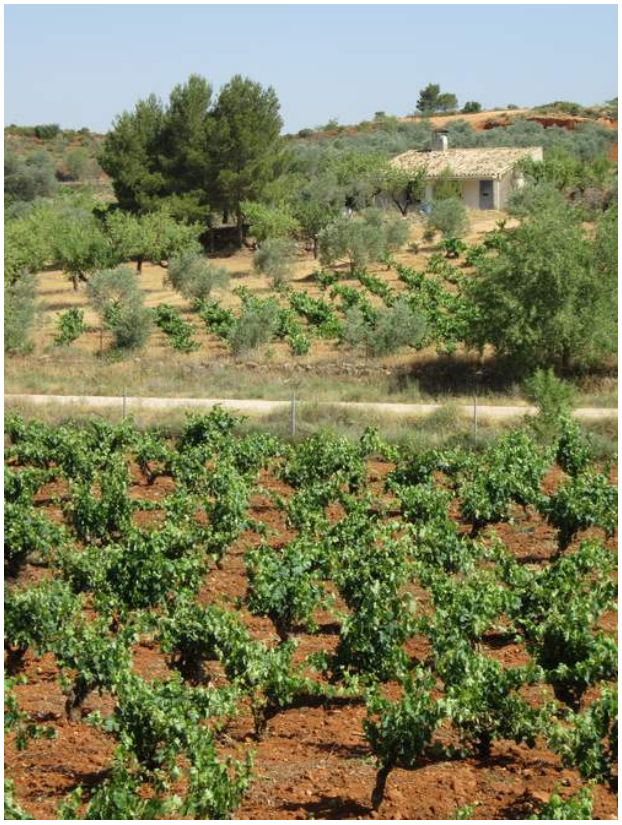
# 8th International Workshop for Grapevine Trunk Diseases, Spain



FINAL REPORT to
**GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION**

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# TABLE OF CONTENTS

**1. ABSTRACT ..... 2**
**2. EXECUTIVE SUMMARY ..... 2**
**3. BACKGROUND ..... 4**
**4. 8TH INTERNATIONAL WORKSHOP FOR GRAPEVINE TRUNK DISEASES ..... 5**
4.1 Pathogen identification and characterisation ..... 5
4.2 Grapevine nurseries ..... 6
4.3 Epidemiology ..... 8
4.4 Host-pathogen interactions ..... 9
4.5 Disease management ..... 9
4.6 Workshop field trip to Utiel-Requena ..... 10
4.7 Committee Meeting of the International Council on grapevine Trunk Diseases ..... 11
**5. POLYTECHNIC UNIVERSITY OF VALENCIA ..... 13**
**6. INSTITUTE FOR FOOD AND AGRICULTURAL RESEARCH AND TECHNOLOGY (IRTA), CABRILS ..... 14**
**7. MAIN OUTCOMES ..... 16**
7.1 Collaboration ..... 16
7.2 Networking ..... 16
7.3 Knowledge ..... 16
7.4 Communication ..... 16
**8. RECOMMENDATIONS ..... 17**
**9. ACKNOWLEDGEMENTS ..... 17**
**10. APPENDICES ..... 18**

## 1. ABSTRACT

With funding from the Grape & Wine Research & Development Corporation and the South Australian Research & Development Institute, Dr Mark Sosnowski attended the 8th International Workshop on Grapevine Trunk Diseases (IWGTD) in Valencia, Spain and visited researchers at the Polytechnic University of Valencia and the Institute for Food & Agricultural Research & Technology near Barcelona. 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 9th IWGTD in 2014.

New knowledge on grapevine disease research has been acquired and will be incorporated into future GWRDC research along with ideas for modification of procedures which will improve the efficiency of current research. For example, there is need for further research to optimise timing of spray application of pruning wound protection and to continue seeking alternative treatments along with elucidating the effects of environmental and production stress on disease. Collaborative links were strengthened with researchers from organisations around the world, particularly in Spain. The next IWGTD will be held in Adelaide, Australia in Nov 2014, providing both researchers and industry and opportunity to be exposed to the latest international research on grapevine trunk disease.

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

## 2. EXECUTIVE SUMMARY

Dr Mark Sosnowski was funded by GWRDC and SARDI to attend the 8th International Workshop on Grapevine Trunk Diseases (IWGTD) in Valencia, Spain, 18-21 Jun 2012. 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 9th IWGTD in 2014. The IWGTD provided an excellent opportunity to gather information on recent developments in grapevine trunk disease research. Meetings, visits and discussions with researchers from around the world, in particular Spain, have opened new doors for information exchange and collaboration as well as strengthening existing links and current collaborative research. Dr Sosnowski also met with researchers at the Polytechnic University of Valencia and the Institute for Food and Agricultural Research and Technology (IRTA) to discuss grapevine research.

The IWGTD attracted 125 delegates from 22 countries, who presented 107 papers comprising research updates on botryosphaeria dieback, eutypa dieback, Petri disease and esca amongst other diseases. Presentations were grouped into Pathogen detection and characterization, Grapevine nurseries, Epidemiology, 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 poster discussion session on Epidemiology, Host-pathogen interactions and Disease management. As Regional Coordinator for Australasia on the Executive Council, he also attended the annual general meeting for the International Council for Grapevine Trunk Diseases and presented a bid to host the 9th IWGTD in Adelaide in 2014.

Highlights of particular relevance to the Australian grape and wine industry were:

- Botryosphaeria dieback has been proposed as the name to describe the wide range of different grapevine trunk disease symptoms disease symptoms such as leaf spots, fruit rots, shoot dieback, bud necrosis, vascular discoloration of the wood and perennial cankers associated with species of the Botryosphaeriaceae.
- *Botryosphaeriaceae* species move endophytically beyond the lesions which could have implications in the nursery industry since the young, symptomless canes may be infected.
- *Phomopsis viticola* has been reconfirmed as an important grapevine trunk disease pathogen, and thus the name Phomopsis dieback is proposed to describe the disease.
- Preventative controls for Phomopsis cane and leaf spot may not completely control the disease since *Phomopsis fukushii* and *Diaporthe eres* are also responsible for wood cankers.
- Genetic studies in California suggest that grapevines are more at risk from inoculum from other grapevine and fruit crops than from riparian areas or ornamentals like willows.

