SARDI
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10th International Workshop on Grapevine Trunk Diseases, Reims, France

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FINAL REPORT to

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1. ABSTRACT

Mr Matthew Ayres attended the 10th International Workshop on Grapevine Trunk Diseases (IWGTD) in Reims, France, with support from a Wine Australia travel bursary and the South Australian Research and Development Institute (SARDI). He visited researchers at the French National Institute for Agricultural Research (INRA) in Bordeaux, France and the Spanish Institute of Grapevine and Wine Sciences (ICVV) in Logrono, Spain, and was invited to attend the "WI**NET**WORK" industry workshop on Grapevine Trunk Diseases and Flavescence Dorée held in Santiago de Compostela, Galicia, Spain. The objectives of the travel were to: i) obtain the latest results of research on grapevine trunk diseases from around the world and apply it to benefit the Australian wine industry, ii) disseminate new knowledge from Australian grapevine trunk disease research, iii) continue the development of professional relationships and provide direct interaction with new researchers and technicians and iv) gain a greater understanding of the latest techniques and methodologies being utilised in the development of control strategies for trunk diseases. Recommendations include expansion of current research programs including spore trapping and pruning wound susceptibility studies in Australian wine growing regions with different climates, increasing the evaluation of rootstock and scion material in the search for resistance to or tolerance of trunk disease and continuing with the evaluation of alternative products for the protection of wounds against GTDs and assess potential products for spray application

2. EXECUTIVE SUMMARY

Mr Matthew Ayres, South Australian Research and Development Institute (SARDI) principle investigator on trunk disease research, was funded by a Wine Australia (WA) travel bursary, and with funding from SARDI, to attend the 10th International Workshop on Grapevine Trunk Diseases (IWGTD) in Reims, France, 3-7 July 2017. Mr Ayres, along with Dr Mark Sosnowski (SARDI), who leads national grapevine trunk disease management programs for SARDI, and Dr Regina Billones Baaijens of the National Wine and Grape Industry Centre (NWGIC), who is co-principle investigator on trunk disease research, presented research results from the WA funded SARDI project; "Practical management of grapevine trunk diseases" (SAR1205) in both oral and poster formats. 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 WA funded grapevine trunk disease research (SAR 1205) to international trunk disease researchers, iii) continue the development of professional relationships and provide direct interaction with new researchers and technicians and iv) broaden Mr Ayres' knowledge of the latest techniques and methodologies being utilised in the development of control strategies for trunk diseases.

Following the workshop, Sosnowski and Ayres visited Dr Patrice Rey at the French National Institute for Agricultural Research (INRA) in Bordeaux, France, where they presented a seminar to researchers and students at INRA providing an overview of SARDI led trunk disease management research. Following this, they visited Dr David Gramaje and his research team at the Spanish Institute of Grapevine and Wine Sciences (ICVV) in Logrono, Spain for collaborative discussions and research activities. They were also invited to attend and present at the "WI**NET**WORK" industry workshop on Grapevine Trunk Diseases and Flavescence Dorée held in Santiago de Compostela, Galicia, Spain.

The workshop attracted 220 delegates from 29 countries. One hundred and fifty abstracts encompassing research updates on botryosphaeria dieback, eutypa dieback and esca amongst other diseases, were delivered in oral presentations and posters. The workshop was divided into five sessions; (i) Pathogen characterisation and identification, (ii) Epidemiology, (iii) Plant pathogen interaction, (iv) Microbial ecology and (v) Disease management in both nursery and vineyard.

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

- Some rootstocks significantly reduce trunk disease symptoms
- Some grafting types appear to significantly affect trunk disease symptoms
- Biological agents (*Bacillus spp.* and *Trichoderma spp.*) may provide similar control of pathogens to synthetic fungicides, although timing of application to wounds may affect the efficacy
- *Trichoderma* can successfully be introduced into propagation material but it remains to be seen if this will provide protection against trunk disease pathogens
- Spore trapping studies are increasingly being conducted around the world, with early results showing vast differences between regions with different climates

- Tractor mounted optical sensors may provide fast, efficient and relatively cheap options for evaluating trunk disease and monitoring vineyard decline
- Poor mother-block practices may be contributing to the prevalence of trunk diseases in nursery material
- Australian native plants may be hosting a wider range of trunk disease pathogens than previously realised
- Some Australian native plants may also contain organisms with potential biocontrol properties
- Australian research is at the forefront of practical management strategies for GTD, leading the international effort in preventative wound protection and remedial control.

Attendance at the 10th IWGTD has provided Mr Ayres with new scientific and technical knowledge which will be incorporated into current and future trunk disease RD&E in Australia. Travel to the French National Institute for Agricultural Research (INRA) and the Spanish Institute of Grapevine and Wine Sciences (ICVV) has allowed Mr Ayres to gain first-hand experience on the latest trunk disease research techniques being used in these leading European research institutions, which will have direct application in both field and laboratory components of current Australian research.

Recommendations include;

- Continue and expand on the evaluation of rootstock and scion material in the search for tolerance to trunk disease
- Evaluate alternative products for protection of wounds against GTDs, timing of application and potential products for spray application
- Assess the effectiveness of unmanned aerial vehicles to quantify and monitor GTDs in vineyards
- Improve our understanding on the thresholds of infection in nursery material that lead to disease in the vineyard
- Examine the role of grafting type on GTDs in the nursery
- Evaluate biological control agents antagonistic to trunk disease pathogens
- Continue and expand on spore trapping and pruning wound susceptibility studies to include other Australian climatic zones.

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 Mr Matthew Ayres to attend the 10th International Workshop on Grapevine Trunk Diseases (IWGTD) in Reims, France 4-7 July, 2017. Every 2 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, of which Dr Mark Sosnowski (SARDI), was the outgoing Chairperson at the conclusion of the workshop. Mr Ayres was a member of the organising committee of the Adelaide workshop where he had the invaluable opportunity to meet with key researchers from around the world and form important collaborative links. At the Reims workshop, Ayres, Sosnowski and Dr Regina Billones Baaijens (NWGIC) presented research results from the WA funded project on "Practical management of grapevine trunk diseases" (SAR1205) in both oral and poster formats.

Following the workshop, Sosnowski and Ayres visited ex SARDI intern and currently winemaker and viticulturist, Ms Jéromine Bailly, in Medoc, near Bordeaux. This was followed by a visit to Dr Patrice Rey and his research team at the French National Institute for Agricultural Research (INRA) in Bordeaux, France and Dr David Gramaje and his research team at the Spanish Institute of Grapevine and Wine Sciences (ICVV) in Logrono, Spain for collaborative discussions and research activities. Ayres and Sosnowski were also invited to attend and present at the "WI**NET**WORK" industry workshop on Grapevine Trunk Diseases and Flavescence Dorée held in Santiago de Compostela, Galicia, Spain.

The objectives of attending the workshop and subsequent visits to key French and Spanish research organisations 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 1205) to international trunk disease research industry members,
iii) Continue the development of professional relationships initiated at the previous workshop and provide direct interaction with new researchers and technicians.
iv) Broaden Mr Ayres' knowledge of the latest international techniques and methodologies.

