection at all times of the year, elucidating relationships between spore dispersal and climatic variables, developing spore trapping techniques with a view towards real time monitoring in the future, investigating the interaction between pathogens in grapevine trunks and their impact on disease progress and severity, continuing research to better understand infection thresholds in propagation material and monitor the micriobiome of nursery material to assist with development of quality assurance systems.

2. EXECUTIVE SUMMARY

Dr Mark Sosnowski, South Australian Research and Development Institute (SARDI), was co-funded by Wine Australia (WA) to attend the 11th International Workshop on Grapevine Trunk Diseases (IWGTD) in Penticton, British Columbia, Canada, 7-12 July 2019. Dr Sosnowski, who leads a grapevine trunk disease (GTD) management program at SARDI, and project team member Dr Regina Billones Baaijens of the National Wine and Grape Industry Centre (NWGIC) presented research results from project SAR1701-1.1 "Grapevine trunk disease management for vineyard longevity in diverse climates of Australia". The objectives of the travel were to; i) obtain the latest results of research into grapevine trunk diseases from around the world, and apply it to benefit the Australian wine industry, ii) disseminate new knowledge from Wine Australia grapevine trunk disease research to international trunk disease research industry members, iii) continue to develop and maintain professional relationships with international experts to promote collaboration with Australian research, and iv) attend the biennial meeting of the ICGTD and undertake official duties at the IWGTD as a science committee member.

The 11th IWGTD attracted 146 delegates from 21 countries. One hundred and twelve abstracts on current research on GTDs were delivered in oral presentations and posters. The workshop was divided into four sessions; (i) Pathogen detection and identification, (ii) Epidemiology, (iii) Host-pathogen interactions and (iv) Disease management in the nursery and vineyard.

Research highlights of particular relevance to the Australian grape and wine industry included;

- A variety of non-destructive GTD detection methods are being developed
- Varying inoculum dispersal patterns are emerging from climatically diverse environments, with rainfall appearing not to be the limiting factor for spore release.
- Co-infection of grapevine with multiple pathogen combinations affect GTD disease severity
- Xylem morphological traits correlate with disease susceptibility
- A number of biocontrols are showing promise for the management of GTDs in the nursery
- There are positive reports on the success of preventative and curative management strategies for GTDs in commercial vineyards
- Pruning management influences development of disease symptoms
- Early intervention and regular management of GTDs will maximise longevity and profitability of vineyards

Following the workshop, Sosnowski visited with Dr José Ramon Úrbez Torres (Agriculture and Agri-Food Canada) who convened the 11th IWGTD and leads a GTD research program at the Summerland Research Centre in the Okanagan Valley, BC. They discussed collaborative research between SARDI and Agri-Food Canada focussing on understanding infection thresholds in grapevine planting material, inoculum surveillance and disease control in mature vines. The outcomes of the collaborative research will assist in maintaining high standard nursery material for the industry, enhance research for monitoring risk of infection and improve strategies for managing grapevine trunk disease in mature grapevines.

Recommendations for future Australian research are to;

- continue the search for alternative natural compounds or biocontrols for wound protection
- better understand the risk of wound infection at all times of the year
- elucidate the complex relationships between spore dispersal and climate variables
- continue developing spore trapping techniques with a view towards real time monitoring in the future
- investigate the interaction between pathogens in grapevine trunks and their impact on disease progress and severity
- continue research on infection thresholds in propagation material and monitor the micriobiome of nursery material to assist with development of quality assurance systems
- continue the evaluation of rootstock and scion material in the search for tolerance to trunk disease

3. BACKGROUND

Grapevine trunk diseases (GTDs), including eutypa and botryosphaeria dieback, and the esca disease complex, cause the decline and eventual death of vines. GTDs threaten the sustainability of the Australian wine industry through loss of production, reduced fruit quality and vine renewal or replacement costs. Grapevine trunk disease research funded by Wine Australia (WA) and conducted at the South Australian Research and Development Institute (SARDI), the National Grape and Wine Industry Centre (NGWIC) and University of Adelaide (UA) is providing practical management strategies for Australian growers and contributing significantly to the worldwide understanding of the best methods to control this insidious problem.

The primary purpose of this travel was for Dr Mark Sosnowski to attend the 11th International Workshop on Grapevine Trunk Diseases (IWGTD) in Penticton, British Columbia, Canada 7-12 July, 2019. Every 2-3 years, researchers from around the world gather to discuss and share the latest research findings of relevance to the management of GTDs. The workshop was organised by the International Council on Grapevine Trunk Diseases (ICGTD), of which Dr Mark Sosnowski (SARDI) is a regional councillor for Australasia and past Chairperson. At the workshop, Sosnowski and project team member Dr Regina Billones Baaijens (NWGIC) presented research results from the Wine Australia funded project "Grapevine trunk disease management for vineyard longevity in diverse climates of Australia" (SAR1701-1.1) in both oral and poster formats.

Sosnowski was invited as a keynote speaker on the "Industry Day" (12 July) to speak on management of GTDs in mature vines. He also conducted official duties as the regional councillor for Australasia on the ICGTD, attending the biennial meeting, and was a member of the 11th IWGTD Scientific Committee, which involved reviewing submitted papers and chairing a session at the workshop.

Following the workshop, Sosnowski visited with Dr José Ramon Úrbez Torres (Agriculture and Agri-Food Canada) who convened the 11th IWGTD and leads a GTD research program at the Pacific Agri-Food Research Centre in Summerland, BC to discuss collaborative research between SARDI and Agri-Food Canada.

The objectives of this travel were to;

i) Obtain the latest results of research into grapevine trunk diseases from around the world, and apply it to benefit the Australian wine industry,
ii) Disseminate new knowledge from Wine Australia grapevine trunk disease research (SAR 1701-1.1) to international trunk disease research industry members,
iii) Continue to develop and maintain professional relationships with international experts to promote collaboration with Australian research,
iv) Attend the biennial meeting of the ICGTD and undertake official duties at the IWGTD as science committee member.

4. 11TH INTERNATIONAL WORKSHOP ON GRAPEVINE TRUNK DISEASES

The 11th International Workshop on Grapevine Trunk Diseases (IWGTD) was held at the Penticton Lakeside Resort, British Columbia, Canada, 7-12 July 2019. It was convened by Dr José Ramón Úrbez Torres of Agriculture and Agri-Food Canada on behalf of the International Council on Grapevine Trunk Diseases (ICGTD). The workshop attracted 146 delegates from 21 countries (Figure 1a). One hundred and twelve abstracts were delivered in oral presentations and posters. The workshop was divided into four sessions; (i) Pathogen detection and identification, (ii) Epidemiology, (iii) Host-pathogen interactions and (iv) Disease management in the nursery and vineyard.

The workshop was an excellent opportunity to present results from ongoing research on the practical management of GTDs in Australia (Figure 1b). Seven abstracts were presented, highlighting the breadth of Wine Australia funded research being undertaken in this area. Following is a summary of information presented by other international speakers that has relevance to current Australian research on management of grapevine trunk disease, particularly Eutypa (ED) and Botryosphaeria dieback (BD) with reference to how the information could be incorporated into future research for the benefit of the Australian viticulture industry.

