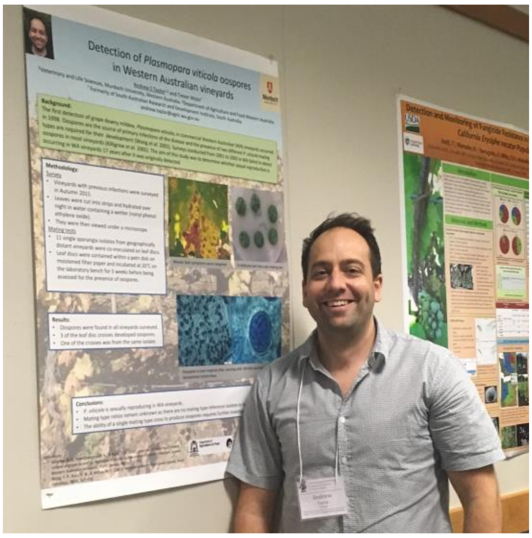
Murdoch
UNIVERSITY

Australian Government
Australian Grape and Wine Authority

Attend and present PhD research findings at the 8th International Workshop on Grapevine Downy and Powdery Mildew in Oregon



FINAL REPORT to
AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AGT 1612**

Principal Investigator: **Andrew Taylor**

Research Organisation: **Murdoch University**

Date: **25 August 2017**

Project Number: AGT 1612

Project Title: Attend and present PhD research findings at the 8th International Workshop on Grapevine Downy and Powdery Mildew in Oregon

Project Supervisor: Andrew Taylor
PhD candidate
Murdoch University
School of Veterinary and Life Sciences
90 South Street, Murdoch
Western Australia 6150

Acknowledgements

The activities in this report were financially supported by Wine Australia and Murdoch University travel bursaries.

Contents

1. Abstract 4
2. Executive Summary 4
3. Summary of workshop 5
    3.1 Disease management 6
    3.2 Breeding and host resistance 7
    3.3 Fungicide resistance 7
    3.4 Plant microbe interactions and population genetics 7
    3.5 Disease management and decision support systems 7
    3.6 Epidemiology 9
    3.7 Posters 9
4. Field visits 9
    4.1 Bethel Heights with Ted Casteel 9
    4.2 Willakia with Geoff Hall. 11
    4.3 Stoller Family estate with Jason Tosch 12
    4.4 Knudsen vineyard with Allen Holstein. 13
    4.5 Lumos winery. 13
5. Professional development 14
6. Outcomes 15
7. Communication 16
8. Recommendations 16

1. Abstract

Andrew Taylor attended the 8th International Workshop on Grapevine Downy and Powdery Mildew in Corvallis, USA, 17-19 July 2017 and provided an oral presentation and poster on his current PhD project on downy mildew in Australian vineyards. A total of 19 presentations and five posters were delivered over the two day workshop with the program being available at http://gdpm2017.org/congress/program/. Training in the use of the population analysis package Poppr was undertaken post workshop. New networks and potential collaborations were established with international researchers. An article is in preparation in an industry journal.

2. Executive Summary

Andrew Taylor, PhD student at Murdoch University and Research Officer at DPIRD Western Australia attended the 8th International Workshop on Grapevine Downy and Powdery Mildew in Corvallis, USA, 17-19th July 2017.

This international workshop, co-hosted by Oregon State and Washington State Universities, provided the attendees a forum to present current research on downy and powdery mildew that has been established in the four years since the previous workshop. Attendees at the workshop represented research institutes and members of industry in Oregon and Washington. Powdery and downy mildew are considered the top two diseases for industry wide economic impact on grapevines in Australia.

A total of 19 presentations and five posters were presented over two days with sessions including disease management, breeding and host resistance, fungicide resistance, population genetics, plant microbe interactions, decision support systems and epidemiology. Andrew presented information regarding his PhD studies on the genetic population of downy mildew in Australia. He also presented a poster detailing the detection of downy mildew oospores in Western Australian vineyards. The complete program of presentations can viewed at http://gdpm2017.org/congress/program/.

Vineyard visits to the Willamette valley enabled workshop participants to gain an understanding of the production systems established in the region as well as current research being undertaken within them.