4. 10TH INTERNATIONAL WORKSHOP ON GRAPEVINE TRUNK DISEASES

The 10th International Workshop on Grapevine Trunk Diseases (IWGTD) was held at the Centre des Congrès, in Reims, France, 4-7 July 2017. It was hosted by the University of Reims Champagne-Ardenne (URCA) and co-organised by the COST Action FA 1303 on behalf of the International Council on Grapevine Trunk Diseases (ICGTD). The workshop attracted 220 delegates from 29 countries (**Figure 1**). One hundred and fifty abstracts were delivered in oral presentations and posters. The workshop was divided into five sessions; (i) Pathogen characterisation and identification, (ii) Epidemiology, (iii) Plant pathogen interaction, (iv) Microbial ecology and (v) Disease management in both nursery and vineyard. The workshop also provided an opportunity for Dr Sosnowski to present a tribute to the late Dr Trevor Wicks, an internationally regarded research scientist, who was instrumental in the promotion of research into trunk disease, and mentored many young scientists in the course of his illustrious 42-year career.

The workshop was an opportunity for Mr Ayres, project leader Dr Mark Sosnowski, and project collaborator, Dr Regina Billones-Baaijens (NWGIC) to present results from recently completed Wine Australia funded research (SAR 1205) on the practical management of GTDs in Australia. Mr Ayres' presentation comprised an oral seminar and a poster (Appendix 2). Following is a summary of information presented by other international speakers that has relevance to current Australian research (SAR 1601) on management of grapevine trunk disease, with reference to how the information could be incorporated into future research for the benefit of the Australian viticulture industry. The information summarised below is from the session focussed on pathogen characterisation and identification and largest workshop session, which was focussed on disease management in nurseries and vineyards, whilst information from sessions focussing on diagnostic tools and epidemiology is presented in the travel report AGT 1614 by project collaborator Dr Regina Billones-Baaijens.



Figure 1. Delegates of the 10th International Workshop on Grapevine Trunk Diseases in Reims, France, 2017.

4.1 Pathogen characterisation and identification

Szabina Lengyel from Eszterházy Károly University, Hungary presented results from a study aimed at determining the causal agents of GTDs in Hungary, of which, relatively little had previously been determined. Samples of grapevine trunks exhibiting GTD symptoms including dieback, lesions and cankers were collected from Hungarian wine regions in 2015. More than 100 symptomatic trunk samples were included, and approximately 700 fungal strains were isolated from them. The isolates were identified by comparing their ITS (internal transcribed spacer), EF (transcription elongation factor) and BT (β-tubulin) sequences with those retrieved from GenBank. Among the isolated strains, *Eutypa lata, Fomitiporia mediterranea, Phaeoacremonium minimum, Phaeomoniella chlamydospora*, as well as some species belonging to *Botryosphaeria* and *Phomopsis* genera were identified. Pathogenicity tests were performed by inoculating young potted grapevines with fifteen representative isolates. The results of the study showed that *Eutypa lata* was the most commonly isolated pathogen (80%) with higher levels of infection in cordon-pruned than umbrella-pruned vines. The most susceptible variety was Sauvignon Blanc, with 24% of 15 year-old vines in one region being affected, followed by Cabernet Sauvignon with

19%. This supports the findings of research conducted in Australia by SARDI scientists where Sauvignon Blanc and Cabernet Sauvignon are often amongst the varieties showing the highest level of GTD symptoms.

Christoffel Spies from ARC Infruitec-Nietvoorbij, and the University of Stellenbosch, South Africa presented findings from a study into *Phaeoacremonium* species diversity and host-range in the Western Cape province of South Africa. The study found 156 previously unreported isolates from 29 hosts in South Africa. The 156 isolates comprised 31 species including thirteen new species and three known species not previously reported in South Africa. Although *Phaeoacremonium* species are found in Australia, they do not currently appear to be having a very severe impact on grapevines with regards to trunk disease. Of relevance to Australia however, is the recovery of *Phaeoacremonium* from species of *Callistemon* in the South African study. *Callistemon* (bottlebrush) are Australian native plant species, some of which are now classified as invasive weeds in South Africa. Future studies could target *Callistemon* species as potential hosts of trunk disease causing fungal species in Australia.

4.2 Epidemiology

Olivier Yobregat from the French Wine and Vine Institute (IFV) presented results from a "study of the contamination kinetics of young plants by fungi responsible for grapevine trunk diseases, from plant material produced free from pathogens." This work examined the contamination of young grafted plants just issued from nurseries and, more broadly, the role of plant material in the spread of pathogens. They compared Sauvignon Blanc on 110 Richter rootstock, obtained by green grafting and containing no pathogenic fungus, with the same material produced by grafting lignified material. Immediately after planting, *Botryosphaeriaceae* species rapidly colonised the plants, and, after two years, the health status of the two grafting types was similar. The fungi were found both on the surface (bark) and inside the woody tissues. The results questioned the efficacy of extensive disinfection in the nurseries, and suggested infection occurred from the external environment. The practice of "green" grafting is worth considering to reduce the spread of disease into the vineyard. As the practice uses young, actively growing plant material, as opposed to calloused and stored material, it may be less likely to become infected with pathogens.

4.3 Plant-pathogen interactions

Maria Pilar Martínez-diz of the Viticultural and Oenological Station of Galicia in Spain, evaluated grapevine germplasm collections from three regions in Spain for resistance to GTD pathogens using potted grapevine cuttings that were wounded with a cork borer and inoculated with a mycelial PDA plug of each fungus. Inoculated cuttings were immediately planted in the field or placed in the greenhouse. Six months later, cuttings were collected and inspected for lesion development. It was found that all cultivars were susceptible to infection by *E. lata*, *N. parvum* and *P. chlamydospora*, but the severity of internal wood discolouration varied significantly, suggesting possible disease tolerance in some accessions. These cutting assays are being repeated, and pathogen isolation will be performed, to confirm whether some cultivars are less susceptible to colonization by fungal trunk pathogens. Ms Martinez-diz will spend 3 months working with the research team at SARDI in winter 2018, and one of her objectives will be to expand on Australian research to screen germplasm against the Esca pathogen, *P. chlamydospora*.

4.4 Trunk disease management in nursery and vineyard

Loris Ouadi of French National Institute for Agricultural Research (INRA) presented results of a study into the monitoring of sap flow in vines affected by Esca disease that showed that when water demand by the vine was at its highest, sap flow density in Esca-symptomatic vines was half that of asymptomatic vines, similar to a trend for stomatal conductance and leaf transpiration rates. It was suggested that these parameters could be useful indicators to assess the health status of a vine before the development of Esca-foliar symptoms. Whether these findings can be translated to Eutypa and Botryosphaeria dieback affected vines would need to be the subject of future research.