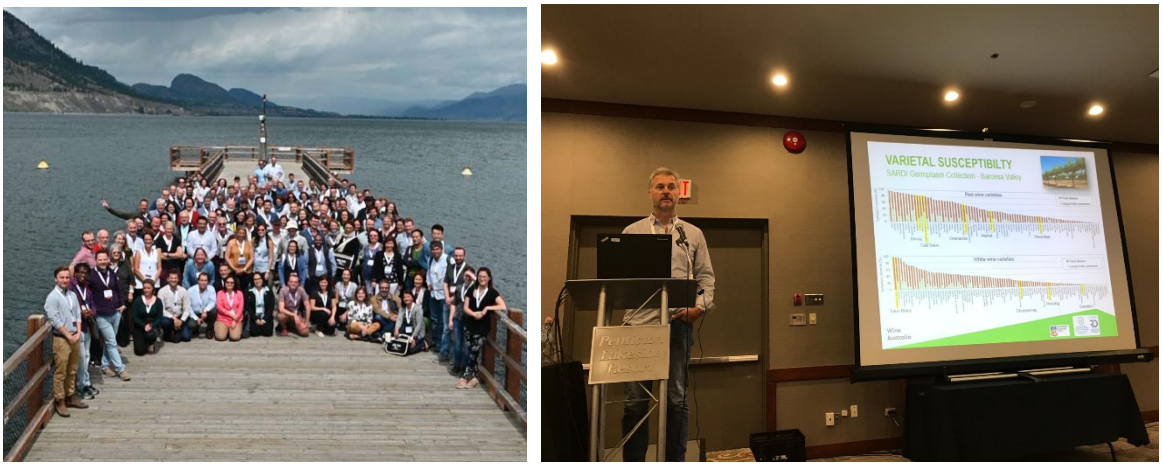


Figure 1. a) Delegates of the 11th International Workshop on Grapevine Trunk Diseases in Penticton, Canada and b) Dr Sosnowski presenting results from Australian research.

4.1 Disease detection

Cédric Moisy from Montpellier in France presented on the monitoring of fungal pathogen development and impact on wood tissues through non-destructive imaging. His research team are evaluating µ-MRI and X-ray µ-CT imaging approaches for detection of GTD to monitor the dynamic colonization of wood by fungi when artificially inoculated under controlled conditions. They were able to follow the progression of fungal growth in different tissues and quantify different types of degraded tissues in grapevine wood. These technologies are very expensive, hazardous to the operator and pose logistical challenges in the vineyard. However, if a more cost effective, safe and versatile system could be developed to scan grapevines non-destructively in the field, it may provide an alternative to the only current strategy of monitoring internal disease, cutting through trunks with a chainsaw.

James Woodhall, from the University of Idaho in the USA and Felipe Sáez Cortez from the University Andrés Bello in Chile both spoke separately on the use of loop mediated isothermal amplification (LAMP) assays for the detection of grapevine trunk disease pathogens. Molecular tests like real-time PCR are sensitive and accurate but confined to the laboratory and take several hours to complete. LAMP offers a rapid and portable alternative for molecular testing. LAMP assays were developed for the main causal agents of ED and BD as well as Esca. The assays were sensitive, and provided results within 10 minutes. Detection in grapevine wood was possible using an on-site DNA extraction method where wood was sampled using a drill, and transferred to a small tube containing buffer and a ball bearing. After vigorous shaking by hand, it was diluted tenfold and used in the LAMP reaction. Wood samples using the in-field extraction method were positive within 10-30 minutes. LAMP offers a rapid and portable diagnostic and detection tool for use when on-site testing is necessary. Current Wine Australia research is developing LAMP assays for rapid detection of ED and BD pathogens on spore tape and in the field, which will improve decision making on GTD management for the industry.

Christopher Wallis from the U.S. Department of Agriculture (USDA) in California, presented an alternative rapid and inexpensive technique for detection and identification of grapevine fungal pathogens using Fatty Acid Methyl Ester analyses (FAME). Fingerprinting of fatty acids that comprise cell membranes of each pathogen was performed using gas chromatography on methyl esters. Profiles for over 20 fungal species were created. A total of 20 samples from culture could be analyzed with FAME in as little as 2 hours, and cost less than 10 cents a sample. Ongoing work is examining ways to extract and analyze fungal FAMEs directly from plant tissues to avoid the need for culturing. FAME provides an alternative to DNA-based identification and could be employed in cases where nucleic acid degradation is a concern, or if an alternative methodology is required for regulatory actions.

4.2 Inoculum dispersal

Mónica Berbegal of the Polytechnic University of Valencia presented results from spore trapping to monitor inoculum dispersal of the esca pathogen *Phaeomoniella chlamydospora* in Spanish vineyards and relate to climatic conditions. Microscope slides coated with silicone were replaced weekly during two seasons and DNA concentration of the pathogen was determined using real time PCR. Pathogen DNA was detected at high levels in 71 of the 139 sampling periods with rain, and at low levels in 20 of the 31 periods without rain. The dynamic of inoculum detection was best described by a Gompertz equation when time was expressed as hydro-thermal time (i.e. a combination of accumulated temperature and relative humidity). The information obtained and the equation developed will help to identify the periods with high risk. Berbegal's research group, who collaborate with the Australian trunk disease research program, have offered to run Australian spore detection data of ED and BD pathogens through their model to determine if it can also predict risk patterns in these pathogens. The Australian data was presented at the IWGTD by project collaborator Dr Regina Billones Baaijens of the National Wine and Grape Industry Centre in Wagga Wagga.

Daniel O'Gorman of Agri-Food Canada used droplet digital PCR technology to quantify BD pathogen spores captured in Burkard spore traps located in commercial vineyards. Results showed spore release events occurring from late March (spring) to October (autumn) and were absent or greatly reduced through the winter, where temperatures were below zero, and snowfall occurred. Generally, the first spore release was detected at the end of winter when average daily temperatures climbed above zero to around 5°C or higher and often correlated, though not always, with rainfall.

Eline Van Zijll De Jong of Linnaeus Laboratory in New Zealand, presented on emerging patterns in the dispersal of Botryosphaeria and Eutypa dieback pathogen spores in New Zealand vineyards from a project led by Dr Sosnowski at SARDI. Burkard spore traps have been deployed in two vineyards in Marlborough and Hawke's Bay. Spore tape samples collected daily from the two sites have been analysed by quantitative real-time PCR (qPCR) for the target spores, and compared against daily temperature and rainfall data. Spores of BD and ED pathogens were detected throughout the year in both regions and tended to be detected during and after rain events, but occasionally when no rainfall occurred. Different patterns are emerging between the two regions in the frequency and quantity of spores detected for each of the pathogens during different seasons.

The presence of spores even when rain is not present is common between these studies and Australian data being collected in Wine Australia research. More research is required to understand the factors leading to spore release in the absence of rain.