- Sucker wounds on grapevines can be naturally infected by a range of trunk disease pathogens.
- Grapevine trunk disease has been attributed, in part, to grapevine nursery propagation procedures world-wide and there is a need to develop guidelines to implement practical propagation procedures.
- Contrary to previous results in other regions of the world, Spanish vineyards have been recorded with greater inoculum levels of the pathogens causing botryosphaeria dieback and esca in spring than in winter.
- NZ research showed that remove of inoculum sources such mummified berries and infested vineyard cuttings and fungicide sprays may be required before bud burst to manage botryosphaeria dieback.
- Olive trees may be an important source of inoculum for grapevine trunk disease pathogens having implications on the management of trunk diseases
- Arthropod species such as ladybirds, ants, beetles and earwigs can carry spores of trunk pathogens and they may infect pruning wounds, where they feed on bleeding sap
- Aerial imagery using Normalised Differential Vegetation Index (NDVI) was shown to be highly correlated to Esca symptoms
- Around 11% of French vineyards are unproductive due to trunk diseases
- In a long-term French study, cane-pruned vines had less foliar symptoms of eutypa dieback but more dead plants than for cordon-pruned, indicating the importance of the proximity of infection sites to the trunk.
- A *Chaetomium* sp. was shown to be antagonistic against various trunk disease pathogens and may provide an alternative biocontrol for trunk disease management

Discussions and visits with researchers from Spain and several other countries have greatly increased the opportunity for collaboration. Several initiatives including a study visit to SARDI by Georgina Elena from the University of Barcelona and IRTA have been implemented. Plans were also made to build on collaboration with IRTA on the effects of stress on trunk diseases following a recent joint publication. In New Zealand collaboration with Lincoln University will build on the linkage with Plant & Food Research in a proposal for SARDI to lead a trunk disease research project with NZ Wine. 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. The bid by Dr Sosnowski to host the 9$^{th}$ IWGTD in Adelaide in 2014 was successful so plans to organise the event which intends to involve industry members in Australia will commence.

## 3. BACKGROUND

Grapevine trunk diseases such as eutypa dieback, botryosphaeria canker and esca all contribute to grapevine decline, reducing productivity and longevity, causing considerable economic loss to the $8.3 billion Australian 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 is delivering 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 8th International Workshop on Grapevine Trunk Diseases (IWGTD) in Valencia, Spain, 18-21 June 2012. Every 2 years, the IWGTD brings together researchers from around the world to share information and combine forces in the challenge of managing grapevine trunk disease. It is organised by the International Council on Grapevine Trunk Diseases (ICGTD; www.icgtd.org) of which Dr Sosnowski is on the executive committee and a regional coordinator for Australasia. The workshop covers topics such as diagnostics, epidemiology, host-pathogen interactions and management 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 and the Polytechnic University of Valencia on the control of other trunk pathogens implicated in the Eutypa dieback syndrome was also presented. Field trips were also made to the Utiel-Requena and Fontanars dels Alforins wine regions where experience with disease issues was gained along with general viticultural practices. Whilst in Valencia, Dr Sosnowski also visited with researchers from the Polytechnic University of Valencia.

Following the workshop, Dr Sosnowski travelled to Barcelona to visit researchers at the Institute for Food and Agricultural Research and Technology (IRTA) in nearby Cabrils, which included visits to vineyards in the Penedès wine region. Furthermore, Dr Sosnowski was invited to present SARDI/UA research on eutypa dieback and other grape diseases common in Australia at the IRTA Research Centre in Cabrils.

The objectives of attending the workshop were to;

i) Acquire the latest information on grapevine trunk diseases from around the world, and use it to develop further research initiatives through GWRDC and industry investment,
ii) Disseminate new knowledge from GWRDC eutypa dieback research (SAR 1001) 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 9th IWGTD in 2014

# 4. 8TH INTERNATIONAL WORKSHOP FOR GRAPEVINE TRUNK DISEASES

The 8th International Workshop on Grapevine Trunk Diseases (IWGTD) was held in Valencia, Spain, 18-21 June 2012. One hundred and twenty five delegates (Figure 1) attended the workshop representing 22 countries, with 56 oral seminars and 51 posters presented. The workshop was divided into five sessions; (i) Pathogen detection and characterization, (ii) Grapevine nurseries, (iii) Epidemiology, (iv) Host-pathogen interactions and (v) Disease management. Dr Sosnowski was on the scientific committee and also chaired the poster discussion session on Epidemiology, Host-pathogen interactions and Disease management.

Presentation of information from SARDI/UA collaborative research comprised an oral seminar and a 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.



Figure 1. Delegates of the 8th International Workshop on Grapevine Trunk Diseases in Valencia, Spain, 2012.

## 4.1 Pathogen identification and characterisation

Regina Billones-Baaijens of Lincoln University investigated 114 isolates representing seven *Botryosphaeriaceae* species isolated from New Zealand grapevine nursery plant material. Pathogenicity results showed that all species were able to produce necrotic lesions on green shoots and one-year-old rooted canes of Sauvignon blanc but with significant variation. *Neofusicoccum parvum* was the most pathogenic, followed by *N. luteum* and *N.australe* while *Diplodia mutila* and *D. seriata* were highly pathogenic on canes but not on green shoots. Except for *D. seriata* which appeared to be necrotrophic, most isolates of the six other *Botryosphaeriaceae* species were shown to move endophytically beyond the lesions. This could have implications in the nursery industry since the young, symptomless canes may be infected.

Jose Ramon Urbez-Torres of Agriculture and Agri-Food Canada presented a review of research on the Botryosphaeriaceae species in grapevine over the past decade. Twenty one different species are currently known to occur on grapes (eight of which occur in Australia) and causing disease symptoms such as leaf spots, fruit rots, shoot dieback, bud necrosis, vascular discoloration of the wood and perennial cankers. Much progress has been made in the development of novel diagnostic and detection techniques as well as in understanding the biology and epidemiology of Botryosphaeriaceae species, leading to the development of management strategies for the disease. The name Botryosphaeria dieback has been proposed to describe the wide range of different grapevine trunk disease symptoms associated with the disease.

In another presentation, Úrbez-Torres explained that since the discovery of *E. lata* as a trunk pathogen in the 1970s, *P. viticola* has typically been associated with Phomopsis cane and leaf spot and not the dead arms it was associated with in the early 1900s. Recently, *P. viticola* was the third most prevalent fungus isolated from grapevine cankers during field surveys in US grape-growing regions. Pathogenicity of the isolates was confirmed by re-inoculation causing dark-streaking of the wood, wedge-shape cankers, shoot dieback and cane bleaching. This study presents sufficient evidence that P. viticola is in fact a grapevine trunk disease pathogen, and thus; the name Phomopsis dieback is proposed here to describe the disease.

