SARDI

SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Government of South Australia
Primary Industries and Resources SA

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Grape and Wine Research and Development Corporation

# 7th International Workshop for Grapevine Trunk Diseases, Chile



## FINAL REPORT to

**GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION**

Project Number: **GWT 09/02**

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# 1 ABSTRACT

With funding from GWRDC and SARDI, Dr Mark Sosnowski traveled to Chile to attend the 7th International Workshop on Grapevine Trunk Diseases in Santa Cruz, Chile and to visit researchers at the University of Talca and the Catholic University of Chile. The objectives of the travel were to acquire the latest information on grapevine diseases, disseminate eutypa dieback research findings from Australia, develop and enhance international collaborations and promote a bid for Australia to host the 8th IWGTD in 2012.

New knowledge on grapevine disease research has been acquired to incorporate into GWRDC projects along with ideas for modification of procedures which will improve the efficiency of current research and strengthen future proposals. For example, there is need for further research to optimise application of pruning wound protection and provide data for registration of fungicides along with elucidating the effects of stress, caused by increasing temperatures and decreased water availability, on disease. Collaborative links were strengthened with researchers from organisations around the world particularly at the Universities of Talca and the Catholic University of Chile.

Recommendations include the incorporation of acquired information on grapevine diseases into GWRDC projects and proposals for new collaboration, particularly on eutypa dieback. Several opportunities for international collaboration in Chile, Spain and the US have been identified and proposals are being initiated.

# 2 EXECUTIVE SUMMARY

Dr Mark Sosnowski was funded by GWRDC and SARDI to travel to Chile to attend the 7th International Workshop on Grapevine Trunk Diseases in Santa Cruz, Chile, 17-21 Jan 2010. This provided an excellent opportunity to gather information on recent developments in grapevine trunk disease research internationally. Meetings, visits and discussions with researchers from around the world, in particular Chile, have opened new doors for information exchange and collaboration as well as strengthening existing links and current collaborative research. Whilst in Chile, Dr Sosnowski also met with researchers at the University of Talca and the Catholic University of Chile to discuss grapevine research. The objectives of the travel were to; i) acquire the latest information on grapevine trunk diseases, ii) disseminate new knowledge from SARDI grapevine research, iii) develop and strengthen collaborative linkages with key international researchers and organisations, and iv) promote a bid for Australia to host the 8th IWGTD in 2012.

The IWGTD attracted 130 delegates from 17 countries, who presented 81 papers comprising research updates on Petri disease, esca, eutypa dieback, Botryosphaeria (Bot) canker and Cylindrocarpon black foot amongst other diseases. Presentations were grouped into pathogen identification and characterisation, disease detection and losses, host-pathogen interactions and disease management. Dr Sosnowski presented an overview of Australian research on eutypa dieback through oral and poster presentations and was invited to chair the disease management session at the workshop. As Regional Councilor for Australasia, he also attended the annual general meeting for the International Council for Grapevine Trunk Diseases.

Of particular relevance to the Australian grape and wine industry was the following highlights:

From the USA:

- Ten different species of Botryosphaeriaceae have been isolated throughout the USA and all have been shown to be pathogenic to grapevine. The distribution of species is based on their ability to cope with cooler or warmer conditions, similar to that seen in Australia.
- In an evaluation of fungicides, thiophanate-methyl (a benzimadazole) was most effective for control of bot canker and myclobutanil was most effective for control of eutypa dieback. A combination myclobutanil, thiophanate-methyl, febucaonazole and Pentrabark provided 100% control of eutypa dieback.

From New Zealand:

- Spores of Botryosphaeriaceae were most abundant in water on the surface of vines from December to February.
- Bacterium such as *Paenibacillus* sp.may be playing a role in the trunk disease complex.
- Biofumigation with brassicaceous plants may be an effective method of reducing *Cylindrocarpon* inoculum in soil.

From Spain:

- Typical vineyard weeds such as Bindweed (*Convolvulus arvensis*) and Greater Beggar's Ticks (*Bidens subalternans*) have been implicated as alternative hosts of *Cylindrocarpon* spp. (black foot) and *Phaeomoniella chlamydospora* (esca).
- Species of *Phaeoacremonium*, *Botryosphaeriaceae*, *Cylindrocarpon*, *Phaeomoniella* and *Cadophora* were isolated from and may be responsible for grafted Syrah (Shiraz) vines showing decline.
- Vines under water stress that were infected with pathogens of both eutypa dieback and bot canker showed greater necrotic staining than controls.
- In a fungicide screening, methyl thiophanate controlled eutypa dieback and bot canker, hydroxyquinoline controlled eutypa dieback and esca and copper sulphate only controlled esca.

From France:

- Incidence of black dead arm (bot canker) symptoms was greatest with increased rain and lower evapotranspiration in spring.
- In an evaluation of rapid bioassays for the screening of grapes for eutypa dieback symptoms, the shadehouse/ potted vine method developed at SARDI, was found to be the most effective.
- Trichoderma was not effective in preventing infection by *E. lata* when infection occured within 24 hours of treating wounds.

From Chile:

- Laboratory experiments showed that tebuconazole, prochloraz and prochloraz + carbendazim were effective at reducing mycelial growth of species of *Phaeomoniella*, *Phaeoacremonium*, *Botryosphaeria*, *Phomopsis* and basidiomycetes.

Discussions and visits with researchers from Chile and several other countries have greatly increased the opportunity for collaboration. Several new initiatives have already been initiated including a study visit to SARDI by David Gramaje from the University of Valencia in Spain, a potential PhD candidate Martin Merino from Dow AgroSciences in Chile, two joint publications with USA and Spanish researchers and project collaborations with Bernardo Latorre from the Catholic University of Chile, Philippe Rolshausen from UC Riverside in USA and Francois Halleen from ARC-Infruitec in South Africa. SARDI is recognised as a leading centre for grapevine research and is addressing several internationally recognised issues of grapevine diseases. As a result, researchers in other countries are keen to collaborate and develop new areas of research in grape diseases as well as share new information. Such collaboration will enhance our efforts to develop effective integrated pest management strategies for the Australian wine industry.

# 3 BACKGROUND

Grapevine trunk diseases such as eutypa dieback, botryosphaeria canker and esca all contribute to grapevine decline, reducing productivity and longevity, thereby causing considerable economic loss to the wine industry. It was estimated that eutypa dieback is costing Australian growers up to $3000 per hectare through lost production alone. In California, the total cost of eutypa dieback and botryosphaeria canker to the industry has been estimated at US$260 million. Eutypa dieback research at SARDI and the University of Adelaide (UA) aims to deliver sustainable vineyard management systems, improved viticultural production and an expanded professional skill base for the industry.

The primary purpose of this travel was for Dr Mark Sosnowski to attend the 7th International Workshop on Grapevine Trunk Diseases in Santa Cruz, Chile, 17-21 Jan 2010. It brought together researchers from around the world to report on epidemiological, management and diagnostic aspects of trunk diseases. Dr Sosnowski presented results from studies of Eutypa dieback in SARDI/UA research, which have provided important information to improve management strategies for management of the disease. Collaborative research between SARDI, the University of California Davis and the United States Department of Agriculture on the diversity of trunk pathogens was also presented. A field trip was also attended as part of the workshop to the Lolos Valley wine region where experience with disease issues was gained along with general viticultural practices.

Whilst in Italy, Dr Sosnowski visited with researchers from University of Talca and the Catholic University of Chile which included visits to vineyards in the Talca and Casablanca wine regions. Furthermore, Dr Sosnowski was invited to present SARDI/UA research on eutypa dieback and other grape diseases common in Australia at two workshops attended by pathologists, vineyard managers and growers from central Chile.

The objectives of this travel were to;

i) Acquire the latest information on grapevine trunk diseases from around the world, which will be used to develop further research initiatives through GWRDC funded research,

ii) Disseminate new knowledge from GWRDC eutypa dieback research (SAR 06/01) to international trunk disease research industry members,

iii) Develop and strengthen collaborative linkages with key international researchers and organisations, and

iv) Promote a bid for Australia to host the 8th IWGTD in 2012.