Post workshop training in population statistical analysis with students in the Grünwald lab, USDA/Oregon State University, was undertaken using the Poppr package in R. This training will aid in current PhD activities and potential new projects. Networks established here and at the workshop will provide for future research collaboration potential. Funding for the travel was provided by Wine Australia and Murdoch University. An article is in preparation for publication in an industry journal.

3. Summary of workshop (co-written between Dr Suzanne McKay and Andrew Taylor)

Thirty delegates from seven countries, including six Australians, attended the workshop, at Oregon State University, Corvallis, Oregon, USA (Fig 1). The delegate number was considerably smaller than usual because of a last minute change of host country. However, the smaller numbers allowed for a lot of positive interaction, lively discussion, problem solving and idea sharing. A tour visiting five vineyards in the northern Willamette Valley was held on the final day of the workshop. A number of internationally renowned grape pathology experts have retired in recent years and further retirements were announced at the conclusion of the workshop. A summary of the presentations is outlined below.

Fig 1. Delegates from the 8th International Workshop on Grapevine Downy and Powdery Mildew (top) and Australian delegates, Bob Emmett, Peter Magarey, Suzanne McKay, Andrew Taylor, Eileen Scott and Barbara Hall (bottom).

3.1 Disease management

Dr Marc Raynal from the French wine and vine institute (IFV) presented the initial findings on research into studying the variability in sensitivity of vineyards over large scales (100 hectares) using information from sensors placed within a vineyard in combination with geographical information systems (GIS) data. These zones within vineyards are known as 'Physiological behaviour units' (PBUs). His study aims to use the PBUs to explain differences within a vineyard for vine physiological development and their susceptibility to disease. With a French national policy to reduce pesticide usage by 50% by 2025 and 80% of fungicides are applied for downy (DM) and powdery mildew (PM) control there is a need to allow for precision techniques in combating these diseases. The GIS maps represent both soil and plant information and therefore provide information of the variability of both the physiological behaviours of the vines and their susceptibility to disease on a spatial scale. The initial results of the study have shown promise but data from several seasons is required before a complete understanding of the stability of the criteria studied is known. The group are also investigating the addition of meteorological data. The long-term goal is to add the PBUs as another factor in existing models/decision support system to assist with decision making and reduce pesticide applications.

LIFE FITOVID is a project aimed at implementing demonstrative and innovative strategies to reduce the use the of phytosanitary products in viticulture in Spain. Ana Diez is project manager and explained that current applications for control of DM in some growing regions of Europe can range from 6-19 whilst for powdery they can be between 7-10. Using two high disease pressure sites the grower spray schedule was compared to that of different disease management schedules, using phenology and weather based information, and those of organic labelled fungicides. A disease management system based on degree-day accumulation, phenology and conventional fungicides reduced the number of applications compared to that of the grower applications. Disease risk based on the Gubler-Thomas model for downy mildew showed a similar result.

Brent Warneke is a master student in the Mahaffee lab investigating fungicide selection and timing. His research is investigating the protective, systemic, translaminar and vapour phase mobility of fungicides registered for PM in Oregon and applied during the stages of inflorescence. Leaf disc bioassays were conducted and compared to that of a control and a sulphur application. A small field trial with spray applications at BBCH stage 55 (Inflorescence swelling, flowers closely pressed together), 65 (Full flowering: 50% of capfall fallen) and 69 (End of flowering) was conducted in both 2015 and 2016. It was discovered that most modern chemistries have some form of mobility and vapour activity. Luna Privilege, Quintec and Flint were most efficient when applied at the end of bloom. Luna Privilege is not registered in Australia for PM on grapes.

The evolution of PM with daily light cycles is being investigated as a potential method of suppressing the disease by Dr Gadoury and researchers at Cornell University. Initial studies using UV applications found that applications were successful on PM control by disrupting several processes but had a deleterious effect on the vines due to radiation. New technologies with solid-state lighting and the reduction in costs for LED technology has led to advancements in this research. Long-wavelength red lighting has been found to suppress PM and in combination with night time applications of UVB and UVC has shown promising results with no side effects. Further work is looking at pulsing different wavelengths of light to determine optimum timing of dosage and any impacts it has on photosynthesis. Although promising an issue will be how to commercialise field applications of the technology so that each plant gets the right dosage of light for suppression over large scale vineyards.