Alessandro Zapatta from BASF Italy reported on the lack of effective chemical wound treatments in Europe since the banning of sodium arsenite for use in grapevines. BASF have developed a new product, to be known as "Tesslor" in Europe, which is reported to be highly effective in reducing new

infections of GTDs via pruning wounds by combining physical and chemical activity of components in its formulation. The Physical activity is provided by a polymer that hardens after being sprayed on the wound surface, providing a barrier to the entry of fungal spores. In addition to the physical barrier, the fungicides pyraclostrobin and boscalid are included in the formulation, to provide chemical control of fungal spores. The product is reported to have been tested at a number of European research institutions which confirmed a high level of protection against GTDs. The product will be marketed as a ready to use formulation together with a specially developed application device that allows accurate point-spray applications to wounds at temperatures as low as 3ºC. Although the product cannot be used as a broad scale spray for use in tractor-driven sprayers, if it becomes registered and distributed in Australia in the future, there may be uses for the product, particularly for smaller vineyard operations or for dressing wounds created during vine reworking.

Stefano Di Marco of the Institute of Biometeorology of the National Research Council in Italy presented on the use of electrolysed acid water (EAW) for the control of trunk disease pathogens in the nursery. Infection of propagation material may occur during the grafting process, particularly during hydration, and promising preliminary results have been obtained in the nursery using EAW, characterised by low pH values of around 2.5, high oxidation reduction potential, and some free chlorine, for the hydration of cuttings. EAW appears to have an effect on the germination of conidia that may be situated on the surface of the propagation material, although it had no effect against mycelial growth *in vitro*. The purchase cost of the EAW generation equipment may be prohibitive for nurseries but in Europe it is available to rent, and the cost has been estimated to be 1 Euro cent per cutting. Although there may be limitations to the use of this technology, it may warrant further investigation as a potential tool for Australian grapevine nurseries.

Joachim Eder of the Institute for Plant Protection, State Education and Research Centre DLR, Germany, presented information on trials conducted to assess the application of *Trichoderma* to propagation material immediately after grafting and before waxing, in order to prevent infection by GTD pathogens. Up to 100% colonisation of the grafted plants was achieved and no negative effects of treatment were observed in nursery fields. The trial was established to observe the long term effects of the treatment, therefore future results will determine whether the colonisation of propagation material with *Trichoderma* is an effective method for the protection of young grafted vines against GTDs.

Presentations by Rana Haidar and Awataf Rezgui of INRA, assessed the antagonistic effect of bacteria on GTD pathogens. The *in planta* assays used cuttings or young plants that were inoculated with the bacteria and pathogens, then disease severity was measured as the length of stem necrosis thought to be associated with the pathogen. Research conducted at SARDI has shown that there is no correlation between length of necrosis and progression of the pathogen, as the pathogen is often found at varying distances ahead of the necrosis. Discussions in question time impressed the importance of reisolating pathogens from vines, rather than just relying on staining of wood.

Eirian Jones of Lincoln University, New Zealand, presented on the biological control of the Botryosphaeriaceae using endophytes from the native Tea Tree, *Leptospermum scoparium*, which is known for its antimicrobial properties. They found that 17% of 230 cultured endophytic bacteria from *L. scoparium* inhibited the *in vitro* growth of *Neofusicoccum luteum*. Of these, ten strains showed broad inhibition of multiple species within the Botryosphaeriaceae by 20-65%. One strain, *Pseudomonas* sp. I2R21, was successfully transmitted to 1-year-old Sauvignon Blanc canes where it colonised the wound site and decreased lesion length and *N. luteum* colonisation of tissue beyond the lesion by approximately 40%. Their work showed that endophytes with biocontrol activity reside as a natural resource in *L. scoparium* and can provide biocontrol activity. The results of this work suggest that Australian *Leptospermum* species, also known for their antimicrobial properties, may also host endophytes with biocontrol potential, which could be the subject of future research.

Mauricio Lolas from the Universidad de Talca, Chile discussed the results of a study where chemical and biological protection sprays and pastes were evaluated on pruning wounds of cvs. Cabernet Sauvignon and Sauvignon Blanc, for their efficacy to control *P. chlamydospora*, one of the most important causal agents of trunk disease in Chile. Treatments included chemicals: pyraclostrobin (Comet, BASF), fluazinam (Shirlan, Syngenta), tebuconazole (Apolo, Anasac) and fluopyram + tebuconazole (Luna Experience, Bayer) applied as sprays; pyraclostrobin (Podexal, BASF) and tebuconazole + kresoxim methyl (Pasta Poda Full, Anasac) applied as pastes; and biologicals: *Trichoderma virens* + *Bacillus licheniformis* + *Bionectria ochroleuca* (Coraza, Bionsumos Nativa) applied as paste; *T. virens* + *T. gamsii* + *B. ochroleuca* (Mamull, Bionsumos Nativa) and *T. virens* native strain (Universidad de Talca) applied as sprays.. Wounds were inoculated a day after treatments were applied using mycelial plugs colonised with *P. chlamydospora*. After 4 months, the length of the necrotic lesion

caused by the pathogen was measured and the results showed that all treatments in both cultivars significantly reduced the length of the necrotic lesion compared with the control treatment. Although only the necrotic lesion was used as a measure of the control of the pathogen, without assessment of the tissue beyond the necrotic staining for the presence of the pathogen, products that have been shown to be effective in controlling GTDs in Australia, including tebuconazole, pyraclostrobin and fluazinam, also proved effective in studies in Chile. The trials are being repeated using fungal conidia, instead of the mycelium plug and it was suggested that they may wish to consider also assessing recovery of the pathogen as well as necrosis length to confirm control. The results also showed that the biological agents provided a similar reduction in lesion length to the synthetic fungicides. Similar biological agents (*Bacillus spp.* and *Trichoderma spp.*) have been, or are currently being assessed in Australian trials and it remains to be seen if they can provide control similar to the synthetic fungicides.

Mark Sosnowski from SARDI, presented results from trials conducted in New Zealand that used detached cane assays and field trials to test a range of fungicides for their efficacy in reducing infection of pruning wounds by Eutypa and Botryosphaeria dieback pathogens. He also presented results of field trials on spur and cane-pruned vines that assessed a number of commercial sprayers for their ability to control infection of pruning wounds by *E. lata* and *N. luteum* using carbendazim. These trials confirmed previous research in Australia (SAR 1001) that commercial sprayers can achieve good wound coverage to provide effective control of GTDs when applying registered fungicides. Future research in NZ will evaluate the curative and preventative properties of fungicides, and the duration of susceptibility to infection of pruning wounds, also expanding on Australian research (SAR1205). Spore trapping studies are also being expanded to New Zealand, and it will be interesting to see whether the spore dispersal patterns differ from those in Australian regions.