# 4 7TH INTERNATIONAL WORKSHOP FOR GRAPEVINE TRUNK DISEASES

The 7th International Workshop on Grapevine Trunk Diseases (IWGTD) was held in Santa Cruz, Italy, 17-21 January 2010. One hundred and thirty delegates attended the workshop representing 17 countries from 5 continents, with 46 oral seminars and 35 posters presented. The workshop was divided into four sessions; (i) pathogen identification and characterisation, (ii) Disease detection and losses (iii) host-pathogen interactions and (iv) disease management. Dr Sosnowski was on the scientific committee and also invited to chair the poster discussion session on disease management.

On 18 January the Annual General Meeting of the International Council of Grapevine Trunk Diseases (ICGTD) was held, of which Dr Sosnowski is on the Executive Council and Regional Coordinator for Australasia. At the meeting Dr Sosnowski demonstrated the new website he developed for the Council and also nominated to host the 8th International Workshop for Grapevine Trunk Diseases in 2012 in Adelaide. With a nomination also coming from Spain, the final decision on the successful nomination will be decided later in the year.

Presentation of information from SARDI/UA collaborative research comprised three oral seminars and one poster (Appendix 3). Summarized below is information presented by other international organizations, that is relevant to current SARDI research on eutypa dieback and botryosphaeria canker and how some of this information may be integrated into future research for the benefit of the Australian viticulture industry. This includes discussions held between Dr Sosnowski and other researchers. Relevant information will also be included in the eutypa dieback module of the Innovators Network series.

## 4.1 Pathogen identification and characterisation

- Rufina Hernandez-Martinez (CICESE Mexico) presented results on surveys of Botryosphaeriaceae species associated with grapevine decline in Mexico. *Diplodia seriata* was most commonly isolated from wedge shaped stained sections and *Neofusicoccum vitifusiforme*, *D. corticola* and *N. australe* were isolated, less commonly, from black streaks and brown-red wood. Pathogenicity studies showed that each species was able to cause necrotic lesions on green grapevine shoots, but to a lesser extent than *Lasiodiplodia theobromae*, which was included in the study as a positive control.
- Nicola Wunderlich (Charles Sturt University, Wagga Wagga) discussed the association of Botryosphaeriaceae species with bunch rot in south-eastern Australia. In a survey of the lower Hunter Valley region, seven different species were isolated; *D. seriata*, *N. parvum*, *N. luteum*, *Dothiorella viticola*, *L. theobromae*, *D. mutila* and *Botryosphaeria dothidea*. A large number of isolates were found on dormant buds and wood along with berries at harvest. Smaller numbers were isolated from flowers and pea-sized berries. All species were pathogenic on berries and 1-year-old canes.
- Jose Urbez-Torres (University of California Davis, USA) provided an update on the status of grapevine canker in the USA. Ten different species of Botryosphaeriaceae have been isolated throughout the country and all have been shown to be pathogenic to grapevine. *E. lata*, *Phomopsis viticola*, *Eutypella vitis*, *Diatrypella* sp., *Diatrype* sp., *Pestalotiopsis* spp., *Truncatella* spp. and *Paraconiothyrum* sp. were also isolated from grapevines and each was pathogenic to grapevine.
- Jose Urbez-Torres also presented studies on the optimal temperature for spore germination of all ten species of Botryosphaeriaceae in USA. *L. theobromae*, which is prevalent in warm, dry regions, preferred 40°C for spore germination, whereas *Dothiorella. iberica* and *Spencermartinsia viticola* prevalent in cooler areas, preferred 15°C. Results provide some explanation for the distribution of Botryosphaeria species in the USA, and a similar distribution is seen across the climatic regions of Australia.
- Nicolas Amponsah (Lincoln University, NZ) sampled rainwater from the surface of grapevines for spores of Botryosphaeriaceae species. He found *Neofusicoccum and Diplodia* spp. in 60 and 40% of samples, respectively. Although present throughout the year, spores were most abundant from December to February.
- Ian Harvey (PLANTwise, NZ) proposed the possibility of bacterium playing a role in the trunk disease complex. Two vineyards submitted trunk samples for diagnosis which, in cross-section, presented as an unfamiliar staining pattern which had a light brown blotchy appearance, unlike that seen with other common trunk diseases. A bacterium was consistently isolated from these samples and was characterized as *Paenibacillus* sp., not previously associated with grapevines. Pathogenicity tests on green and 1-year-old shoots led to the same symptoms, from which the bacterium was reisolated again. It was suggested that bacteria should not be ignored as potential trunk pathogens but further investigation is necessary to confirm these results.

- Carlos Agusti (University of Valencia, Spain) surveyed weeds in the vineyard for pathogens which cause black foot and Petri disease. The pathogens *Cylindrocarpon macrodidymum*, *C. luteo-olivacea* and *Phaeomoniella chlamydospora* were isolated from Bindweed (*Convolvulus arvensis*) and Greater Beggar's Ticks (*Bidens subalternans*), confirming the potential of some weeds in the vineyard as alternative hosts.
- A poster by Wayne Pitt (Charles Sturt University, Wagga Wagga) described the distribution of Botryosphaeriaceae associated with grapes in NSW and SA. Eight species have been identified; *Diplodia seriata*, *D. mutila*, *Lasiodiplodia theobromae*, *Neofusicoccum parvum*, *N. australe*, *Botryosphaeria dothidia*, *Dothiorella viticola*, *D. iberica*. *Diplodia and Dothiorella* spp. were most commonly isolated from all regions with *Neofusicoccum* spp. less frequently isolated and limited in geographic range, with only one isolate of *L. theobromae* found in northern NSW.

## 4.2 Disease detection and losses

- Annemiek Schilder (Michigan State University, USA) discussed the development of a crop loss model for eutypa dieback and showed that yield loss was associated with a reduction in shoots and clusters due to infection by *E. lata*. Using economic data, cost-benefit tools are being developed to aid growers in decision making for eutypa dieback management. A preliminary model was developed in SARDI research, based on decision making for remedial surgery. There is potential to form some collaboration between SARDI and MSU.
- David Gramaje (University of Valencia, Spain) found a number of trunk pathogens associated with decline of grafted Syrah vines in Spain. Species of *Phaeoacremonium*, *Botryosphaeriaceae*, *Cylindrocarpon*, *Phaeomoniella* and *Cadophora* were isolated from affected vines and suggested that these fungal pathogens should be considered as potential causal agents of the decline. With a prevalence of Shiraz in Australian vineyards and the presence of most of these fungi, this may have implications for the industry. Discussions with Gramaje have led to a 3-month visit to SARDI planned from October 2010, where he will work alongside Dr Sosnowski on trunk disease research and the Horticulture Pathology group with other pathology projects.

## 4.3 Host-pathogen interactions

- Eliane Abou-Mansour (University of Fribourg, Switzerland) has extracted and identified the toxins hydroxymellein and *trans* hydroxymellein, which were produced by *N. parvum* which causes Bot canker.
- Jordi Luque (IRTA, Spain) presented research on the effects of water stress on young Tempranillo vines inoculated with *E. lata* and *N. parvum*. Results of this study revealed greater necrotic staining in vines under water stress, although the difference was not significant. These results were similar to that of SARDI research, when comparing vines that were under water stress at 22°C. However different trend occurred when water stress was coupled with temperature stress in SARDI experiments. Discussion between Drs Luque and Sosnowski may lead to a joint publication on the results and future collaboration with further proposed research in this area.
- Philippe Larignon (IFVV, France) discussed the climatic conditions favourable to the expression of symptoms of black dead arm (bot canker) in a French vineyard. Incidence of symptoms was greatest with increased rain and lower evapotranspiration in spring. Previous research at SARDI showed that rainfall and temperature in spring correlated to symptom expression of eutypa dieback.
- Pascal Lecomte (INRA, France) presented an evaluation of rapid bioassays for the screening of grapes for eutypa dieback symptoms. It was concluded that a method based on a 1-month *in vitro* screening of plantlets was not a reliable method since no foliar symptoms were observed and stem necrosis only extended up to 3 mm from the site of inoculation, not providing a significant difference from controls. Another method was evaluated, similar to that developed in SARDI research, where symptoms are detected on potted vines within 12 months of inoculation with *E. lata* and staining difference is significant between inoculated and control vines.