3.2 Breeding and host resistance

Dr Sabine Wiedemann-Merdinoglu presented updated information on the breeding program for resistant vine cultivars to DM and PM. Known as INRA-ResDur, the program began in 2000 with the aim to produce vines with durable resistance with high quality wine traits. The breeding program has used both genetic markers and traditional breeding from mildew resistant *Vitis* species (American and Asian species) and cross bred them with traditional varieties to produce new cultivars with the same wine quality. The genetic markers identified are known as RPV, resistant to *Plasmopara viticola*, and REN, resistant to *Erysiphe necator*. Like most horticulture breeding programs it takes several years from breeding to commercial release. The breeding program has many collaborators including CSIRO in Australia. Two red varieties, IJ58 and IJ134 and a white variety COL-2007G have shown promise. A number of other varieties are slated for release in 2017 and 2024.

3.3 Fungicide resistance

Dr McKay and Mrs Hall from SARDI presented updated information about fungicide resistance in Australia for PM and DM. Resistance to the Quinone outside Inhibitor (QoI) fungicide pyraclostrobin has been detected in around 50% of the powdery mildew samples tested. Although resistance was found to the Demethylation Inhibitor (DMI) fungicide, myclobutanil, it was not widespread, with only 14% of powdery samples resistant. Resistance was not detected to the DMIs penconazole or tetraconazole. For DM, metalaxyl resistance has now been found in New South Wales and Western Australia. The G143A allele, linked with QoI resistance, was detected in one sample of DM from New South Wales.

3.4 Plant microbe interactions and population genetics

Andrew Taylor from Murdoch University presented preliminary results of his PhD study investigating the population genetics of DM in Australia. There were two original detections of DM in Western Australia, a 1997 detection in a remote location in the states northern regions and a 1998 detection in the Swan Valley. Using microsatellite primers on a number of isolates collected, it has been determined that they are genetically the same and therefore only a single introduction event has occurred. Further work will investigate the Australia population as a whole and compare those of the European and American populations.

3.5 Disease management and decision support systems

VitiMeteo (VM) is a modelling platform, which is a cooperative project between Institutes in Germany and Switzerland and a private German company, GEOsens. Gottfied Bleyer, from State Institute for Viticulture and Enology, Freiburg, Germany, outlined the recent additions and improvements to VM, in particular the addition of information about trellising systems and growth stage data (BBCH phenology scale and canopy leaf area). When using VM, these additions have resulted in a decrease in severity and incidence of downy mildew under high disease pressure when the leaf area is between 400 to 600 cm^2 following the use of both potassium phosphonate and Folpet. This occurred on both leaves and bunches. When the model suggests there is no infection risk for DM it automatically moves to modelling the risk of PM infection. Dr Pierre-Henri Dubuis, from Agroscope Changins, Switzerland, reported the success of the use of VM in reducing the average number of sprays for powdery by one/year with no reduction in efficacy. VM was more effective on DM with a reduction of up to 4 sprays a season depending on pressure. When a tool called 'Crop adapted spraying' (CAS), which estimates foliar volume, is added to VM, it was possible to reduce the quantity of fungicide applied per season by 20% or 163 Swiss francs/ha. VM and related tools like CAS are available to Swiss and German viticulturists on the platform http://www.agrometeo.ch free of charge.

DM is a significant problem in Italy and growers are currently spraying with 18-20 spray applications a season, usually on a calendar based approached. Dr Tito Caffi, from the Università Cattolica del Sacro Cuore, Italy, described the success, particularly for Organic growers, of the web-based support system, Vite.net®. Vite.net® is based on grapevine phenology and canopy development, soil water status as well as meteorological data, risks for DM, PM, Bot outbreaks as well as for insect pests are delivered to growers via alerts. Vite.net® forecasts risk and it is up to the growers to make informed decisions about spraying. The system was validated over a network of 21 organic vineyards in Italy, where disease control and costs were compared for parts of the vineyard that used Vite.net® with those that did not. Although disease control was not different the amount of copper applications were reduced when using Vite.net® by almost 40% with a saving of 195€/ha each year. Since its development in 2011 the grower usage has risen from 20 to 300 in 2017. There has been good grower uptake of the system because of it has very simple graphic interfaces, the system is continually updated based on grower feedback and regular grower contacts are maintained.