Sosnowski also presented results from an economic study, based on an extensive survey undertaken on Sauvignon Blanc vineyards in New Zealand. Results showed that early adoption of preventative wound treatments in vineyards will minimise the cost of trunk disease and provide a net present value (NPV) future benefit over the life of a vineyard. Application of pruning wound treatments with a tractor-driven sprayer will further minimise costs compared with hand-application, and ensuring good coverage will maximise efficacy and hence the NPV future benefit. If preventative treatment does not commence until after trunk disease is evident, there will be costs for future crop loss and remediating or replacing vines. The cost of remediating vines by reworking or regrafting will be less than the cost of replacing vines. The sooner the remediation treatment is commenced, the greater the NPV future benefit. It was concluded that the potential “national” value of an effective annual spray treatment to the New Zealand industry is estimated to be at least NZ$20m per annum, increasing by a further NZ$20m per annum when combined with remediation treatments. This information is highly relevant to the Australian wine industry and highlights the importance of early adoption of GTD management.

Furthermore, Sosnowski presented results from Australian trials that assessed the effectiveness of using tractor mounted optical sensors (Greenseeker®) in determining grapevine canopy decline due to eutypa dieback. Comparing visual assessments of two different personnel, there was no significant difference. There was a strong correlation between visual assessments and the Greenseeker data, with R2 value of 0.73 when data from both vineyards were combined. Sosnowski recommends that further research be conducted to evaluate its effectiveness in different environments. The use of tractor-mounted devices such as Greenseeker, as well as unmanned aerial vehicles, may provide fast, efficient and relatively cheap options for evaluating productivity loss, and to monitor vineyard decline.

Laura Martín of the Centre of Wine Technology (VITEC), Spain presented a poster showing results from detached cane assays (DCA), based on methods developed by SARDI (SAR1001), where 10 products were assessed for efficiency as pruning wound protectants. Natural formulations, fungicides and biological control agents were included in the assay. Each product was tested against three GTD pathogen species to determine if they could prevent mycelial colonization of pruning wounds. After two months of incubation, re-isolation of both pathogen and biological agents was performed, and the percentage and length of vascular discoloration estimated. Protection with *Trichoderma spp.* resulted in failure to recover *Phaemoniella chlamydospora* and reduced re-isolation of *Phaeoacremonium minimum* and *Diplodia seriata*. Chitosan and copper oxychloride decreased the infection of *D. seriata* and *Phaeoacremonium minimum*, respectively.

José Ramón Úrbez Torres of Agriculture Canada also presented results of a DCA evaluation of chemical and biological pruning wound protectants against grapevine trunk diseases. Eight chemicals and four biological products were screened in the greenhouse against 10 GTD pathogens. Several triazole and thiophanates were able to achieve a high level of control. Mean percent disease control (MPDC) of chemical products decreased when pruning wounds were challenged with pathogens either 7 or 21 days

after treatment, compared with 1 day. Conversely, MPDC increased after 21 days when pruning wounds were treated with a *Trichoderma*-based biological product, suggesting longer term protection than with chemicals. In personal discussions at the workshop, Úrbez Torres indicated that he encountered difficulties when trying to assess the effect of the *Trichoderma*-based product against *E. lata* as *Trichoderma* is a very fast growing organism on agar plates and overgrows the pathogen. Botryosphaeria dieback pathogens are much faster growing on agar than *E. lata*, so are able to compete with *Trichoderma* on agar, making assessment easier. In recent DCAs conducted at SARDI (SAR 1601), similar problems have been encountered. The trials are currently being repeated with Botryosphaeria dieback pathogens. Meanwhile, field trials using *Trichoderma* against GTD pathogens introduced at different times after pruning have been established in South Australia, with the hope of reducing issues experienced in DCAs.

The use of “alternative” products for the control of GTDs has been the subject of on-going research in Wine Australia funded research programs (SAR 1001 and SAR 1205) with mixed results. Martín found chitosan to be effective in reducing wound infection by *D. seriata*, one of the most virulent GTD pathogens in Australia. Current Australian research (SAR 1601) will assess, chitosan as a pruning wound protectant against pathogens including *D. seriata* and *E. lata*, The detached cane assay, developed at SARDI, is being adopted in research throughout the world and is proving to be a useful, high-throughput *in vivo* method for screening products for their ability to prevent infection of pruning wounds.

Severine Mary from Université de Bordeaux, France presented results from a 4-year experiment on Cabernet Sauvignon grafted onto four different rootstocks (Gravesac, Millardet 101-14, 3309 Couderc, Riparia Gloire), showing varying levels of Esca symptom development. They also investigated the role of three grafting methods: Omega, Whip and Tongue and Full cleft graft on Esca foliar symptom expression on cvs Cabernet-Sauvignon and Mourvèdre A higher incidence of Esca was observed on the Whip and Tongue grafted vines. It was concluded that that the spread of infection during mechanical grafting may be one of the factors explaining the increase of the incidence of Esca in vineyards. Preliminary SARDI research has shown that there may be effects of rootstock and clone on expression of GTD symptoms. Current research (SAR1601) is further examining this with visual assessments of historical rootstock and clonal trials, but further scientific evaluation will be required to confirm any possible resistant or tolerant germplasm. The effect of grafting on GTD in an Australian context may also be worth addressing in future.

4.5 Field trip to Champagne.

On Thursday July 6th, workshop delegates were taken on a field tour of Champagne. The Champagne region spans 34,000 ha with 280,000 individual vineyard plots, tended by 15,000 growers and supplying 300 Champagne Houses. In 2015, 312 million bottles were produced with a turn-over of 4.7 billion euros. Currently, the main focus of the region is sustainable development; controlling inputs and their potential impact on health and the environment; the preservation of terroirs, biodiversity and landscapes; along with accountable management of water, wastewater, by-products and waste; and the need to address the energy and climate challenges. The Interprofessional Committee on Champagne, hosted workshop delegates at their main research vineyard near Epernay. They showcased research into the breeding of new cultivars for possible future use in the Champagne region, the impact of various trellising and vine spacing techniques on yield and disease management, including GTDs, and the importance of "terroir" on varietal expression (**Figure 2**). This was followed by a tour of the Mercier cellars to learn about the unique process of Champagne production concluding with a guided tasting.



Figure 2. a) presentation by experimental vineyard manager on the importance of terroir in the Champagne region; b) workshop delegates touring the vineyard; d) discussion of different trellising techniques and e) immature fruit on a newly developed cultivar.

5. VISIT TO MEDOC, FRANCE

Following the workshop, Ayres and Sosnowski visited Ms Jéromine Bailly in Médoc, near Bordeaux. Ms Bailly was a French Masters student who undertook an internship with Dr Sosnowski at SARDI in 2015. She is now viticulturist and winemaker at Chateau Nouret in Médoc, France. A visit with Ms Bailly provided the opportunity for Sosnowski and Ayres to experience grape and wine production on this unique region in France, located on a peninsula between the Gironde Estuary and the Atlantic Ocean.

The Médoc region contains approximately 5,700 hectares of vineyards, producing 300,000 hectolitres of wine per annum. The soils are Garonne gravel, Pyrenees gravel and clayey limestone with extreme variation in character. Vineyards are less densely packed than further south in Bordeaux. The main grape varieties are Cabernet Sauvignon and Merlot, and to a lesser extent Petit Verdot and Malbec, Cabernet Franc and Carménère are also grown in the region. No white varieties are grown in Médoc.