## 4.4 Disease management

- Doug Gubler (UC Davis, USA) presented information on pruning wound prevention fungicides for bot canker and eutypa dieback. Thiophanate-methyl (a benzimadazole) was most effective against the bot canker fungus, *L. theobromae*, and myclobutanil was most effective for *E. lata*. They also showed that both fungicides were effective for controlling the esca causing fungi. Furthermore, the

use of double pruning to delay the final pruning cut enhanced the fungicide effectiveness. Pruning wound susceptibility was found to decrease significantly three weeks after pruning. Myclobutanil is being evaluated in SARDI/GWRDC trials. Further to these results Dr Gubler also showed that they achieved 100% control of eutypa dieback by applying a combination of Rally (myclobutanil) at 150 g/ha, Topsin M (thiophanate-methyl) at 1.7 kg/ha, Enable (febucaonazole) at 56 g/ha and Pentrabark at 16 mL/L.

- Philippe Larignon (IFVV, France) studied the effect of *Trichoderma* spp. for the protection of pruning wounds from *E. lata.* Wounds were treated with *Trichoderma* spp. following pruning and then wounds were inoculated with *E. lata* the following day. Five weeks after inoculation, although 87-100% of wounds contained *Trichoderma* spp., *E. lata* was also isolated from 90-100% of wounds. It was concluded that Trichoderma was not effective in preventing infection by *E. lata* when infection occurs within 24 hours of treating wounds.
- Javier Legorburu (NEIKER, Spain) tested the effect of fungicide application on pathogen reisolation from dormant grapevine canes inoculated with trunk disease fungi. It was shown that methyl thiophanate (benzimidazole) reduced incidence of reisolation of *E. lata* and *D. seriata* (bot canker), hydroxyquinoline reduced incidence of reisolation of *E. lata* and *P. chlamydospora* (esca) and Cubiet (copper sulphate) only reduced the incidence of reisolation of *P. chlamydospora*.
- Carolyn Bleach (Lincoln University, NZ) presented findings which showed that the incorporation of brassicaceous plants into the soil lead to reduced infection by *Cylindrocarpon* spp. in cuttings planted into the soil. These results were confirmed by laboratory tests growing the fungi on plates amended with ground mustard plants. It was concluded that biofumigation may be an effective method of reducing *Cylindrocarpon* inoculum in soil.
- Jaime Auger (University of Chile) evaluated the efficacy of fungicides for the protection of pruning wounds against the main pathogens associated with trunk disease in Chile; including species of *Phaeomoniella*, *Phaeoacremonium*, *Botryosphaeria*, *Phomopsis* and basidiomycetes. *In vitro* experiments showed that tebuconazole, prochloraz and prochloraz + carbendazim were effective at reducing mycelial growth of all species compared to controls. However, subsequent field trials showed that these fungicides were only effective on *P. chlamydospora* and not any of the other pathogens.
- Cheusi Mutawila (University of Stellenbosch, South Africa) investigated the susceptibility of pruning wounds to colonization by *Trichoderma* spp. followed by natural infection with *Phaeoacremonium*, *Phomopsis*, Botryosphaeriaceae and Diatrypaceae. Variable control was achieved by *Trichoderma* spp. and it was revealed that wound protection was a result of antagonist-grapevine-pathogen interactions rather than just the suppressive effects of the antagonist on the pathogens.
- Cristophe Bertsch (University of Haute-Alsace, France) evaluated the efficacy of saponins, which naturally occur in roots and leaves of healthy plants, against *D. seriata* and *N. parvum* (bot canker). *In vitro* experiments showed that an increase in saponin concentration led to a decrease in mycelial growth of both pathogens on agar.

Following a call for interest for PhD students by Dr Sosnowski following a presentation, Mr Martin Merino (Dow AgroSciences, Chile) offered his interest in undertaking a project in Australia on the effect of water and temperature stress on eutypa dieback. Mr Merino has since followed up and is looking in to the application process with University of Adelaide with Dr Eileen Scott selected as an ideal primary supervisor.

A request was made by several delegates at the workshop for Dr Sosnowski to present SARDI research results on pruning wound protection, of which he did for a small side group. Discussion with Benjamin Valiente and Ruben Santa Maria (Bayer Crop Science) were based on the industry partnership commitment by Bayer (Australia) to the project application to GWRDC (BCX 3085). The potential for further collaboration with Bayer (Chile) will be considered.

Discussions with Dr Philippe Rolshausen (UC Riverside, USA) and Mr Francois Halleen (ARC-Infruitec, South Africa) revealed they were planning to initiate trials to look at the efficacy of spray application based on the preliminary data from SARDI trials. They will look at a range of different trunk pathogens so it will be useful to maintain collaboration with both of them to build on the outcomes in the proposed SARDI project 'Optimising management of eutypa dieback' (BCX 3085).

Prof Doug Gubler also responded to Dr Sosnowski's presentation on remedial surgery with the possibility that some data may also be available from California and offered it to be combined with SARDI results in a join publication.

## 4.5 Vineyard field trip

On Tues 19 Jan, a field trip was made to Viña Santa Cruz, where the first vines were planted in 1997 and is situated in the Lolol Valley, within the greater Colchagua Valley, 150 km south of Santiago, 42 km from the Pacific Ocean. Vineyards occupy 160 ha and consist of six red cultivars, Cabernet Sauvignon, Syrah, Malbec, Merlot and Petit Verdot. Other than trunk diseases, Botrytis bunch rot is a concern and so preventative chemical control is used.

Following an introduction presentation (Figure 1a), different blocks on the property were inspected for different trunk diseases. Four stations were set up for us to visit and Dr Jaime Auger (workshop convenor) presented the symptoms observed via a poster (Figure 1b). A vine with foliar symptoms typical of eutypa dieback (Figure 1c) was spotted by Dr Sosnowski but on cutting the trunk (Figure 1d), no wedge of stained tissue was evident (Figure 1e). Chlorotic leaf symptoms were commonly seen on different cultivars (Figure 1f) as was staining in trunk cross-sections (Figure 1g). Also typical in trunks of vines with these foliar symptoms is the staining shown on the poster at Stations I and IV (Figure 2). Isolations from these symptoms yield Botryosphaeriaceae, *Phaeomoniella chlamydospora* and Basidiomycetes. No *Eutypa lata* has been isolated in Chile thus far. The symptoms observed in this vineyard have been locally referred to as chlorotic leaf roll.

Preventative action against trunk disease pathogens is recommended and used by growers to stop airborne spores entering pruning wounds. Paints or pastes with fungicides such as tebuconazole and carbendazim added are generally applied to wounds of all sizes (Figure 1h). With labour costs in Chile substantially less than that in Australia, this practice is practical and based on research at SARDI, very effective.

