“Oral presentation at the 10th International Workshop on Grapevine Trunk Diseases in Reims, France”
4-7 July 2017

FINAL REPORT to
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1. ACKNOWLEDGEMENTS

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2. ABSTRACT

Dr. Regina Billones-Baaijens attended the 10th International Workshop on Grapevine Trunk Diseases in Reims, France. The workshop was held at the Centre des Congres, sale Clovis, Reims, France on 4-7 July 2017. She presented orally the new molecular tools she developed to detect and quantify Eutypa dieback (ED) and Botryosphaeria dieback (BD) pathogen inoculum from the environment. She further presented the preliminary results of the spore dispersal patterns of ED and BD pathogens in four wine growing regions in Australia using the above-mentioned molecular tools. These studies were part of the recently completed research on Practical Management of Grapevine Trunk Diseases (SAR 1205) funded by Wine Australia (WA). Dr. Billones-Baaijens was also co-author of four additional oral presentations and four poster presentations at this workshop. Key plant pathologists and specialists in grapevine trunk diseases participated in the workshop and the information gathered provided her with the latest information on the diagnostic tools and epidemiology of grapevine trunk diseases. The information gained from this workshop will be transferred to Australian grape growers and industry personnel via future oral presentations and industry articles.

3. EXECUTIVE SUMMARY

Eutypa dieback (ED) and botryosphaeria dieback (BD) are serious diseases of grapevines worldwide causing cankers, dieback and eventually the death of vines. These grapevine trunk diseases rank in the top five priority diseases of the Australian wine grape industry (GWR08/04). The diseases persist in affected vines from season to season, causing long term decline. They are becoming more prevalent as vineyards age and are a threat to the Australian wine industry, which contributes $40 billion to the economy.

The International Workshop on Grapevine Trunk Diseases (IWGTD) is organised by the International Council on Grapevine Trunk diseases (ICGTD) every two years to discuss recent research on the etiology, epidemiology and control strategies of different grapevine trunk diseases including BD, ED, young vine decline and other trunk disease pathogens. Key plant pathologists and specialists in grapevine diseases and other grapevine industry stakeholders participated in the workshop. This forum is an important opportunity for Australian researchers to exchange information on grapevine trunk diseases and to keep updated on the latest research findings in grapevine trunk disease (GTD) research.

The workshop attracted ~220 participants from 27 countries particularly European countries including France, Italy and Spain. There were also representatives from the USA, Canada, South America, China, Middle East and New Zealand. Of the four participants from Australia, three delegates Dr. Mark Sosnowski (SARDI), Dr. Billones-Baaijens and Mr. Matthew Ayres (SARDI) are on the project team of the recently Wine Australia-funded research “Grapevine Trunk Disease Management for Vineyard Longevity in Diverse Climates of Australia” (SAR 1601). Ayres and Billones-Baaijens received Wine Australia travel bursaries to attend the workshop.
The sessions at the workshop covered pathogen characterisation and identification, epidemiology, plant-pathogen interactions, microbial ecology and disease management in nurseries and vineyards. A total of five invited lectures of the main achievements and future prospects in GTD research, 49 papers and 92 posters were presented at the workshop. Dr. Billones-Baaijens presented two papers on the new molecular tools she developed to detect and quantify ED and BD pathogens in Australian vineyards and the preliminary results of spore trapping studies using these molecular tools. These studies were part of the recently completed research on Practical Management of Grapevine Trunk Diseases (SAR 1205) funded by Wine Australia. Dr. Billones-Baaijens was also a co-author of four oral presentations and four poster presentations at the workshop.

The workshop participants visited experimental vineyards near Epernay in the Champagne region. Some of the research conducted in these experimental vineyards include improving viticultural practices to increase yield and reduce impact of GTDs and breeding programs for varietal improvement and disease management. The field tour also included a visit to the cellar door and wine tasting at the Laison de Champagne Mercier.