Of the trunk diseases, Esca is the most prominent in this region and one of the most serious diseases in much of Europe and can lead to considerable crop loss. Esca is caused by a complex of fungi that includes several species of *Phaeoacremonium*, and *Phaeomoniella chlamydospora.* Foliar symptoms (apoplexy and typical tiger-striped leaf symptoms; **Figure 3** a-b) were most visible in vineyards, with cankers also observed regularly on mature vines (**Figure 3** c). Also observed in a vineyard was a suspected symptom of leafroll virus (**Figure 3** d).

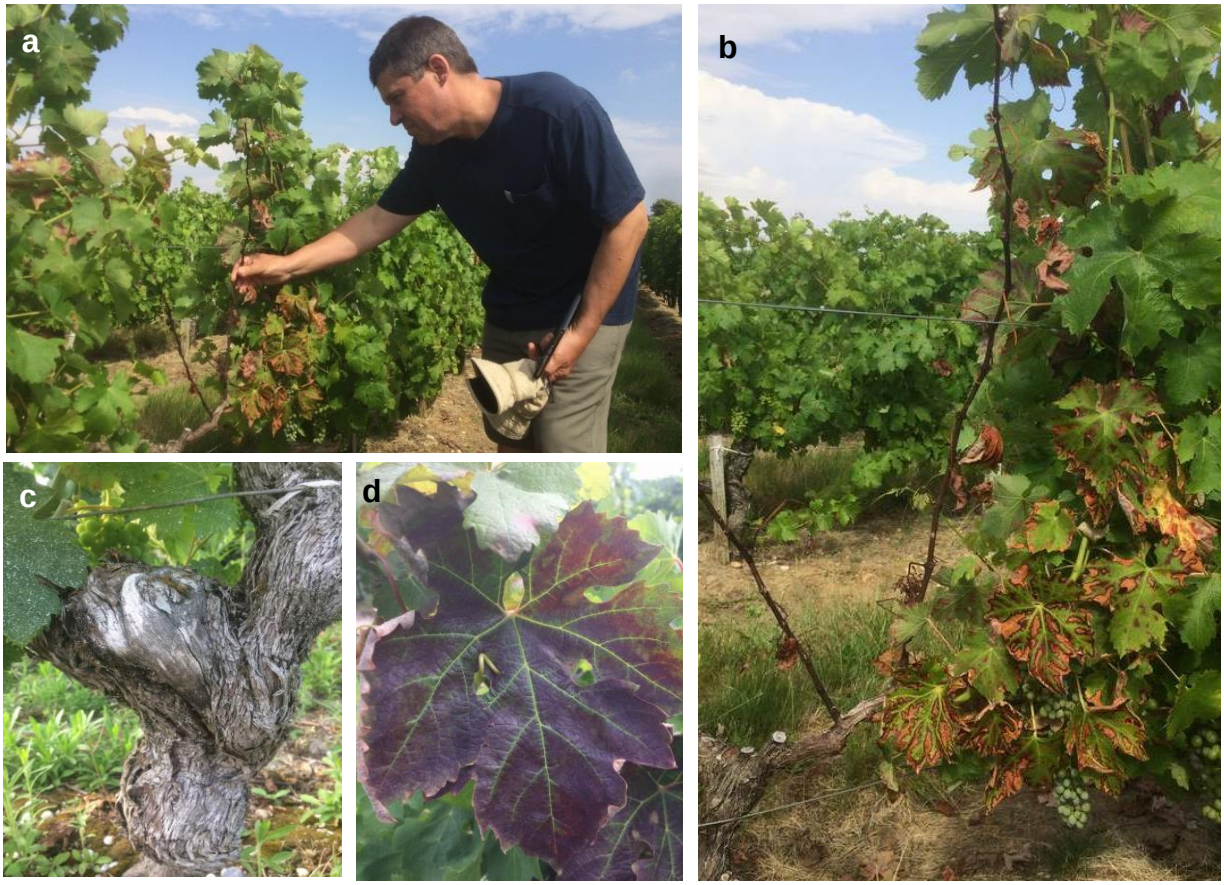


Figure 3. a) Mr Ayres inspecting Esca symptoms in Médoc, b) tiger-stripe symptoms and apoplexy caused by Esca, c) trunk cankers on mature Cabernet Sauvignon vines and d) suspected symptom of leafroll virus.

6. FRENCH NATIONAL INSTITUTE FOR AGRICULTURAL RESEARCH (INRA) IN BORDEAUX, FRANCE.

A visit was made to the French National Institute for Agricultural Research (INRA) in Bordeaux, where Sosnowski and Ayres met with Dr Patrice Rey. Dr Rey is leading a European Union Horizon 2020 proposal, on which Dr Sosnowski has been invited to join the advisory board. Demonstration trials will be established around the world, with one each planned for Australia and New Zealand, to which Dr Sosnowski will likely play a greater role. The project aims to develop and validate practical strategies for a wide range of environments. Involvement in this large project will ensure the Australian wine industry remains abreast of the latest research being undertaken in Europe.

Sosnowski and Ayres presented a seminar to researchers and students at INRA providing an overview of SARDI led research trunk disease management research program. This prompted discussions on research in both countries, particularly with view to opportunities of closer collaboration between the groups.

7. SPANISH INSTITUTE OF GRAPEVINE AND WINE SCIENCES (ICVV) LOGRONO, SPAIN

Ayres and Sosnowski visited Dr David Gramaje, who leads a major research program on control of grapevine trunk pathogens, and his research team at the Spanish Institute of Grapevine and Wine Sciences (ICVV) in Logrono, Spain for collaborative discussions and research activities (**Figure 4**). Dr Sosnowski, Dr Gramaje and Dr Jose Urbez Torres (Agriculture and Agri-Food Canada), a highly regarded scientist internationally, who leads a program at the Pacific Agri-Food Research Centre in Summerland British Columbia, developing molecular approaches for rapid and reliable diagnostic methods for grapevine trunk pathogens, used the visit to progress a collaborative research project. The project, which is linked with objectives of Wine Australia project SAR1601, is focussing on determining thresholds of trunk pathogen infection in grapevine propagation material. The outcomes of the collaborative research will be used 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.

Mr Ayres had the opportunity to interact with research staff and to see first-hand the techniques and protocols being used and which will be adopted in the SARDI led research program. Mr Ayres was shown the research station vineyards and orchards, trials being conducted in greenhouses investigating the effect of water deficit on grapevine trunk diseases and laboratory methods including non-destructive techniques of pathogen recovery from diseased grapevine wood.