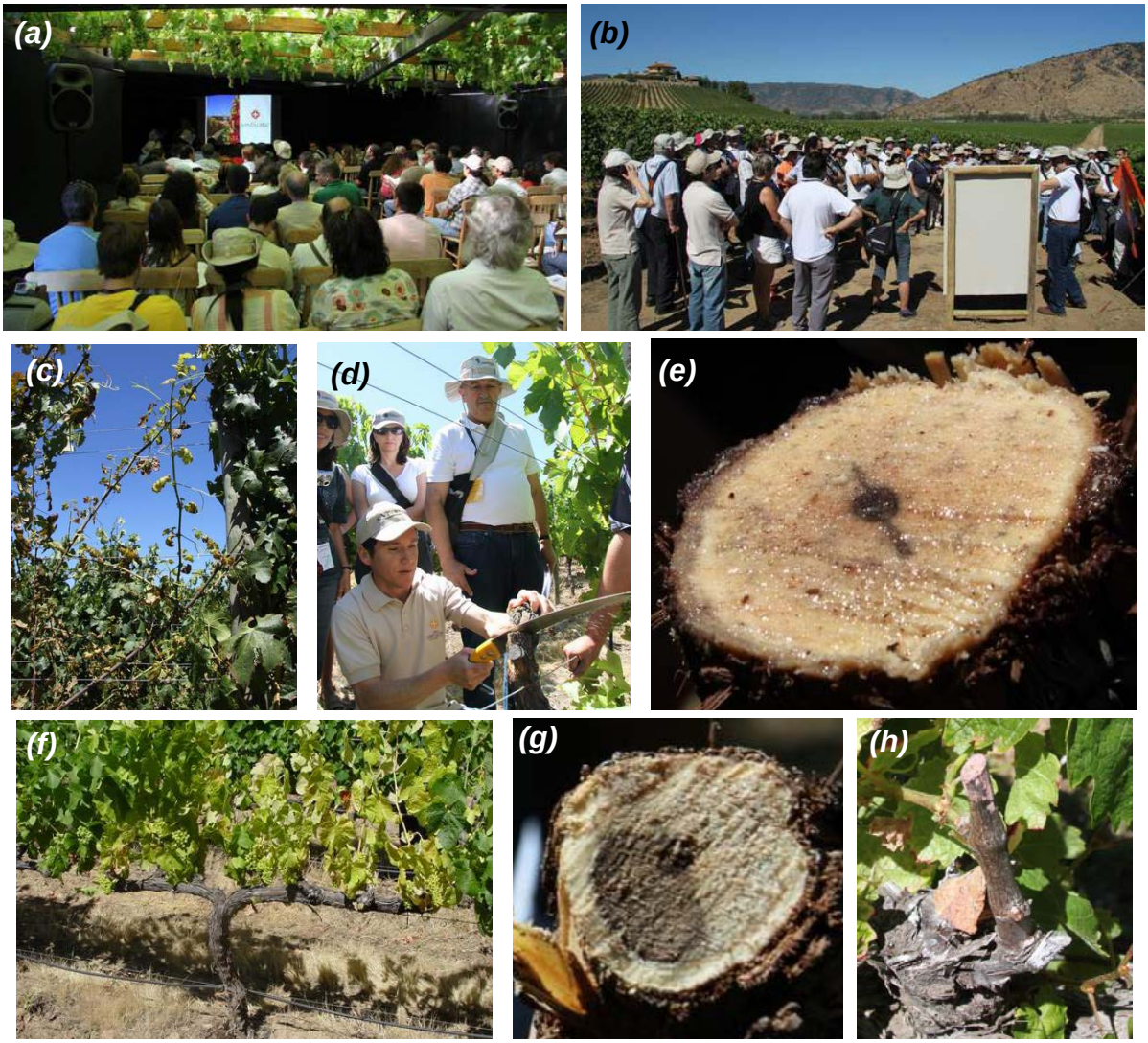



Figure 1. Photos taken on the vineyard field trip at the Viña Santa Cruz vineyard in the Lolos Valley. (a) introductory seminar, (b) vineyard demonstration, (c) foliar symptoms resembling eutypa dieback, (d) cutting a trunk for internal inspection, (e) no staining in trunk cross-section of vine with eutypa-like symptoms, (f) chlorotic leaf symptoms, (g) staining in trunk cross-section of vines with stunted shoots and (h) painted pruning wound.

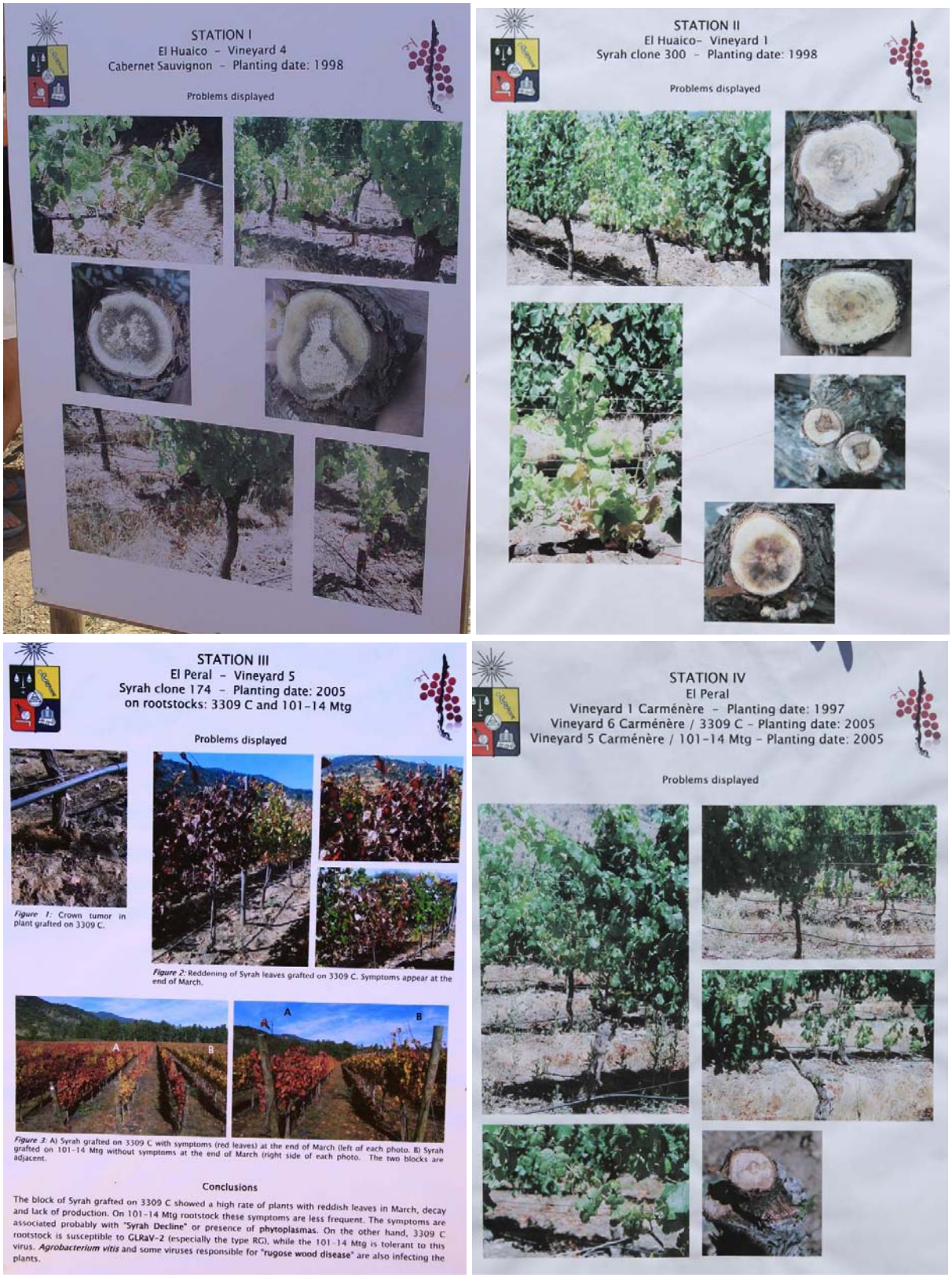



Figure 2. Station posters in the vineyard at Vina Santa Cruz showing typical trunk disease problems being experienced in different blocks and cultivars.

# 5 UNIVERSITY OF TALCA

A visit was made to the University of Talca and hosted by Dr Mauricio Lolas (Department of Agricultural Production - Phytopathology) and Dr Yerko Moreno (Vine and Wine Technology Centre). Talca is a large town 250 km south of Santiago which lies in the Maule Valley region. Maule Valley is the largest and one of the oldest wine regions in Chile. Old-bush, dry-farmed vineyards of Carignan, Cabernet Sauvignon and Malbec predominate the region. Newer plantations include Merlot, Cabernet Franc, and Carmenere. A short tour of the Vine and Wine Technology Centre located on the university campus revealed the significant investment that is being put into the Chilean wine industry. Research is particularly aimed at improving wine quality but there is room for expansion in viticultural research, which appears to have limited funding currently. Efforts are being made to increase investment in this area and our visit and potential for future collaboration will be used as leverage for attempts at future funding proposals to the government and industry.