Mr Magarey from Australia introduced GrowCare, a web-based tool designed to alert growers to better manage DM and PM. Based on weather-data, subscribed growers receive an infection alert via sms. Growers can access GrowCare to determine likely occurrences of downy and powdery to help decide on pre-infection sprays.

Dr Odille Carisse from Agriculture and Agri-Food, Canada, has been using a network of spore traps to detect PM and DM airborne inoculum among the small-sized but rapidly expanding number vineyards in Ontario and British Colombia. Inoculum density was determined using qPCR and disease risk was related to inoculum density A PM model was developed with spray intervals based on a threshold of 15-20 conidia/m^3/day combined with growth stage. Initial studies indicate a reduction of between 25-50% of the required fungicide applications. The DM population has shown a shift in cryptic species during the season.

Fungicide resistance monitoring is also carried out using pooled samples from several vineyards and pyrosequencing is used to detect resistance alleles from SDHIs and QoIs and other fungicides. Their findings suggest that botryticides are starting to show signs of resistance, by both detection of particular resistance alleles and decreasing field performance. Interestingly, results of grower interviews indicate that they do not want models, they want a spore trap on their property, someone to visit and to interpret the data. One other important issue observed is that growers don't understand the concept of fungicide resistance as it is a very complex area to understand and develop solutions.

Dr Mahaffee, USDA, USA, compared the Caffi, Carrise, Moyer and Gubler/Thomas models to assess their suitability to predict powdery mildew ascospore availability during the season in the maritime climate of the Willamette valley in Oregon. All models over-predicted cleistothecia dehiscence and inoculum availability. It is still unknown why cleistothecia develop in Oregon as studies on the effect of climate found it did not impact initiation. Overall findings suggest that empirical models are not necessarily suitable for environments where neither the host nor pathogen is native.

3.6 Epidemiology

Contradicting evidence of the susceptibility of grapevine clusters to DM infection in the laboratory compared to that seen in the field at led Swiss researchers to investigate disease host-interactions through artificial inoculations. Infections were conducted at BBCH 55 (inflorescence swelling, inflorescence fully developed, 65 (full flowering), 75 pea-sized berries) and 81 (veraison) and microscopically examined. It was determined that on a susceptible variety infection occurs at all growth stages except veraison. There is no evidence of systemic development of DM within clusters.

3.7 Posters

5 posters on subjects including DM population biology, fungicide resistance testing and disease monitoring were presented at the workshop. Further detail can be found in the proceedings.

4. Field visits (co-written between Dr Suzanne McKay and Andrew Taylor)

Low input viticulture and enology (LIVE) certification scheme. The LIVE certification scheme is a whole of farm certification system implemented in Pacific Northwest vineyards that supports environmentally and socially responsible winegrowing. The program is based on standards of international protection with working-group committees deliberating on regional standards. These standards are accredited by the International Organisation for Biological and Integrated Control and compliance with the standards are audited by a third party annually. At present 328 vineyards and 42 wineries are members.

<u>Northern Willamette Valley</u>

4.1 Bethel Heights with Ted Casteel

Founded in 1977 by the Casteel and Dudley families, Bethel Heights is one of the original vineyards established in the Willamette valley (Fig 2). From an original 14 acres the vineyard has grown to 100 acres with pinot noir representing 70% of the plantings. In the 1990's the introduction of phylloxera to the region meant a program of systematic replanting from own roots to grafted vines was required. As only 8000-9000 cases are produced a year they sell at a high price point which is enabling them to continue at a strategic replanting. Forty percent of sales are through the cellar door.

This vineyard is certified by the LIVE scheme. They use inter-row flowering plants to maintain predator biodiversity to control insect pests (Fig 3) and as a result have never sprayed with an insecticide, except for a pest-oil for rust mite. Many areas of the vineyard still have narrow rows and tight within row spacing's which poses some problems for mechanisation. They have some years with high disease pressure, but in general their spray program consists of sulphur every 7-8 days unless they have used a synthetic e.g. Quintex, Vivando or Turino. They have their own rotor-rod spore trap in the vineyard, owned by a private company, who collect the rods weekly and process them using qPCR. The result are an estimate of the number of powdery mildew spores on each rod, which may be indicative of the overall the spore load in the vineyard. Spray scheduling is based around these figures. At present red blotch virus is the biggest concern and money is being spent on researching vectors of the virus. In southern Oregon vineyards have become unproductive in 3-4 years post infection so the industry is harmonising quarantine rules.