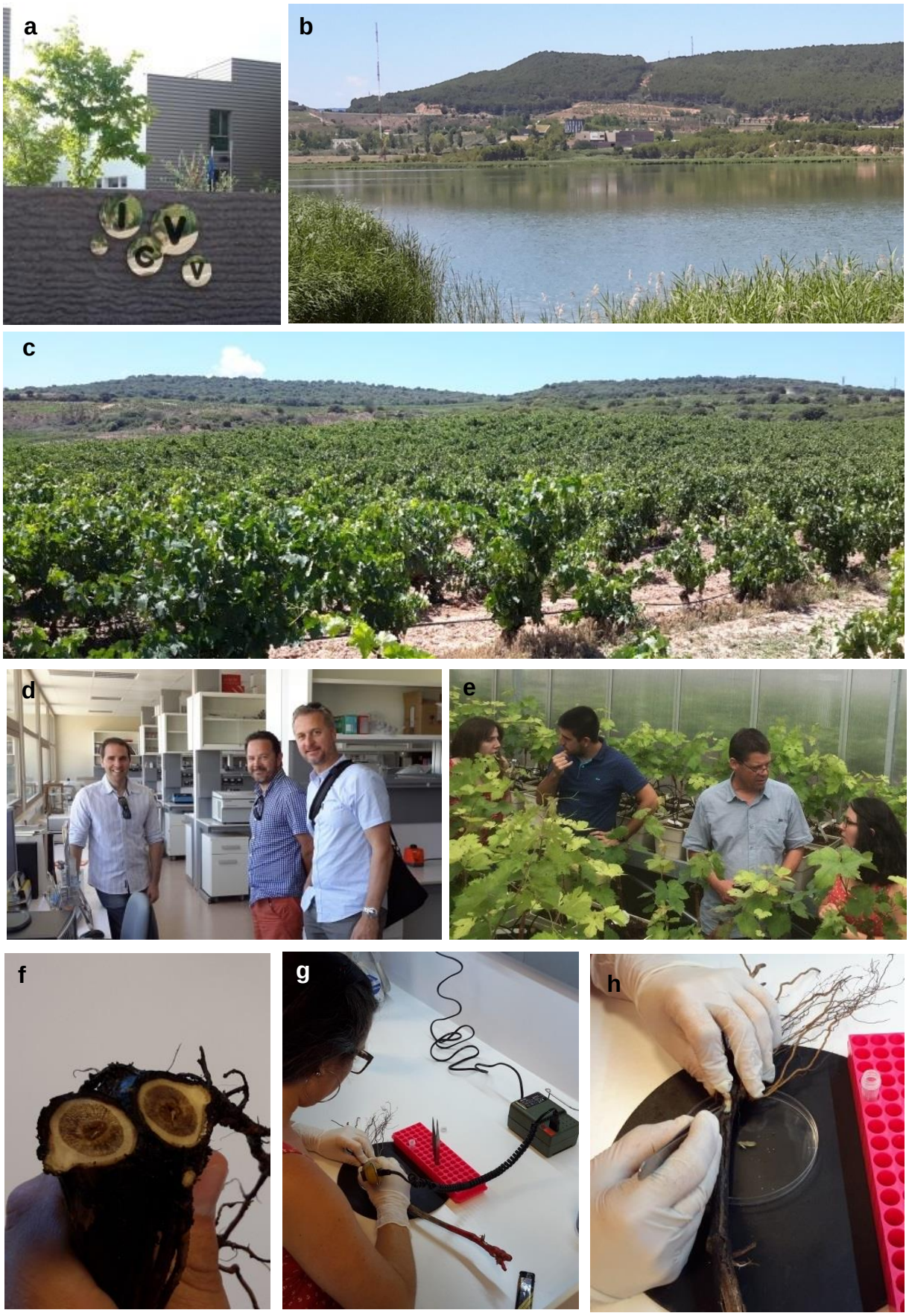


Figure 4 (a & b) Institute of Grapevine and Wine Sciences (ICVV) in Logrono, Spain; (c) experimental vineyard; (d) Drs Gramaje, Urbez-Torres and Sosnowski in Dr Gramaje's research laboratory; (e) Mr Ayres with research staff in the experimental greenhouses; (f) young grapevine showing internal trunk disease symptoms and (g & h) extracting samples from diseased grapevine stem for analysis.

8. VISIT TO DEMENCIA WINES, VILLAFRANCA DEL BIERZO, SPAIN

A visit with Mr Nacho León at Demencia Wines provided insight into a progressive way of making wine in Spain. Mr León is a winemaker and viticulturist who acquired 3 ha of 80-100 year-old vines of the cv. Mencia (**Figure 5**). He is revitalising the vineyard which has significant levels of trunk disease, in particular Esca. Vines are being remediated by cutting out dead arms and retraining low sucker shoots. He is using a sustainable program of management, including reduction of the carbon footprint, and is progressing to a fully organic vineyard with no mechanisation. The wine making process is based on natural yeast fermentation and a tasting revealed the depth and complexity of fruit from the historical vineyard.



Figure 5. a & b) visit to 80-100 year-old vineyard of the local variety Mencia with Mr Nacho León and c) old vine with trunk disease canker.

9. "WINETWORK" INDUSTRY WORKSHOP ON GRAPEVINE TRUNK DISEASES AND FLAVESCENCE DORÉE, SANTIAGO DE COMPOSTELA, GALICIA, SPAIN

Dr Sosnowski and Mr Ayres were also invited to attend and present at the "WINETWORK" industry workshop on Grapevine Trunk Diseases and Flavescence Dorée held in Santiago de Compostela, Galicia, Spain. The workshop brought together 63 growers and industry personnel to hear the latest European and international knowledge on trunk disease management. From University of Reims in France, Dr Florence Fontaine provided an overview of European research, focussing on metabolic studies being undertaken in France. David Gramaje, from Rioja presented on Spanish research, with a focus on nursery propagation and germplasm evaluation for resistance. Dr Jose Ramon Úrbez Torres provided an overview of Californian and Canadian research, with a focus on development and use of sensitive and rapid molecular diagnostic tools. Sosnowski and Ayres presented an overview of SARDI research in Australia and New Zealand, with a focus on practical strategies of wound protection and remedial surgery. Sosnowski and Ayres were also included on an expert panel where workshop participants asked questions pertaining to management of grapevine trunk diseases.

At the meeting, discussion with Ms María del Pilar Martínez Diz from Estación de Viticultura e Enoloxía de Galicia (EVEGA-INGACAL) was had about her upcoming visit to SARDI in winter 2018, when she will conduct some studies as part of her PhD research. Ms Martinez will also assist the SARDI research team in establishing field trials in South Australia and New Zealand.

Following the workshop, Sosnowski and Ayres were invited to attend a field trip to Martin Codax vineyards where they joined a discussion on management issues relevant to the area, were shown various experimental trellising techniques and witnessed a range of symptoms of the Esca disease complex (**Figure 6**). In the vineyards of Martin Códax, there has been significant evaluation of different trellis systems. Traditionally Albariño has been grown using an 'Emperrado' trellis system. This system is difficult to adapt to mechanisation, compared with modern vertical trellis systems. However, the evaluations have revealed that the 'Emperrado' system produces superior quality fruit, mainly due to the high exposure of leaves to sunlight. This region, south of Santiago de Compostela, has an annual rainfall of 1600 mm, leading to high pressure for foliar diseases, particularly downy mildew. An average of 12 sprays need to be applied annually, but thanks to a network of meteorological stations, a disease alert system is in place throughout the region allowing less applications in low disease pressure seasons. Esca was evident in the vineyards with a remedial surgery program in place to rejuvenate infected vines, with reports of success with this management program.