The first vineyard visit was to Via Wines. As with the field visit near Santa Cruz, chlorotic leaf roll appears to be of major concern here also. Typical symptoms were seen on Malbec and Cabernet Sauvignon (Figure 3a-d) including stunted shoots, chlorosis (yellowing) and curling of leaves. Eutypa dieback foliar symptoms typically look slightly different with a greater level of necrotic leaf margins. The cross sectional wedges show typical symptoms of bot canker or eutypa dieback as well as the cream coloured soft rot appearance of basidiomycete infection, only in this instance the affected wood was hard.

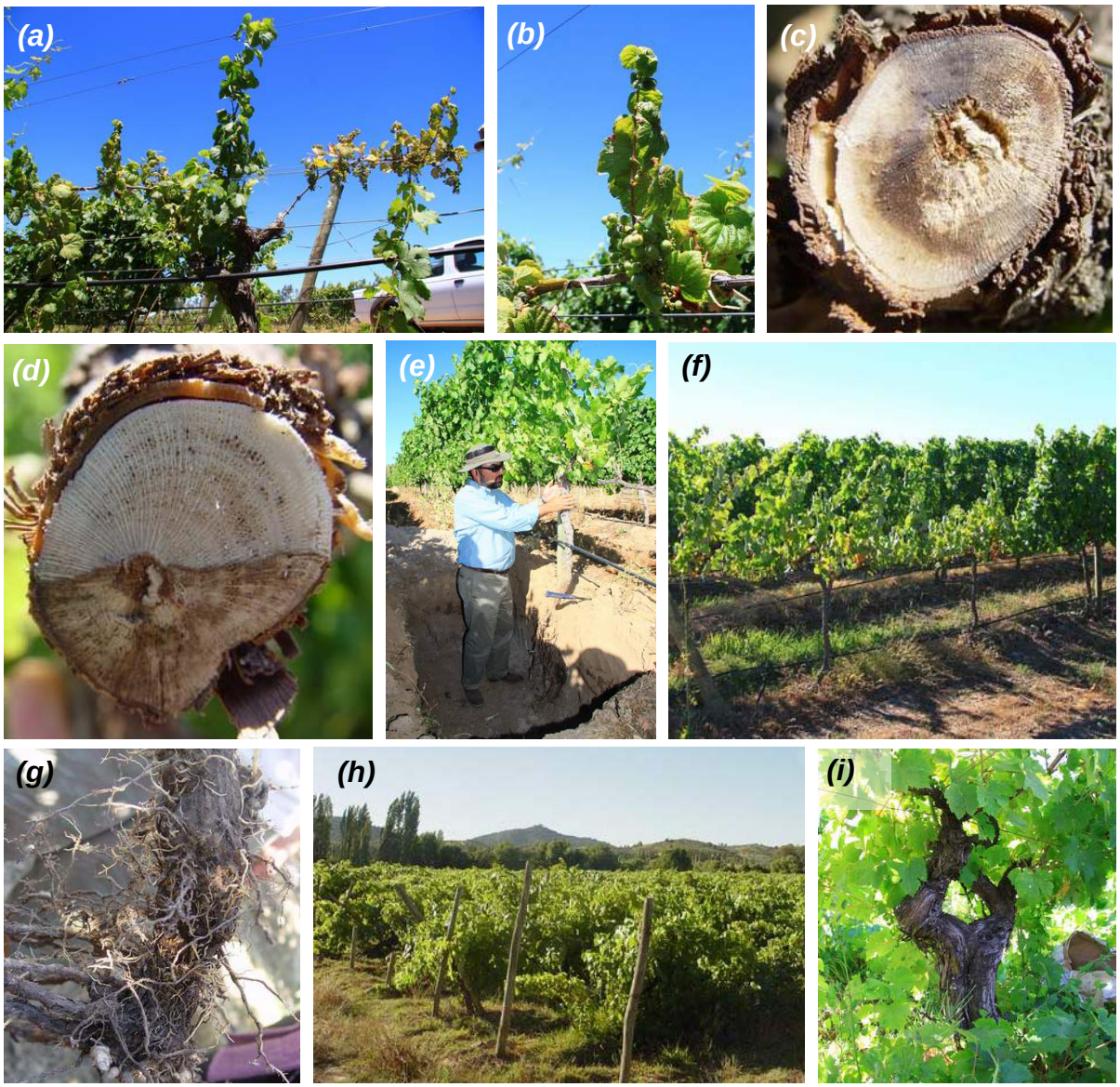



Figure 3. Symptoms of chlorotic leaf roll on Malbec (a-c) and Cabernet Sauvignon (d) at Via Wines; Dr Yerko Moreno (e) showing symptoms of Syrah decline (f-g) at Viña Alquihue and an over 80-year-old Cabernet Sauvignon vineyard (h-i) in the Maule Valley region of central Chile.

In the second vineyard at Viña Alquiheu, Dr Moreno showed us the other major concern for grapes in this region, Syrah decline (Figure 3e-g). Syrah (Shiraz) decline has also been reported in France and USA and is characterized by the development of swollen and cracked graft unions followed by leaf reddening in mid to late summer. In Chile, the canopy of affected vines begins to lack vigour in the first year, progressing each year until the vine is dead, which can be within 4 years. Affected vines occur sporadically around the block and in this particular vineyard up to 5% of vines were affected. Inspection of the roots revealed significant lateral root death and the production of new roots to replace them. There is no known cause or control for this syndrome, although it is believed to be virus related.

A third vineyard visited in the Maule Valley was Cabernet Sauvignon which was over 80-years-old (Figure 3h-i). Interestingly, this vineyard is minimally pruned by hand and only sulphur is applied for disease control. It is this type of management which has been attributed to the lack of trunk disease observed in such old vines, however the quality of grapes is low as a consequence of this management style.

# 6 CATHOLIC UNIVERSITY OF CHILE

A visit to Pontifica Universidad Católica de Chile was hosted by Prof Bernardo Latorre and a PhD student Mr Gonzalo Diaz. In the laboratory (Figure 4a-b), techniques were demonstrated for the isolation of; Diatrypaceous (eutypa dieback) fungi by Dr Sosnowski, Botryosphaeriaceae (bot canker) fungi by Dr Jose Urbez Torres (UC Davis, USA) and Basidiomycete (soft rot) fungi by Mr Francois Halleen (ARC-Infruitec, South Africa). Mr Diaz has subsequently forwarded photographs of the results of isolations enabling discussion and feedback by email. It was agreed that we would continue to collaborate together on the project as Mr Diaz plans to survey vines from regions in central to southern Chile in order to establish the distribution of trunk pathogens in the country. Furthermore through pathogenicity studies he will also attempt to determine the causal agent/s of chlorotic leaf roll symptoms.

Prof Latorre took us on a visit to Veramonte Vineyard in the Casablanca Valley and met with the vineyard manager (Figure 4c). Casablanca is a cool climate region 100 km north of Santiago, near the coast, and is predominantly planted to white varieties such as Chardonnay and Sauvignon Blanc. Again the predominant disease concern here was chlorotic leaf roll and similar symptoms were observed as in previous vineyards (Figure 4d-e). As a control measure, paint with fungicides such as tebuconazole added is applied to larger wounds in this vineyard.

In another vineyard in Casablanca, Viña Casas del Bosque, we again observed symptoms of chlorotic leaf roll. This has confirmed the fact that these symptoms are widespread in central Chile and like many of the other trunk diseases, is causing significant yield loss and death of vines. Vines as young as 6-years-old were observed with severe symptoms of this disease and the industry are understandably concerned. The research of Diaz and Latorre are very important in the hope of finding some answers and it will be important for the Australian industry to be aware of the findings. In Australia we certainly have all of the4 same causal agent but just have not yet seen the foliar symptoms.