4.3 Host-pathogen interactions

Christopher Wallis of the USDA in California, presented on multiple pathogen infections. Despite being located on different branches, a first infection may induce systemic changes in host physiology that could alter progress of an independent infection elsewhere on the vine. To investigate the interactions of two separate infections in a single vine, a trunk pathogen was inoculated two months after initially infecting the vine with a trunk pathogen on a different branch. Initial inoculation with the BD pathogen *Diplodia seriata* was associated with reduced lesion lengths of subsequent inoculation with the same pathogen, the BD pathogen *Neofusiccocum parvum* and the Esca pathogen *P. chlamydospora*. Preliminary results suggested these reductions were associated with changes in host physiology including shifts in amino acid, sugar, phenolic compound, and terpenoid levels. Follow-up studies are underway to confirm this phenomenon and examine other pathogen combinations. Studies investigating the effect of multiple trunk disease pathogen infections on grapevine are lacking and may provide some insight into the variability of disease progress in vineyards. Knowing that ED and BD pathogens co-exist

in many Australian and New Zealand vineyards, it is important to understand how they impact each other in regard to development and progression of disease symptoms.

Jerome Pouzoulet of INRA in France is studying the genetic basis of xylem morphology in grapevine using the esca pathogen *P. chlamydospora*. Previous studies reported on the role of xylem vessel diameter in the ability of some commercial cultivars to respond to infection. Using a grapevine rootstock experimental progeny (F2, *V. riparia* cv. 'Gloire de Montpellier' x *V. vinifera* cv. 'Cabernet Sauvignon'), 261 genotypes from the progeny were characterized for various xylem morphological traits, and a quantitative trait locus (QTL) analysis was performed over 2 years of observation. Results showed that there were strong and stable QTLs associated with various xylem morphological traits, including diameter of vessels. Based on this analysis, subsets of genotypes were further characterized for functional traits (hydraulic conductivity and pathogen susceptibility). A QTL found for vessel diameter was seen to impact xylem hydraulic conductivity significantly. In addition, results confirmed that within this progeny, the density of vessels of wide diameter was positively correlated with the level of susceptibility to the pathogens. In Australia, ED and BD susceptibility of germplasm has been linked to xylem size, and this provides an opportunity for QTL analysis to be utilised to develop markers for traits of disease susceptibility which could be adopted for screening in breeding programs.

4.4 Stress

Aurélie Songy of the Université de Reims Champagne-Ardenne in France presented research on the effect of water and heat stress on the physiology of Ugni blanc infected with the BD pathogen *N. parvum*, based on specific transcriptomic and metabolomic profiles. The impact of infection on plant metabolism was greater after heat stress, compared to water stress, but greatest when they occurred simultaneously, due to reduced plant growth and increased aggressiveness of *N. parvum*. Lipids and secondary metabolites were impacted by infection. It was concluded that these results may help explain the seasonal variability of GTD symptom expression in vineyards, which may be influenced by abiotic stresses that can occur separately or simultaneously. This result concurs with results of previous Wine Australia research which showed that seasonal variation of ED foliar symptoms was linked with temperature and water availability. More recent Australian research, however, has shown that water stressed field vines are not more susceptible to colonisation by ED and BD pathogens.

4.5 GTD Management in the nursery

María Del Pilar Martínez-Diz of Estación de Viticultura e Enoloxía de Galicia (AGACAL-EVEGA) presented results from screening of biocontrol agents (BCAs) for propagation material from the nursery against black-foot and Petri disease. Her study evaluated five BCAs; two *Streptomyces* sp., *Pythium oligandrum*, and commercial products containing *Trichoderma atroviride*, *T. koningii* and *Pseudomonas fluorescens* + *Bacillus atrophaeus*. Prior to dispatch and planting, 1-year-old dormant grafted vines were hot-water treated at 53ºC for 30 min and roots were immediately soaked in BCA suspensions for 24 h. Two additional applications of BCAs were applied by drip irrigation in the field. Other than *T. koningii* and *P. fluorescens* + *B. atrophaeus*, BCAs were able to significantly reduce incidence and severity of infection by the pathogens.

In a similar study, Isidora Silva of UC Davis Chile, reported control of BD pathogens by *Trichoderma* sp., *Clonostachys* sp. and *Epicoccum* sp. in grapevine cane bioassays. Australian research is investigating the role of nursery production in BD along with the microbiome of microorganisms present in propagation material. Research in New Zealand has eliminated the use of HWT for GTD control due to vine mortality, so viable alternatives are required. With further evaluation, the information from Spanish and Chilean research may assist future recommendations for best practice management in local grapevine nurseries.

Ana Romeo-Olivan from the University of Toulouse, France reported on ozone dissolved into water as an innovative tool in the grapevine nursery for young plants production. Using esca pathogens, *in vitro* studies revealed that no spores germinated in the ozone treated water. Inoculated plants treated with ozonated water were recorded with lower levels of esca pathogens, and increased bud burst and root growth, suggesting some potential for management of GTDs in the nursery.

4.6 GTD Management in the vineyard

George Karaoglanidis of the Aristotelian University of Thessaloniki in Greece reported on evaluation of the fungicide Tessior® (boscalid and pyraclostrobin) for control of Esca and BD pathogens. Applications of Tessior resulted in significant reduction of disease incidence and severity when applied either on the

day of pruning or 6 days after pruning. Delayed applications of 13 or 29 days after pruning and inoculation did not contribute to pathogen control, with disease incidence similar to that observed in the untreated controls. Long-term trials were also established in newly planted vineyards in 2015, where applications of Tessior were initiated from the 1st year of planting, and artificial inoculations with either *P. chlamydospora* or *D. seriata* were initiated 2 years after establishment. Continued monitoring of the trial will determine longer-term efficacy of regular application of Tessior to wounds. SARDI research has identified pyraclostrobin as an effective fungicide for control of ED and BD pathogens, but to date no label registration for pruning wound protection has been forthcoming. Long-term pruning wound trials like this are lacking and would be worth establishing in Australia using locally registered products to demonstrate the economic benefits of regular pruning wound protection program adopted from planting.

Mark Sosnowski spoke about the factors affecting progress of grapevine trunk disease in New Zealand vineyards based on surveys he conducted in 2013 and 2018 for the Bragato Research Institute. The overall mean incidence of dieback increased from 8 to 20% and foliar symptoms 0.1 to 0.3% over the 5 years, which corresponded with an increase in average vine age from 12 to 17 years. Overall trends in 2018 revealed variation in dieback incidence between vine ages, varieties, clones, rootstocks and pruning styles. There were positive signs of short-term success for the control GTDs using remedial surgery and wound protection.

Jinxz Pollard-Flamand from Agri-Food Canada has been screening a range of *Trichoderma* species alone and in combination for pruning wound protection against BD pathogens using a detached cane assay technique developed at SARDI as part of Wine Australia research. His results showed 100% reduction of infection from 1d to 21d post treatment with combined BCAs compared to inoculated controls. At SARDI evaluation of a commercial *Trichoderma* product has revealed difficulties with evaluation using the detached cane assays, which are assessed after 4 weeks incubation. Due to rapid colonisation of agar plates with *Trichoderma*, the pathogen doesn't have time to colonise agar from the wood samples. Field trials are underway to evaluate the product in the vineyard with 12 month incubation before assessments are made, in the hope it will provide clearer results.