Fig 2. Dr Suzanne McKay (SARDI) overlooking the Bethel Heights vineyard.

Fig 3. Inter-row flowering cover crops to encourage biodiversity to encourage predators for biological control of insect pests.

4.2 Willakia with Geoff Hall.

The Willakia vineyard is a member of the Ste. Michelle Wine Estates group of vineyards (Fig 4). The Willakia estate consists of a total 300 acres with 100 acres of pinot noir and 19 acres of chardonnay. They produce medium to premium wines with their flagship wine being a Pinot Noir, selling at $50-60/bottle. Their main challenges are the different row spacings throughout the vineyards and the implications this has for mechanisation and controlling vigour due to the high rainfall, clay soils and high organic matter, which results in high disease pressures. Majority of blocks are caned pruned but some are spur pruned. Vines are leaf plucked by hand on the side that receives the morning sun. Yields vary depending on target market with some blocks cropped at 2.5-3t/acre and others at 7-8t/acre. In the past they were not highly mechanised as there was a ready supply of cheap labour but now this situation has changed they have to become increasingly mechanised. They use a harvester to shake trash from bunches to decrease the Botrytis risk. This stimulated much discussion amongst conference participants about the efficacy of this method to open bunches and therefore mitigate disease.

Fig 4. The Willakia vineyard, Willamette Valley showing the narrow row spacing's.

4.3 Stoller Family estate with Jason Tosch

Located in the Dundee hills and was originally a turkey farm but was converted to a vineyard in 1995. The estate now consists of 373 acres with 215 under vines. Of the 215 acres of vines, Pinot Noir consists of 121 acres, Chardonnay 53 acres, Pinot Gris 10 acres with the remainder a mix of Riesling, Syrah, Pinot Blanc. Of the 400 ac property ~ 25% is set aside for environmental compensation area to remain as natural woodland. This vineyard is certified by the LIVE scheme. Stoller vineyards and winery are aiming to become zero net energy, by becoming fully solar powered and having their own water collection and recycling all on-site. They were the first in Oregon to be leadership in energy and environmental design (LEED) accredited. They use pest and disease scouts weekly to look for PM, insects, virus and water stress. OSU Masters student, Brent Warneke (Fig 5), told us about his research carried out at this vineyard, about fungicide mobility and how this affects timing of application to be most effective. A research tower with multiple growth and pathogen sensors from the Mahaffee lab (USDA) is also located at this vineyard (Fig 6).

Fig 5. Brent Warneke, master's student from Oregon State University, describes his research at the Stoller Family estate vineyard.

Fig 6. USDA tower with sensors (left) and Peter Magarey inspecting the tower (right)

4.4 Knudsen vineyard with Allen Holstein.

The Knudsen vineyard was first planted in 1974 and now consists of 127 acres of planted vines, predominantly Pinot Noir and Chardonnay. The vineyard is planted on a slope and this means an elevation differential of approximately 150 meters that influences ripening. The steep nature of the slope means erosion can be an issue and cover cropping with oats is used to maintain top soil. Originally started with Californian production systems, which in time, were not suited to the growing conditions of Oregon, the vineyard was then taken over by French growers who changed some of the vineyard to narrower spacings, in some blocks the planting density is approximately 3000 vines per acre. This has meant some issues with botrytis as the vines can be constantly shaded but applications of botryticides at bloom and bunch closure prevent epidemics. Given the small spacing specialty tractors are required for vineyard work with the first only being purchased in 1998. After a brief ownership by Australians, who put in the first drip irrigation system in the area, it now has new owners who are now slowly transitioning the vineyard to production systems more suited to Oregon where both canopy vigour and slow ripening needs to be controlled. . Phylloxera appeared around 25 years ago and has slowly spread but they do not see it as a major problem.

4.5 Lumos winery.

Workshop dinner was held at Lumos winery. A toast was made in memory of Dr Trevor Wicks who contributed many years of research to the control of both powdery and downy mildew in vineyards.