An investigation of wood diseases in table grapes in the Coachella and San Joaquin Valleys of California was presented by Philippe Rolshausen of UC Riverside in the USA. Vineyards ranged from 2 to 35 years old and 94% had esca disease while 65% had botryosphaeria canker with at least 12 different fungal species isolated from symptomatic wood. The occurrence of pathogens differed between the two regions which have different climatic conditions, a phenomena which is also occurs in Australia, with eutypa dieback predominating in cooler, southern regions and botryosphaeria in the warmer, northern regions.

Renaud Travadon of UC Davis in USA studied the genetic diversity of *Eutypa lata* in vineyards, stone fruit orchards and riparian areas of California. Two hundred and sixteen isolates were collected from grapevine, apricot, cherry, almond, pear and willow at four locations. The incidence of *E. lata* ranged from 30-50% in apricot orchards and vineyards, and from 4-24% in willows in riparian areas. Most isolates had unique genotypes, confirming that ascospores are the main infection propagules on all hosts. No differences in measures of genetic diversity were found among locations. In contrast, populations from willow were smaller and significantly less diverse than those from grapevine. Our findings suggest that *E. lata* spores are spread bilaterally between stone fruit orchards and vineyards, whereas infections in riparian areas are more likely originate from stone fruit and grapevine. This results suggest that grapevines are more at risk from infection from other grapevine and fruit crops than from willows.

Kendra Baumgartner of the United States Department of Agriculture in California studied the association between the cane and leaf spot symptoms and the wood-canker symptoms of Phomopsis disease in eastern North American vineyards. *Phomopsis* isolates were recovered from wood cankers in 23 vineyards with symptoms of Phomopsis cane and leaf spot (caused by *Phomopsis viticola*), in eight northeastern US states and Quebec, Canada. Species identified were *P. viticola*, *P. fukushii,* and *Diaporthe eres*. Spore inoculations to wounded, woody stems of potted Concord (*Vitis labruscana)* and Chardonnay showed that *D. eres* and *P. fukushii* were pathogenic (with mean lesion lengths of 7 mm, at one year post-inoculation), but significantly less so than *P. viticola* (mean lesion length of 14 mm). The co-occurrence of foliar symptoms and wood cankers and the fact that cankers can be caused by the additional species *P, fukushii,* and *D. eres*, indicates that controls for preventing the foliar symptoms of Phomopsis cane and leaf spot may not prevent wood cankers.

Francois Halleen of ARC Infruitec-Nietvoorbji in South Africa showed that sucker or spring wounds posed an entry port for several trunk disease pathogens in addition to *E. lata*, which has been demonstrated in the past. A survey was carried out to determine the incidence of trunk disease pathogens in sucker wounds of one and two-year-old wood. Isolations were made from 130 sucker wounds on both wine (Chenin blanc and Cabernet Sauvignon) and table grapes (Crimson Seedless and Thompson Seedless). Wine grape cultivars had a higher pathogen incidence than table grape cultivars. *P. viticola*, species of Botryosphaeriacae and *Phaeomoniella chlamydospora* were the most commonly isolated fungi at incidences of 43%, 35% and 28% from wine grape cultivars and 24%, 14% and 5% from table grapes, respectively. Other pathogens isolated included species of *Phaeoacremonium* and *Eutypella*. The infection of sucker wounds by trunk pathogens was further confirmed by reisolation of *P. chlamydospora* and *E. lata* on sucker wounds following artificial inoculation on actively growing one-year-old grapevine canes in the glasshouse. These results confirm that sucker wounds can be naturally infected by a range of trunk disease pathogens.

## 4.2 Grapevine nurseries

Increasing concern world-wide regarding the contribution of grapevine nurseries to trunk diseases led to a special session devoted to nursery practices in managing trunk diseases. Experiences from different countries were summarized, and all led to similar conclusions, that trunk disease management must begin in the nursery.

**France**

Philippe Larignon of the French Institute of Viticulture and Wine discussed the quality of nursery plants with regard to esca and Petri disease, botryosphaeria dieback and phomopsis leaf and cane spot. In Isolated from grapevine nursery samples taken in Burgundy, Provence-Alpes-Côte d'Azur and Midi-Pyrénées, were *P. chlamydospora* from up to 61%, 34% and 19%, respectively, *P. aleophilum* from up to 22%, 4% and 6.4%, respectively, *Phomopsis* spp. from up to 90%, 88% and 45%, respectively, and species of the Botryosphaeriaceae from up to 82%, 68% and 77%, respectively. In general, plant quality varied with nursery practices. Other French studies revealed that scion mother vines were a source of trunk disease fungi with the various species detected in up to 5%

of propagation material. In another study, the surface of scion or rootstock cuttings were detected with up to 46% *P. chlamydospora*, 26% *P. aleophilum* and 28% Botryosphaeriaceae. In another study, trunk disease species were detected in propagation material collected from vineyards with good and poor sanitary status. In terms of control, hot water treatment (50°C, 45 min) provided the best efficacy, compared with other chemical treatments on several fungi (*P. chlamydospora*, *D. seriata*, *Phomopsis* spp.), but rootstock and scion cuttings were sometimes contaminated in the field nursery. In conclusion, even though nursery material was detected with varying levels of infection by trunk disease pathogens, optimal sanitary nursery processes could not be clearly identified and further studies are necessary.

**Spain**

David Gramaje of the Institute of Sustainable Agriculture in Spain presented information from surveys of grapevine trunk pathogens in Spanish grapevine nurseries. Previous investigations led to the conclusion that planting material used in young vineyards is infected, either systemically from infected mother vines or by contamination during the propagation process (e.g. soil and planting material from rootstock mother fields, post storage hydration tanks, grafting tools, callusing media and soil and planting material from open-root field nurseries). This research did not lead to improved propagation practices in Spain so an intensive survey of nurseries was undertaken covering all aspects of the propagation cycle. All nurseries used hydration, and some soaked cuttings in water for more than 4 days. Registered source blocks are inspected for disease status at least once a year. Most nurseries sell a significant percentage of second quality vines to grape growers. Approximately 50% of survey respondents did not use any individual sanitary practices. The majority reported that they knew about hot-water treatment (HWT), but had never used it. The reliability and efficacy of HWT was questioned by most nursery operators who argued that significant losses are still being attributed to HWT. Barriers to adoption include the ineffectiveness to eliminate the pathogens, the logistics involved in applying these treatments and the lack of studies in commercial batches. It was concluded that research to demonstrate practical propagation procedures and development of guidelines for nurseries to produce high quality vines is required.