Trichoderma was the subject of many other oral and poster presentations, showing the potential this biological control has for GTD control. However the challenge remains, of *Trichoderma* rapidly colonising the agar plates, possibly reducing the chances of isolating the pathogen and giving a false result. Molecular tools can detect if DNA of the pathogen is present, but even if the fungus is dead, the DNA will still be present. In discussion with Jermome Pouzelet of INRA, France, he suggested that qPCR is still a valid tool for this as it will differentiate the large amounts of pathogen DNA present when it has survived and colonised the wood versus the small amount of DNA from the dead pathogen that did not. Therefore it may be worth considering for future evaluations of *Trichoderma* wound protection against EU and BD pathogens in Australia.

4.7 Pruning management

Emilie Bruez of INRA Bordeaux presented on the effect of the position of pruning wounds in relation to esca by studying the impact of the cones of desiccation on sap flow, using Ugni blanc grapevines. Vines were pruned directly below or directly above the node position to investigate the impact of the natural dieback on sap flow to the new shoots. Varying extent of desiccation was reported depending on the position of the cut, with low cuts having desiccation cones impeding the vascular tissue of the new shoots. There has not been any assessment on the impact of infection success by esca pathogens, but this should be investigated for ED and BD pathogens in Australia in the future.

Christian Kraus of the University of Hohenheim in Germany evaluated the influence of minimal (Semi minimal pruned hedge) vs intensive (Vertical shoot position) pruning on the occurrence of esca. Over the four year study, symptoms varied, with pruning style having no consistent effect on the appearance of symptoms.

Chloe Delmas of INRA Bordeaux presented on the effect of training systems on esca in French vineyards. They studied four pairs of 'Ugni blanc' vineyards ranging in age from 30-46 years, where each pair consisted of vineyards located in similar environments and comparable age, with one trained as 'espalier cordon' (spur-pruned) and the other 'espaliers Guyot-arcure' (cane-pruned). Cane-pruned vines generally had more esca symptoms than spur-pruned vines. This supports previous research on ED in France where greater disease and mortality was recorded in cane-pruned vines that were older than 20 years of age.

A PhD study by Bruce Henderson at the University of Adelaide and SARDI showed that overall surface area of pruning wounds on spur-pruned vines is significantly greater than on cane-pruned vines, leading to more visual symptoms of ED in the spur-pruned vines. It is understood that disease symptoms can progress internally in cane-pruned vines due to the proximity of wounds to the trunk, and therefore not be evident externally, whereas spur-pruned vines have propensity to express foliar symptoms, and spur and arm dieback are very obvious from a relatively young age.

4.8 Economics of managing GTDs

Jonathan Kaplan from California State University in Sacramento has undertaken extensive economic analysis and reported on the financial consequences of grapevine trunk diseases and the potential gains from adopting management strategies. If GTDs are left unmanaged in vineyards, they lead to falls in revenue and significantly shortened profitable lifespans. By simulating alternative scenarios depicting their adoption on symptomatic vines in a Cabernet Sauvignon vineyard, he found that preventative practices adopted early (≤5 years old) and remedial surgery adopted in mature vineyards (>10 years old) significantly reduced yield losses, raised revenue, and extended the profitable lifespan of the vineyard (approximately 25 years) by over 100%. Adoption of preventative practices from year 10, however, showed limited economic value. He also showed that remedial surgery alone outperformed early-adopted preventative practices, but in tandem they performed best, with greater gains the earlier preventative practices began. Further, the greatest gains from remedial surgery occurred when performed on all symptomatic vines beginning in year 11 (when no preventative practices are adopted earlier) and up to year 14 (when adopting preventative practices in year 3). As such, growers are recommended to adopt preventative practices in young vineyards and then remedial surgery after symptoms appear in approximately 20% of vines or before year 15, to maximize vineyard profitability and longevity. These assumption based outcomes correspond with recommendations resulting from modelling of data collected from Sauvignon Blanc vineyards in New Zealand as part of SARDI led research.

All abstracts presented at the 11th IWGTD are available at http://iwgtd2019.ca/program/ and will be published in the European journal *Phytopathologia Mediterranea*.

4.9 Field trip to the Okanagan Valley.

A tour of the Okanagan Valley wine region provided insight into the BC wine industry (Figure 2). With a variety of climates and soils throughout British Columbia, 4,400 hectares are planted in 186 vineyards to over 60 different grape varieties such as Merlot, Pinot Noir, Cabernet Sauvignon, Pinot Gris, Chardonnay and Riesling, with a 50% split between red and white varieties. There was a big move away from the American native hybrid varieties with mass planting of *Vitis Vinifera* varieties during the 1990's meaning the most vineyards are up to 25 years old. The main diseases affecting the industry currently are trunk diseases and viruses. The tour provided the opportunity to hear from local viticulturists and winemakers and see first-hand the way the industry is tackling trunk disease management as well as other general viticultural practices. Growing grapes in this region presents logistical challenges and abiotic stresses during the winter freeze. In summer, hot, dry conditions and bushfires present similar challenges to those experienced in Australia.

4.10 Pacific Agri-Food Research Centre (PARC), Summerland

Research lead by Dr José Ramón Úrbez Torres at the Summerland Research Centre in the Okanagan Valley is tackling grapevine trunk diseases and viruses. Dr Sosnowski visited Dr Úrbez Torres and together they visited some vineyard trial sites to see Burkard spore traps. This provided Dr Sosnowski an opportunity to receive an update on how these were performing, and compare with spore traps deployed in Australian and New Zealand vineyards. Another major field trial in a commercial vineyard is evaluating remedial surgery as a management strategy to extend the profitable life of the vineyard, even if the disease has reached ground level. This research compliments past and current research led by SARDI to evaluate remedial surgery under different scenarios and in different climates.

Dr Úrbez Torres is a collaborator on SARDI research projects in both Australia and New Zealand. Discussions with Dr Sosnowski provided a comparison of preliminary results from all three countries, and assisted with the development of plans for future complimentary trials between SARDI and Agri-Food Canada.



Figure 2. Vineyard tour of Okanagan Valley (a-c) and internal symptoms of trunk disease in the cross-section of a grapevine trunk (d).

5. MAIN OUTCOMES

5.1 Knowledge

Attendance at the 11th International Workshop on Grapevine Trunk Diseases provided Dr Sosnowski with the opportunity for interaction with scientists and industry representatives from across the world. He was able to present the latest results from Australian research trials and obtained the latest information and results of international GTD research, much of which is outlined in this report, which can be applied here to benefit the Australian wine industry.