Dr. Billones-Baaijens’ attendance at the 10th IWGTD was supported by the travel bursary from Wine Australia, the National Wine and Grape Industry (NWGIC) travel grant and her personal funds. Dr. Billones-Baaijens’ oral presentations showcased the research capabilities of the Australian researchers in the field of molecular biology and GTD epidemiology. Her attendance also strengthened her existing networks with international researchers and assisted in developed new links for future potential collaborations. She discussed with Dr. Jose Urbez-Torres (Summerland Research and Development Centre, BC Canada) and Dr. David Gramaje (Instituto de Ciencias De Vid Y Vino, Spain) potential collaborative research looking at GTD infection thresholds in propagation materials. She also established contact with Dr. Dario Cantu from the University of California – Davis and Dr. Moustafa Selim from Geisenheim University, Germany that may also lead to future collaborations between NWGIC and their respective institutions. Dr. Cantu is an expert in the field of bioinformatics and comparative genomics while Dr. Selim specialises in nuclear magnetic resonance spectroscopy, an imaging system used for the detection GTDs in plants. She was further invited to conduct a short seminar later in 2017 on her current research on GTDs in Australia by Assoc. Prof. Eirian Jones from Lincoln University, New Zealand.

4. Summary of 10th International Workshop on Grapevine Trunk Diseases (IWGTD) Sessions

The IWGTD is organised by the ICGTD every two years to discuss recent research on the etiology, epidemiology and control strategies of different grapevine trunk diseases including Botryosphaeria dieback, Eutypa dieback, young vine decline and other trunk disease pathogens. Approximately 220 participants from 27 countries including Europe, the USA, Canada, South America, China, Middle East, New Zealand and four representatives from Australia attended the workshop.

This report will cover the selected presentations on diagnostic tools and epidemiology that relate to the Australian wine industry through Wine Australia funded research. The sessions on disease resistance/tolerance and management strategies will be covered in a separate report by Mr. Matthew Ayres.
1) Molecular diagnostic tools to detect and quantify GTD pathogens for young vines. These presentations focused on different DNA-based techniques to detect pathogens from nursery plants and other environmental samples. Such information is relevant to Dr. Billones-Baaijen’s current AGWA-funded research (SAR 1601) which aims to quantify levels of GTD pathogens from nursery propagation material and determine different stress conditions that trigger disease expression in vineyards. Some new approaches presented at the workshop that can complement the current project may be potentially explored.

a. **R. BILLONES-BAAIJENS, J. URBEZ-TORRES, M. AYRES, M.R. SOSNOWSKI and S. SAVOCCHIA.** Development of molecular tools for the detection and quantification of Eutypa and Botryosphaeria dieback pathogen inoculum in **Australian vineyards.** This presentation focused on the molecular tools developed to detect and quantify ED and BD pathogen inoculum in Australian vineyards (Figure 2). The two qPCR assays using multi-species primers was shown to detect multiple species of the ED and BD pathogens known to be present in Australian vineyards. These tools are essential for the investigations on spore dispersal patterns of these pathogens in the vineyard as one of the objective of the recently completed AGWA-funded research (SAR1205). To her knowledge, these qPCR assays are the first DNA-based techniques developed to detect and quantify multiple ED and BD pathogen inoculum from the environment. The qPCR developed in this study were shown to be rapid and sensitive in detecting ED and BD pathogens from environmental samples and are currently being used to analyse spore trap samples from different viticultural regions in Australia.

b) **Dr. Jose Urbez-Torres - DNA-macroarray to determine the health status of the grapevine nursery propagation material in British Columbia.** This DNA-based diagnostic technique is a rapid and specific tool to detect and identify plant pathogens in a single test. This technique was successfully developed by Dr. Urbez-Torres to detect young vine decline (YVD) pathogens from nursery plant materials in British Columbia. Dr. Baaijens conducted hands-on training on the DNA macro-array with Dr. Urbez-Torres in Summerland, British Columbia in March-May 2016. This travel was financially supported by Wine Australia (GWT 1421) and NWGIC. Dr. Billones-Baaijens can potentially conduct collaborative work with Dr. Urbez-Torres in the development of DNA macro-array for ED and BD pathogens that can complement the analyses of spore trap samples and nursery plant materials as this will allow the identification of individual species trapped in vineyards. However, it requires some funding to purchase specialised equipment, particularly the hybridisation oven and the chemiluminiescent imaging system, which is currently not funded in the new project.