An evaluation of factors that influence the viability of hot-water treated planting material in Spanish nurseries was also presented by David Gramaje. Hot water treatment (HWT) at 53ºC for 30 min has been shown to be an effective control method for black-foot and Petri disease pathogens in Spanish grapevine nurseries but anecdotal reports of unacceptably high rates of mortality have been associated with HWT. Several critical factors, such as plant hydration and cold storage, have emerged in the production of quality grapevine planting material. The research presented indicated that long time cold storage could be detrimental to planting material, especially when plants have not been previously hydrated following HWT.

**South Africa**

Francois Halleen of ARC Infruitec-Nietvoorbji presented the current situation of grapevine trunk diseases in South African nurseries. Increased complaints of poor establishment in new vineyards led to investigations which identified the source of infection to be rootstock mother vines for Petri disease, botryosphaeria dieback and phomopsis. It was confirmed using molecular techniques that infections occurred during the hydration, grafting and callusing processes. Hot water treatment (HWT; 50°C for 30 min) of grafting material was identified as the most effective method to reduce Petri disease pathogens. Further research developed an integrated management strategy for the nursery industry which included pruning wound protection and sanitation in mother blocks, incorporation of chemicals and/or biological control agents in hydration and soak water during all stages of preparation and grafting of propagation material, HWT of propagation material prior to grafting, as well as HWT of dormant nursery plants. A protocol where graft material is soaked in a carbendazim solution prior to cold storage, HWT and Sporekill soak before grafting, followed by a Trichoderma soak after grafting and once more before planting reduced incidence of *P. chlamydospora*, *Phaeoacremonium* spp. and Botryosphaeriaceae spp. It was concluded that the challenge is to convince local nurseries to implement and maintain strategies to eliminate or reduce trunk pathogen infections.

**Australia**

Helen Waite of the NWGIC gave a perspective of the nursery propagation practices affecting grapevine trunk diseases in Australia. Young vine decline (YVD) has been associated with the pathogens Botryosphaeriaceae spp., *Cylindrocarpon destructans*, *P.chlamydospora* and *Phomopsis viticola*. Although HWT was shown to be an effective control for these pathogens, it was not routinely used to treat cuttings in propagation because of concerns about vine failure associated with this technique. Nursery practices contributing to cross contamination, physiological stress and ultimately vine failure included poor nursery sanitation, prolonged hydration of cuttings (sometimes exceeding 12

hours) and poor cold storage conditions. A downturn in the wine industry led to reduced plantings from the mid 2000s, stalling research developments in nursery propagation practices. A recent survey of Australian nurseries identified inconsistent propagation protocols and continued reluctance to use HWT. Continued reports of YVD, even in HWT vines, led to further research implicating nursery soils and contaminated water as sources of re-infection following HWT. It was concluded that the adoption of standard operating procedures incorporating HWT and good nursery sanitation are necessary to manage YVD and the efficacy of the standard HWT regime (50°C for 30 min) may need to be reviewed.

## 4.3 Epidemiology

Jordi Luque of the Institute of Food and Agricultural Research and Technology (IRTA) in Spain studied the incidence of natural infections by trunk pathogens in two mature vineyards. Results revealed that for *D. seriata* and *P. chlamydospora*, higher frequencies were detected in spring (after the late pruning) than in winter (after the early pruning). *E. lata* was detected infrequently so no differences were observed. This is in contrast to previous research in other regions of the world with differing climatic conditions, which suggested reduced inoculum levels in late winter and early spring.

In another study, Javier Legorburu of the Basque Institute for Agriculture Research & Development in Spain investigated pruning wound susceptibility to infection by *E. lata* ascospores. Vines pruned in early, mid and late winter were inoculated with ascospores either on the same day or one, two, three and four weeks afterwards. Analysis of data from isolations made 2 weeks after each inoculation showed the diminution of susceptibility both over the winter and with time after pruning date. Models were developed to provide quantitative measures of wound susceptibility diminution that allow comparison between experiments. The model can be used to quantify other factors affecting the susceptibility and/or infection pressure, such as weather conditions.

There is no recent information on inoculum levels and wound susceptibility over the pruning season in different climatic regions of Australia. Studies are required to ensure that recommendations of late pruning are appropriate for pathogens causing both Eutypa and Botryosphaeria dieback in Australia.

Javier Legorburu also investigated the relationship between foliar symptoms of Eutypa dieback and the presence of *E. lata* in the vine. In previous research they observed a weak relationship and in the current investigation no relationship was found between the fungus and foliar symptoms. This supports findings from research at SARDI, which went on to determine that foliar symptoms are related to environmental conditions such as rainfall and temperature.

Marlene Jaspers of Lincoln University in NZ presented a study investigating the susceptibility of different wounded tissue types to conidial inoculation with *N. luteum* (Botryosphaeria dieback). The fungus was reisolated predominantly from trunks, pruned cane ends, shoots, buds and berries, but not from roots. Results from this research indicate that *N. luteum* can infect all aerial parts of grapevine and progresses to young stem tissues where it eventually causes wood necrosis. It was concluded that it is important to remove inoculum sources such mummified berries and infested vineyard trash and fungicide sprays may be required before bud burst in order to manage the disease.

A study of almond trees on the island of Mallorca in Spain was a part of the PhD studies of David Gramaje at the Polytechnic University of Valencia. Observation of almond tree decline and wood decay on the island led to a study in order to determine the cause. *N. parvum* was the dominant species, followed by *E. lata*, *D. olivarum* and *N. australe*. First reports from almond include *D. olivarum* and *Pm. iranianum* and two new species were described, *Collophora hispanica* sp. nov. and *Phaeoacremonium amygdalinum* sp. nov. These results confirm that almond trees may serve as potential inoculum sources for trunk disease pathogens, with implications in the epidemiology and control of these diseases.

Olive trees are a potential alternative host for grapevine trunk disease pathogens in California according to Jose Ramon Úrbez-Torres. During a study of olive twig and branch dieback in California, 16 fungal species known to be involved in grapevine trunk diseases were isolated from symptomatic wood of olive trees. They included species associated with Eutypa dieback, Botryosphaeria dieback Petri disease, esca, black foot and wood decay of grapevines. Fruiting bodies for most of the species were also found on symptomatic olive twigs and branches. Olive trees are often grown near vineyards throughout grape-growing regions with Mediterranean climate, including Australia. These findings suggest that olive trees may be an important source of inoculum for grapevine trunk disease

pathogens and may have important implications in the epidemiology, and consequently the management of these diseases.

Evidence was presented by Francois Halleen that arthropods can disseminate grapevine trunk disease pathogens. Over 5600 samples were collected by washing fungal spores from a range of different arthropod species including ladybirds, ants, beetles and earwigs. Pathogens detected were associated with Petrie disease, esca, botryosphaeria dieback, eutypa dieback and phomopsis. Several of the arthropod species were associated with infected pruning wounds. Research is continuing to confirm whether arthropods are able to infect vines through pruning wounds.