5.2 Professional links

At the workshop, Sosnowski met with Spanish PhD student Marcos Andres-Sodupe, and his supervisor Dr David Gramaje, who leads a major research program on control of grapevine trunk diseases at the Instituto de Ciencias de la Vid y del Vino (ICVV; Research Centre of Vine-and-Wine-related Science) in Logrono, La Rioja, Spain. Marcos has requested to spend 3 months at SARDI in 2020, to undertake experiments complimentary to his PhD and the Wine Australia trunk disease program. Past study visits with the grapevine trunk disease program at SARDI by international students has led to industry outcomes linked to Wine Australia research, a number of peer reviewed journal publications and development of international collaborative projects

Collaborative links were strengthened with researchers from organisations around the world whose main focus is grapevine trunk diseases. Of particular note was Dr Gianfranco Romanazzi of the Marche Polytechnic University in Italy, who has proposed an international collaboration to evaluate GTD management strategies in long-term trials and Mónica Berbegal, from the Polytechnic University of Valencia, Spain, regarding applying spore trapping data from Australia to the model her group has developed based on spore trapping in Spain. Dr Romanazzi also works with chitosan, a natural product which has shown some efficacy against GTDs in preliminary Wine Australia trials. With no commercial products available in this country, a new link with a European commercial producer may provide an avenue in future.

This travel also provided an opportunity for Dr Sosnowski to progress collaborative research with Dr José Ramón Úrbez Torres (Agri-Food Canada) and Dr David Gramaje (ICVV Spain), who lead major research programs on control of grapevine trunk pathogens in Canada and Spain, respectively. Úrbez Torres and Gramaje are collaborators on the Wine Australia project led by SARDI, with current joint research focussing on understanding infection thresholds in grapevine planting material. The outcomes of the collaborative research will be to assist in maintaining high standard nursery material for the industry, enhance research for monitoring risk of infection and improve strategies for managing grapevine trunk disease in mature grapevines.

5.3 Communication

An article will be submitted to an Australian industry magazine reporting on the main outcomes of this travel. In addition, knowledge from this travel will be disseminated through various industry workshops presented in Australia by the SAR1701-1.1 project team.

6. RECOMMENDATIONS

The workshop highlighted that Australian research is at the forefront of delivering practical outcomes to assist growers in management of grapevine trunk diseases. It is, therefore, important to continue this research which contributes significantly to the sustainability of the Australian wine industry.

Future Australian research should;

- continue the search for alternative natural compounds or biocontrols for wound protection
- better understand the risk of wound infection at all times of the year
- elucidate the relationships between spore dispersal and climatic variables
- continue developing spore trapping techniques with a view towards real time monitoring in the future,
- investigate the interaction between pathogens in grapevine trunks and their impact on disease progress and severity
- continue research on infection thresholds in propagation material and monitor the micriobiome of Australian nursery material to assist with development of quality assurance systems
- continue the evaluation of rootstock and scion material in the search for tolerance to trunk disease

It is further recommended that Wine Australia continue to support Australian researchers' attendance at future International Workshops on Grapevine Trunk Diseases, as they contribute significantly to professional development, expand collaboration with key researchers from around the world and aid in the advancement of research programs that will benefit the Australian wine industry.

7. ACKNOWLEDGEMENTS

Thanks to Wine Australia, SARDI, New Zealand Winegrowers and the British Columbia Ministry of Agriculture for contributing funds for this travel.

8. APPENDICES

Appendix 1. Travel Itinerary

July 2019	**Activities**
Sat 6	Travel from Adelaide to Penticton, BC Canada
Sun 7	Biennial meeting of the International Council on Grapevine Trunk Diseases, Penticton Lakeside Resort, BC Canada
Mon 8 – Fri 12	11th International Workshop on Grapevine Trunk Diseases, Penticton Lakeside Resort, BC, Canada
Mon 15 – Tue 16	Collaborative visit with Dr José Ramón Úrbez Torres, Pacific Agri-Food Research Centre in Summerland, BC, Agriculture and Agri-Food Canada.
Wed 17 – Fri 19	Travel from Penticton to Adelaide

Appendix 2. Abstracts

11th International Workshop for Grapevine Trunk Diseases

Oral presentations

Disease Management 101: Grapevine trunk diseases, control in mature vineyards.

MARK R. SOSNOWSKI

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Grapevine trunk diseases (GTDs) have become a major concern worldwide, causing significant economic impact by reduced production and vineyard longevity. Eutypa, Botryosphaeria and Phomopsis dieback, and esca affect mature vineyards. These diseases are caused by a wide range of fungal pathogens producing a range of symptoms including: leaf and shoot distortion and discolouration, wood cankers and dieback, internal wood necrosis and staining, poor growth, and eventually vine death. The prevalence of GTDs has significantly increased with changes in production practices, loss of effective chemicals, predominance of susceptible cultivars and ageing of vineyards. Pruning wounds are the main infection portal for these pathogens and inoculum sources include a wide range of alternative hosts such as fruit crops and many introduced and/or native tree species. A good understanding of the etiology, biology and epidemiology of GTD fungi has led to the development of effective management strategies in the vineyard. Cultural practices, such as the timing of pruning, can influence the likelihood of infection and disease control can be achieved through early adoption of preventative strategies to protect pruning wounds. Paints and pastes are used for protection of large reworking wounds. Fungicide and biocontrol products, used for annual pruning wounds, can be applied most efficiently with spray machinery. Removing infected wood by remedial surgery and retraining grapevines can provide curative control.

Standardizing Botryosphaeriaceae infection levels in experimental grapevine plant materials.

REGINA BILLONES-BAAIJENS[1], MARK SOSNOWSKI[2,3], MATTHEW AYRES[2] and SANDRA SAVOCCHIA[1].

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Grapevine nursery plants have been reported with latent infections of grapevine trunk disease (GTD) pathogens. However, the link between these latent infections and GTD symptoms observed in vineyards is still unclear. Studies have also shown that water-stress can increase the susceptibility of young vines to GTDs. Accurate quantification of infection levels from naturally-infected young vines is difficult since latent infections have no internal or external symptoms and may be randomly distributed within a vine. Thus, investigations on the effects of water stress on young vines artificially inoculated with three different conidial concentrations of GTD pathogen may provide insight on the infection thresholds that result in disease expression in vines. The objective of this study was to develop an inoculation method to standardize GTD infection levels in young vines. A published vacuum-inoculation method was evaluated for inoculating Shiraz cuttings with 300 (low), 3,000 (moderate) and 30,000 (high) conidia of *Neofusicoccum luteum* while Ringer's solution was used to inoculate control vines. The vacuum-inoculation was a reliable method as evidenced by qPCR analyses, resulting in the conidia infecting the basal, middle and apical part of the inoculated canes. The qPCR analyses further differentiated the low, moderate and high infections with the highest amount of pathogen detected from canes inoculated with 30,000 conidia and lowest from those inoculated with 300 conidia. No pathogen was detected in any of the control canes. This study showed the vacuum-inoculation method was suitable for introducing different concentrations of *N. luteum* spores into dormant canes without significant impact on plant viability. The method was used to standardize infection levels of *N. luteum* in ~400 Shiraz rootlings in newly established glasshouse and shade house experiments. Observations on the effect of water stress on Botryosphaeria dieback symptom expression is on-going.

Spore dispersal patterns of Diatrypaceae and Botryosphaeriaceae species in Australian vineyards.