5. Professional development

Post workshop I spent a week with PhD and post-doctoral students at the Grünwald laboratory in Corvallis, Oregon learning aspects of computational population biology. The group included Niklaus Grünwald, Brian Knaus, Javier Tabima, Shankar Shakya and Zachary Foster. Poppr is an R package (below) developed by researchers at the Grünwald laboratory for the purposes of genetic population analysis (Kamvar *et al*. 2014). Poppr is ideally suited to my PhD (project GWR Ph1301) study on grape downy mildew as it provides analysis for populations that are either sexual, clonal or a combination of both, for which grape downy mildew has the potential to be. As part of my PhD project I have collected genetic microsatellite data from the grape downy mildew populations in Australia, Europe and America and using the tools within Poppr I can determine whether the populations are defined by geographic locations. Other aspects of Poppr allow populations to be assessed for genetic diversity and population structure within vineyards, regions or at larger scales.

R is a coding language, used amongst other things for statistical analysis and computing, that has risen in popularity in recent years. R is available for free (https://www.r-project.org/) and allows programmers to develop their own statistical and graphical packages by writing their own code and lodging them in a number of searchable repositories, for example CRAN and GitHub. R can run numerous types of code and can be run on different computer operating systems. The packages are often maintained by the original designer or laboratory for which they are created and therefore improvements can be ongoing. This allows other users with similar statistical requirements to download packages and analyse their own data without having to write large amounts of new code. The ability to produce graphical plots of publishable quality is a key feature that makes R highly extensible.

Grape downy mildew is not present on *Vitis vinifera* in Oregon but is present on related species Boston Ivy (*Parthenocissus triscuspidata*) and Virginia creeper (*Parthenocissus quinquefolia*). Research has recently indicated that although the species is the same, *Plasmopara viticola*, there are some genetic differences between that which is found on grape and those of other hosts. A survey of the local area revealed some plants of Boston Ivy (Fig 7) that were infected and I was able to collect DNA samples using the quarantine import permit I have obtained and can use this DNA for comparison against the Australian samples I have collected.

Whilst surveying for samples I came across the Pixie™ grape that are dwarf *Vitis vinifera* vines (Fig 8). Bred by the USDA for research purposes these vines have now been commercialised for retail sale. They grow to approximately 60cm and produce fruit clusters. They would be ideal for research on obligate biotrophs such as downy and powdery mildew as they produce functional leaves but take up less area than conventional *Vitis vinifera* vines. At present Pixie™ cultivars include Pinot Meunier, Cabernet Franc and Riesling.

Kamvar ZN, Tabima JF, Grünwald NJ. (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281. doi: 10.7717/peerj.281

Fig 7. Downy mildew infection of Boston Ivy (Parthenocissus triscuspidata) plants in Oregon.

Fig 8. Pixie™ grapes are dwarf vines ideal for research.

6. Outcomes

- Increased understanding of the issues and solutions surrounding downy and powdery mildew in northern hemisphere grape production and how these could be transferred to Australian production systems. In particular modelling and decision support systems.
- Collaboration with the Grünwald laboratory.
- Developed new techniques and skills for analysing data with the use of new statistical packages. These skills can be transferrable to other projects.
- Obtained isolates of downy mildew to be added to the genetic analysis of Mr Taylor's PhD study.
- Increased biosecurity awareness and challenges associated with exotic pathogens to Australia. In particular threats such as red blotch virus and phylloxera.
- A number of pathology experts have announced their retirements.

7. Communication

A joint article from Australian workshop participants is being prepared for the Australian and New Zealand Grapegrower and Winemaker magazine.

A small article will be submitted for the September 2017 Wine Industry Newsletter (WIN) prepared by the Department of Primary Industries and Regional Development WA.

8. Recommendations

1. Maintain linkages with international researchers and organisations. A number of experienced grapevine pathologists worldwide have or are in the process of retiring, taking a great deal of knowledge with them. Linkages developed at workshops can be mutually beneficial to Australian and overseas industries.
2. Wine Australia to continue to support travel bursaries, either for researchers in Australia to head overseas or for overseas researchers to come to Australia.
3. That the feasibility of importing the Pixie™ grape to Australia to assist researchers of grape diseases be determined.
4. Maintain related skills in grapevine pathology in Australia. With the number of retirements of internationally renowned researchers there is a need to maintain expertise in this field.