The minimum spore inoculum concentration required to infect pruning wounds with *Diplodia seriata* (Botryosphaeria dieback)and *Phaeomoniella chlamydospora* (esca) was presented by Georgina Elena of the University of Barcelona and IRTA in Spain. Five-year old potted vines were pruned and inoculated with spore suspensions of 4000, 2000, 1000, 100 and 10 conidia per wound. Four months later, canes were assessed and it was shown that inoculation with 10 conidia per wound led to cane infection (<50%). Infection by *D. seriata* was 100% with 1000+ conidia per wound, while for *P.chlamydospora* the mean infection percentage ranged between 80-95 % at the highest concentration. Recent SARDI/UA research showed that 10 spores of *E. lata* per wound led to 20% infection in detached canes in the glasshouse and further investigation is required to determine whether lower concentrations, reflecting closer to natural disease pressure than the 500-1000 spores currently used in field trials, could be used in future research. Georgina will travel to Adelaide and expand her research to *E. lata* at SARDI during a sabbatical planned for next year.

Laura Mugnai of the University of Florence in Italy presented interesting information on the use of unmanned aerial vehicles (UAVs) for the detection, development and spatial distribution of grapevine trunk diseases with remote sensing. The preliminary results obtained in the pilot experiment, showed a high level of correlation between Normalised Differential Vegetation Index (NDVI) and Esca symptoms. The method has demonstrated both the ability to discriminate symptomatic from asymptomatic plants but also to detect infected but asymptomatic plants, which had expressed symptoms in the past. The use of aerial imagery for detection of grapevine diseases may have potential for surveillance of exotic disease incursions. Proposed research by SARDI and the Grapevine Phylloxera Board of SA aims to develop surveillance methods for grapevine disease using eutypa dieback as a model and based on current surveillance methods for Phylloxera.

A survey of French vineyards was conducted by J. Grosman of DRAAF-SRAL for grapevine trunk diseases during the period 2003 to 2008. It was reported that up to 83% of vineyards showed symptoms of esca and/or black dead arm and eutypa dieback was recorded in up to 53% of vineyards. It was estimated that around 11% of French vineyards are unproductive due to these three diseases and in the Loire-et-Cher region alone, the economic impact was estimated at 5-6 million euros per year. These findings highlight the economic impact of trunk diseases in France and that it is important the Australian wine industry is aware of the full impact of trunk diseases in this country to ensure appropriate management of vineyards for long-term sustainability.

## 4.4 Host-pathogen interactions

Cheusi Mutawila from the University of Stellenbosch in South Africa demonstrated the expression of defence related genes through the response of *V. Vinifera* cells to culture filtrate of *E. lata* and *Trichoderma atroviride*. The research investigated the pathogenesis related proteins class 2, 3, 4, 6, chitinase class IV and secondary metabolism genes of the phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL), Coumaroyl Co-A (CCo-A) and stilbene synthase (STS), chalcone synthase (CHS). Results indicated that both the pathogen and biocontrol triggered a defence response by the host and it was concluded that *T. atroviride* may have potential to enhance plant defence against pathogen infection.

## 4.5 Disease management

Vincent Dumot from the Bureau National Interprofessionnel du Cognac in France has been evaluating the long-term effects of different cultural practices on the development of eutypa dieback and esca diseases. The study evaluated three factors: i) training system (cane- or cordon-pruned); ii) nutrition (no fertilizer, 50-0-100 NPK or 100-0-100) and iii) rootstock (41B, Fercal, RSB and Rupestris du Lot.) in a 2 ha vineyard of cv. Ugni blanc planted in 1991 by assessing foliar symptoms and mortality each year over a 20-year period. The cane-pruned system resulted in less foliar symptoms of eutypa

dieback but more dead plants than for cordon-pruned. This suggests less infection on the cane-pruned vines due to less surface area of wounds, however the closer proximity of wounds to the trunk may have led to increased death of vines. The higher the nitrogen input, the more severe the foliar symptoms and death of plants was. This confirms preliminary controlled environment studies at SARDI, showing increased growth of *E. lata* in vines with higher nutrition, suggesting the pathogen growth may be enhanced with higher nutrient availability in the plant. Of the rootsocks, 41B showed less symptoms of eutypa dieback, but more symptoms of esca than the other rootstocks which were similar to each other. There were more dead plants on Rupestris du Lot than for the others. There is little information available on the susceptibility of rootstocks to trunk diseases.

In a second presentation by Vincent Dumot, the long-term effect of pruning wound protection was also evaluated in the same experiment described above. Each season over the 20 years, pruning wounds were treated with either; ATEMICEP (carbendazim + cyproconazole), ESCUDO (carbendazim + flusilazole), LAC BALSAM (mastic), copper based spray at bud-break or late pruning without wound protection. All treatments, except copper, resulted in reduced symptoms of eutypa dieback compared with controls. Foliar symptoms of esca disease were reduced for both chemical wound protectants but not for any other treatments. Vines subjected to late pruning expressed less symptoms of eutypa dieback than the control, but no differences were observed for esca symptoms. This supports worl-wide research on pruning wound treatments for control of eutypa dieback.

These studies represent the first long-term experiment to evaluate cultural and chemical management strategies for trunk diseases. Results provide some insight into the efficacy of the strategies evaluated, however conclusions are limited by the use of foliar symptom assessment rather than assessment of infection by pathogens in vines. A similar study in Australia evaluating incidence of infection would provide information on the long-term effects of strategies being implemented currently and provide the basis for recommendations in the future.

Doug Gubler of the University of California Davis in the USA summarized control of grapevine trunk diseases in California. In recent years myclobutanil and thiophanate methyl have been registered as a tank mix that can be applied by tractor as an over the vine spray on pruning wounds. These treatments are effective for only 2-3 weeks while pruning wounds remain susceptible for up to 6-8 weeks during the winter. Double pruning provides 90-95% control of botryosphaeria and eutypa dieback based on decreased susceptibility during late winter and early spring in California. Recently a highly antagonistic strain of *Trichoderma viride* has been isolated from a grapevine pruning wound and is currently being developed into a biocontrol product. In another recent development, Vitiseal paste (certified organic product in California) sprayed onto wounds at a concentration of 10% acts as a physical barrier and has anti-fungal properties. Finally, spray application of lime sulphur during dormancy has resulted in a 75% reduction in fruiting bodies on bark of pathogens involved in Botryoshphaeria dieback. A request has been made for Vitiseal paste to be provided to SARDI for inclusion in pruning wound treatment evaluations. It may be included in future planned evaluation of polymers and silicates sprayed onto wounds to prevent infection by trunk diseases.