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Eutypa dieback (ED) and botryosphaeria dieback (BD) are considered the two most important grapevine trunk diseases in Australia, causing significant yield reduction and threatening the sustainability of Australian vineyards. Spores of the causal pathogens are generally dispersed by rain splash and wind and infect pruning wounds resulting in cankers, dieback and eventually death of vines. Thus, understanding the spore dispersal patterns of these pathogens will help determine the critical times of the year when spores are abundant in Australian vineyards. This will assist growers in making decisions on optimal timing of pruning and wound treatment. This study investigated the spore dispersal patterns of ED (Diatrypaceae) and BD (Botryosphaeriaceae) pathogens in four wine growing regions in Australia. Burkard spore traps were deployed in South Australia (Barossa Valley and Coonawarra) and in New South Wales (Hunter Valley and Griffith) between 2014 and 2016. Spore trap tapes were collected and replaced monthly at each site and analysed for Diatrypaceae and Botryosphaeriaceae spores using qPCR assays. The 3-year study showed spore dispersal of ED and BD pathogens was sporadic and varied between regions, season and year. In South Australia, ED and BD spores were primarily detected in late winter and early spring while in New South Wales, a high number of spores were trapped over summer. Spores were generally recorded during or immediately after rain but not all rain events resulted in spore detection. The spore numbers and frequency of detection varied between years with the highest number of spores being recorded in 2016, particularly for Diatrypaceae species. Additional spore traps were deployed in 2017 across Australian wine regions and will be monitored for a further 3 years. Computer modelling of data will also investigate the role of other weather factors (temperature, relative humidity, dew point, wind direction) on the detection of these spores.

Poster presentations

Evaluating techniques to induce water-shoot production following remedial surgery to control grapevine trunk diseases.

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Remedial surgery (also known as vine surgery or trunk renewal) is an effective method of controlling the grapevine trunk diseases Eutypa and Botryosphaeria dieback. It involves cutting visibly affected wood from vine cordons and trunks, and retraining a basal shoot (aka watershoot) into a new canopy. However, it has been reported that, for some own-rooted cultivars, shoot production may be limited to as few as 60% of vines, and even fewer in grafted vines. In 2017, a trial was established to evaluate techniques anecdotally reported to improve water-shoot production in an own-rooted 'Cabernet Sauvignon' vineyard planted in 1995 in the Clare Valley, South Australia. Vines were assessed with 100% incidence of external symptoms of Eutypa dieback and Botryosphaeria dieback. All vines underwent remedial surgery by cutting trunks with a chainsaw to just above the irrigation wire. Beforehand, cross-sectional cuts starting at the top of the trunk revealed wood symptoms of Eutypa dieback and Botryosphaeria dieback in 87% of vines, with far fewer vines, 28%, with wood symptoms at the base. Treatments for inducing water-shoots were undertaken in an area on the lower trunk predesignated by painting a white circle. They included hitting with a hammer, making an X-shaped cut with a tomahawk, rubbing bark with a wire brush and application of a hormone used to break dormancy (Dormex®). In September 2017, a spring remedial surgery was undertaken, and treatments applied, as described above. Grafting treatments were conducted in October. Assessment in February 2018 revealed water-shoots were readily produced on 80-92% of treated and untreated control trunks, but rarely (<18%) originated within the painted white circle, 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%. This research shows that, of the treatments tested, only grafting significantly improved water-shoot production following remedial surgery. Trials continue to investigate seasonal variability of water-shoot production and effectiveness of grafting.

Evaluating spray coverage and fungicide efficacy when using adjuvants for pruning wound protection.

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Eutypa dieback, which causes significant impacts to vineyards worldwide, can be controlled by applying fungicides to pruning wounds using tractor-driven sprayers, with efficacy correlated to wound coverage. In winter 2017, a vineyard trial was established in McLaren Valley, South Australia to evaluate the effect of using spray adjuvants with fungicide on wound coverage, using fluorescent dye and fungicide efficacy as assessment criteria. A recycle sprayer was used to apply two fungicide treatments; fluazinam (Emblem®) and tebuconazole (Gelseal™) with or without a wetter (trisiloxane ethoxylate; DuWett®) or a sticker (di-1-p-menthene; Flextend®). In order to determine if adjuvants can increase coverage, spray treatments were applied at a low water rate of 200 L/ha to simulate poor coverage. Following treatment, wounds were inoculated with *Eutypa lata* ascospores. To assess wound coverage, fluorescent dye was added to the treatments and water sensitive papers were attached to four positions on a post and six randomly selected positions on cordons and spurs. A selection of treated canes was immediately removed from vines and returned to the laboratory for assessment under UV light. Digital images were captured and analysed using Image J software, revealing little difference between treatments for coverage. Image analysis of water sensitive paper indicated relatively higher wound coverage compared with fluorescent dye. After 12 months, the inoculated canes were removed from vines and returned to the laboratory for isolation on agar media. There was no difference between treatments for recovery of *E. lata*. This preliminary data suggests there may be no advantage in using adjuvants when applying fungicides to pruning wounds with tractor-driven sprayers for protection against infection by *E. lata*. The use of fluorescent dye in treatments may be a more accurate tool than water sensitive paper for evaluating spray coverage on the pruning wound surface. The trial was repeated in winter 2018 to confirm the results.

Evaluating natural alternative products for the control of eutypa dieback in grapevine.

MATTHEW AYRES[1] and MARK SOSNOWSKI[1,2]

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Eutypa dieback is a major grapevine trunk disease throughout the world, and the most effective means of control is to prevent infection of pruning wounds. Research has shown that synthetic fungicides are effective, and a number of products are now registered in Australia and New Zealand for this purpose. However, there are limited natural alternative products registered for pruning wound protection against Eutypa dieback. Detached cane assays using single node Shiraz canes were used to evaluate vanillin (at 5 and 10 mg/ml), chitosan (at 12.5 and 25 mg/ml), previously reported with activity against trunk disease pathogens, and the yeast-like fungus *Aureobasidium pullulans* (Botector®), registered for control of botrytis bunch rot. The fungicide tebuconazole (Gelseal™), registered for control of Eutypa dieback, was included as a reference, as well as negative (no treatment, no inoculum) and positive (no treatment, inoculated) controls. All treatments (except negative control) were inoculated with 100 ascospores of *Eutypa lata*, the primary causal agent of eutypa dieback, and the canes grown in tubs of water for 5 weeks prior to their removal to the laboratory for isolation on agar medium. Vanillin had no effect on recovery of *E. lata* compared with the positive control, while chitosan, particularly at the higher concentration, significantly reduced recovery, to a similar level to canes treated with Gelseal. No *E. lata* was recovered from the canes treated with Botector, and all agar plates contained *A. pullulans.* The results of this experiment indicate that chitosan and Botector may have potential as pruning wound protectants against infection by trunk disease pathogens. Ongoing research is evaluating these products against *N. luteum* (Botryosphaeria dieback) and Botector will be further evaluated in vineyard trials.

Grapevine cultivar variability in colonization by *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*.