c) **Marion Sineux et al. - Sampling method of young plant tissues in grapevine nurseries for GTDs.** This study focused on the development of sampling method on young grafted plants to detect different trunk disease pathogens in French nurseries. The method used qPCR to detect and quantify five pathogens (*Phaeoacremonium aleophilum, Phaeomonieila chlamydospora, Diplodia seriata, E. lata and Neofusiccom parvum*). For testing, entire young grafted plants were cut in several fragments and used for DNA extractions and each fragment was analysed by qPCR to quantify the amount of pathogen DNA present in each sample. Their results showed that low concentrations of either *P. aleophilum, P. chlamydospora, D. seriata* and *N. parvum* were detected in all samples but none of the wood samples tested positive to *E. lata.* Higher concentrations of these pathogens were associated with wounds such as graft union or disbudding injuries and basal end of cuttings. This study allows detection of GTD pathogens in grafted plants but still involved destructive sampling.
c) Dr. Eline Van Zil de Jong et al. - Molecular diagnostic assays for evaluating the impact of hot-water treatment on grapevine trunk pathogens. The study developed a qPCR method to detect fungal pathogens associated with BD, ED, Black foot disease and Petri disease of grapevines in New Zealand. These assays were shown to detect the target pathogens from grapevine tissues to nanogram levels. A low number of grafted plants subjected to pre-grafting hot-water-treatment (HWT, 48°C and 50°C for 30 minutes) were tested positive to some GTD pathogens indicating minimal benefits to pre-grafting HWT. The authors further reported reduction in nursery recovery and initial vine fitness when vines were subjected to pre-grafting HWT.

d) Cedric Moisy et al. - Quantitative assessment of grapevine wood colonization by fungal pathogens for association genetic studies. The objective of this study was to develop a qPCR method to identify varieties resistant to GTDs. The authors studied the grapevine genome to understand the variation in susceptibility of different grapevine genotypes to GTDs. This allowed them to identify new genetic markers associated with susceptibility and tolerance to GTDs that will assist and fast-track selection for breeding and varietal improvement.

e) Erin R. Galarneau et al. - Whole-plant response during the early stage of infection to the wood-canker pathogen Neofusicoccum parvum (Botryosphaeria dieback). This study developed a detection tool for the early stage of BD infections from nursery materials using leaves instead of wood. The authors initially identified plant defense genes that are expressed at early stage of infection. By quantifying the expression of these defense genes from young leaves, the authors were able to detect the early stage of N. parvum infections. The use of leaves to detect infection, therefore, reduced the need for destructive sampling to detect GTDs from nursery plant materials. This technique can be potentially explored and incorporated into the on-going research (SAR 1601) aimed at detecting and quantifying latent infections in nursery plant materials.

f) Ales Eichmeier et al. – Spatial and temporal variation of active fungal communities on grapevine propagating material after hot-water-treatment. This study investigated the effect of HWT on the active microflora and GTD pathogens present in young vines. Dormant grafted vines were subjected to HWT at 50 and 53°C for 30 minutes before planting in vineyards. Fungal communities within the grafted plants were evaluated immediately after HWT and after one growing season using conventional techniques and metatranscriptomic sequencing of the ribosomal DNA region. The metatranscriptomic approach was able to detect more diverse and complex microflora compared to conventional techniques. The results showed that HWT reduced the number of fungal microflora including the GTD pathogens but these fungal communities increased after one growing season. This study also demonstrated that HWT does not completely eliminate fungal species within the wood tissue of grafted vines.
2) **Molecular tools to study the epidemiology of GTD pathogens.** These presentations focused on different DNA-based techniques to detect and quantify different GTD pathogens from vineyards and other environmental samples. The information is relevant to Dr. Billones-Baaijen’s on-going AGWA-funded research (SAR 1601) looking at the spore dispersal patterns of ED and BD pathogens in Australian vineyards. Some new approaches presented at the workshop that can complement the current project and be potentially explored.