Allessandro Spagnolo from University of Reims in France has demonstrated the possible efficacy of a species of *Chaetomium*, isolated from an asymptomatic grapevine. In a preliminary *in vitro* screening against pathogens causing esca and botryosphaeria dieback, the *Chaetomium* sp. was antagonistic against *Phaeomoniella chlamydospora, Phaeoacremonium aleophilum, Neofusicoccum parvum* and *Diplodia seriata*. Further evaluation of this potential biocontrol agent is planned using potted grapevines.

## 4.6 Workshop field trip to Utiel-Requena

On Wed 20 June, a field trip was made to Utiel-Requena, which is about 70 km west of Valencia. The region is planted to 40,000 ha of predominantly red wine cultivars, with 80% planted with cv. Bobal (Figure 2a), a native cultivar to the region, and second most widely planted to cv. Tempranillo. Bobal is well adapted to the local environment of altitude 700m and with short and dry summers and cold winters. Bud break is later in Bobal, helping it avoid spring frosts which occur regularly in this region. With high levels of anthocyanins in the skin, wines made from Bobal have an intense colour and character, and a high level of tannins that is good for ageing. It is often blended with other varieties to provide more intense red colour.

Prof Josep Armengol hosted the tour starting with a presentation on the region and the School of Viticulture and Oenology of Requena (Figure 2b). Visits were made to a number of vineyards in the

region and provided an opportunity for workshop delegates to observe grapevine trunk disease symptoms common to the region. These included foliar symptoms of eutypa dieback (Figure 2c), chlorosis of young vines (Figure 2d) along with associated wood staining from the same vine (Figure 2e). Another interesting symptom in some very old Bobal vines were stunted shoots with interveinal chlorosis of leaves (Figure 2f). It had similarities to iron deficiency symptoms, but a range of trunk disease pathogens has been isolated from the vine. Also evident in these vineyards were esca-like tiger-striped symptoms (Figure 2g). Trunks of these vines when cut were observed with wedge shaped staining (Figures 2h-i) consistent with botryosphaeria and eutypa pathogens. There was much discussion about the different symptoms amongst delegates (Figures 2j-k).

## 4.7 Committee Meeting of the International Council on grapevine Trunk Diseases

On 18 June the Annual General Meeting of the International Council of Grapevine Trunk Diseases (ICGTD) was held, of which Dr Sosnowski is on the Executive Council as Regional Coordinator for Australasia. At the meeting, Dr Sosnowski nominated to host the 9$^{th}$ International Workshop for Grapevine Trunk Diseases in 2014 in Adelaide. The nomination was accepted and it was agreed that the next meeting would be held in Adelaide. This workshop is likely to attract around 100 international researchers to Australia, and will give the viticulture industry access to all of the latest information around trunk disease management. This will also build on SARDI's international reputation as a centre of excellence in grapevine trunk disease management research.