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The use of tolerant cultivars is a key element in an Integrated Pest Management program and would be one of the most effective means of controlling grapevine trunk diseases, not only reducing disease losses, but also decreasing the need for chemical control strategies. The aim of this study was to evaluate the extent of colonization by fungi associated with esca disease in a detached cane assay with twenty grapevine cultivars from the South Australian Research and Development Centre germplasm collection. In June 2018, one-year-old canes were harvested and stored at 4°C. Two weeks later, dormant grapevine canes were cut into 10 cm single-node sections and placed into holes in polystyrene boards which were floated on water in plastic tubs in a greenhouse. Wounds on top of twelve canes per cultivar were inoculated with spore solutions (800 spores per wound) of either *Phaeomoniella chlamydospora* or *Phaeoacremonium minimum*. Non-inoculated controls were also included. Canes were harvested seven weeks after establishment and the extent of colonization by the fungi was assessed by isolation onto potato dextrose agar. Both pathogens colonized the xylem vessels of all cultivars but the extent of fungal growth within woody tissue varied significantly. Canes of all cultivars were colonized between 3 and 34 mm (*P. chlamydospora*) and between 9 and 48 mm (*P. minimum*) from the wound site. This variation could suggest different disease progression depending on the cultivar and pathogen tested, indicating possible disease tolerance in some cultivars. Further investigation is required to confirm the results and better understand the way that fungi colonize the tissues and how some cultivars are able to restrict this colonization.

Evaluating techniques to induce watershoot production following remedial surgery to control grapevine trunk disease

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Introduction

Remedial surgery is an effective method of controlling the grapevine trunk diseases (GTDs) Eutypa and Botryosphaeria dieback. It involves cutting visibly affected wood from vine cordons and trunks, and retraining watershoots to renew vines. However, it has been reported that, for some own-rooted cultivars, shoot production may be limited to as little as 60% of vines, and even less in grafted vines. In 2017, a vineyard trial was established in the Clare Valley, South Australia to evaluate techniques anecdotally reported to induce watershoot production.

Methods

- Used own-rooted Cabernet Sauvignon vineyard planted in 1995
- Remedial surgery undertaken in early winter (late June 2017) by cutting trunks with a chainsaw to just above the irrigation wire
- Treatments for inducing watershoots were undertaken in an area on the lower trunk predesignated by painting a white circle
- 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® (Fig 1)
- In spring (late September), remedial surgery was undertaken on a second set of vines, and treatments applied as described above
- Grafting treatments were conducted later in spring (late October)



Figure 1. Treatments applied to grapevine trunks post-remedial surgery; a) hitting with a hammer, b) X cut with tomahawk, c) scratching by wire brush, d) hormone application and e) bud graft.

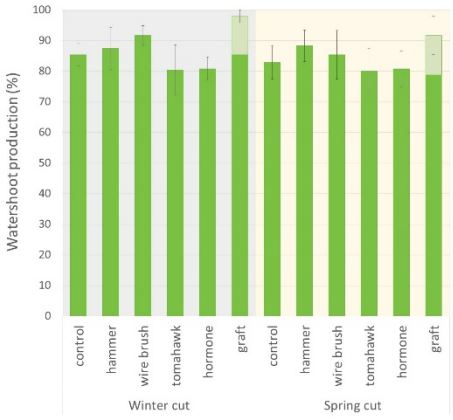


Figure 2. Incidence of watershoots produced following various induction treatments applied after remedial surgery in winter and spring. Grafting conducted in late spring and assessments made in summer. Dark green bars represent watershoots and light green extension bars represent successful grafts where no watershoots were produced.

Results

- Vines were initially assessed pre-surgery with 100% incidence of GTD symptoms
- Cross-sectional cuts of trunks revealed 87% disease staining at the top of the trunk and 28% at the bottom
- Assessment in late summer (mid February 2018) revealed watershoots were readily produced on 80-92% of treated and untreated control trunks
- No significant differences in watershoots were observed between winter and spring remedial cuts
- Watershoots rarely (<18%) originated within the painted white circle, suggesting little influence of most treatments
- No significant differences were observed between treatments, other than grafting, which increased reworking success from 85 to 98% following the winter cut (Fig 2)

Conclusion

This research shows that, of the treatments tested, only grafting significantly improved success of regrowth following remedial surgery. Trials continue to investigate seasonal variability of watershoot production and effectiveness of grafting.

Wine Australia

Acknowledgements

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SOUTH AUSTRALIA

Government of South Australia Primary Industries and Regions SA

SARDI RESEARCH AND DEVELOPMENT

Evaluating spray coverage and fungicide efficacy when using adjuvants for pruning wound protection

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Introduction

Eutypa dieback, which causes significant impacts to vineyards worldwide, can be controlled by applying fungicides to pruning wounds using tractor-driven sprayers, with efficacy correlated to wound coverage. A trial was conducted in 2017 in McLaren Vale, South Australia, to evaluate the effect of using spray adjuvants (wetter and sticker) with fungicide on wound coverage, using water-sensitive papers, fluorescent dye and fungicide efficacy as assessment criteria.

Methods

- Recycle sprayer used to apply two fungicide treatments at 200 L/ha; fluazinam (1 ml/L, 500 g/L a.i; Emblem®) and tebuconazole (0.3 ml/L, 430 g/L a.i; Gelseal™) with or without a wetter (trisiloxane ethoxylate; 0.75 ml/L, 500 g/L a.i; DuWett®) or a sticker (di-1-p-menthene; 0.75 ml/L, 859 g/l a.i; Flextend®) to fresh pruning wounds (Fig 1a&b)
- Spray coverage assessed using water-sensitive papers (Fig 1c), and fluorescent dye added to treatments (Fig 1d-f)
- Wounds were inoculated with *Eutypa lata* ascospores following treatment (Fig 1g)
- Fluorescent dye treated wounds photographed in laboratory under UV light (Fig 1d-f)
- Digital images analysed using Image J software
- After 12 months, inoculated canes and controls were harvested and assessed for pathogen recovery

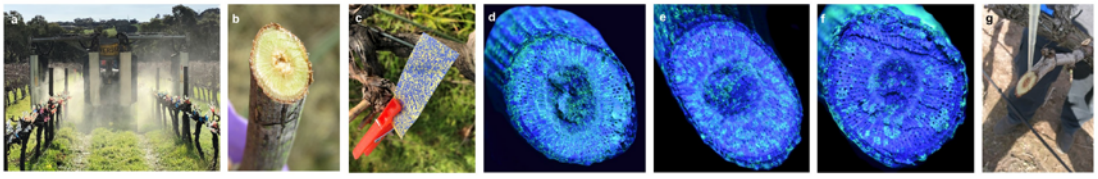


Figure 1. a) Recycle sprayer applying treatment to pruning wounds; b) pruning wound following spray treatment; c) water sensitive paper following spray treatment; d-f) digital images of fluorescent dye on pruning wound under UV light showing differences in spray coverage and g) inoculating wound with *Eutypa lata* ascospores.