Figure 6. **a) vines using a double trellis system, b) Emperrado trellis system, c) Esca foliar symptoms on a white grape variety, d) Esca foliar symptoms on a red grape variety in Medoc, France, e) shrivelled fruit caused by Esca and f) the sudden dieback or apoplexy of an entire shoot caused by Esca.**

10. MAIN OUTCOMES

10.1 Knowledge

Attendance at the 10th International Workshop on Grapevine Trunk Diseases provided the Mr Ayres 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, which can be applied here to benefit the Australian wine industry.

The direct interaction with key researchers and technicians conducting grapevine trunk disease research will allow for new techniques and protocols to be incorporated into current and future Australian GTD research programs.

10.2 Professional links

At the workshop, Sosnowski and Ayres met with Spanish PhD student Ms María del Pilar Martínez Diz from Estación de Viticultura e Enoloxía de Galicia (EVEGA-INGACAL). She will spend 3 months at SARDI from June until August 2018, undertaking experiments complimentary to her PhD and the SARDI trunk disease program, including the establishment of pruning wound protection trials and germplasm evaluation for disease tolerance. Past study visits with the grapevine trunk disease program at SARDI by international students has led to 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. The post-workshop visits provided an opportunity to establish professional relationships with researchers at the French National Institute for Agricultural Research (INRA) in Bordeaux, and strengthen existing ties with researchers at the Spanish Institute of Grapevine and Wine Sciences (ICVV) in Logrono, Spain.

10.3 Communication

An article will be published in collaboration with Dr Regina Billones-Baaijens, for submission to an Australian industry magazine to report 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 SAR1601 project team.

11. 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 and expand on the evaluation of rootstock and scion material in the search for resistance to or tolerance of trunk disease
- Evaluate alternative products for protection of wounds against GTDs, the timing of application and assess potential products for spray application
- Assess the effectiveness of unmanned aerial vehicles to quantify and monitor GTDs in vineyards
- Examine the implication of grafting on GTDs in the nursery
- Evaluate biological control agents antagonistic to trunk disease pathogens Continue spore trapping and pruning wound susceptibility studies and expand to include other Australian climatic zones.

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.

12. ACKNOWLEDGEMENTS

Thanks to Wine Australia for their generous support in providing funds for Mr Ayres to undertake this travel and for the hospitality provided by hosts in France, Ms Jéromine Bailly, and Spain, Dr David Gramaje, and Ms María Jesús Fanjul Alonso of WiNETwork and **Instituto Galego da Calidade Alimentaria (INGACAL)**.

13. APPENDICES

Appendix 1. Travel Itinerary

July	**Activities**
Mon 3 – Fri 7	10th International Workshop on Grapevine Trunk Diseases, Reims, France
Sat 8 – Sun 9	Visit with Jeromine Bailly, Chateau Nouret, Medoc, France
Mon 10	Visit with Dr Patrice Rey, French National Institute for Agricultural Research (INRA), Bordeaux, France
Tue 11	Visit with Dr David Gramaje at the Instituto de Ciencias de la Vid y del Vino (ICVV), Logrono, Spain
Wed 12	Visit with Nacho León at Demencia Wines, Villafranca del Bierzo, Spain
Thu 13 – Fri 14	"WI**NET**WORK" Industry Workshop, Santiago de Compostela, Spain

Appendix 2. Abstracts

10th International Workshop for Grapevine Trunk Diseases

Oral presentation

Critical timing of application of fungicides for the prevention of wound infection by *Eutypa lata* and *Neofusicoccum luteum.*

MATTHEW R. AYRES[1], REGINA BILLONES-BAAIJENS[2], SANDRA SAVOCCHIA[2], EILEEN S. SCOTT[3] and MARK R. SOSNOWSKI[1,3] .*[1]South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001. [2]National Wine and Grape Industry Centre, Charles Sturt University, School of Agricultural and Wine Sciences, Locked Bag 588, Wagga Wagga NSW 2678. [3]School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064.*

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The grapevine trunk diseases eutypa and botryosphaeria dieback are caused by fungal pathogens that infect pruning wounds, leading to vine decline and eventual vine death. Research has shown that fungicides applied to grapevine pruning wounds can prevent infection by trunk disease pathogens, but in order to maximise the efficiency of pruning wound protection, there is a need to determine the critical timing of fungicide application following pruning. Two field trials were conducted each in South Australia (cv. Shiraz) and New South Wales (cv Cabernet Sauvignon) between 2013 and 2015. Three fungicides: tebuconazole (Folicur), pyraclostrobin (Cabrio) and fluazinam (Emblem) were applied to fresh grapevine pruning wounds made in winter 1, 6 or 14 days prior to inoculation with *Eutypa lata* (South Australia) or *Neofusicoccum luteum* (New South Wales), to assess their ability to prevent infection. The same fungicides were also applied 1, 3 and 6 days after inoculation with the trunk disease pathogens to assess their curative effect. In the following winter of each trial, the treated canes were harvested, and assessment of colonisation of the canes by the pathogens was conducted in the laboratory by isolation on agar media. All three fungicides provided preventative control of *E. lata* for at least 1 week and *N. luteum* for up to 2 weeks after pruning and application. The fungicides also exhibited curative properties by reducing infection by both pathogens when applied up to 6 days after pruning and inoculation. Therefore, if applied 1 week after pruning, a single fungicide application could provide up to 3 weeks of wound protection, thereby providing improved trunk-disease management options for growers.

Poster presentation

Susceptibility of grapevine pruning wounds to the trunk disease pathogens *Eutypa lata, Diplodia seriata* and *Neofusicoccum luteum.*

MATTHEW R. AYRES[1], REGINA BILLONES-BAAIJENS[2], SANDRA SAVOCCHIA[2], EILEEN S. SCOTT[3] and MARK R. SOSNOWSKI[1,3]. *[1]South Australian Research and Development Institute, GPO Box 397, Adelaide SA 5001. [2]National Wine and Grape Industry Centre, Charles Sturt University, School of Agricultural and Wine Sciences, Locked Bag 588, Wagga Wagga NSW 2678. [3]School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Glen Osmond SA 5064.*

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Eutypa and botryosphaeria dieback, caused by fungal pathogens that infect grapevine pruning wounds, pose a serious threat to wine-grape production throughout the world. Limited information exists on the duration of susceptibility of wounds to infection at different times during the pruning season. Field trials were conducted in South Australia (cv Shiraz) and New South Wales (cv Cabernet Sauvignon) between 2013 and 2015 to evaluate grapevine wound susceptibility following early (June), mid (July) and late (Aug) winter pruning, by inoculating with spores of *Eutypa lata*, *Diplodia seriata or Neofusicoccum luteum* up to 16 weeks after each pruning time. The following winter of each trial, canes were assessed for infection by isolation on agar media. Wounds were generally more susceptible to infection by *E. lata* when pruned early in June compared with the later pruning times, and were most susceptible to infection for 2 weeks regardless of pruning time, after which susceptibility decreased sharply. For *D. seriata*, there was no difference between pruning times but susceptibility was extremely high in the first 2 weeks compared with the other pathogens, followed by a steady decline thereafter. With *N. luteum*, susceptibility decreased at the late pruning time, and the most susceptible period was the first week, with a sharp decline in the following weeks. Detached cane assays using *E. lata* and *N. luteum* were also established in the greenhouse to compare wound susceptibility between six cultivars: Shiraz, Chardonnay, Cabernet Sauvignon, Merlot, Sauvignon Blanc and Semillon, and revealed no difference following infection by both pathogens 1, 7 and 14 days after pruning. This study highlights the importance of protecting pruning wounds, regardless of the pruning time or grapevine cultivar.