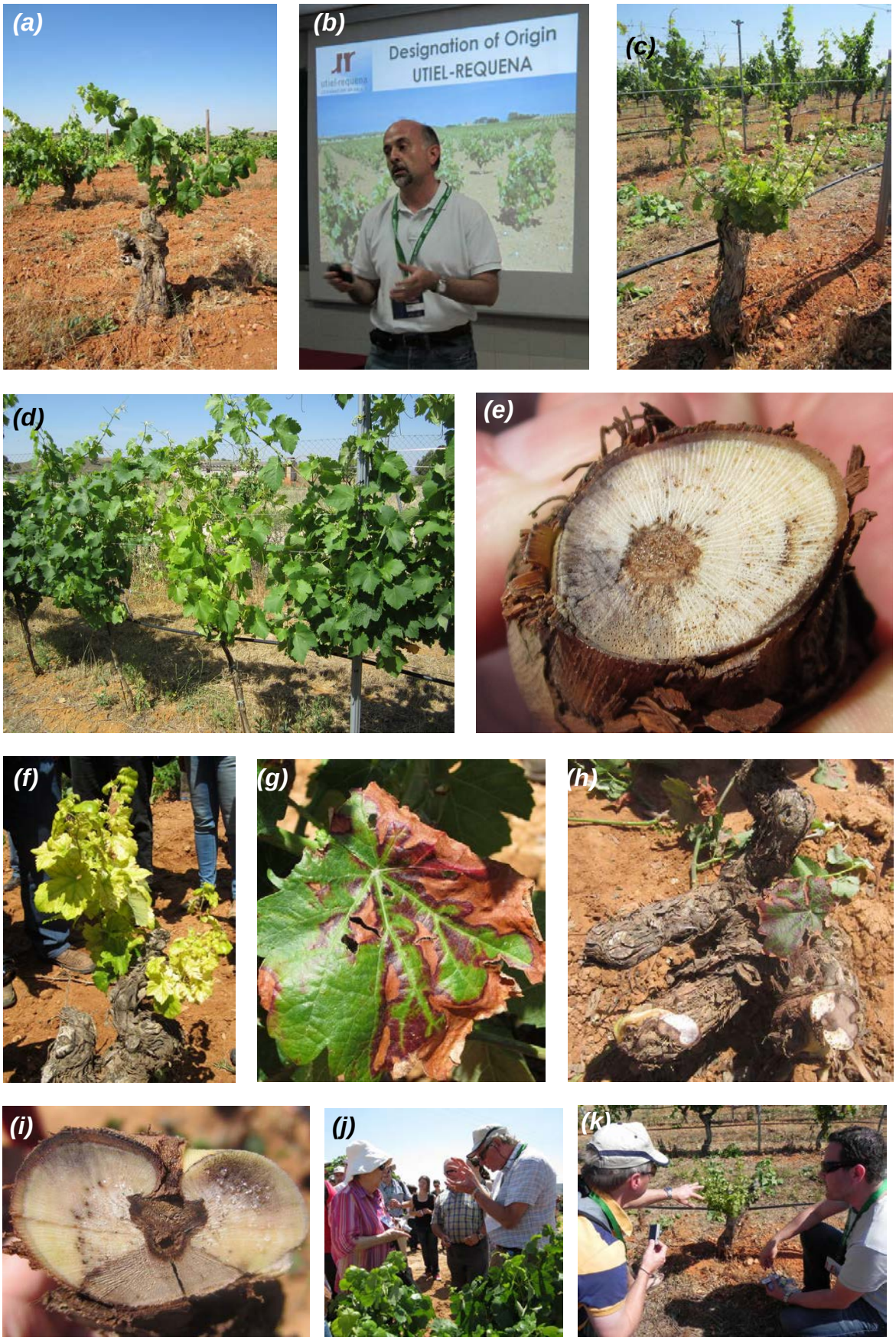



Figure 2. Photos from the vineyard field trip to the Utiel-Requena wine region of Spain. (a) introductory seminar by workshop convenor Dr Josep Armengol (Polytechnic University of Valencia), (b) 100 yo cv. Bobal vine, (c) foliar symptoms of eutypa dieback, (d) stunted, chlorotic foliage observed on a young vine, (e) staining observed in cross-section of trunk of young chlorotic vine, (f) chlorotic leaves with green veins, (g) leaf symptoms resembling esca, (h-i) top view of Bobal bush vine trunks with cross-sectional staining, (j) Dr Richard Smart (Smart Viticulture) imparts his knowledge to Dr Marlene Jaspers (Lincoln University, NZ) as other delegates look on, and (k) Dr Sosnowski and Dr Jose Urbez Torres (Agri-Food Canada) discuss eutypa dieback symptoms.

## 5. POLYTECHNIC UNIVERSITY OF VALENCIA

A visit was made to the Polytechnic University of Valencia on 22 June where Prof Josep Armengol (Faculty member of the Research Group on Plant Pathogenic Fungi) hosted a tour of their facilities (Figure 3a). The group conducts research on fungal plant pathogens which cause diseases on vegetable, fruit, ornamental and forest plants. They also hold the Spanish Reference Laboratory for the identification of fungal plant pathogens, being responsible for the identification of quarantine pathogens, and new and uncommon diseases in Spain. More recently, citrus diseases have been the main focus of the group, but trunk disease aspects of grapevine nursery production have also been investigated, particularly through the PhD research of Dr David Gramaje, who spent 3 months working with the eutypa dieback research team at SARDI in 2010.

Prof Armengol and Dr Gramaje also hosted Dr Sosnowski and several other delegates on a visit of vineyards in the Valencia region of Fontanars dels Alforins (Figure 3b). It provided some incite into viticultural and wine production in this part of Spain and the highlight was the winery, Celler del Roure, where wine is being fermented under ground in traditional tinajas (buried clay jars or amphoras) dating from the early seventeenth century (Figure 3c-d).




Figure 3. Visit to the Polytechnic University of Valencia (a) J Urbez-Torres, M. Sosnowski, J Armengol & D Gramaje (L to R) and the Valencian region of Fontanars dels Alforins with (b) a typical vineyard and (c-d) a visit to the underground tinajas (dating back to early 17$^{th}$ century) and where wine is still produced today.

# 6. INSTITUTE FOR FOOD AND AGRICULTURAL RESEARCH AND TECHNOLOGY (IRTA), CABRILS

A visit to the Institute for Food and Agricultural Research and Technology (IRTA), Cabrils (Figure 4a) provided the opportunity for Dr Sosnowski to meet with Dr Jordi Luque and PhD candidate Ms Georgina Elena (Figure 4b) to discuss collaboration planned between SARDI and IRTA.

Similarly to SARDI in South Australia, IRTA is a research institute owned by the Government of Catalonia and falls under the Department of Agriculture. IRTA's mission is to contribute to modernising, improving, boosting competitiveness, and fostering sustainable development in the sectors of agriculture, food, agroforestry, aquaculture, and fishing, as well as in all areas of activity directly or indirectly related to the supply of healthy, high-quality food to consumers, while also contributing to food safety and safe processing of foodstuffs and in general enhancing the health and well-being of the population. Its general objectives are to promote research and technological development in the area of agri-food, to facilitate the transfer of scientific advances and to evaluate its own technological advances whilst seeking the utmost coordination and collaboration between the public and private sectors. IRTA, like SARDI, has a strong focus on building links with international research organisations.

A tour of the IRTA facility at Cabrils showed the extent of research being conducted there, particularly in grapevine and a range of other fruit tree crops and cut flowers. Dr Luque has been conducting research similar to that at SARDI, investigating the effect of stress on grapevine trunk disease development. A joint paper on this topic was recently published between SARDI, IRTA and the Uni of Adelaide (*Phytopathologia Mediterranea*, 2011, 50, S127−S138). Currently at IRTA the main focus is botryosphaeria dieback and at SARDI it is eutypa dieback. With plans for more research on botryosphaeria dieback at SARDI in future this collaboration will be very useful.

Ms Elena, who is undertaking a PhD through the University of Barcelona and based at IRTA is investigating epidemiological aspects of the trunk diseases botryosphaeria dieback and esca. She has been offered a scholarship to work at SARDI with the trunk disease research group for 3 months in 2013. Plans were discussed for the scope of research she will undertake in South Australia, which will include aspects of her PhD thesis focussing on eutypa dieback and drawing on expertise at SARDI, as well as assisting the SARDI team in their project activities.

A visit was made to the Penedès region, east of Barcelona. IRTA field experiments are conducted on commercial vineyards in this region and several were visited. Ms Elena is developing a new technique to protect pruning wounds from natural infection whilst maintaining conditions as near to naturally as possible in order to study infection periods throughout the dormant season. The technique currently consists of a clear, flexible rubber tube over the cane with a cotton wool stopper at the top to allow air flow but prevent spores from reaching the wounds (Figure 4c). Condensation on the inside of the tube suggests the humidity is high around the wound so adjustments to the technique will be necessary. Also observed in the vineyards were apoplexy (Figure 4d) associated with Petri disease and typical tiger-striped leaf symptoms of esca (Figures 4e-f). Eutypa dieback was also evident in vineyards with dieback of cordons and spur positions, stunted shoots with characteristic cupped leaves and necrotic margins and cross-sectional wedge-shaped staining in cordons (Figures 4g-i). Also spotted in vines were new downy mildew infections (Figure 4j) and in another vineyard, old infections which had been sprayed with copper (Figure 4k). Phylloxera is common in Spain and leaf galls were observed on vines (Figure 4l). Finally, the disease armillaria root rot is also present in this region and is causing significant losses in vineyards (Figures 4m-n). With no effective control strategies and the inability to replant in affected soil, this disease poses a significant threat in the region.




Figure 4. (a) Visit to the Institute for Food and Agricultural Research and Technology (IRTA) in Cabrils, (b) Dr Jordi Luque and Ms Georgina Elena, (c) novel method for protecting wounds against natural infection by wound pathogens, (d) apoplexy symptoms, (e-f) foliar symptoms of esca, (g-h) foliar symptoms of eutypa dieback, (i) trunk staining in cross section of vine with eutypa dieback, leaf lesions of downy mildew (j) pre and (k) post spraying with copper, (l) phylloxera galls and (m-n) vine loss due to armillaria root rot.