a. **R. BILLONES-BAAIJENS, S. SAVOCCHIA, M. AYRES and M.R. SOSNOWSKI** - Detection and quantification of Diatrypaceae and Botryosphaeriaceae inoculum in Australian vineyards. This study is the first comprehensive study to investigate the spore release patterns of ED and BD pathogens in Australian vineyards. Dr. Billones-Baaijens presented the preliminary results of the spore trapping studies conducted in New South Wales (NSW) and South Australia (SA) using the qPCR assays she developed to quantify and detect these pathogens from vineyards. The results showed the ED and BD spores were released sporadically all year round. However, seasonal release and no. of spores differ between regions and pathogens with high spore release in winter for SA while high spore numbers were released in summer in NSW. Rainfall was found to be the primary factor in the spore release with as little as 0.2 mm resulting in spore release. However, not all rain events resulted in spore release. Furthermore, spores were released a week after rainfall on a few occasions. The number of ED spores trapped were also higher (up to 7,500 spores) compared to BD spores (up to 1,300 spores). The difference is mainly due to the size and mode of discharge of these pathogens. ED pathogens are known to eject their spores actively while BD spores are primarily rain-splashed and less airborne. These data will determine the critical times of the year when GTD pathogen spores are abundant in vineyards which will assist growers in making decisions on optimal timing of pruning and wound treatment.

b. **Monica Berbegal et al.** - Development of real-time PCR protocol to quantify the airborne inoculum of *Phaeomoniella chlamydospora*. This study developed molecular tools to detect and quantify the inoculum of the Petri disease pathogen *Phaeomoniella chlamydospora* as per to Dr. Baaijen’s studies (SAR1205). The authors developed a DNA extraction protocol and qPCR assay to detect and quantify *P. chlamydospora* spores from Spanish vineyards. Instead of using Burkard spore traps as per SAR1205 project, this study used slide-mounted tapes to collect spores in vineyards.

c. **Dr. Jose Urbez-Torres et al.** - Grapevine trunk diseases epidemiological studies in British Columbia – Implementation of Droplet Digital PCR. The objective of this study was to detect and quantify ED and BD inoculum in British Columbian vineyards using the droplet digital PCR (ddPCR). This new technology allows absolute quantification of DNA or RNA molecules and is reported to be more sensitive and accurate than qPCR. To collect spores from vineyards, five cyclone samplers (Burkard Manufacturing Co. Ltd) were deployed in five different vineyards along the Okanagan Valley. Dr. Billones-Baaijens, Dr. Savocchia and Dr. Sosnowski were co-authors of this paper as the ED and BD multi-species primers designed by Dr. Billones-Baaijens (SAR 1205) were used for ddPCR as part of her collaborative research with Dr. Urbez-Torres (GWT 1421). Dr. Urbez-Torres’ team at SuRDC tested these multi-species primers and they were shown to be highly suitable for ddPCR technology. Preliminary results from their spore trapping studies showed that ED and BD spores were released intermittently throughout the growing season. The ED and BD inoculum were generally detected during late winter and early spring when the temperatures are above freezing and correlated but not always with
rainfall. The results from the BC spore trapping studies are very similar to the data collected from the spore trapping studies in Australia (SAR 1205) confirming that rain is the primary factor for the spore release of these pathogens in vineyards.

d. **Lizel Mostert et al. - Detection and quantification of black foot pathogens in grapevine nursery soil, rotation crops and weeds in South Africa.** This study developed qPCR assays to detect and quantify black foot pathogens from soil. The authors developed genus-specific primers targeting the species belonging to the genus *Cylindrocarpon* which are primary pathogens causing black foot disease. The results showed that black foot pathogens were detected from rhizosphere soils from five nurseries over three years. These pathogens were detected in soil samples collected at a depth of up to 60 cm. The authors also investigated the effect of crop rotation on black foot pathogens. This involved a 3 year survey of plants and soil conducted at five nurseries. Young nursery vines, rotation crops (Triticale, lupins, canola, white mustard and forage radish), weeds and soil were collected from each nursery between 2013 and 2014. Several black foot pathogens were isolated from young vines, one species each were isolated from triticale roots and a weed called Corn spurry. No black foot pathogens were isolated from the roots of rotation crops: lupins, canola, white mustard and forage radish.