Another observation of interest in this region were symptoms of leaf roll virus in a young block (Figure 4h) which is believed to have originated from nursery cuttings from France. It was advised by the visiting group that the vines be removed as soon as possible to reduce the likelihood of spread to other blocks in the vineyard. Finally, an unusual technique of grafting was spotted in a vineyard where the union is protected with a bag of sand causing root growth to occur (Figure 4i-j).




Figure 4. Visit to the Catholic University of Chile laboratory to demonstrate isolation techniques for trunk pathogens (a-b), vineyard visit to Veramonte (c-e) and vineyard visit to Viña Casas del Bosque (f-g) to see symptoms of chlorotic leaf roll, leaf roll virus (h) and an unusual grafting technique (j-k).

## 7 ANASAC WORKSHOPS

ANASAC is a Chilean company that produces, sells and distributes products for Agriculture, Livestock, Forestry and Agroindustry, as well as gardens, parks, environmental health and animal care. Dr Sosnowski was invited by Mr Mauricio Sanchez (ANASAC, Chile) to present information from SARDI research on the management of eutypa dieback as well as presenting an overview of grapevine disease management in Australia. The first workshop, held at the ANASAC headquarters near Santiago (Figure 5a), was attended by about 15 grapevine and tree pathologists from across the country. Dr Sosnowski presented the latest information on eutypa dieback research in Australia. Great interest was shown in the pruning wound protection and remedial surgery control work. Also presenting at the workshop was Dr Jose Ramon Urbez Torres, who presented information on Bot canker research being undertaken at UC Davis in USA.

Following the workshop a vineyard visit was made by the workshop group to Viña Santa Rita, 40 km south of Santiago in the Maipo Valley (Figure 5b). Vineyards are planted in the foothills of the Andes Mountains and include cultivars such as Cabernet Sauvignon, Merlot, Cabernet Franc, Shiraz and Petite Syrah. As with all the vineyards visited so far, chlorotic leaf roll was a significant problem in the old Cabernet Sauvignon vines. Stunted shoots, cupped leaves with reddish margins and chlorotic zones between veins were observed (Figure 5c-f). A demonstration was conducted by Dr Sosnowski successively cutting back trunks until no more necrotic staining (Figure 5g) was visible.

A second workshop was held at Los Lirios Country Club in Requínoa, 100 km south of Santiago. Dr Sosnowski was invited to present research on grapevine disease management in Australia, so he presented information on management strategies for some of the more common disease problems such as powdery and downy mildew, botrytis, eutypa dieback, bot canker and phomopsis. The audience of approximately 40 people consisted of vineyard managers and viticulturists from wine regions of central Chile. Again there was a lot of interest in the presentation with lengthy discussion following and management strategies for trunk diseases were again high on the priority list, especially with the level of chlorotic leaf roll occurring in vineyards.

Following the workshop a vineyard visit was made by attendees to Los Boldos near Requínoa in the Rapel Valley. Again symptoms of chlorotic leaf roll here were similar to that seen already. Interestingly some remedial surgery work had been performed in this vineyard and demonstrated the effect of cutting vines low and taking a shoot from near the ground (healthy foliage) rather than the top of the trunk (symptomatic foliage; Figure 5h). Cuts were made through the trunk of a symptomatic vine exposing severe necrosis at the top of the trunk (Figure 5i) and reduced necrosis closer to the ground (Figure 5j).

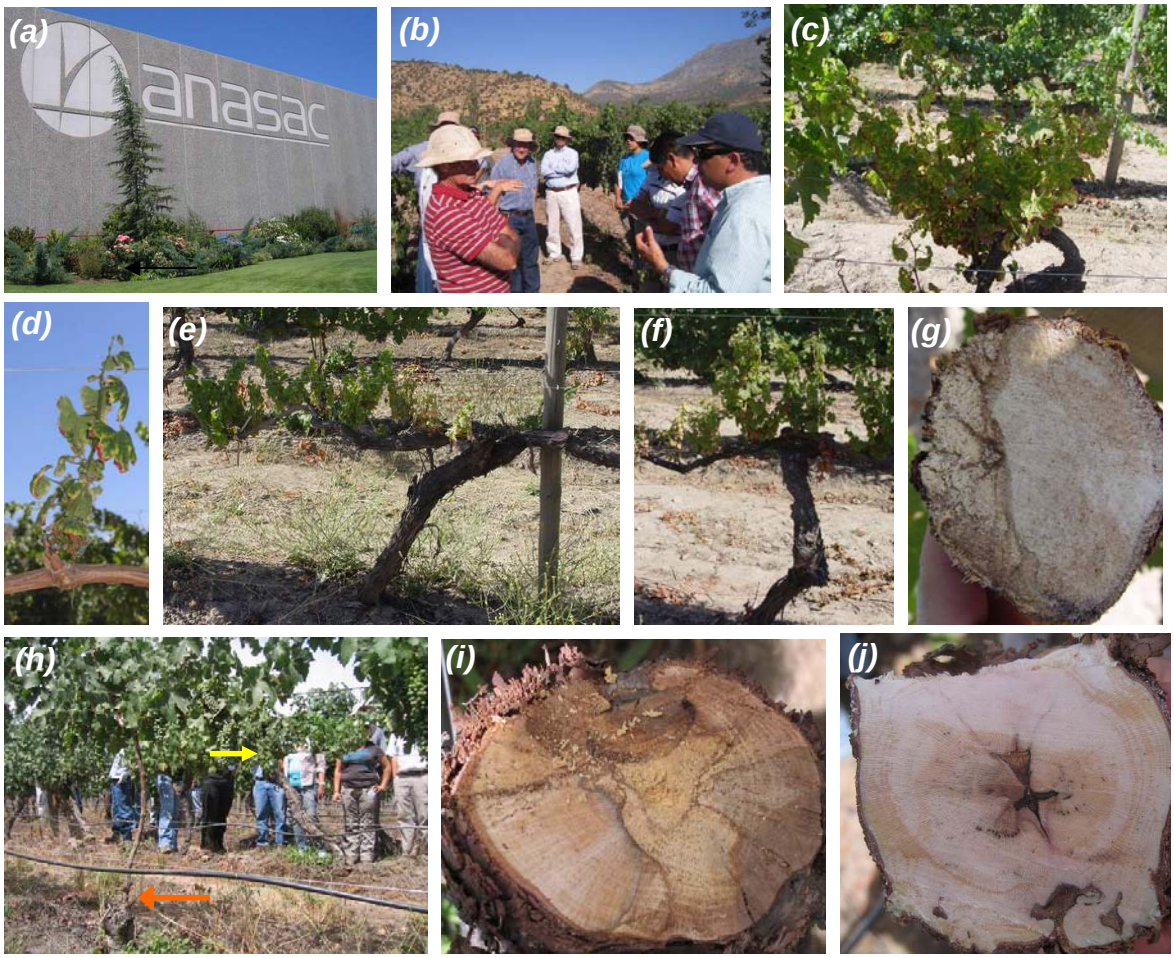



Figure 5. ANASAC Workshop was held at the factory near Santiago (a), vineyard visit to Viña Santa Rita near Buin showing discussions between pathologists (b), foliar symptoms of chlorotic leaf roll (c-f), trunk symptoms and vineyard visit to Los Boldos near Rancagua showing reworked vines (h) with symptoms on shoots originating from top of vine (yellow arrow) and healthy shoots originating from bottom of vine (orange arrow) and symptoms in cross-sections of trunk (i-j).

# 8 MAIN OUTCOMES

## 8.1 Collaboration

Collaborative links were strengthened with researchers from organisations around the world particularly at the University of Talca and the Catholic University of Chile.