Results

- Wound coverage as assessed via fluorescent dye was 19% for the controls and ranged from 21–38% for treatments, and via water-sensitive papers was 48% for the controls and 56–78% for the treatments
- Spray coverage was significantly greater than controls when Gelseal was applied without adjuvant and with a sticker, as well as when Emblem was applied with a wetter
- Although spray coverage assessed by water-sensitive papers and fluorescent dye were different, the trends between treatments were similar (Fig 2)
- *E. lata was* recovered from 79% of inoculated controls and between 58 and 76% for all treatments (Fig 3)
- Gelseal without adjuvant and Emblem with wetter significantly reduced recovery of *E. lata* compared with the control

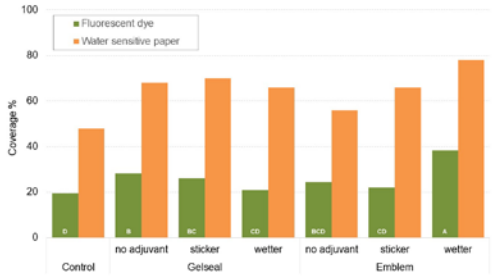


Figure 2. Spray coverage on wounds as indicated by fluorescent dye (green bars) and water-sensitive papers (orange bars). Control wounds were sprayed with fluorescent dye in water. Values within a column with the same letter are not significantly different from one another ($P = 0.05$)

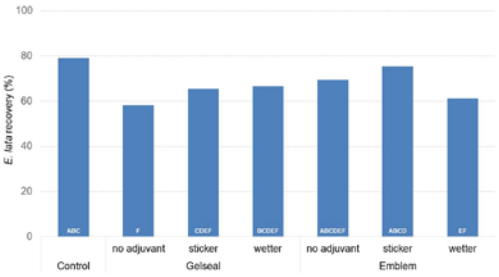


Figure 3. Incidence of *Eutypa lata* recovery from Shiraz canes following harvest, 12 months after pruning and inoculation. Control (positive) wounds were inoculated but received no treatment. Values within a column with the same letter are not significantly different from one another ($P = 0.05$)

Conclusion

The use of fluorescent dye in treatments may be a more accurate indicator of spray coverage on the pruning wound surface than water-sensitive papers. These preliminary data, based on one field experiment, suggest there may be little advantage in using adjuvants when applying fungicides to pruning wounds with tractor-driven sprayers for protection against infection by *E. lata*. The high recovery of *E. lata* from treated wounds may reflect the relatively low spray coverage. The trial was repeated in winter 2018 to confirm the results.

Acknowledgements
This research was funded by Wine Australia. Thanks to Ian Bogisch, Pilar Martinez-Diz, Entesar Abood and Brittany Oswald for technical assistance.

Wine Australia · The University of Adelaide · South Australia · Government of South Australia Primary Industries and Regions SA · SARDI Research and Development

Evaluating natural alternative products for the control of eutypa dieback in grapevine

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Introduction

Eutypa dieback is a major grapevine trunk disease throughout the world, and the most effective means of control is to prevent infection of pruning wounds, particularly using synthetic fungicides, with limited natural alternative products registered for pruning wound protection. Research in Australia is ongoing to evaluate a range of natural products, shown to be effective for control of other grapevine pathogens, for their efficacy in preventing infection of pruning wounds by *Eutypa lata*, the causal agent of eutypa dieback.

Methods

- In 2017 a detached cane assay using single node cv Shiraz canes (Fig 1a) was used to evaluate vanillin (Sigma-Aldrich, USA; 5 and 10 mg/ml), chitosan (low molecular weight, Aldrich, USA; 12.5 and 25 mg/ml), and the yeast-like fungus *Aureobasidium pullulans* (Botector®)
- Tebuconazole at 0.3 ml/L (430g/L a.i; Gelseal™) was used as a synthetic fungicide reference for comparison
- Treated wounds were inoculated with 100 ascospores of *E. lata* (Fig 1b)
- Canes were grown in polystyrene boards in tubs of water for 5 weeks prior to removal to the laboratory for isolation on agar medium (Fig 1c-e)
- The assay was repeated in 2018

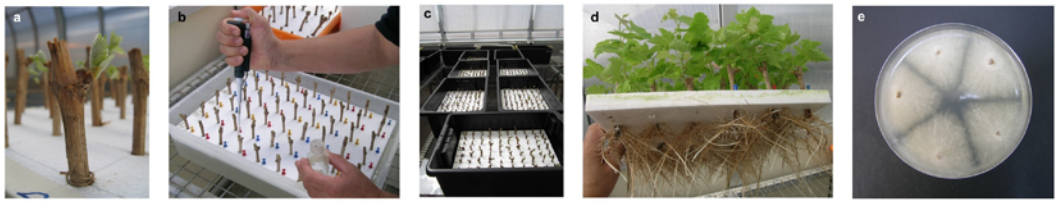


Figure 1. a) Single-node cutting 1 week after establishment; b) Inoculating single node cv Shiraz cuttings with suspension of *Eutypa lata* ascospores following treatment; c) canes grown in polystyrene boards in tubs of water in greenhouse; d) Single-node cuttings 5 weeks after establishment and e) *Eutypa lata* growing on agar following re-isolation from inoculated cane segments.

Results

- *E. lata was* recovered from 46–49% of inoculated controls
- Recovery of *E. lata* from canes was reduced significantly when treated with *A. pullulans* (0%) and chitosan (12–26%), similar to that of tebuconazole (6–9%)
- Vanillin had little effect on recovery of *E. lata*

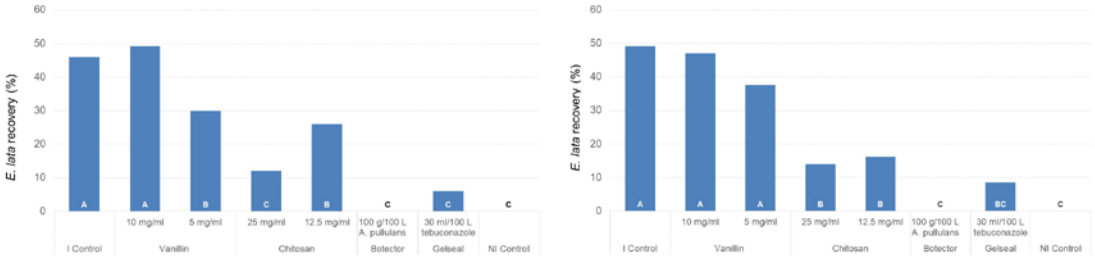


Figure 2. Incidence of *Eutypa lata* recovery from treated single-node Shiraz canes in detached cane assay conducted in 2017 (left) and 2018 (right). I control = inoculated control, NI control = non-inoculated control. Values within a column with the same letter are not significantly different from one another ($P = 0.05$).

Conclusion

The results of these experiments indicate that chitosan and *A. pullulans* (Botector) have potential as pruning wound protectants against infection by trunk disease pathogens. Ongoing research is evaluating these products against *Neofusicoccum luteum* (botryosphaeria dieback). Botector, registered for control of botrytis bunch rot in Australia, is currently being evaluated in vineyard trials to control eutypa and botryosphaeria dieback. 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.

Acknowledgements
This research was funded by Wine Australia.