# 7. MAIN OUTCOMES

## 7.1 Collaboration

Collaborative links were strengthened with researchers from organisations around the world particularly at the Polytechnic University of Valencia and the Institute for Food and Agricultural Research and Technology (IRTA).

- PhD student Georgina Elena (University of Barcelona / IRTA) along with her supervisor Dr Jordi Luque (IRTA) discussed with Dr Sosnowski her plans to visit SARDI for 3 months between May and August in 2013. Funding is already in place through a scholarship provided by the Spanish government. Research will include epidemiological studies on eutypa dieback parallel to the studies she is currently conducting on botryosphaeria dieback and esca. This information will build on our current knowledge of eutypa dieback. Ms Elena will also gain experience working on different aspects of the trunk disease program assisting the research team.
- Further discussions between Drs Luque and Sosnowski regarding research on the effects of stress on trunk diseases led to agreement to develop a collaborative program between IRTA and SARDI to further the results already achieved at each institution.
- Discussions with Dr Marlene Jaspers (Lincoln University, New Zealand) have led to plans of collaborating on trunk disease research being proposed in New Zealand and led by SARDI.

## 7.2 Networking

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

## 7.3 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. Much of this information has been summarised in this report.

## 7.4 Communication

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

## 8. 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.
- Develop new research to:
  - (i) confirm highest risk periods of infectivity for pathogens causing both eutypa and botryosphaeria dieback
  - (ii) determine optimal timing of application for pruning wound protectants for both eutypa and botryosphaeria dieback
  - (iii) evaluate alternative wound protection treatments that can be applied with a sprayer, such as polymer and silicate based physical barriers
  - (iv) demonstrate long-term control of trunk disease using wound protection strategies being implemented currently which will provide the basis for future recommendations
  - (v) do cost/benefit analysis the full impact of trunk diseases and control in Australia to ensure appropriate management of vineyards for long-term sustainability
  - (vi) develop aerial surveillance methods for exotic grapevine disease such as Pierce's disease using eutypa dieback as a model and based on current surveillance methods for Phylloxera
- Finalise arrangements to host a research study visit during May-Aug 2013 by Georgina Elena of the University of Barcelona and IRTA in Spain. Travel, accommodation and living expenses will be funded by the Spanish government and SARDI will provide in kind support through laboratory and office facilities and supervision.
- With the successful bid to host the 9$^{th}$ International Workshop for Grapevine Trunk Diseases in Adelaide in November 2014, an organizing committee should be formed and workshop arrangements commenced. Australian industry members should also be invited to participate in order to maximise the benefit of hosting this event in Australia.

## 9. 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 Spain, Drs Josep Armengol, David Gramaje and Jordi Luque and Ms Georgina Elena.

# 10. APPENDICES

***Appendix 1. Major contacts***

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## *Appendix 2. Travel Itinerary*

| July 2012 | |
|---|---|
| Sat 16 – Sun 17 | Travel from Adelaide to Valencia, Spain |
| Mon 18 – Thu 21 | 8th International Workshop on Grapevine Trunk Diseases |
| Fri 22 – Sat 23 | Visit with Prof Josep Armengol at Polytechnic University of Valencia & vineyard visits |
| Mon 25 | Travel to Barcelona |
| Tues 26 - Wed 27 | Visit with Dr Jordi Luque at IRTA + vineyard visits |
| Thu 28 – Sat 30 | Return from Barcelona to Adelaide |

## *Appendix 3. Abstracts*

### 8th International Workshop for Grapevine Trunk Diseases

### Oral presentation

**Optimising pruning wound protection to manage eutypa dieback**

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The fungus *Eutypa lata* infects grapevines through pruning wounds, colonising wood of cordons and trunks and causing eutypa dieback. Wound treatments reduce infection by *E. lata*, and benomyl and carbendazim, which were the most effective fungicide treatments, are no longer available for use on grapes in Australia. To provide data to assist registration in Australia, five alternative fungicides, three natural products and two surfactants were selected for field evaluation, based on results from previous research. Treatments at different concentrations were applied by hand and wounds inoculated with ascospores of *E. lata*. Twelve months later, treated canes were removed and assessed by isolation on PDA. Results indicated that three of the fungicides significantly reduced infection by *E. lata* and efficacy increased with dose rate; tebuconazole (59-88% control), fluazinam (21-58%) and pyrimethanil (33-53%). There was no benefit in adding surfactants Pentrabark or Du-Wett and natural products did not reduce infection significantly. The fungicides were also evaluated in the greenhouse using a detached cane assay where treatments were assessed 4 weeks after application and results suggest it may offer a rapid alternative method of evaluation. Commercial sprayers (air-blast, air-shear, fan and recycle) were evaluated with the aim of improving the efficiency of applying pruning wound treatments. Using tebuconazole, some sprayers achieved control of eutypa dieback similar to hand-application, providing deposition was maximised by focussing spray at the pruning wound zone using water volume of 600 L/ha. Results will be discussed in relation to optimising management of eutypa dieback.

### Poster presentation

***In vitro* evaluation of fungicides to control diatrypaceous fungi associated with grapevine trunk disease**

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A number of Diatrypaceous fungi have been isolated from the wood of cankered grapevines and shown to be pathogenic when inoculated in stems of potted vines. Until now management strategies have focused on the development of procedures and products to prevent or reduce *Eutypa lata* infection through pruning wounds. In a study conducted in Australia, six fungicides were evaluated *in vitro* for their efficacy in reducing mycelial growth of two isolates each of *Eutypa lata*, *Cryptovalsa ampelina*, *Diatrypella vulgaris*, *Eutypa leptoplaca* and *Eutypella citricola*, and one isolate of *Eutypella microtheca*. The fungicides carbendazim, fluazinam, tebuconazole, and prothioconazole + tebuconazole, incorporated in PDA at 1 or 10 ppm, were effective at reducing or inhibiting mycelial growth of all Diatrypaceae spp. tested. Pyraclostrobin reduced mycelial growth of most of the fungal species tested by at least 50%. Pyrimethanil was least effective at reducing mycelial growth of these pathogens. Five of the six fungicides evaluated in this *in vitro* study have demonstrated efficacy against Diatrypaceae spp. but require further evaluation in the field. This study is the first approach for fungicide evaluation against mycelial growth of diatrypaceous fungi, other than *Ea. lata*. It will contribute to the development of integrated control strategies for grapevine trunk diseases.