- David Gramaje (University of Valencia, Spain) approached Dr Sosnowski at the IWGTD for the opportunity for him to spend 3 months at SARDI gaining experience and building collaboration between UV and SARDI, following the completion of his PhD. Mr Gramije plans to arrive in early Oct 2010 and will collaborate on eutypa dieback research and work with other grape disease researchers at SARDI.
- Mr Martin Merino (Dow AgroSciences, Chile) responded to Dr Sosnowski's call for interested PhD candidates for a project at SARDI/UA looking at the effects of water and temperature stress on eutypa dieback. Dr Eileen Scott (UA) has offered to be the primary academic supervisor. Mr Merino plans to apply to UA for an international scholarship and if successful will aim to begin the project in 2011.
- At the IWGTD discussions were held with Prof Doug Gubler (UC Davis, USA) on pruning wound protection and remedial surgery for management of eutypa dieback. It is important for us to continue our collaboration and communication with Prof Gubler's team on the proposed SARDI project aiming to optimise management of eutypa dieback (BCX 3085). Furthermore there is potential for a joint publication on the effects of remedial surgery.
- A discussion with Dr Jordi Luque from the IRTA, Spain following his presentation of research on the effect of water stress at the IWGTD may lead to collaboration. Potential of a joint publication on our preliminary research will be followed up. Dr Luque plans to continue his research and plans are in progress to establish a PhD project at SARDI/UA to extend the research on water and temperature stress in Australia. Collaboration between Australia and Spain will be beneficial to the wine industries in both countries.
- Discussions between Dr Philippe Rolshausen (UC Davis USA), Mr Francois Halleen (ARC-Infruitec, South Africa) and Dr Sosnowski will lead to collaboration on planned research to apply pruning wound protection with spray machines. This will enable more data to be collected for eutypa dieback control and the research to be extended to other trunk diseases such as bot canker and esca.
- Prof Bernardo Latorre and PhD candidate Mr Gonzalez Diaz will continue to consult Dr Sosnowski, Dr Urbez Torres (USA) and Mr Halleen (South Africa) on the project surveying Chilean vineyards for trunk disease pathogens and pathogenicity studies to better understand the cause of chlorotic leaf roll.

## 8.2 Networking

Informal meetings and discussions took place throughout the visit with researchers from Chile, USA, Italy, Spain, France, NZ and South Africa and it was excellent opportunity to share information and ideas, and strengthen ties between various research groups. New relationships have been built and the opportunity for future contact greatly enhanced, particularly with several Chilean researchers.

## 8.3 Knowledge

New knowledge and information has been acquired to incorporate into GWRDC projects along with ideas for modification of procedures which may improve the efficiency of current projects and strengthen future proposals.

## 8.4 Communication

An article will be drafted for submission to an Australian industry magazine to report on the main outcomes of this travel.

# 9 RECOMMENDATIONS

- SARDI/UA have established a centre of excellence in eutypa dieback research and with no other research occurring on spray application for pruning wound protection or climatic stress on eutypa dieback, it is important to continue this research for the sustainability of the Australian wine industry.
- Finalise arrangements to host a research study visit during Oct-Dec 2010 by David Gramaje (University of Valencia, Spain). Travel, accommodation and living expenses will be funded by University of Valencia and SARDI will provide in kind support through laboratory and office facilities and supervision.
- Follow up and progress plans for PhD candidate Martin Merino (Dow AgroSciences, Chile) to apply for an international scholarship with UA.
- Follow up on potential collaborative joint publications with Jordi Luque (IRTA, Spain) and Doug Gubler (UC Davis, USA).
- Establish collaboration with Philippe Rolshausen (UC Riverside, USA) and Francois Halleen (ARC-Infruitec, South Africa).
- If the bid to host the 8th International Workshop for Grapevine Trunk Diseases in Adelaide in February 2012 is successful, an organizing committee should be formed and workshop arrangements commenced.
- Continue communication with Bernardo Lattore (Catholic University of Chile) and PhD candidate Gonzalez Diaz on their research on trunk diseases in Chile with view of potential collaboration in the future between Chile and Australia.
- Contact Annemiel Shilder (Michigan State University) on the potential for collaboration with economic modeling of eutypa dieback disease.

# 10 ACKNOWLEDGEMENTS

Thanks to GWRDC and SARDI for their generous support in providing funds for Dr Sosnowski to undertake this travel and for the hospitality provided by hosts in Chile, Drs Jaime Auger, Mauricio Lolas, Bernardo Latorre and Mr Mauricio Sanchez. Very special thanks to Dr Jose Ramon Urbez Torres for helping with arrangements for visits and acting as tour guide and interpreter throughout the visit.

# 11 APPENDICES

## Appendix 1. Major contacts

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## Appendix 2. Travel Itinerary

| January 2010 | |
|---|---|
| Sat 16 | Travel from Adelaide to Santa Cruz, Chile |
| Sun 17 – Wed 21 | 7th International Workshop on Grapevine Trunk Diseases |
| Thu 22 | Visit with Dr Mauricio Lolas at University of Talca + vineyard visits |
| Sat 23 | Travel to Santiago, Chile |
| Mon 25 - Tues 26 | Visit with Dr Bernardo Latorre at Catholic University of Chile + vineyard visits |
| Wed 27 | Visit to Agricom Avocado Farm |
| Thu 28 | ANASAC workshop I (grapevine pathologists) and vineyard visit |
| Fri 29 | ANASAC workshop II (growers/vineyard managers) and vineyard visit |
| Sun 31-Tues 2 Feb | Return from Santiago to Adelaide |

## Appendix 3. Abstracts

### *7th International Workshop for Grapevine Trunk Diseases*

#### *Oral presentations*

**Controlling eutypa dieback by remedial surgery**

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Eutypa dieback is a disease of grapevine caused by the fungus *Eutypa lata,* which infects through wounds and gradually colonises wood tissue, causing stunted shoots with cupped, chlorotic and necrotic leaves, dieback of cordons and eventually death of vines. Once infection is established control is difficult as shown by the lack of success using fungicides, biocontrol agents and nutrients. Twelve long-term trials were established in South Australia to evaluate two methods of remedial surgery to control eutypa dieback; i) low-cut, which involves removing the trunk 30 cm above ground and training a watershoot to replace the canopy and ii) high-cut, where trunks are cut at the crown and the lowest shoot is trained to form a new canopy, followed by removal of excess trunk. Between 42 and 100% of vines produced watershoots, generally reaching a plateau within 3 years. More shoots developed on high-cut vines than on low-cut vines, with considerable variation among cultivars. Recurrence of stunted shoots ranged from 0 to 11% up to 9 years after surgery on low-cut vines, compared with 9 to 54% on high-cut vines. On high-cut vines, the lowest watershoot suitable for reworking emerged from anywhere between the ground and the crown and recurrence of stunting increased with height above ground. The frequency of stunting in high-cut vines was probably due to infected tissue remaining in the trunk. Findings support the need to remove all *E. lata*-infected tissue from diseased vines to restore long-term productivity to vineyards.

**Diatrypaceae associated with *Vitis vinifera* in New South Wales and South Australia**

F.P. TROUILLAS[1*], W.M. PITT[2], **M. SOSNOWSKI[3]**, F. PEDUTO R. HUANG[2], A. LOSCHIAVO[3], S. SAVOCCHIA[2], C. STEEL[2], E. SCOTT[4] AND W.D GUBLER[1]
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Diatrypaceae are common pyrenomycetous fungi occurring on a wide range of woody angiosperms around the world. *Eutypa lata*, the causal agent of Eutypa dieback of grapevine, is the most common diatrypaceous fungus associated with grapevine cankers and it is found in all major grape growing regions worldwide. Recent studies in California have shown that additional Diatrypaceae can infect grapevine wood and possibly cause canker diseases similar to Eutypa dieback. During 2007-08, a number of surveys for fungi associated with canker diseases in grapevine were conducted in New South Wales and South Australia. In many instances, fungal colonies displaying morphological characteristics typical of Diatrypaceae were isolated from grapevine cankers. Fruiting bodies of Diatrypaceae were also found on dead grapevine wood. Morphological studies and phylogenetic analyses of the complete sequence of the internal transcribed spacer (ITS) region of the rDNA and partial β-tubulin gene identified *Cryptovalsa ampelina*, *Diatrypella* sp., as well as two species of *Eutypella* from grapevine cankers, in addition to *E. lata*. Similar species occurred on other agricultural host plants and ornamentals adjacent to vineyards. Surveys also documented *Eutypa leptoplaca* on *Fraxinus*, *Populus* and *Schinus* spp., however it was not isolated from grapevine. Pathogenicity studies of Diatrypaceae were conducted by drilling a 5-mm-diameter hole into the wood of potted Cabernet Sauvignon grapevines and inserting an agar plug with fresh mycelium obtained from the margin of 8-day-old colonies. Plants were maintained in a shade house for 10 months before being inspected for developing lesions and fungal recovery. Preliminary results for pathogenicity are discussed.