e. **Olivier Lobregat - Study on the contamination kinetics of young plants by fungi responsible for grapevine trunk diseases, from plant materials produced free from pathogens.** This study investigated the role of plant materials in the spread of GTD pathogens. Green grafted plants that are free of GTD fungal pathogens and standard lignified grafted plants of the same root-stock/scion combination were planted in nursery beds. Two years after planting, wood samples were collected from both type of materials and tested for GTD contamination. Disease assessment two-years post-planting showed both the disease-free and standard grafted plants were positive to Botryosphaeriaceae species. These pathogens were both isolated from the surface (bark) and inside the woody tissues. These results indicate that infections of BD pathogens likely originated from the external environment rather than the propagation system.

5. **Main Outcomes**

Dr. Billones-Baaijens greatly benefitted from taking part in the workshop. Her oral presentations on new molecular tools developed for spore trapping studies in Australian vineyards showcased the capabilities of Australian researchers in the field of molecular biology and GTD epidemiology. Her presentations were well received by the workshop participants, attracting much interest. She gained further knowledge on new developments in molecular techniques for pathogen detection and epidemiological studies of GTDs. The majority of the papers presented at the workshop included DNA-based techniques in their methodology, demonstrating the importance of molecular expertise in the field of GTD research. Some of these molecular techniques can potentially complement her current research and can be further explored and incorporated into her studies. Dr. Billones-Baaijens' attendance at the workshop also strengthened her links with researchers in Europe, Canada, USA and New Zealand. She also gained more knowledge in the field of disease management and disease resistance. This information will be reported by her fellow participant and colleague Mr. Matthew Ayres.
6. Communication

A summary of the workshop will be collectively prepared for submission to an Australian industry journal by Mr. Matthew Ayres, Dr. Billones-Baaijens and Dr. Sosnowski. This information will increase the knowledge base of the wine industry in the field of GTD diagnosis and management. All abstracts from the workshop will be further published in the international journal Phytopathologia Mediterranea later this year.

7. Recommendations

It is recommended that the established links with other overseas GTD researchers be maintained in order to keep Australian scientists and grape growers up to date with research and development in GTDs. This includes:

a) Utilising the knowledge gained from Dr. Jose-Urbez-Torres and Dr. David Gramaje in identifying GTD pathogens and to determine infection thresholds from young vines. This involves the adoption of a DNA macroarray and ddPCR but requires acquisition of equipment for these technology to be used for the Australian wine industry. Financial support to acquire these equipment from the industry and the university will assist in ensuring that the Australian wine industry remains at the forefront of the latest molecular technology in GTD research.

It is further recommended that Wine Australia continue to support early career researchers to attend conferences to aid professional development and establish links and collaboration with other researchers from overseas.

a) Establish stronger links with Dr. Dario Cantu at UC-Davis to enhance Dr. Baaijens' skills in bioinformatics and genomic studies.

b) Develop collaborative work with Dr. Moustafa Selim at Geisenheim University, Germany for Dr. Baaijens' to gain experience in the field of imaging technologies to diagnose and study GTDs.
8. FIGURES

Figure 1. Participants in the 10th International Workshop on grapevine Trunk Diseases, Reims, France.

Figure 2. Experimental vineyard at Epernay, visited by workshop participants.
Figure 3. Regina Billones-Baaijens during her presentation on the molecular tools to detect Eutypa and Botryosphaeria dieback pathogen inoculum in Australian vineyards.

Figure 4. Dr. Jose Urbez-Torres’ presentation on the DNA-macroarray to determine the health status of grapevine nursery materials.
Figure 5. Marion Sineux’s presentation on the diagnostic tool and sampling method of young plants in grapevine nurseries.

Figure 6. Regina Billones-Baaijens presenting her paper on the detection and quantification of Eutypa and Botryosphaeria dieback pathogen inoculum in Australian vineyards