Susceptibility of grapevine pruning wounds to the trunk disease pathogens *Eutypa lata, Diplodia seriata and Neofusicoccum luteum*

M. R. Ayres[1], R. Billones-Baaijens[2], S. Savocchia[2], E. S. Scott[3] and M. R. Sosnowski[1,3]

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Introduction

Grapevine trunk diseases, caused by fungal pathogens that infect pruning wounds, affect vine health and longevity. Trials were conducted in McLaren Vale and Wagga Wagga, Australia, to evaluate the duration of pruning wound susceptibility throughout the pruning season, and to compare commonly planted cultivars in terms of wound susceptibility.

Field trials

Methods

- Vines were pruned early June, mid July and late August (Fig 1)
- Wounds were inoculated with *Eutypa lata* (eutypa dieback – McLaren Vale) or *Diplodia seriata* or *Neofusicoccum luteum* (botryosphaeria dieback – Wagga Wagga) spores at intervals up to 16 weeks after pruning
- After 12 months, canes were harvested and pathogens re-isolated

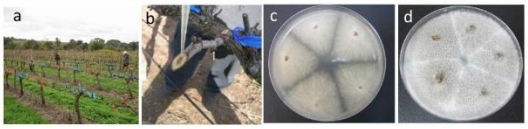


Figure 1. Field trial; a) pruning; b) inoculating pruning wounds; c) *E. lata* isolated from canes on agar medium; d) *D. seriata* isolated from canes on agar medium.

Results

- Regardless of pruning time, wounds were highly susceptible to infection by all pathogens for first 2 weeks after pruning (Figs 2, 3 & 4)
- Inoculation 4 or more weeks after pruning led to lower incidence of infection, particularly by *E. lata* and *N. luteum*

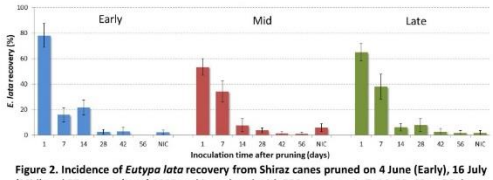


Figure 2. Incidence of *Eutypa lata* recovery from Shiraz canes pruned on 4 June (Early), 16 July (Mid) and 27 August (Late) 2013 and inoculated with 500 spores at 1, 7, 14, 28, 42 or 56 days after pruning. NIC = non-inoculated control. Bars = standard error of the mean.

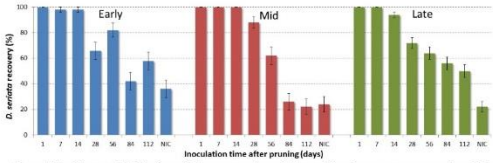


Figure 3. Incidence of *Diplodia seriata* recovery from Cabernet Sauvignon canes pruned on 12 June (Early), 22 July (Mid) and 22 August (Late) 2014 and inoculated with 1000 spores at 1, 7, 14, 28, 42, 56, 84 or 112 days after pruning. NIC = non-inoculated control. Bars = standard error of the mean.

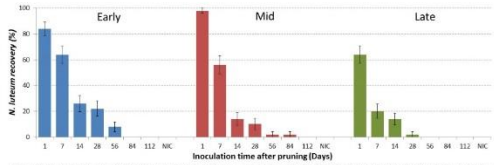


Figure 4. Incidence of *Neofusicoccum luteum* recovery from Cabernet Sauvignon canes pruned on 19 June (Early), 20 July (Mid) and 17 August (Late) 2014 and inoculated with 1000 spores at 1, 7, 14, 28, 42, 56, 84 or 112 days after pruning. NIC = non-inoculated control. Bars = standard error of the mean.

Detached cane assays

Methods

- In the greenhouse, single node cuttings of cvs Shiraz, Cabernet Sauvignon, Merlot, Chardonnay, Sauvignon Blanc and Semillon were inoculated with *E. lata* or *N. luteum* at intervals up to 14 days after pruning (Fig 5)
- After 4 weeks, canes were harvested and pathogens re-isolated



Figure 5. Detached cane assay; a) inoculating cuttings inserted into polystyrene boards floating on water; b) single node cuttings; c) *N. luteum* isolated from cane segments on agar medium.

Results

- All cultivars were susceptible to infection by both pathogens
- For all cultivars, susceptibility decreased with time of inoculation after pruning (Figs 6 & 7)
- There was no difference between cultivars

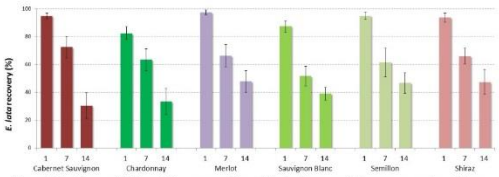


Figure 6. Incidence of *Eutypa lata* recovery from detached canes which were pruned and then inoculated with 500 spores at 1, 7 or 14 days after pruning. Bars = standard error of the mean.

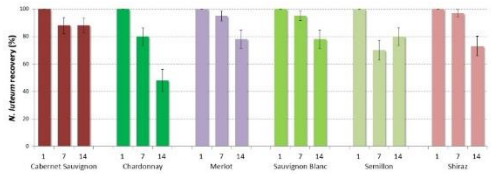


Figure 7. Incidence of *Neofusicoccum luteum* recovery from detached canes which were pruned and then inoculated with 500 spores at 1, 7 or 14 days after pruning. Bars = standard error of the mean.

Conclusion

Grapevine pruning wounds were susceptible to infection by trunk disease pathogens, regardless of the pruning time, particularly for the first 2 – 4 weeks. Duration of susceptibility was less for Shiraz inoculated with *E. lata*, and Cabernet Sauvignon inoculated with *N. luteum* than for Cabernet Sauvignon inoculated with *D. seriata*. All the cultivars commonly grown in Australia assessed were susceptible to infection. Field trials are being repeated, and future research will evaluate wound susceptibility in other regions of Australia. Results will provide decision support for managing grapevine trunk diseases.

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

Acknowledgements

This research was funded by Wine Australia. Thanks to Cathy Todd, Ian Bogisch, Ian Riley, Lee Bartlett and Georgina Elena for technical assistance.