**Intercontinental genetic structure of the fungal grapevine pathogen *Eutypa lata***

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The ascomycete fungus *Eutypa lata*, causal agent of Eutypa dieback of grape (*Vitis vinifera*), impacts all vineyard production systems worldwide. Our objectives were to characterize the population structure of *E. lata* at different geographical scales to identify migration patterns through ascospore dispersal, centers of diversity and new introductions areas. We first genotyped 145 isolates from four vineyards in each of two locations (California, South Australia) using nine polymorphic microsatellite loci. Results showed high levels of gene (*H*=0.56 to 0.62) and genotypic (*G*=0.85 to 1) diversity among the eight vineyards examined. No linkage disequilibrium among loci ($P<0.01$) was detected. We found that >93% of genetic variance was found within vineyards ($P<0.01$) and 3.24% of variance could be attributed to differences between California and South Australia ($P<0.05$). Three pairwise comparisons revealed significant genetic differentiation between populations from these two continental regions ($F_{ST}$=0.03-0.10, $P<0.05$). When isolates were pooled by continental regions, genetic differentiation between the two populations was low but significant ($F_{ST}$=0.03; $P<0.05$). These preliminary findings suggest that gene flow prevents genetic differentiation at the regional scale within continents, spanning distances up to 410 km, whereas continental populations are somewhat isolated. An additional collection of 156 isolates from Europe and South Africa, representing 12 vineyards populations, was recently obtained, and are being genotyped using the same nine microsatellite loci.

***Poster presentation***

**The effect of water and temperature stress on eutypa dieback**

**M.R. SOSNOWSKI[1*]**, A.P. LOSCHIAVO[1,2], T.W. WICKS[1], AND E.S. SCOTT[2]

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Eutypa dieback is caused by the fungus *Eutypa lata* which invades wood tissue causing dieback, stunted shoots, and the gradual death of grapevines. Yearly variation of foliar symptoms is thought to be due to environmental conditions. The effect of temperature and water stress on symptom expression in inoculated vines was evaluated. Potted grapevines were grown in controlled environment rooms at 14, 22 and 30ºC for 7 months. Within each room, soil was maintained at low, medium and high water content. Foliar symptoms tended to be most severe on inoculated vines subjected to the upper and lower extremes of both soil water content and temperature. The most severe symptoms developed on vines grown at 30ºC with high soil water content. Conversely, growth of *E. lata* from the inoculation site, assessed by isolation, appeared to be less for vines kept in extreme conditions, suggesting fungal growth may be impeded under stressful conditions. The effect of deficit irrigation on wound infection by *E. lata* was investigated in field experiments in the Barossa Valley and Riverland wine regions of South Australia. Control vines were irrigated according to annual allocation whereas deficit-irrigated vines received 0 - 60% of the volume applied to controls. The frequency of recovery of *E. lata* from wounds increased from 74 to 95% in vines under severe water deficit in the Riverland, yet it remained unchanged in the Barossa Valley. As the Riverland is drier and warmer than the Barossa Valley, these results suggest that vines under severe stress are more susceptible to infection by *E. lata*.

# The effect of water and temperature stress on eutypa dieback

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Figure 1. Controlled environment experiment

## Introduction

Eutypa dieback, caused by the fungus *Eutypa lata,* infects through pruning wounds and causes stunted shoots with chlorotic, cupped leaves with necrotic margins, dieback of wood and the gradual death of grapevines. Annual variation in symptom expression is thought to be influenced by environmental conditions. The influence of temperature and soil water stress, which are known to affect the severity of other diseases, were investigated.



Figure 3. Deficit irrigation trial

## Controlled environment experiment

### Methods

- Grapevines (cv. Grenache) in a shadehouse were inoculated with a mycelial plug of *E. lata* in February 2008.
- From June, pots were maintained at low (5-20%), moderate (10-25%) or high (20-40%) volumetric soil water content.
- In September pots were moved to controlled environment rooms (Fig 1) set at low (14ºC), moderate (22ºC) and high (30ºC) temperature and irrigation regimes maintained.
- Foliar symptoms were assessed in November 2008 and in April 2009 colonisation of stems by *E. lata* was assessed by isolation.

### Results

- Foliar symptoms were more severe for the extreme treatments (e.g. high or low temperature coupled with high or low soil water), with the greatest mean severity (27%) occurring on vines at 30ºC with high soil water (Fig 2a).
- Mean colonisation ranged from 20 to 60 mm and tended to be less, although not significantly so, in the extreme treatments (Fig 2b).

## Deficit irrigation trials

### Methods

- In the Riverland, South Australia, Cabernet Sauvignon vines planted in 1992 were irrigated at 100, 60 and 25% of water allocation in each year since 2006.
- In the Barossa Valley, South Australia, Ruby Cabernet vines planted in 1993 were either irrigated (100% allocation) or not irrigated (0%) since 2006.
- Mean annual rainfall in the Riverland and Barossa Valley is 261 and 472 mm, and mean maximum temperature 23.8 and 21.5ºC, respectively.
- Vines were spur-pruned to two buds in July 2008 (Fig 3). Wounds were inoculated within an hour with 500 spores of *E. lata* and again 4 days later.
- In June 2009, inoculated spurs were excised and *E. lata* isolated in the laboratory.

### Results

- In the Riverland, *E. lata* was recovered most frequently from vines with reduced irrigation (Fig 4a).
- In the Barossa Valley, there was no difference in infection between irrigated and unirrigated vines (Fig 4b).

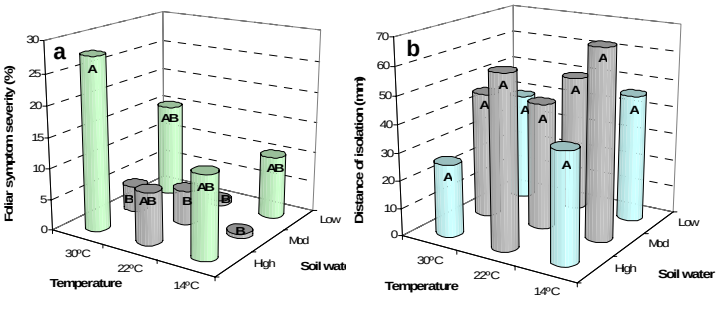



Figure 2. Effect of temperature and soil water on (a) foliar symptoms and (b) the spread of mycelium of *E. lata* along stems (treatments with the same letter are not significantly different from each other)

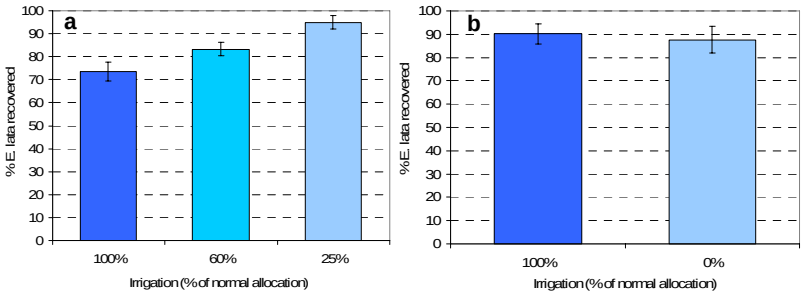



Figure 4. Effect of vine irrigation on susceptibility of pruning wounds to infection by *E. lata* in (a) the Riverland and (b) the Barossa Valley regions of South Australia (bars represent standard error of the mean)

## Conclusion

Extreme temperature and moisture stress may lead to increased expression of foliar symptoms of eutypa dieback but reduced growth of *E. lata*. In a warm, dry region, deficit irrigation increased the susceptibility of pruning wounds to infection by *E. lata*. Further research is required to substantiate these findings.

Australian Government
Grape and Wine Research and Development Corporation

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